Novel insights in the transmission of *Rice yellow mottle virus* in irrigated rice

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CHAPTER 1

INTRODUCTION

Rice production in West Africa

Rice is the major food source for about 40% of the world's human population (Ng *et al*., 1988, WARDA, 1993). While the principal rice producing countries are found in Southeast Asia, rice is also produced in all West African countries with a production amounting 900,000 tons in 1998, corresponding with approximately 1% of the global rice production.

Cultivars of the rice species *Oryza sativa* are widely grown in Asia, whereas cultivars of the species *O. glaberrima* have been grown for ages in Africa. In the last century several *O. glaberrima* cultivars were replaced by higher yielding *O. sativa* cultivars in Africa (Pinto *et al*., 1999). Both species, belonging to the genus *Oryzae* in the family Poaceae, are annual grasses, which can grow for more than a year under favourable conditions, and are well adapted to aquatic habitats.

Both the growth of the human population and changes in food preferences have led to a greater demand for rice in West Africa, resulting in an imbalance between regional rice supplies and need. This has partially been compensated by an increased importation of rice at the expense of scarce foreign exchange (WARDA, 1996). Demand for rice in West Africa grew at an annual rate of 6% between 1973 and 1992. Rice imports grew at an annual rate of 8.4% and averaged 2.6 million tons in the early 1990s. The rice production in West Africa, amounting to 60% of the total production in the sub-Saharan countries, has annually grown 8.5% between 1983 and 1992, as a result of expanding the rice production area and an increase of 1.9% in yield per year (WARDA, 1996). Seventy two percent of the West-African rice is produced in Mali (Hirsch, 2000).

Rice production systems in West Africa

Rice is mainly a wetland crop by origin and is as such preferred by farmers. Its cultivation depends on the presence of water, which can be supplied by rain, by seepage of ground water, by flooding or by irrigation. A classification adopted by the West African Rice Development Association (WARDA) recognises three main ecologically distinguishable habitats, viz. an upland/lowland swamp continuum, the Sahel zone, and the mangrove swamps (WARDA, 1993).

The upland/lowland continuum ranges spatially from areas where rice is strictly rain-fed, via slopes where rice cultivation depends on seepage of groundwater, to swamp areas where the land is flooded. Temporary, this continuum changes from year to year as rainfall patterns differ, and land management alters the supply of water. A farm will often include parcels in the upland area and parcels in the valley swamp zone. The upland/lowland continuum, the major system of rice cultivation in West Africa, is restricted to areas with a high rainfall and suitable rainfall distribution over the year. Water conditions in the lowlands are usually not uniformly optimal for rice cultivation. Levelling and bounding fields to prevent water from flooding, and storage of rain water

or seepage water can improve rice production in these areas.

In the Sahel, most rice is grown in governmental development projects by creating large irrigated areas. Rice is usually the only crop cultivated in the rainy season. Farmers may cultivate other crops during the dry season. Outside the irrigated polders farmers may grow other crops, like millet and sorghum, during the rainy season.

Rice is produced on at least 180,000 ha of cleared mangrove swamps in West Africa (Fomba, 1988). These swamps are found along the banks of rivers and creeks and are subjected to flooding twice a day. Rice can here be grown when sufficiently refreshed during the rainy season by flood water. Successful rice cultivation in any area is strongly influenced by the length of the salt free period. Where the mangrove forests have been replaced by small polders, the land is irrigated by rain-fed rivers. Salinity of these rich soils forms a real problem in these polders.

Lowland production, including mangrove and deep water systems, covers about 60% of the total rice cropping area in West Africa. These systems comprise different levels of water management and land development. In some locations water supply can not be controlled, whereas in other locations rainwater can efficiently be retained or rice crops are well irrigated.

Irrigated production systems, with one or two crops per year, are found in all agro-ecological zones in the Sahel and in the upland/lowland swamp continuum of the humid forest zone. The average rice yields vary between 4 and 5 tons/ha in most West African countries, but individual farmers in the Sahel achieve yields close to the biological yield potential of 10 tons/ha in a single season (Dingkuhn and Sow, 1995).

Many technical, economical and social constraints exist in the rice production in West Africa. Shortage of farm labour is a major constraint. Field preparation, transplanting seedlings, weeding, bird scaring and harvesting are all either delayed or performed inefficiently because of the conflicting labour demands of other crops, sometimes with grave consequences.

The major constraint in the Sahel is water management. Whereas Asian farmers have centuries of experience in irrigation, the technique is relatively new to African farmers. Inadequate assessment of initial water resources, neglect of overall watershed management (leading to sedimentation of soil) or poor drainage (leading to salinization) create also large problems. In addition, poor water management stimulates the growth of weeds on dry fields. Yields are occasionally limited by adverse soil conditions like salinity problems, which affect large areas of the Niger delta.

The climate is another major physical constraint for rice production in the Sahel. Low temperatures and dust-laden winds discourage the growing of a second crop during the cold dry season. These dry conditions also cause post-harvest losses, as brittle rice grains shatter during milling.

Rice culture in Mali

Mali is a landlocked country in the interior of West Africa. This country is bound to the north by Mauritania and Algeria, to the east by Niger, to the south by Burkina Faso, Ivory Coast and Guinea, and to the west by Senegal. The southern part of Mali

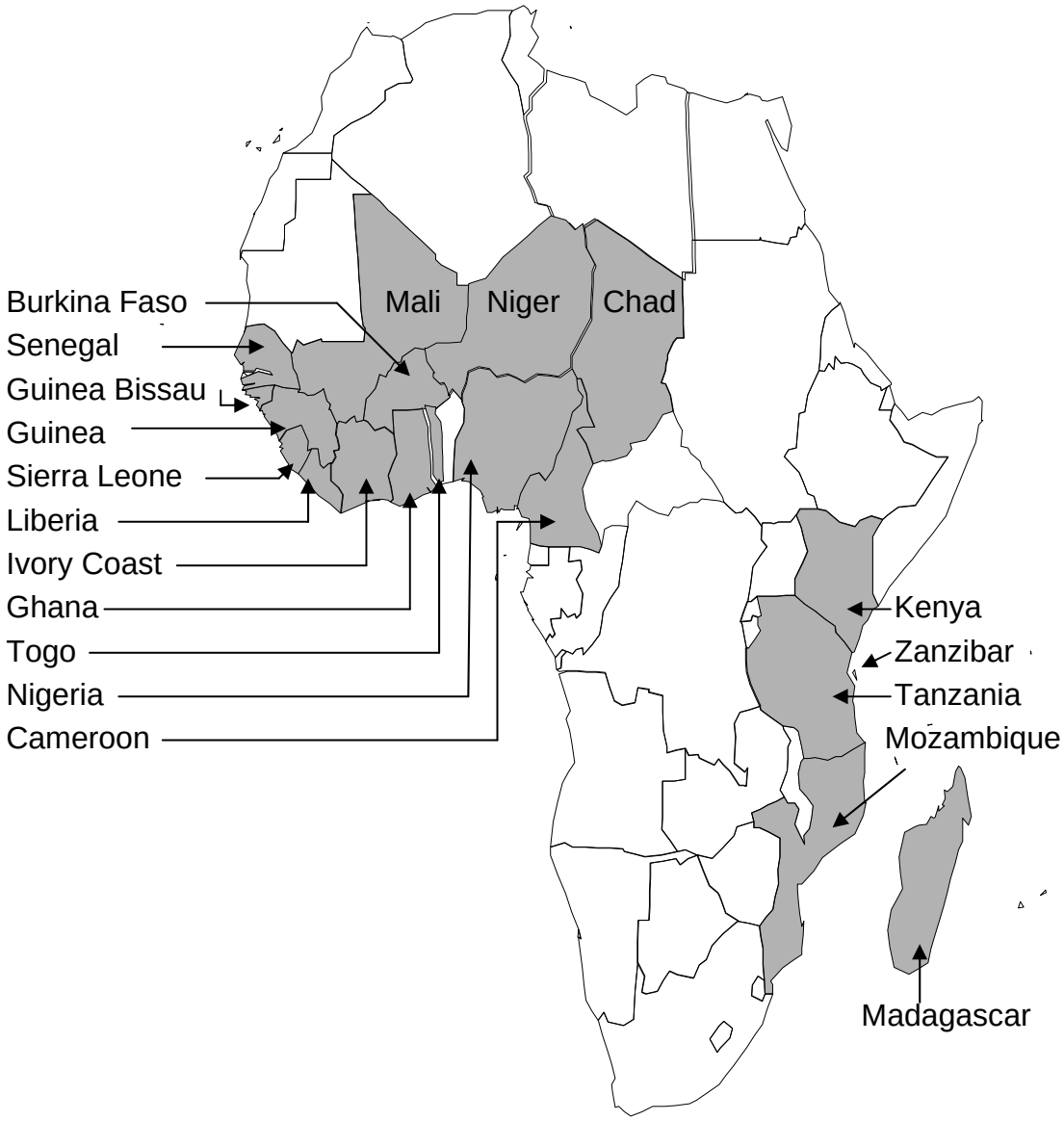

Figure 1. The distribution of African countries with reported areas with *Rice yellow mottle virus* infected irrigated rice crops.

represents a semi-arid transition zone from the wooded savannah in the far south to the beginning of the Sahara desert, which covers much of the northern half of the country. The rainy season from May to September provides an average annual rainfall of 1100 mm in the South to a low of 200 mm in the North (FAO, 1998).

Cultivation of rice in Mali was well established by the first century A.D. as part of the farming, herding and fishing complex that remains dominant to this day (McIntosh and McIntosh, 1984) and has to date expanded to large areas within the country. Deep flooded rice is cultivated on more than 184,000 ha in the Niger valley in the regions of Mopti and Ségou. This type of cultivation yields 1000 to 1500 kg rice/ha. Large rice production areas of more than 80,000 ha have been developed in the last decades north of Ségou around Niono. These newly-created polders are irrigated by the Niger. In this area, rice yields can reach 6 ton/ha, a value which compares well with the yields

obtained in the high ranking rice production areas in Asia (Hirsch, 2000). Especially, the rice yield in the ‘Office du Niger’ region can reach values as high as 7 to 8 ton/ha with an average of 5.5 ton/ha. This region is the main zone for irrigated rice culture in Mali and is followed in importance by the 'périmètre de Baguinéda' and the ‘périmètre de Sélingué’. In Mali, 320,000 ton of rice is consumed, equalling 33 kg per capita. Paddy production ranged between 280,000 and 520,000 ton with an average of 425,000 ton in recent years. The fluctuation in yield varies primarily from year to year as a function of water supplied by the Niger.

The cattle farmers are allowed to let graze their herds in the stubble fields during the intercropping seasons as food becomes scarce in the savannah in this period. The straw left in the fields after harvesting is also given to cattle. In return, the rice farmers appreciate the droppings left by the cows as dung for the next crop.

Irrigated rice production in Mali

In Mali, irrigated rice is mainly grown in the ‘Office du Niger’, and in the ‘périmètres de Baguinéda’ and ‘de Sélingué’. The ‘Office du Niger’ is divided in five zones (Niono, Molodo, N’Débougou, Kouroumari, and Kolongo/Macina). In 1932, the French started to build a barrage in the Niger near Markala, a small village north of Ségou. The reservoir created could be flooded in 1947 and an area of 960,000 ha, originally aimed for cotton production, will finally be irrigated when all infrastructural operations are finished. Regular extension of the infrastructure resulted in an irrigated area of 55,000 ha in 1960 and has by now reached an area of 80,000 ha. Most of this irrigated area has well been levelled and rice cropping has generally attained a high level of crop management.

The rainy and dry season can be sharply distinguished in the region of the ‘Office du Niger’. The rainy season starts normally at the end of May or in the beginning of June, and lasts until the end of September or the beginning of October. The rainfall measures approximately 500-600 mm, but annual differences can be as large as 300-400 mm. At the beginning of the season, showers are accompanied by thunderstorms, and less strong showers occur later in this season. The showers are often preceded by strong winds, which take along large quantities of dust and sand, especially at the beginning of the rainy season or after a couple of dry days during the season.

The average relative humidity and temperatures during the winter months (January and February) are low. In the rainy season, the average daily relative humidity fluctuates between 55 and 80% in June, reaches values of 80-90% in August, remains high (70-90%) in September during which the last showers occur, declines to 50-60% in November and to values below 40% in December. During the rainy season, the average daily temperatures are around 31^{o}C in June and slowly decrease to 27^{o}C in August. After these rains the average daily temperature increases to 29^{o}C in October, and decreases to 26^{o}C in November and around 22^{o}C in December. After March, the temperatures can reach values over 40^{o}C in April and May.

The rice cropping season starts with the beginning of the rainy season in June, and ends in November or December. Each zone can be considered as a large mono-cropped rice cultivation area. After the first rains in West Africa, the water level rises in the Niger, and with this increase of water the main canals are opened to let in water.

Depending on the level in the main canals, water will be supplied to the fields that can then be irrigated and prepared for the next crop to be grown.

From June to August, farmers start to prepare their parcels. A seedbed for seedling production is prepared in each parcel. To transplant 1 ha, a seedbed of 500 m^2 suffices and requires 75 kg of seed. The seedlings are primarily grown for the farmer's own need. Any surplus is sold to other farmers or any shortage is bought. In a time interval of three to four weeks between sowing and transplantation the fields are equalised, rearranged into basins, ploughed and irrigated. The basins can vary in size from 1000 to 2500 m^2 depending on the management strategies applied by the farmers in the different zones. Four to 10 basins can be found on a 1 ha large parcel. The approximately four weeks old seedlings are transplanted. One or two days before transplanting, the weeds are, when possible, cleared from the basins. Otherwise they are removed during the transplanting. Transplanting seedlings knows several phases. First, they are uprooted and then, depending on the distance between seedbed and basin, transported manually in baskets, by a donkey-cart or by an ox-drawn sledge. The seedlings are transplanted in a bundle of 2 to 4 plants. The distance between each bundle is about 20-25 cm. When the seedlings are too long, the plants are sometimes decapitated.

Four to six weeks after transplanting, the basins are usually weeded manually. The weeds are pulled out and thrown aside on dikes, levees or on roadsides, or are bundled and stamped into the soil. Herbicides are occasionally used in the weed control in the 'Office du Niger'. The most important weed species growing in the 'Office du Niger' rice fields belong to *Cyperaceae* and *Poaceae* and a few dicots like *Sphenochlea zeilanica, Eclipta prostrata,* and *Ludwigia spp*. The weeding activities are continued until the plants start to flower. During flowering and ripening of the crop, no farmer's activities take place within the fields. One week before the harvest, the water is released from the parcels. Pest problems other than weed infestations have generally been minor under the dry Sahelian conditions, although in the last decade *Rice yellow mottle virus* (RYMV) became a serious constraint in the 'Office du Niger' region and the other périmètres. The wild rice species *Oryza longistaminata* gave such large problems that farmers were forced to change their habit of direct sowing the seeds to producing seedbeds and transplanting seedlings. Densities of *O. longistaminata* of more than 150 plants/m^2 have been reported on some on-farm sites in the Niger delta (Dembélé *et al*., 1990). Rice grain losses of 85% have been demonstrated in fields heavily infested by *O. longistaminata* (IER, 1989). The expansion of both perennial and annual wild rice species can result in the abandonment of land. In the Macina zone, an area of 900 ha has been abandoned by the farmers due to their failure to control *O. longistaminata* (ON 1990).

During the dry season or contra season the focus is towards fruits and vegetables, like onion and tomato, although some rice is grown.

Périmètre de Baguinéda

Located in the Sudan zone, this polder (périmètre) constitutes a zone between the ' 'Office du Niger' and the 'Sélingué' region. This périmètre consists of many 'bas-fonds'

(moor-like areas). The wild rice species *O. longistaminata* can be found the whole year round in these 'bas-fonds'. Cattle, donkeys and rats often have freely access to the fields in this périmètre due to the absence of levees between the roadside and the fields. The farmers in this périmètre are often more interested in the production of fruits, maize and vegetables like tomatoes, onion and cabbage for the Bamako market than in the cultivation of rice. The rice gall midge *Rosella oryzivora* and the rice caseworm *Nymphula depunctalis* form serious pests in this area.

Périmètre de Sélingué

This approximately 1000 ha large périmètre is situated in the so-called pre-Guinea zone. The view of this polder from the dam in the Sankarani river differs completely from that of the two other main irrigated rice growing périmètres in Mali. The well-growing stubble and the numerous weeds, mostly grass and wild rice species form a green lavishly growing cover in the periods that no rice is grown. This cover favours the development of large insect populations. The beetle *Trichispa sericea*, also known as a vector of *Rice yellow mottle virus* (RYMV) (Bakker, 1974), can often be found in large numbers in this polder (Sarra and Peters, unpublished observations).

The rice-producing farmers in this périmètre are more interested in the production of millet and sorghum. The farmers start with the production of rice when the millet and sorghum have been sown. As a consequence, farmers start late with cultivating rice compared to the 'Office du Niger'. Another peculiarity in farming practices is that the seedling density in seedbeds is here notably higher than in the other regions. As a consequence, the plants will make leaf contact at an earlier stage in their development and may offer in this way more shelter to beetles. They are transplanted at a younger stage and will therefore be more susceptible to RYMV infection.

The genus *Sobemovirus* and *Rice yellow mottle virus*

One of the main constraints of rice production in sub-Saharan Africa is the increasing incidence of *Rice yellow mottle virus* (RYMV). This virus has first been found in Kenya (Bakker, 1970) but has now been reported from most African countries with ecological systems where rice has to be irrigated or lowland is watered by seepage or flooding (Fomba, 1990; Awoderu, 1991a,b; Traoré *et al*., 2001; Fig. 1). The virus has been found under hydromorphic and swamp conditions in Sierra Leone (Raymundo and Buddenhagen, 1976; Fomba, 1984; Fomba, 1988), in irrigated rice crops in Nigeria (IITA, 1978; Rossel *et al*., 1982a,b), and Côte d'Ivoire (Fauquet and Thouvenel, 1977; Awoderu *et al.,* 1987; Yoboué, 1989), in both lowland and irrigated rice crops in Niger, Burkina Faso, Senegal and in the deep water polders of Mali (John *et al*., 1984), in lowland irrigated rice areas of Madagascar (Reckhaus and Randrianangaly, 1990; Yassi *et al*., 1994), and in valley bottoms of Tanzania and Zanzibar (Rossel *et al*., 1982b; Ali and Abubakar, 2001).

Table 1. Recognized and tentative *Sobemoviruses*, their vectors and other biological factors playing a role in their spread in a crop.

Species	Sigla	Taxon of vector	Vector species	Tm[3]	Transmission[4] by						R[5]
					Mt	Gr	Pc	Se	P	Su	
Blueberry shoestring[1]	BSSV	Aphididae	*Masonaphis pepperi*	S	+	+	–	–	–		a
Cocksfoot mottle	CfMV	Coleoptera	*Lema melanopa*	S	+		+	–	–		b
			L. lichensis								
Lucerne transient streak	LTSV				+			–	–		c
Rice yellow mottle	RYMV	Coleoptera	*Chaetocnema pulla*	S	+		+	–		+	d
			Sesselia pusilla								
			Trichispa sericea								
Rottboelia yellow mosaic	RYMoV				+			–			e
Solanum nodiflorum mottle	SNMoV		*Epilachna sparsa*[x]		+			–			f
			E. doryca australica[y]								
			E. guttatopustulata								
			Psylliodes sp								
		Hemiptera	*Cyrtopeltis nicotianae*								
Southern bean mosaic	SBMV	Coleoptera	*Ceratoma trifurcata*	S	+	+		+	+		g
			Epilachna variestis								
Southern cowpea mosaic	SCPMV		*Ootheca mutabilis*		+			+	+		h
Sowbane mosaic	SoMV	Aphididae	*Myzus persicae*	N	+	+		+	+		i
		Diptera	*Liriomyza langei*								
		Homoptera	*Circulifer tenellus*								
		Hemiptera	*Halticus citri*								
Subterranean clover mottle	SCMoV				+			+			j
Turnip rosette	TRoV	Coleoptera	*Phyllotreta nemorium*		+						k
Velvet tobacco mottle	VTMoV	Hemiptera	*Cyrtopeltis nicotianae*	P	+			–			l
		Coleoptera	*Epilachna spp*								
Cocksfoot mild mosaic[2a]	CMMV	Aphididae	*Myzus persicae*		+		–	–	–		m,n
		Coleoptera	*Lema melanopus*								
Cynosurus mottle	CyMoV	Aphididae	*Rhopalosiphum padi*	N	+		+				o
		Coleoptera	*Lema melanopus*								
Ginger chlorotic fleck	GCFV				+						p
Ryegrass mottle[2b]	RgMoV				+						q

[1] Recognized species in italics; [2a] Tentative and [2b] possible species in regular; [3] Tm = transmission mode, S = semi-persistent; N =non-persistent; P = persistent; [4] Mt = mechanical transmission, Gr= grafting, Pc = plant contact, Se = transmission by seeds, P = transmission by pollen. Su = transmission in substrate; [5]R = references: [a]Ramsdell, 1996; Munthe, 2004; [c]Forster, 1996; [d]This publication; [e]Thottappilly *et al*., 1992; [f]Greber and Randles, 1996; [g]Sehgal, 1996; [h]Tremaine and Hamilton, 1983 ; [i]Teakle, 1996; [j]Francki, R.I.B., 1996; [k]Brunt, 1996c; [l]Randles and Francki, 1986; [m]Huth, 1996; [n]Lapierre, 2004; [o] Signoret, 2004; [p]Thomas, 1996;[q]Toriyama, 2004.

RYMV is a member of the genus *Sobemovirus*. This genus, with *Southern bean mosaic virus* (SBMV) as type member, encompasses 11 established species (van Regenmortel *et al.*, 2000; Table 1). Three tentative members and one possible species of this genus are, pending additional data, Cocksfoot mild mosaic virus, Cynosurus mottle virus, Ginger chlorotic fleck virus, and Ryegrass mottle virus (Table 1) (van Regenmortel *et al*., 2000; Tamm and Truve, 2000).

The sobemoviruses share several basic physico-chemical and molecular properties, such as capsid architecture, genome organisation and replication strategy. The sobemoviral genome is a single, undivided positive sense ssRNA molecule of approximately 4100 to 4500 nucleotides in size (Brunt *et al*., 1996; Tamm and Truvi, 2002). The viral capsid is composed of 180 sub-units of a 37 kDa coat protein species

Table 2. Susceptibility of poaceous species for sobemoviruses naturally infecting species of the Poaceae. The number of species tested for their susceptibility for RYMV by Bakker (1974) is considerably larger than listed here. Only those species are listed, which are also used by other authors in tests for the susceptibility of one or more of these sobemoviruses. **N** = naturally infected host, E = experimentally infected host, I = non-susceptible host.

Species	CfMV[1,2]	CMMV[3]	CnMoV[4]	RoMoV[5]	RYMV[6]	RyMoV[9]
Agropyron intermedium		I				
Agropyron repens	I	I	I			
Agrostis puchella			E			
Agrostis stolonifera		N	**N**		I	
Agrostis tenuis			**N**		I	
Alopecurus pratensis	I[1]				I	
Anthoxanthum odoratum	I	I				
Avena fatua			E		I	
Avena sativa	E	E	E		I	E
Avena strigosa		E				
Brachypodium pinnatum	I[1]				I	
Bromus arvensis	I				I	
Bromus commutatus				I	I	
Bromus inermis		E				
Bromus mollis	I	**N**	I			
Bromus racemosus		E			I	
Bromus secalinus		E	E		I	
Bromus sterilis				I	I	
Bromus tectorum		E			I	
Cynisurus cristatus	I		**N**			
Cynodon dactylon					I[6,7]	
Dactylis glomerata	**N**	**N**	I		I	**N**
Dinebra retroflexa					E[6,7]	
Diplachne caudata					E	
Echinochloa colona			I		I[6]/**N**[8]	
Echinochloa crus-galli			I		I	
Eleusine indica					E[7]	
Eragrostis ciliaris					E	
Eragrostis namaquensis					E	
Eragrostis tenuifolia					E[7]	
Festuca arundinacea	I					
Festuca pratensis		**N**	I		I	
Festuca rubra					I	E
Holcus lanatus	I	**N**				
Hordeum vulgare	E	E	E	I	I	E
Ischameum rugosum					**N**[6]	
Lagurus ovatus			E			
Lamarkia aurea		E	I			
Lolium multiflorum		E	I		I	**N**
Lolium perenne	I	I	**N**		I	E
Lolium perenne x L. multiflorum			**N**			
Lolium persicum		E				
Lolium temulentum		E			I	
Oryza australiensis					E	
Oryza barthii					E[6]/**N**[8]	
Oryza brachyantha					E	
Oryza glaberrima					E	

Oryza longistaminata					**N**[8]	
Oryza nivara					E	
Oryza punctata					E	
Oryza ridleyi					E	
Oryza rufigopon					E	
Oryza sativa	I		I	I	**N**	
Oryza spontanea					E	
Panicum miliaceum		E	E			
Panicum repens					I[6]/**N**[9]	
Panicum virgatum						
Paspalum membranaceum		E				
Phalaris arundinaceae		E		I		
Phleum arenarium		E	E		E	
Phleum pratense		**N**		I	I	
Poa annua		I	E			
Poa compressa		E			I	
Poa pratensis	I					
Poa trivialis	I	**N**			I	
Rottboellia cochinchinenesis				**N**	I[7]	
Secale cereale		E	E	I	I	E
Setaria italica		E	I			E
Setaria viridis		E	I		I	
Sorghum bicolor					I	
Sorghum halepense	I[1]					
Sorghum vulgare var saccharatum	I[1]					
Triticum aestivum	**N**	E	E	I	I	E
Triticum durum				I		
Zea mays	I	E	I	E	I	

[1]Serjeant, 1967; [2]Jeyanandarajah, 1991 ([a]only by Serjeant); [3]Huth, 1991; [4]Brunt, 1996a; [5]Thottapilly *et al*, 1992; [6]Bakker, 1974; [7]Okioma *et al*, 1983; [8]Konaté *et al*., 1997; [9]Brunt, 1996b.

arranged in a T=3 icosahedral symmetry (Francki, 1985; van Regenmortel *et al*., 2000). The coding region of the compact sobemovirus genome contains four overlapping open reading frames (ORFs). Contrary to the other analysed sobemoviruses, the small ORF 1 encoding the coat protein at the 3' end of the RYMV and *Cocksfoot mottle virus* genome has no overlap with ORF 2 (Tamm and Truve, 2000).

Host range, symptomatology and pathogenicity of RYMV

The natural host range of most sobemoviruses is often restricted to a few species within a botanical family. For instance, SBMV infects only some species within the *Leguminosae* (Sehgal, 1981, 1996) while LTSV and TRoSV infect plant species belonging to three or four families. TRoSV is restricted to the families of the *Cruciferae*, *Resedaceae*, *Solanaceae* and *Asteraceae* (Broadbent and Heathcoate, 1958; Hollings and Stone, 1973), the principal hosts of SoMV are found in the family of the *Chenopodiaceae* (Dias and Waterworth, 1966; Sehgal, 1981; Teakle, 1996). The natural hosts as well as the experimental host of six out of the 15 recognised and tentative species all belong to the *Poaceae* (Table 2). Only two of these grass-infecting viruses (CMMV and CfMV) have apparently one natural host (*Dactylus glomerata*) in common. These markedly distinct and non-overlapping host ranges of most sobemoviruses signify a high degree of biological specificity and host plant adaptation.

Sobemovirus infections typically result in the production of mottling or mosaic

symptoms often without any necrosis in most systemic hosts (Bakker, 1970; Attere and Fokum, 1983) Symptoms persist in most cases or become more severe when the infection ages. The symptoms of LTSV disappear soon after infection. Effects of RYMV are more severe and consist of a persistent mottling or streaking giving the plant a yellow or orange colour. Infected plants become stunted and may die when the plants are infected soon after germination. Infected plants, especially those, which are infected early in the growing season, often fail to produce grains (Bakker, 1974; Taylor, 1990). The presence of small or large spots with unmowed plants in harvested fields refers to severe RYMV infections and the failure of the plants to produce grains.

SBMV and SoMV are both geographically rather widespread. They are found on the American continent as well as in Europe and Africa. RYMV, endemic in sub-Saharan Africa, seems widely spread in regions where rice is grown as irrigated and rain-fed crops. LTSV has been reported in Australia and Canada. The distribution of the other species is more restricted. CfMV has been described in the United Kingdom, Norway and Russia (Tamm and Truve, 2000), while TRoSV seems to occur only in Scotland (Brunt, 1996c).

The sobemoviruses are highly infectious and can experimentally as well as naturally be transmitted in different ways. The ease with which plants are inoculated using extracts from infected plants is undoubtedly due to the marked stability of sobemoviruses and the high titres they reach in infected plants. Longevity *in vitro* can last for at least 30 days at room temperature (Bakker, 1970) and infection can still be detected when extracts from RYMV, SBMV or TRoSV-infected plants are being diluted 10^6 times or more (Fauquet and Thouvenel, 1977). Extracts prepared from young RYMV-infected Sidano plants 2-3 weeks after inoculation were still infectious after diluted 10^{10} times (Bakker, 1970).

The sobemoviruses are highly immunogenic and high titred antisera can readily be obtained. No major serological cross-reactions occur amongst the various sobemoviruses. Some intraspecies differences exist between RYMV isolates found in Kenya and in Ivory Coast (Fauquet and Thouvenel, 1977).

All sobemoviruses can efficiently be inoculated by mechanical ways (Table 1). Spread by leaf abrasion or mechanical leaf contact during strong breezes has been considered to be a real possibility for these viruses to spread in a crop (Sehgal, 1981). Dissemination of CfMV by treading or grazing animals has already been suggested in the sixties (Upstone, 1969). Transmission of SCMoV could readily be demonstrated by sheep grazing and treading in subterranean clover (*Trifolium subterraneum*) (McKirdy *et al*., 1998). Using this mechanism of transmission resistance to SCMoV could efficiently be demonstrated by grazing experimental plots by sheep (Ferris *et al*., 1996). Rapid spread of CnMoV and CfMV has been observed in *Cynosurus cristatus* lawns and cocksfoot (*D. glomerata*) leys after mowing (Huth and Paul, 1977; Upstone, 1969). Soil on which infected SBMV plants have been grown seems also to be a source for infection of germinating bean seeds (Teakle and Morris, 1981). Similar results were obtained when bean seeds were soaked in extracts from SBMV infected plants. A high rate of seedling infection was consistently obtained by planting healthy bean seeds previously soaked in an SBMV inoculum and then dried (Hamilton, 1978). Apparently,

SBMV can invade and subsequently infect germinating seedlings without the help of a vector or without abrasive contact with infected plants. The ease, by which contact between virus and plants can result in an infection, was shown by deposition of freshly damaged sites with CfMV-infected fecal material of its vector (A' Brook and Benigno, 1972). Bakker (1970) suggested that RYMV exuded from rice plants in guttation water might contaminate irrigation water, which could thus serve as inoculum source.

Vectorial transmission

Probably all sobemoviruses are insect-transmissible. Several sobemoviruses are transmitted by *Coleoptera* (Table 1). Chrysomelid beetles transmit CfMV, RYMV, SBMV and TRoV, while SNMV and VToMV are transmitted by coccinellid beetles (Table 1). The plant virus-transmitting beetles are commonly known as leaf beetles. They have a rather restricted host range and feed usually on closely related plants species (Selman, 1973). They possess biting mouthparts with which they ingest leaf tissue of interveinal areas, consequently, the damaged leaves have a tattered or skeletonized appearance.

SNMV and VToMV, two viruses infecting *Nicotiana* species, are also transmitted by the myriad bug, *Cyrtopeltis nicotianae*. SoMV is not transmitted by beetles, but by members of four different orders, i.e. the leafminer *Liriomyza langei*, the aphid *Myzus persicae*, and the hoppers *Circulifer tenellus* and *Halticus citri*. Some other sobemoviruses are also transmitted by aphids (Table 2). BSSV is known to be transmitted by the aphid *Masonaphis pepperi*, while *Rhopalosiphum padi* apparently transmits CyMV (Table 2). No insect vectors have thus far been reported for GCFV, LTSV, and RyMoV.

Several chrysomelid beetles can transmit – or carry at least - RYMV (Bakker, 1970, 1971, 1974). This author lists *Apoplylia* spp., *Chaetocnema abyssinica, C. kenyensis, C. pallidipes, C. pulla, Dactylispa bayoni, D. paucispina, D. viridicyanea, Monomolepta flaveola, M. haematura, Oulema dunbrodiensis (nigripennis), Sesselia pusilla*, and *Trichispa sericea* as possible vectors. The beetle species *Dicladispa gestroi* has been described as a RYMV-vector on Madagascar (Reckhaus and Andriamasintseheno, 2001), whereas *Dactylispa lenta* (Banwo *et al.*, 2001a) and *Chaetocnema varicornis* (Banwo *et al*., 2001b) appear to be transmitters of RYMV in Tanzania. Transmission of RYMV has also been reported for the grasshopper *Conocephalus merumontanus* when an acquisition feeding period of 24 h was given (Bakker, 1974). This insect inflicts severe feeding damage on plants.

In all, the relationships between sobemoviruses and their vectors are rather diverse (Walters, 1969; Fulton *et al*., 1987; Sehgal, 1981; Hull, 1988). Definitely, it can be concluded that these viruses are not propagated in the vector. However, they circulate either or not in the vector and are transmitted in a semi-persistent or a non-persistent fashion. A real latency period does not exist (Hull, 2002). The insects can transmit as soon as they acquire the virus, but the efficiency to transmit increases with longer feeding, as does retention time (Fulton *et al*., 1987; Hull 1988). The persistence of the virus in the vector is extremely variable. Certain vectors loose their infectivity within a day, while others retain the ability to transmit as long as two weeks. Some sobemoviruses are transmitted by one and the same species, but the biokinetics by

which these different viruses are transmitted may differ. The significance of the infective vectors in the spread and the epidemiology of the different sobemoviruses have not extensively been studied.

Only the beetle species *T. sericea* and one or more *Chaetocnema s*p seem to occur in Mali. *T. sericea* is a known pest on rice in Africa. The species can often be encountered in the périmètre de Sélingué. To which extent this species and the other species really contribute to the spread of RYMV has remained unknown. A study made on Madagascar showed that an average of 18% of the plants became infected in 196 crops without any *Dicladispa gestroi* infestation or damage caused by beetles, while 30% of the plants was infected in 53 crops with *D. gestroi* infestation. A similar infection rate was also found in 75 crops with severe feeding damage (Reckhaus and Andriamasintseheno, 1997).

Transmission by seeds

The sobemoviruses SBMV, SCMoV, SCPMV, and SoMV have been shown to be transmittable through seeds (Zaumeyer and Harter, 1943; Wroth and Jones, 1992; Shepherd and Fulton, 1962; Dias and Watersworth, 1967). The efficiency of seed transmission of sobemoviruses appears to decline with age and maturation of the seeds. Only, five percent of the plants were infected after sowing seven-month-old SBMV-infected bean seeds (Zaumeyer and Harter, 1943). In case of SoMV efficiency of seed transmission can be as high as 83% (Bennet and Costa, 1961; Dias and Watersworth, 1967). Transmission of PMV through *Setaria italica* seeds seems to be restricted to a single isolate, whereas seed transmission of the other PMV isolates could not been demonstrated (Niblett and Paulsen, 1975). CfMV and RYMV are reported not to be seed borne (Catherall, 1970; Bakker, 1975; Fauquet and Thouvenel, 1977). Seeds of RYMV-infected rice plants contain virus as shown by ELISA and infectivity assays. The infectivity drops rapidly when seed matures and dries (Konaté *et al*., 2002), whereas the ELISA values are evidently not affected.

Pollen from some sobemovirus infected plants can be infected and transmission by pollen has been demonstrated for some viruses (Table 1).

Scope of the investigations

The main objective of this study was to gain more knowledge about the epidemiology of *Rice yellow mottle virus* (RYMV) in the irrigated rice cultures in Mali. This virus, first reported in Kenya (Bakker, 1970), emerged after the introduction of *O sativa* cultivars from Asia into Africa. Due to its rapid spread the virus has become a major limiting factor in the rice production in lowland and irrigated crops in entire sub-Saharan Africa. RYMV is known as a beetle vectored and mechanically transmitted virus, but the epidemiology of the disease is still poorly understood and apparently the main mode of transmission explaining the dynamic nature of RYMV is yet to be fully elucidated (Nwilene, 1999).

Before the nineties of the last century the incidence of RYMV in Mali was low. In the rainy season of 1995, 200 ha were entirely wasted in Mali with an estimated loss of 900,000 kg. Yield losses ranged among individual farmers from 25-97%. In the

périmètre de Sélingué, more than 80% of the fields were infected, and yield losses of 25 up to 80% were recorded in crops depending upon the cultivar and time of infection (Sy, 1994).

It became evident that spread of RYMV in a crop could not be explained by beetle transmission, as the number of beetles caught in the 'Office du Niger' always remained low. In addition, several infection patterns were observed that could not be explained by the behaviour and prevailing infestation of beetle vectors. Completely infected crops occurred side by side with crops in which infections were virtually absent. Small strips, in which all plants were infected can occasionally been found in the first one or two rows along levees, dikes and roadsides. Fields, in which cattle were stalled during the night in the contra-season, often show large spots with severe infections in the next cropping season. These observations suggested strongly that other mechanisms than beetle-mediated transmission contributed to the spread of the virus. To understand the development of RYMV infections in the field, the current study was aimed to analyse the transmission of RYMV in field and greenhouse experiments with the ultimate goal to define cultivation practices and means by which virus spread can be prevented.

As a first step in this study, a new enzyme linked immuno-sorbent assay (ELISA) based on the use of leaf disks instead of leaf extracts was developed to by-pass the time-consuming maceration of the sclerophyllous leaves of rice (Chapter 2). As there were indications that RYMV spreads readily during farmer's operations, the effect of transplantation of seedlings from infected seedbeds on the development of the infection in the field was studied (Chapter 3). The effects of other common practices, such as weeding crops and applying fertilizers, on the spread of the RYMV have also been described in this chapter. Next, possible wind-mediated spread by leaf contact between healthy and infected plants was analysed in both field and greenhouse experiments (Chapter 4). Chapter 5 focuses on alternative transmission routes, i.e. by animals grazing on rice. Chapter 6 describes the survival of infectious virus in living and dead plant material, in soil from infected fields, as well as the release of virus from infected roots, respectively. Finally, the impact of a possible transmission of RYMV through seeds was re-evaluated in Chapter 7. The general discussion presented in Chapter 8 places the major findings in a broader context and presents suggestions for improving RYMV control in rice cropping.

CHAPTER 2

A NOVEL ELISA TECHNIQUE TO DETECT *RICE YELLOW MOTTLE VIRUS* BASED ON VIRUS RELEASE FROM INFECTED LEAF DISKS

To avoid tiresome maceration of the sclerophyllous rice leaves a novel enzyme-linked immunosorbent assay (ELISA) protocol for *Rice yellow mottle virus* (RYMV) was developed based on virus release from leaf disks. Statistically, no significant differences were found between the recorded ELISA values using leaf disks or extracts from well-growing infected plants, showing that RYMV is efficiently released from the disks. The amount of virus released did not differ among variable incubation periods of few minutes to some hours. The disks continuously released virus in successive immersions in fresh sample buffer, but the readings gradually decreased. The incubation temperature did not affect virus release. The release could be better correlated with the periphery than with the surface of the disks, whereas the volume in which the virus can be released had a negative effect on the readings. It is concluded that this leaf disk technique is reliable and useful for at large scale monitoring of RYMV. Efficient release from leaf disks was also found for *Cowpea mosaic virus* and *Brome mosaic virus*, indicating that the leaf disk assay may be used for other viruses.

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INTRODUCTION

Rice yellow mottle virus (RYMV) forms a serious threat to the African rice cultivation in rainfed and irrigated rice crops. The disease was first discerned in Kenya in 1966 (Bakker, 1970) but has now widely spread in the lowland and irrigated rice cultivation areas of sub-Saharan Africa. RYMV is transmitted by beetles (Bakker, 1974; Banwo, 2001c), but also in other ways, like wind-mediated leaf contact between healthy and infected plants (Chapter 4; Sarra *et al*., 2004), and by foraging cattle, donkeys and rats (Chapter 5; Sarra and Peters, 2003).

To study the epidemiology and spread of this virus, numerous plants have to be assayed. The most appropriate choice is the use of the enzyme linked immunosorbent assay (ELISA), as powerful antisera against this virus are available (Bakker, 1974; Fauquet and Thouvenel, 1977; this Chapter). Since the development of this technique by Clark and Adams (1977), ELISA has become a widely applied method to detect and assay plant viruses in their hosts. The method is economical in the use of reactants and can readily be adapted to qualitative and quantitative measurements (Tijssen, 1985). Many variants of the basic procedure have been described with the objective of optimising the detection level and reliability. All procedures applied use either extracts from infected plants or virus suspensions. To avoid the time consuming process of grinding plant tissue the use of intact leaf disks has been proposed in the past (Romaine *et al*., 1981). Unfortunately, this approach was not widely adapted as, unquestionably, less virus was released from the supposedly injured cells at the periphery of the leaf disk than could be detected in tissue homogenates. Especially, grinding the sclerophyllous leaves of rice plants is a tiresome process, requiring much time, force and patience to get a total extract. To overcome these practical difficulties and considering that the virus is readily released in guttation water (Bakker, 1974), the use of leaf disks for an ELISA-based detection of RYMV has been re-investigated with infected rice plants.

This chapter shows that the release of RYMV from leaf disks results in quantitative detection levels comparable with the use of extracts from infected rice plants. In addition, we demonstrate that this technique can well be applied to other beetle-transmitted viruses like *Cowpea mosaic virus* and *Brome mosaic virus* and can be used to sample infected plants in the field.

MATERIALS AND METHODS

RYMV isolate

The RYMV isolate used in this study was collected from infected cv. BG 90-2 leaf material after the harvest of the rice growing season of 1999 near Niono in the 'Office du Niger', Mali. This isolate was maintained and propagated on 'BG-90-2' or 'Kogoni 91-1' seedlings, two varieties widely grown in Mali. Seedlings were inoculated with an extract of 0.5 g infected plant material in 10 ml phosphate buffered saline (PBS), pH 7.0, and kept at 25ºC either in a climate room or in a greenhouse at 25 to 30ºC.

Preparation of RYMV antiserum

Five g infected 'BG 90-2' leaf material was macerated in 200 ml PBS with a blender

and then triturated with a mortar and pestle after adding some carborundum powder. The macerate was strained through cheesecloth and clarified by centrifuging at 10,000 rpm for 10 min at 4°C. A 1:1 mixture of butanol and chloroform (40 ml) was added to the supernatant. After gently stirring for 15 min, the emulsion was centrifuged at 10,000 rpm for 10 min. The virus was precipitated by adding 4% polyethylene glycol and 0.2 M sodium chloride to the water phase, while gently stirring the suspension for 1 h at 4°C. The precipitate was collected by a 15-min-centrifugation at 10,000 rpm and suspended in 5 ml 0.5x PBS. The denatured material was removed by centrifuging the suspension at 8,000 rpm for 10 min. The virus concentration was determined by assuming that an extinction coefficient of 8.3 at 262 nm corresponded with 1 mg virus/ml.

A rabbit was immunised with two samples of 200 µg at a two-week-interval. Blood was collected three times every two weeks after the last injection. The serum was produced by centrifugation (10 min at 5000 *g*) of the clotted blood after incubating the blood for a night at 4ºC.

ELISA procedure

The double antibody sandwich enzyme-linked immunosorbent assay (ELISA) was used to monitor the presence of RYMV in infected leaf extracts and disks (Clark and Adams, 1977). Leaf extracts were prepared by grinding 1 g of leaf material in 30 ml PBS containing 0.05% Tween-20 (sample buffer). This ratio of weight to volume approximately corresponded with the weight of a leaf disk and 0.2 ml sample buffer, in which the disks were incubated. The disks were either cut with a one-hole perforator (5 mm in diameter) or were cut as 4 x 4 mm squares from leaf blades with a razorblade. In comparative studies, the extracts and the leaf disks were obtained from the same infected leaf. ELISA plates were coated with 1 µg/ml anti-RYMV-IgG using 0.2 ml per well in 0.05 M carbonate buffer, pH 9.6. After coating, the wells were incubated with 0.2 ml leaf extract or with disks immersed in 0.2 ml sample buffer. This incubation was followed by adding 1 µg/ml alkaline phosphatase conjugate (0.2 ml/well) in sample buffer to the wells. The plates were incubated for 2 h at room temperature or overnight at 4ºC. The plates were washed with tap water between the incubations. After this 2-h incubation, the reaction was developed by adding 0.2 ml of 1 mg/ml of a freshly prepared nitrophenylphosphate solution. The absorbencies were read at 405 nm with a Bio-kinetics reader EL312 at intervals of 15 or 30 min.

Release of virus from disks

Release of RYMV from leaf disks was studied using infected leaf material from the highly susceptible cv. BG 90-2 (Konaté *et al.*, 1997) and the more tolerant cv. Kogoni 91-1 (Goulibaly *et al*., 1999). The amount of virus released from intact disks was compared with extracts from the same leaf. ELISA values were also determined with extracts from disks, which were not immersed, and from disks after being immersed once or twice for 2 h.

The same techniques were used to study the release of *Cowpea mosaic virus* (CPMV) from cowpea (*Vigna uniguiculata*) leaf disks and *Brome mosaic virus* (BMV) from oat (*Avena sativa*) leaf disks.

The amount of virus released from rice leaf disks and present in extracts was assayed by titration of the ELISA values against a three-fold dilution series of 1 mg of purified virus. Ten leaf disks were immersed in 2 ml sample buffer for 2 h. Extracts prepared from the same leaf were also diluted in a three fold dilution series.

The optimum time required to release virus from the disks was determined by immersing batches of 10 leaf disks, one per well, for 1, 4, 16, 64 or 256 min. After removal of the disks the plates were incubated for another 255, 252, 240, 192 and 0 min to complete the sample incubation period of 256 min.

Temperature dependence of virus release from disks was measured by incubation of six disks at 4, 15, 22, or 37 ºC for 2 h.

The effect of the size and the periphery of the disks on the release of the virus was studied by incubating 4 x 4 mm (16 mm^2), 2 x 2 mm (4 mm^2), and 1 x 1 mm (1 mm^2) mm leaf squares. Squares of 4 mm were also chopped up in strips of 1 x 4 mm to increase the periphery to 40 mm.

The effect of volume on the release was determined by incubating eight disks of an infected 'BG 90-2' leaf in 1.6 ml, eight disks in 3.2 ml, eight disks in 6.4 ml and eight disks in 12.8 ml sample buffer. After a 2-h incubation, 6 aliquots of 0.2 ml of each sample were assayed by ELISA.

Serial incubation of leaf disks

The release of virus from a disk in subsequent incubations was studied in three differently designed experiments. In the first experiment four leaf disks from an infected 'BG 90-2' leaf were 20 times serially incubated in fresh wells for 1 min.

In the second experiment the amount of virus released was quantified by repeatedly incubating disks in five series with increasing incubation periods. These incubation series consisted of 4 incubations of 10 sec, 10 incubations of 1 min, 10 incubations of 10 min, 10 incubations of 1 h, and 3 incubations of 12 h. The disk was then once incubated for 1 h and then for 24 h.

In the third experiment, a single infected leaf disk from 'BG 90-2' and from 'Kogoni 90-1' were serially incubated 80 times for 1 min. After the last incubations, the disks of the two last experiments were macerated and assayed. The disks in these three experiments were transferred from well to well by a small pin pierced through the disks.

Table. 1. Average enzyme-linked immunosorbent assay (ELISA) readings (A_{405}) for *Rice yellow mottle virus* released from four disks and of extracts prepared from the same infected leaf of the cultivars BG 90-2 and Kogoni 91-1, and their standard deviations. The extracts were prepared in the same weight/sample buffer ratio as used for the disks.

Leaf	BG 90-2		Kogoni 91-1	
	Disk	Extract	Disks	Extracts
1	0.83 ± 0.15	1.05 ± 0.09	1.01 ± 0.07	1.08 ± 0.06
2	0.92 ± 0.03	0.97 ± 0.04	0.93 ± 0.06	1.10 ± 0.04
3	0.59 ± 0.13	0.93 ± 0.05	0.99 ± 0.07	1.08 ± 0.05
4	0.86 ± 0.10	1.01 ± 0.07	1.07 ± 0.11	1.06 ± 0.06

Detection of RYMV in intact leaves, young seed, seed coats, and root fragments

Three to four cm long leaf tips, still attached to the plant, were immersed in 5 ml sample buffer for 24 h. Aliquots of 0.2 ml of the suspension so obtained were assayed by ELISA. Young seeds collected from infected plants and root and stem fragments, 4 mm long and 2 mm thick were incubated in 0.2 ml sample buffer for 2 h. Leaf tips, seeds, root and stem fragments from healthy plants were used as control.

RESULTS

Release of RYMV from leaf disks

Incubation of RYMV-infected leaf disks in IgG–precoated wells with sample buffer and of extracts in the same leaf weight/buffer ratio resulted in values, which did not statistically differ ($f = 15.3$, $df = 3$, $P>0.05$) (Table 1). The ELISA values obtained were slightly lower for the leaf disks, whereas the STD values also were slightly higher. These results show that RYMV can readily and reliably be detected in infected rice plants using either leaf disks or extracts. The rate of virus release from disks was affected by the susceptibility of the cultivar.

Since the amount of virus detected in extracts did not significantly differ from that released from disks, the question arose to which extent the amount of virus released from disks was affected by the incubation. The readings obtained after incubating disks once or twice for 2 h did not differ significantly (Table 2). Extracts from non-incubated disks and from disks incubated once or twice gave similar ELISA readings demonstrating that the plateau values were reached.

Incubation of disks in PBS with or without Tween-20 resulted in almost comparable ELISA readings (1.47 versus 1.31). Incubation in water resulted in approximately 70% (0.39) lower readings. The amount of virus released by incubating leaf disks and in extracts was calibrated using a three–fold dilution series of a virus solution containing original 1 mg/ml (Fig. 1). The amount of virus found in extracts appeared to be at least 100 times larger than found in leaf disk lecheates (Table 3).

Kinetics of RYMV release from disks

The virus was efficiently released from disks in view of the ELISA values obtained. To understand the mechanism of this release in more detail and to test the robustness of this ELISA approach, the effects of incubation time, temperature, the volume in which the disks are immersed, and of the size and form of the disk were studied.

Table 2. Average enzyme-linked immunosorbent assay values (A_{405} nm) after incubation of six *Rice yellow mottle virus* infected 'BG 90-2' or 'Kogoni 91-1' leaf disks which were either once or twice immersed in sample buffer and of the extracts from these immersed disks. Extracts from fresh leaf disks of the same infected leaf served as control for the virus level in the leaf.

Treatment	BG 90-2	Kogoni 91-1
Leaf disks once immersed	2.04 ± 0.26	1.66 ± 0.32
Extracts of once-immersed leaf disks	1.99 ± 0.14	1.79 ± 0.11
Leaf disks immersed for a second period of two h.	1.84 ± 0.13	1.95 ± 0.09
Extracts of twice-immersed leaf disks	2.08 ± 0.18	2.04 ± 0.04
Extracts of non-immersed leaf disks	2.21 ± 0.10	2.18 ± 0.14

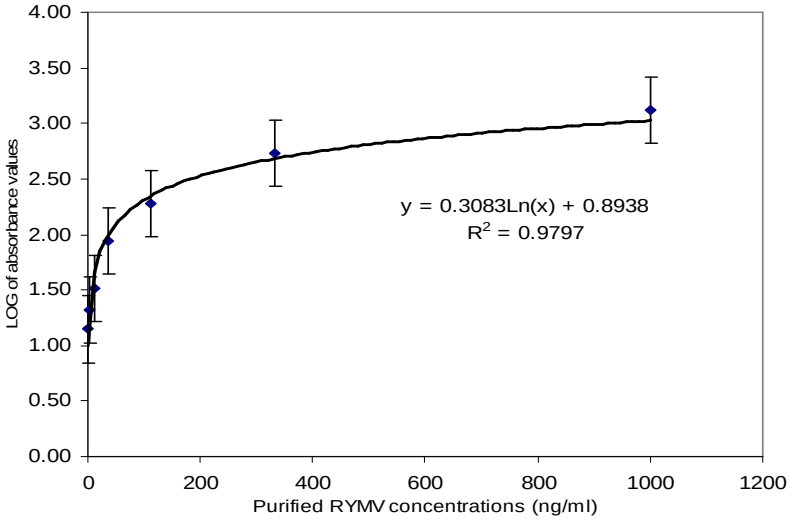

Figure 1. Standard curve by plotting a three-fold virus dilution series against log transformed ELISA readings.

Effect of incubation time. Incubation of disks for 1 min resulted already in a vast release of virus giving only slightly lower ELISA readings compared to disks incubated for longer periods (Table 4). The ELISA values found for five incubation periods tested and for the extract did not significantly differ ($F = 6.24$, $df = 4$, $P>0.05$). The almost similar results obtained after incubating the disks for 1 up to 256 min indicate that the virus is released in an extremely rapid rate.

Table 3. Amount of virus released from leaf disks and detected in an extract incubated in sample buffer for 2 h in a three-fold dilution series. The concentration of leaf disks was determined using the formula y= 0.308ln(x) + 0.894, where y = log of the ELISA values and x = virus concentration. The data given were read 60 min after substrate incubation.

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Dilution	Disk			Extract		
	A_{405nm}	Virus detected (µg/ml)	Virus detected times dilution (µg/ml)	A_{405nm}	Virus detected (µg/ml)	Virus detected times dilution (µg/ml)
1	1.56	0.88	0.88	2.15	1.36	1.36
3	1.50	0.82	2.46	1.76	1.03	3.09
9	1.23	0.62	5.54	1.63	0.92	8.27
27	0.67	0.26	7.07	1.49	0.81	21.95
81	0.29	0.08	6.48	1.47	0.80	64.72
243	0.13	0.03	6.32	1.23	0.62	151.39
729	0.07	0.01	7.29	0.80	0.34	247.86
2187				0.39	0.12	266.81
6561				0.19	0.04	282.12
19683				0.09	0.02	314.93
59049				0.07	0.01	708.59

Table 4. Mean enzyme-linked immunosorbent assay (ELISA) values (A_{405nm}) and standard deviation of *Rice yellow mottle virus* released from infected leaf disks immersed in microplate wells for 1, 4, 16, 64, or 256 min[a].

Incubation period of the disks (min)	Average ELISA values (A_{405}nm)
1	1.00 ± 0.19
4	1.17 ± 0.11
16	1.22 ± 0.09
64	1.25 ± 0.09
256	1.20 ± 0.09
Leaf extract	1.21 ± 0.09

[a] After removing the disks, the suspensions were incubated for another 255, 251, 240, 192 and 0 min. Each treatment consisted of 20 leaf disks. The plates were read after 30 min.

Temperature. The effect of temperature on the release of virus was measured by incubating disks at four different temperatures. The average values show that there is no positive temperature-dependent effect on the release of the virus from the disk (Table 5). The negative correlation coefficient (-0.73) found has to be attributed to the lower values found for the disks incubated at 29℃. The reason for these lower values has remained unknown.

Size of the disks. The effect of disk size and periphery on the release of RYMV was studied by incubating 1 x 1 mm (1 mm^2) squares, 2 x 2 mm (4 mm^2) squares, and 4 x 4 mm (16 mm^2) squares, and 4 x 4 mm (16 mm^2) squares which were chopped up in strips of 4 x 1 mm. These squares had peripheries of 4, 8, 16 and 40 mm long. The results show that virus release increased with both surface size and length of the periphery (Table 6). However, a better correlation was found with the periphery than with the surface size.

Incubation volume. The disks were usually incubated in volumes of 0.2 ml and the amount of virus released appeared to be slightly lower when incubation periods of 1 min were used. Reaching rapidly a limit might indicate that an equilibrium exists between the amount of virus released in the suspension and the remaining concentration in the disk. This hypothesis was tested by assaying 0.2 ml aliquots of 1.6, 3.2, 6.4 and 12.8 ml large samples in which eight disks were incubated, respectively. The readings obtained were negatively correlated with the volume in which the virus was released, but approximately similar amounts of virus were released in the respective volumes (Table 7). These results were in contradiction with the expected results. Similar readings and logarithmic increasing amounts of released virus were expected. Further analyses to understand the apparent contradiction between the obtained and expected results were not made.

Table 5. The mean ELISA found after incubation at four different temperatures of 10 disks cut from *Rice yellow mottle virus* infected rice leaves. The readings were made 30 min after substrate incubation.

Incubation temperature (℃)	4	15	29	37
Mean ELISA values	0.72 ± 0.03	0.69 ± 0.07	0.58 ± 0.05	0.65 ±0.07

Table 6. Average ELISA-values virus released from *Rice yellow mottle virus* from leaf disks differing in surface size and periphery length. The plates were read 30 min after substrate incubation.

Surface size (mm^2)	1	4	16	16
Length of the periphery (mm)	4	8	16	40
ELISA values	0.45 ± 0.11	0.61 ±0.17	0.80 ± 0.25	1.62 0.14

Table 7. The average amount of *Rice yellow mottle virus* released by eight disks from infected BG 90-2 plants in 1.6, 3.2, 6.4 or 12.8 ml sample buffer.

Sample volume (ml)	Average ELISA values (N = 8)	Amount of virus released (µg/ml)[a]	Total amount of virus released (µg)
1.6	1.60	0.72	1.15
3.2	0.91	0.32	1.01
6.4	0.45	0.18	1.15
12.8	0.25	0.11	1.46

[a] The virus concentration and the ELISA readings were related by the formula $y = 0.83\ln x + 1.87$ ($R^2 = 0.98$).

Release of virus from leaf disks by serial immersion

Immersing a 'BG 90-2' leaf disk for four periods of 10 sec, ten periods of 1 min, ten periods of 10 min, ten periods of 1 h, three periods of 12 h, a period of 1 h and one of 24 h resulted in a slow decrease of the readings within a series of constant incubation periods (Table 8). Increasing the length of the immersion period in a next series gave higher readings in the first few immersions than the last reading in the previous (Table 8). The high values obtained in incubation periods of 1 sec and of 1 min show that the virus was continuously released at a high rate. The total amount of virus released by the disk in this experiment was estimated to be 18.1 µg. A similar trend of virus release was found in a comparable experiment with a Kogoni 91-1 disk. The amount of virus released by this disk was estimated to be 17.3 µg. The residual amount of virus in the extracts of the macerated disks appeared to be 0.49 and 0.40 µg, respectively. In these series of immersions approximately 20 times more virus was released than the 0.92 µg virus monitored in extracts of directly macerated disks.

Table 8. Enzyme-linked immunosorbent assay (ELISA) values of one *Rice yellow mottle virus* infected BG 90-2 leaf disk serially immersed for 4 periods of 10 sec, 10 periods of 1 min, 10 periods of 60 min, 3 periods of 12 h, one period of 1 h and a period of 12 h. The disk was then ground in 0.2 ml sample buffer. Absorbency values were read at 405 nm 1 h after substrate incubation.

Incubations		Incubation										Virus
Length	Number	1	2	3	4	5	6	7	8	9	10	released (µg)
10 sec	4	2.72	2.27	2.25	19.2							3.30
1 min	10	2.62	2.31	2.26	1.60	1.68	1.64	1.56	1.64	1.41	1.32	6.50
10 min	10	2.60	2.26	2.00	1.82	1.57	1.41	1.23	1.40	1.03	0.89	5.73
1h	10	1.73	1.57	1.26	1.50	1.31	1.05	1.13	0.95	0.90	0.84	0.36
12h	3	1.99	1.36	1.21								1.65
1h	1	0.93										0.34
12h	1	0.63										0.23
Disk extract		1.35										0.49

Continuous release of virus could also be demonstrated in a series in which four 'BG 90-2' disks were analysed in 20 serial immersions in incubation periods of 1 h (Fig. 2). The ELISA values dropped from an average value of 2.29 after the first incubation to a value of 1.03 in the 20th incubation. The values found per single disk fluctuated considerably, resulting also in large fluctuations between the virus amounts among individual disks. Fig. 3 illustrates the results of the immersion of a 'BG 90-2' and of a 'Kogoni 91-1' leaf disk serially in 80 IgG-coated wells for 1 min. The readings decreased gradually and reached asymptotically a low level of virus release (Fig. 3). The final leaf disk, however, still contained a readily detectable amount of virus.

Detection of RYMV released from intact leaves, young seeds, and stem and root pieces

The virus was released from leaf tips when these were incubated for 24 h in 5 ml sample buffer, while the tip was still attached to the plant (Table 9). RYMV was also released from young seeds from infected plants. Release from tips and seeds indicate that the RYMV is released from leaf tissues without wounding. Higher ELISA values were found for root segments than for stem segments. These results might indicate that the virus concentration is higher in roots than in stems as the root and stem segments were approximately similar in length and diameter. Alternatively, the virus was more readily released by root tissue than by stem tissue.

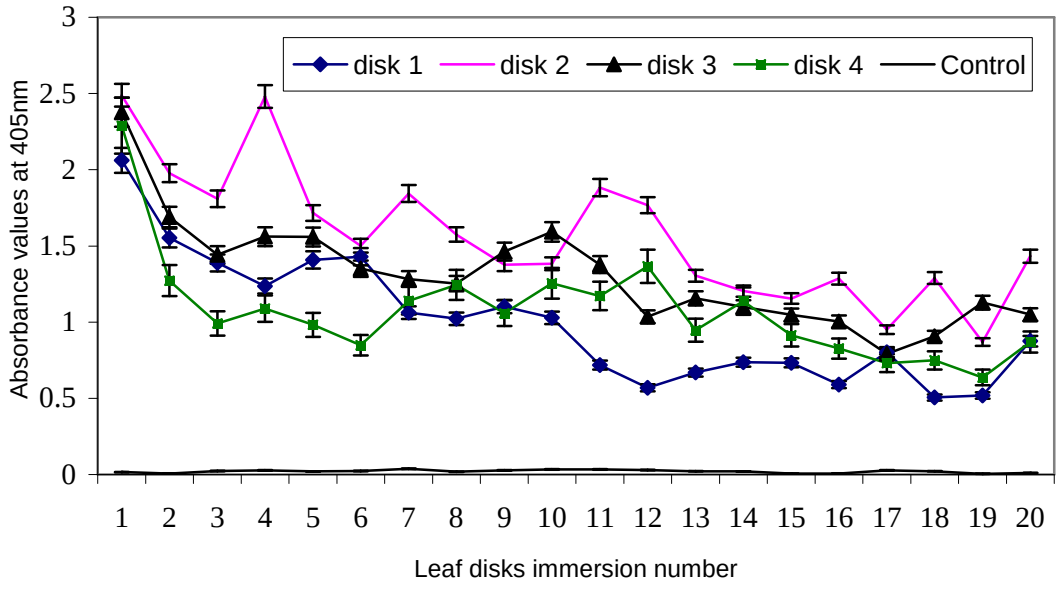

Figure 2. Release of *Rice yellow mottle virus* from four infected BG 90-2 leaf disks which were individually incubated serially in 20 successive wells for 1 h. The enzyme-linked immunosorbent reactions wer done as described.

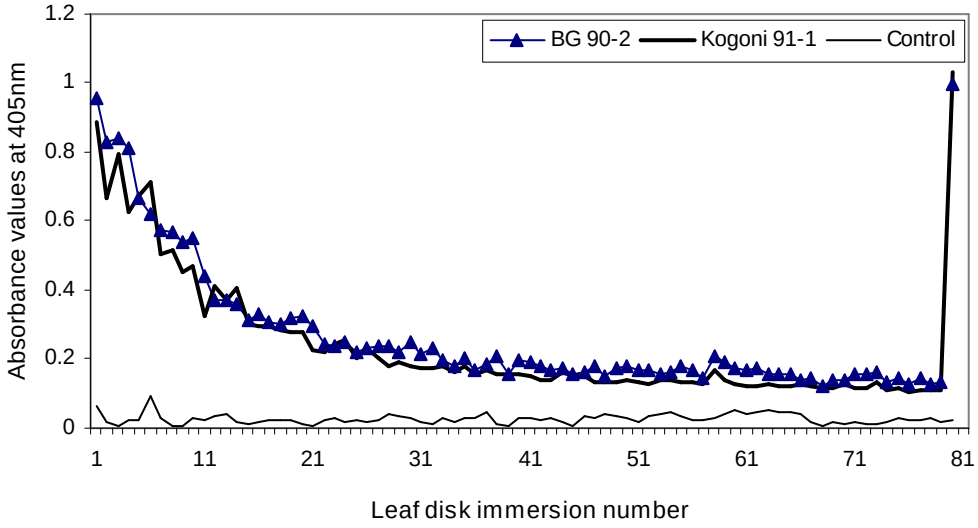

Figure 3. Enzyme-linked immunosorbent assay (ELISA) values obtained after serially incubation of an infected 'BG 90-2' and a 'Kogoni 91-1' leaf disk 80 times for 1min at room temperature. After these series of incubations the disks were incubated for a night in a well (the 81th well) at 4ºC.

Release of other viruses from disks

The successful release of RYMV from leaf disks raised the question whether other viruses could also be released from disks prepared from their respective hosts. Immersion of leaf disks from CPMV-infected cowpea plants and BMV-infected barley plants indeed resulted also in high ELISA readings comparable to those obtained using extracts from this material (Table 10).

Table 9. Release of *Rice yellow mottle virus* from leaf tips, young seeds, and root and stem tissue from infected BG 90-2 plants.

Plant tissue	Infected plant material		Healthy plant material	
	Average ELISA reading	Standard Deviation	Average ELISA reading	Standard Deviation
Leaf disks	2.14	0.16	0.05	0.00
Intact leaf tips	0.65	0.26	0.03	0.01
Intact seeds	1.83	0.58	0.02	0.01
Roots fragments	2.76	0.35	0.05	0.01
Stem fragments	1.43	0.12	0.04	0.00

Table 10. Release of *Cowpea mosaic virus* from 6 infected cowpea plant leaf disks and of *Brome mosaic virus* from 6 infected barley plants in pre-coated ELISA plate wells with ELISA sample buffer.

Virus species	Disks/extracts	Mean ELISA values	STD
CPMV	Disks	2.38	0.14
	Extracts	2.47	0.10
BMV	Disks	0.69	0.07
	Extracts	0.67	0.05

DISCUSSION

The data presented in this chapter demonstrate that *Rice yellow mottle virus* (RYMV) can readily be detected by the enzyme-linked immunosorbent assay (ELISA) by incubating disks of infected leaves in sample buffer. The ELISA readings, obtained with the leaf disk assay procedure developed gave in most experiments values identical to the values when extracts from the leaf disks were used. Since extracts and disks gave similar readings in the same format, the leaf disk incubation technique can substitute the use of rice leaf extracts, thus circumventing laborious maceration of the scleroid rice leaves.

Comparable amounts of virus are released from infected rice leaf disks independent whether they were incubated for 1 min or much longer. It is tempting to assume that the virus is rapidly released from the injured cells at the periphery of the leaf disk. Since almost all cells will be disrupted by macerating the tissue, more virus will be present in extracts than in leaf disk lecheates when similar tissue weight/sample buffer ratios are used. The finding that comparable readings are found in tissue extracts and in leaf disk lecheates suggests that plateau values were obtained due to over-saturating virus amounts. Indeed, a three-fold dilution series demonstrated that the amount of virus in an extract was estimated to be approximately 100 times higher than in a first leaf disk incubate.

Assuming that rice plant cells have a diameter of 20 µm, the ratio between the volume of peripheral ring of damaged cells and that of a 5 mm-diameter disk is 250 times. Forty percent of the virus present in the periphery is released in the first incubation. However, further incubation of a disk revealed that a multitude of this amount is released (Table 8). This suggests that virus was also exuded by other ways than by leakage from the damaged cells at the periphery. Release of virus from leaf tips still connected to leaves and from young seeds as shown in this study (Table 6), and the release of virus from the roots of infected plants in nutrient media (Chapter 6), show that virus can readily be released from intact plant tissues. Possibly, RYMV is released via the pathway used for the exudation of guttation water. The presence of virus in guttation water (Bakker, 1974, Chapter 3) can be interpreted as seepage of virus with water out of xylem vessels and tracheids via hydrathodes into epidermal pores.

RYMV accumulates in most cell types during systemic infection of a rice plant (Opalka *et al.*, 1998). Most of the virus particles appeared to accumulate in xylem parenchyma cells and sieve elements. Co-localisation of cell wall markers and viral antibodies over pit membranes suggests a pathway for virus translocation between vessels after partial digestion of these membranes as a consequence of programmed cell death (Opalka *et al*., 1998). Active virus transport in xylem has also been demonstrated for *Bean pod mottle virus*, *Cowpea severe mosaic virus*, and *Southern bean mosaic virus*, whereas the non-beetle transmitted viruses of *Bean yellow mosaic, Sunn hemp mosaic*, and *Tobacco ringspot* are not transported through the xylem as shown in differently designed experiments (Gergerich and Scott, 1988). Plants became infected when severed plant stems were placed in purified extracts of these beetle transmitted viruses. Plant tissue became also infected above a steam-killed area after

injection of purified virus in sections below this steam-killed stem segment. Similar treatments with the non-beetle transmitted viruses did not infect plants when severed plant stems were placed in a virus suspension or when injected below stem killed portions of the stem. These infections are thought to be the result from active transport in xylem. In our studies virus may be released by transport from the xylem vessels into the sample buffer. More virus is released from disks when their surface increases in size, but periphery had a larger effect on the release than surface. A linear correlation could the expected with the periphery of the disks and an exponential correlation with their surfaces. Such correlations were not found. The absence of a positive correlation of virus release between the periphery and the surface strongly indicates that the virus is not only released from damaged cells at the periphery, but also from undamaged cells in the disks.

The release of RYMV from rice leaf disks differs quantitatively from the process by which *Tobacco ring spot virus* (TRSV) and *Maize dwarf mosaic virus* (MDMV) are released from leaf disks of florist's geranium (*Pelargonium x hortorum*) and corn (*Zea mays*), respectively. Unquestionable, lower readings were obtained when disks were used than extracts prepared from infected plants while the protocol had to be changed by incubating the samples for 18-20 h at 6℃ and pr olonging substrate reaction time for several hours. The lower release of MDMV can be explained by the shape of this virus, viz. 750 nm long thread-like particles

The low rate of TRSV release (Romaine *et al*., 1981), even lower than that of MDMV, is more difficult to explain. This virus has icosahedral morphology like RYMV, CPMV and BMV, and has similar dimensions as these viruses. Exudation of virus from the non-linear xylem vessels of the geranium plants may be more difficult than from linear-running vessels in the leaves of maize and rice. However, the release of CPMV from disks, quantitatively comparable to that of RYMV, demonstrated that this virus is efficiently released from leaves with not linear–running vessels. The efficient release of both viruses and of BMV suggests that the release of large amounts of virus is associated with properties enabling these viruses to be transmitted by beetles or the translocation of these viruses in plants (Gergerich and Scott, 1988).

The disk immersion technique has the obvious advantage that plants can directly be sampled in the field in stead of in the laboratory. It is also an advantage that the material has not to be immersed directly in pre-coated plates. The efficient release of CPMV and the efficient, but lower release of BMV demonstrate that field sampling may not only be restricted to RYMV, but may be extended to the monitoring of plants infected with other beetle transmitted viruses.

CHAPTER 3

SPREAD OF *RICE YELLOW MOTTLE VIRUS* IN IRRIGATED RICE CROPS BY FARMERS' OPERATIONS

The sobemovirus *Rice yellow mottle virus* (RYMV) can be introduced in rice crops by transplanting seedlings from seedbeds with a limited infection. The number of plants that became infected during transplantation was several times larger than the original incidence of infected seedlings. This increase was later followed by a second wave of virus spread due to other transmission mechanisms operating in the crop during the growing season. These mechanisms include wind-contact between plants, treading the crops during farmers' operations like weeding and applying fertilisers, and spread of RYMV from infected to healthy plants via the soil. Infection of plants via roots was confirmed in experiments, in which plants were prevented to make leaf contact or when roots were incubated in a virus suspension. Mowing of the crop also resulted in an increase of the infection. While transplantation and treading affected the incidence in the present crop, mowing enhanced the virus reservoir by which the next crop will be infected. Transplantation of seedlings, treading crops, wind-mediated leaf contact, and release of virus into soil form a cascade by which a limited infection can be spread further.

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INTRODUCTION

Rice yellow mottle virus (RYMV, genus *Sobemovirus*) is endogenous to Africa and probably the only virus of economic importance in rice on this continent (Konaté and Fargette, 2001). Since the first reports on its occurrence in Kenya (Bakker, 1970 and 1971), RYMV has been found in irrigated lowland and water swamped rice crops in all major rice producing zones of sub-Saharan Africa (Banwo *et al*., 2002, John *et al*., 1984). The incidence and distribution patterns of infected plants differ from crop to crop. With respect to its occurrence in the region referred to as 'Office du Niger' (Chapter 1) most crops are virtually not infected. Virus incidence in an infected crop may vary from only a few plants scattered over the field to, incidentally, completely infected crops. These crops can be found next to apparently virus-free crops.

RYMV has been reported to be transmissible by beetles (Bakker, 1974; Banwo *et al.*, 2001c; Reckhaus and Andriamasintseheno, 1997). However, the role of the fifteen or more reported vector species in the epidemiology of RYMV is not well established (Konaté and Fargette, 2001; Peters *et al.*, 1999). Often virus spread can not be correlated with the number of beetles present in crops. Infections may occur in the virtual absence of beetles while crops can remain completely uninfected in the presence of massive numbers of beetles as observed, as in the périmètre de Sélingué (Sarra and Peters, unpublished observations). Field surveys made in the Northwest of Madagascar showed that an average disease incidence of 18% was found in 196 fields in which no *Discladispa gestroi* beetles were observed or damage inflicted by these beetles could be identified. An incidence of 30% was found in 53 crops in which each plant was infested by beetles. A similar percentage was found in 75 crops with severe beetle damage (Reckhaus and Andriamasinthseheno, 1997). The occurrence of completely infected crops next to virus-free crops indicates that other mechanisms than beetle-mediated transmission may underlie RYMV epidemiology. Indeed, recent studies have demonstrated that the virus is readily spread by cattle and other vertebrates grazing on rice (Chapter 5; Sarra and Peters, 2003), and by wind-mediated contact between healthy and infected plants (Chapter 4; Sarra *et al*., 2004). This contact-mediated transmission suggests that mechanical transmission may also occur during farmers' operations.

A number of plant viruses are known to spread readily by farmers' operations. For instance, *Tobacco mosaic virus* (TMV) can readily contaminate hands, clothing, and tools, and can thus be spread by workers in tobacco and tomato crops (Matthews, 1992). *Potato virus X* can also be spread in this way and by contaminated implements or machines, on the clothes of workers and on the fur of animals that have been in close contact with infected plants (Todd, 1958). Crop infection by other contact-transmissible viruses, including the sobemoviruses, has been studied less extensively.

Although seedlings showing RYMV-characteristic symptoms are rarely observed, seedbeds may contain the first infected plants. These infections, which are usually unnoticed at transplantation, may be introduced by beetles, and by grazing vertebrates (Chapter 5) acquiring the virus from infected weeds or volunteer rice plants in and outside the seedbed. Alternatively, the seedlings may also become infected by leaf

contact with infected weeds and volunteer rice plants and by virus released in soil by infected plant remains. Despite the probably low incidence of RYMV in infected seedbeds, the infection rate may increase by several farmers' activities during transplanting seedlings. Transplantation of seedlings encompasses uprooting, bundling, transportation and planting (usually two or three seedlings together) during which abrasive contacts occur between the leaves of plants. Besides this mutual leaf contact, hand and plant contact will be a second way to infect plants during transplantation. After transplantation contact-mediated spread may also occur by treading of crops while weeding or applying fertilisers.

The objective of this study was to evaluate the impact of various treatments and practices as performed by farmers during rice cultivation. To this end we determined the quantitative significance of transplanting seedlings from RYMV-infected seedbeds for virus spread under standard field situations, the effect of treading, and the effects of mowing infected rice crops at harvest. The results obtained in the field are supported by greenhouse experiments.

MATERIALS AND METHODS

Spread of RYMV by transplanting seedlings from infected seedbeds

Twenty 0.5 by 0.5 m wide seedbeds were sown with 400-500 seeds of 'BG 90-2' and twenty of such beds with seeds of 'Kogoni 91-1'. The first of these cultivars, grown widely in the "Office du Niger", is highly susceptible to RYMV (Konaté *et al.,* 1997), whereas the other is considered to be rather tolerant (Goulibaly, 1999). Two weeks after sowing, two seedlings were inoculated in four seedbeds of each cultivar, four seedlings in four seedbeds, eight in four seedbeds, and sixteen in four seedbeds. The inoculated seedlings were marked. An average of 0.20, 0.44, 0.97 and 3.83% of the 'BG 90-2' seedlings, and 0.14, 0.27, 0.61 and 2.43 % of the 'Kogoni 91-1' seedlings became infected. No seedlings were inoculated in the four seedbeds sown either with 'BG 90-2' or with the 'Kogoni 91-1'. They served as blank. Two weeks after inoculation, the seedlings were transplanted in a Fisher's block design (Kawanchai and Gomez, 1983). Each block consisted of five plots with seedlings from one of the seedbeds with different infection levels. Each plot was 2 x 3 m wide and a 1 m wide strip was left between the plots. All seedlings of each seedbed were uprooted, transported to the plots, and transplanted by one technician. He washed his hands very well with soap and 96% ethanol before transplanting a new seedbed.

Weeds were controlled three weeks after transplanting by application of Londax 60DF consisting of 60% bensulfuron-methyl as active ingredient in a dose of 100 g/ha.

Plants were visually recorded for the development of foliar symptoms at 15, 30, 45, 60 and 75 days after transplanting. The paddy loss, determined when the plots were harvested, was related to the initial infection rate of the seedlings in the seedbeds, and to the final incidence of infected plants. The paddy yield was calculated by the formal YL (%) = (Yh – Yi) 100/Yh, in which YL is the paddy yield loss in percentage, Yh is the paddy yield of the plots without virus infection, and Yi is the paddy yield of infected plots. Data were statistically analysed using the ANOVA calculation of STATITCF and

means were separated according to Newman and Keul's test at 5% level.

Spread of RYMV by treading

The effect of treading on the spread of RYMV was studied in plots with different treatments. The experiments were done with both the highly susceptible rice cv. BG 90-2 and the more tolerant Kogoni 91-1 in the rice-growing season of 2001, 2002 and 2003 in four repeats. Each repeat existed of six blocs each with a different treatment. Each bloc consisted of two 5 m long rows each with 33 plants (Fig. 1). The distance between the blocs was 50 cm, and between the rows 25 cm. The plant distance was 15-16 cm in a row. The treatments were randomised in each repeat (Kawanchai and Gomez, 1983). The source plants were mechanically inoculated fifteen days after transplantation of the seedlings, using a 1/10 (w/v) extract from an RYMV-infected plant in 0.01M phosphate buffer, pH 7.0. To prevent infection by leaf contact an 80-cm high plastic screen separated the row with healthy plants from the row with inoculated plants in four treatments. The plants in the control plot were not inoculated, and the rows were not separated by a screen. All plants were inoculated in one of the rows of the five other treatments. In one of these treatments no screen was erected between the inoculated and non-inoculated plants; so that these plants could make leaf contact. In three other treatments blocs were treaded once 15 days after inoculation (DAI), twice 15 and 45 DAI, or three times 15, 45 and 75 DAI. Treading was done by pacing out the length of the bloc between the row of inoculated plants and the screen. No walk was made between the rows in the sixth treatment. The disease incidence in rows with non-inoculated plants was monitored and expressed in percentages. The data have been analysed by STATITCF and GENSTAT Edition 5 (Payne *et al*., 1993).

Spread of RYMV by mowing infected crops

The effect of mowing was studied on the developing regrowth after harvesting the crop. The infected plots in each bloc in the seedling transplantation experiment were first mowed at harvest and then the uninfected plots. Mowing of the healthy plots started in the middle of one side of a plot. Infection of the regrowth was monitored by counting the infected tillers and by measuring the distance between the starting point and the last infected plant. The results were statistically analysed as described above.

Infection of plants via soil

Infection of plants via soil was studied in two experimental designs differing in plant densities in a greenhouse. In one experiment with a high plant density, 20 six-weeks old healthy 'BG 90-2' plants were placed in two rows between three rows of ten infected plants in a 70 x 45 cm wide and 8 cm deep tray. Leaf contact between the healthy and infected plants was prevented by transparent plastic screens. The distance between the plants in the rows was 6-7 cm and in the columns 9 cm providing a space for each plant of 63 cm^2.

In the second design three week old 98 'BG 90-2' seedlings were transplanted in two 1.4 x 1.4 m wide and 30 cm deep basins. After three weeks, the central plant in each basin was inoculated with the HA strain and a week later caged in a transparent

cylinder. The distance between the plants in the rows and columns was 18 cm providing a plant space of 324 cm^2. This is close to the usual plant density in rice crops. However, in this experiment one seedling was planted per 324 cm^2, whereas the farmers plant 2 to 3 seedlings together on such a spot. Development of the infection was monitored twice a week. When the plants started to flower, the infected plants were trimmed with pruning scissors and subsequently the 32 plants in the border rows with the same scissors. The remaining plants were then trimmed with another pair of scissors. Infection in the trimmed plants was monitored twice a week.

Inoculation of plants by immersing roots in a virus suspension

A possible infection of rice plants with RYMV via their roots was studied with extracts from plants infected with three different isolates. These extracts were prepared by grinding 5 g of infected leaf material of a low aggressive (LA), a moderately aggressive (MA), and a highly aggressive (HA) isolate in 50 ml of PBS buffer, pH 7.2 using a mortar and pestle. LA, MA and HA were collected in farmer's fields in the "Office du Niger" in 1999, in the contra-season of 2004 and the season of 2002, respectively. The extracts were strained through cheesecloth to remove large plant debris. The roots of ten days old 'BG 90-2' seedlings, the seeds of which germinated in water, were immersed in the extracts for 24 h. The seedlings were than planted in 20 by 40 cm wide and 8 cm deep plastic trays. Seedlings were monitored for virus infection and tested by ELISA 3 week post-transplantation.

Inoculation of plants with infected guttation water

Three-week old seedlings (10 per bucket) were serially inoculated with a guttation-water-contaminated hand by abrasing a leaf over a distance of approx. of 5 cm after dusting the plants with carborundum powder. The final numbers of infected plants were read three weeks after inoculation.

RESULTS

Effect of transplanting rice seedlings from infected seedbeds

To estimate the effect of transplanting seedlings from a seedbed on the spread of RYMV, a limited number of the seedlings was mechanically inoculated. The first symptoms could be discerned on the inoculated seedlings when they were uprooted. Spread to the neighbouring seedlings within the seedbeds was not observed. RYMV disease symptoms were more pronounced on the 'BG 90-2' seedlings than on the 'Kogoni 91-1' seedlings.

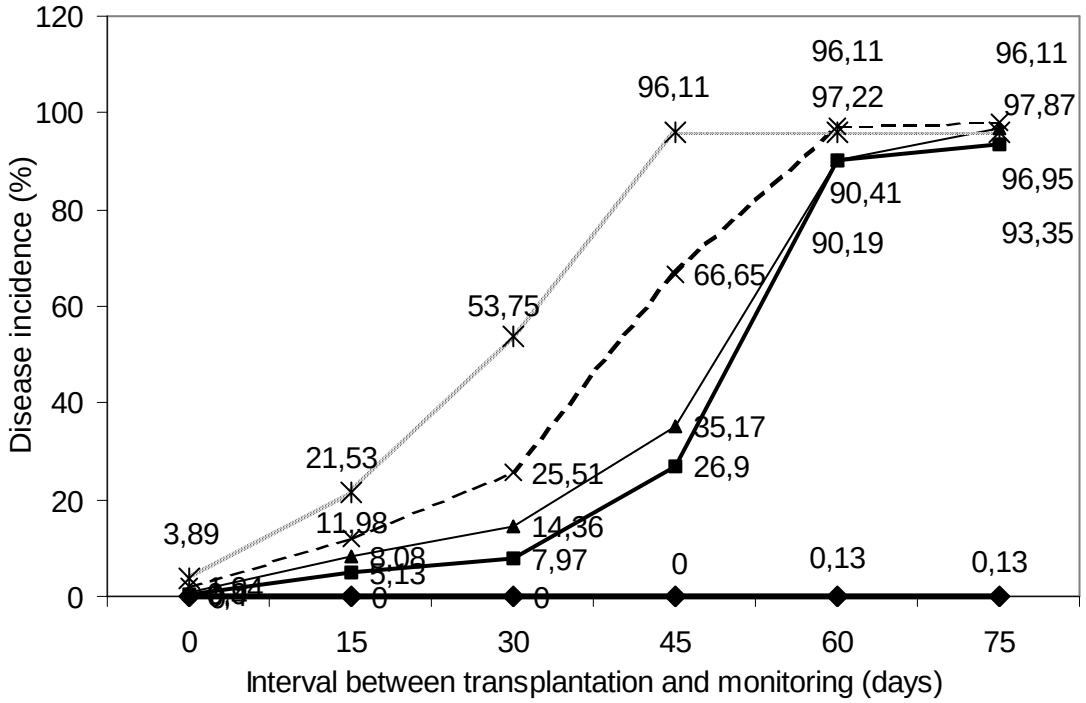

Figure 1. Effect on the spread of *Rice yellow mottle virus* (RYMV) in rice by transplanting seedlings from infected 'BG 90-2' seedbeds in the field. In the seedbeds were 0 (—♦—), 0.2 (—■—), 0.44 (—▲—) 0.97 (—x—) and 3.83 (····□····) % of the seedlings infected.

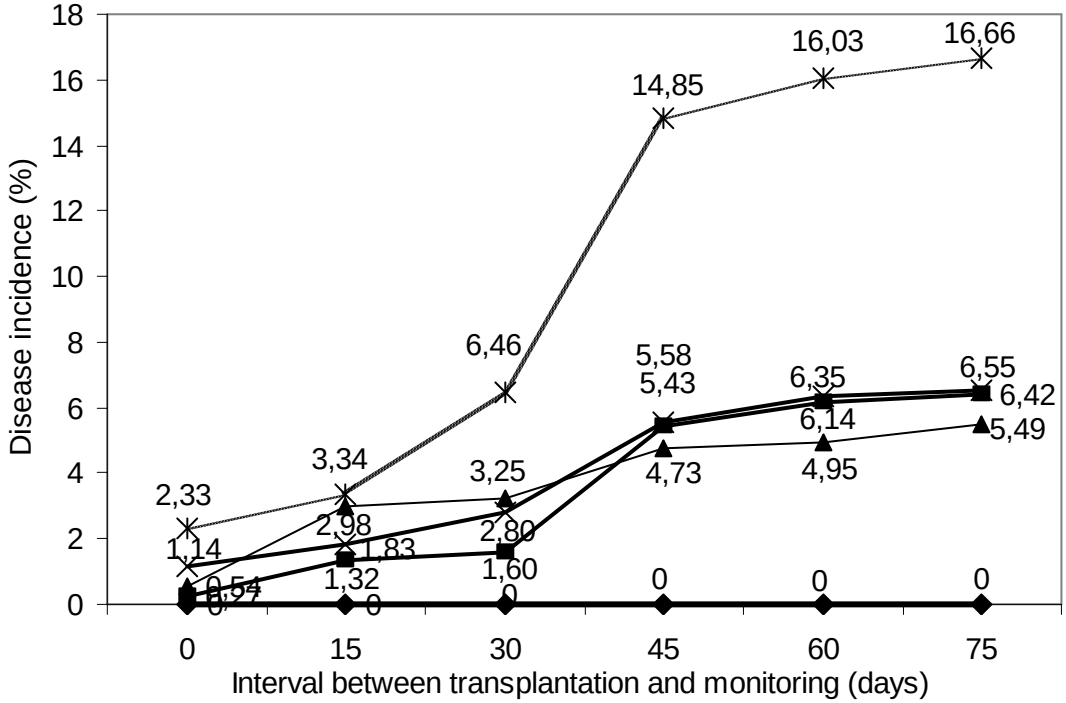

Figure 2. Effect of transplanting seedlings from an infected 'Kogoni 91-1 seedbed on the spread of *Rice yellow mottle virus* (RYMV) in the field. In the seedbeds were 0 (—♦—), 0.14 (—■—), 0.27 (—▲—), 0.61 (—x—) and 2.43 (····✳ ····) % of the seedlings infected.

Fifteen days after transplanting, the number of infected plants had approximately increased 5.5 to 12.8 times in the ‘BG 90-2’ seedling plots, and 2.8 to 5.5 times in the ‘Kogoni 91-1’ plots (Fig. 1). Plants were monitored for infection by visual inspection for the occurrence of typical RYMV symptoms. Between 2 and 4 weeks after transplanting, the disease incidence had roughly doubled in the ‘BG 90-2’ plots and was 1.3 times higher in the ‘Kogoni 91-1’ plots when compared to the original seedbed infection.

A further significant increase of infected plants was noticed in the ‘BG 90-2’ plots in the period between 30 and 60 days after transplantation after which a limit was apparently reached (Fig. 1). This limit, corresponding with approximately 95 % of the plants infected was reached earlier in the plots with the highest numbers of originally infected seedlings. These results demonstrate that an infection of seedlings of only 1 or 2 percent or less in a seedbed can result in an almost complete infection of the plots with plants of the susceptible rice cv BG 90-2.

A pronounced increase was also noticed between 30 and 45 days after transplanting the Kogoni 91-1 seedlings (Fig. 2). The final incidence varied from 5.49% for the plots with originally 0.14% infected seedling to 16.66% for the plots with originally 2.43% infected seedlings (Fig. 2). Only, a single infected plant was found in two of the four blanks with ‘BG 90-2’ plants 60 days after transplantation, whereas in the ‘Kogoni 91-1’ blank plots no infected plants were found.

To correlate the loss of rice with the infection incidence, the paddy yields were determined when the plots were harvested. Higher paddy yield reductions were found in the 'BG 90-2' than in the Kogoni 91-1 plots (Table 1). The reductions ranged between 52.2 to 63.4% in the ‘BG 90-2’ plots and between 12.5 to 27.1% in the ‘Kogoni 91-1’ plots. The reductions increased with the original infection level in the seedbed as well as with the final incidence. A correlation co-efficient of -0.73 was found for the relation between the initial infection level in the ‘BG 90-1’ seedbed and the yield reduction. This value was -0.97 for ‘Kogoni 91-1’.

Spread of RYMV by treading infected fields

The effect of treading on the spread of RYMV in infected fields was studied in plots by placing footsteps once, twice, or three times in plots between a row of inoculated and a row of healthy plants (Table 2 and 3). All inoculated plants in the source rows became infected. The plants that became infected in the healthy rows were randomly distributed within the rows. The number of infected plants in the treatments, in which the rows with inoculated plants were paced out, increased with the number of pacings made as well as with time (Table 2 and 3). A higher rate of treading-mediated virus spread was recorded in the ‘BG 90-2’ plots than in the ‘Kogoni 91-1’ plots. The highest numbers of infected plants were found in the treatment in which no barrier was placed between the rows and no walks were made. A few infected plants were found in
the plots, which were not treaded, while the rows were separated with a screen. No infection occurred in the non-inoculated plots.

Table 1. The relation between paddy yield and the final incidence of *Rice yellow mottle virus* in plots transplanted with seedlings of the varieties BG 90-2 and Kogoni 91-1 from seedbeds with different infection levels.

BG 90-2				Kogoni 91-1			
% infected seedlings	% infection 75 days after transplantation	Paddy yield (kg h^{-1})	Yield reduction (%)	% infected seedlings	% infection 75 days after transplantation	Paddy yield (kg h^{-1})	Yield reduction (%)
0	0	9234 a	0	0	0	8424a	0
0.20	93	4417b	52	0.14	5.5	7371b	13
0.44	97	4159b	55	0.27	6.4	7008bc	17
0.97	98	3433b	63	0.61	6.6	6983bc	17
3.83	96	3383b	63	2.43	16.6	6142c	27

*Numbers followed by the same letter in the yield column are statistically identical.

Table 2. Incidence of RYMV infections after treading BG 90-2 plots in 2001, 2002, and 2003 at one, two or three 15-days intervals after inoculation. Each pl ot consisted of two rows (one inoculated and one healthy) of 33 plants. Inoculated rows were separated from healthy ones by a plastic screen in all treatments except in one in which the inoculated plants and non-inoculated plants could make leaf contact, and the control plot to evaluate natural spread. N = total number of exposed plants (four repetitions with 33 plants a year), dap = days after pacing. Numbers followed by the same letter in a column are not statistically different (P>0.05).

Treatment	Average % of infected plants (N = 396)		
	30 dap	60 dap	90 dap
Control	0.0 ± 0.0 f	0.0 ± 0.0 e	0.0 ± 0.0e
No screen between inoculated and non-inoculated plants	56.8 ± 11.8a	66.4 ± 10.1a	68.4 ± 10.0a
Screen between inoculated and non-inoculated plants, once paced	14.9 ± 5.6d	22.0 ± 5.5c	26.3 ± 6.4c
Screen between inoculated and non-inoculated plants, two times paced	19.9 ± 5.7c	24.7 ± 5.5c	30.0 ± 5.7c
Screen between inoculated and non-inoculated plants, three times paced	24.2 ± 6.0b	31.3 ± 5.2b	38.4 ± 5.8b
Screen between inoculated and non-inoculated plants, no pacing	10.9 ± 2.9e	15.7 ± 3.7d	17.4 ± 3.6d

Table 3. Incidence of RYMV infections after treading Kogoni 90-1 plots in 2001, 2002, and 2003 at one, two or three 15-days intervals after inoculation. Each plot consisted of two rows (one inoculated and one healthy) of 33 plants. Inoculated rows were separated from healthy ones by a plastic screen in all treatments except in one in which the inoculated plants and non-inoculated plants could make leaf contact, and the control plot to evaluate natural spread. N = total number of exposed plants (four repetitions with 33 plants a year), dap = days after pacing. Numbers followed by the same letter in a column are not statistically different (P>0.05)

Treatment	Average % of infected plants (N = 396)		
	30 dap	60 dap	90 dap
Control	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0
No screen between inoculated and non-inoculated plants	45.2 ± 11.9a	52.0 ± 11.4a	53.8 ± 11.2a
Screen between inoculated and non-inoculated plants, one paced	7.1 ± 3.4c	8.6 ± 4.0c	13.9 ± 5.2c
Screen between inoculated and non-inoculated plants, two times paced	5.8 ± 1.9c	7.8 ± 2.3c	14.9 ± 5.1c
Screen between inoculated and non-inoculated plants, three times paced	10.6 ± 3.2b	13.9 ± 3.1b	16.9 ± 4.b
Screen between inoculated and non-inoculated plants, no pacing	0.8 ± 0.4d	1.0 ± 0.4d	4.3 ± 1.3d

The rate at which the plants became infected differed from year to year. The spread of RYMV was considerably higher in 2002 and 2003 than in 2001. An average of 9.8% plants became infected in the 2001 plot in which no screen was placed between the row with infected and healthy plants, while in the 2002 and 2003 plots with the same treatment 84% of all plants became infected in the healthy row. The large spread in 2002 and 2003 at one hand, and the limited spread in 2001 at the other hand explain the large standard errors as shown for all treatments in which plants were inoculated (Table 2 and 3).

RYMV spread by mowing

The effect of mowing on the spread of RYMV was studied in plots with healthy 'BG 90-2' plants at harvest. A decreasing gradient of infected plants was found from the point where the first plants were mowed, using a virus-contaminated infected sickle, to the place where the last infected plant was found (Table 4). Almost no spread occurred 30 days after mowing the plots. The rapid drop of the infected plants from the spot were the first plants were mowed indicates that the sickle is quickly cleaned from the virus acquired by mowing infected plants.

Infection of plants via soil

Spread of virus from infected plants to healthy plants could be demonstrated while leaf contact was prevented by caging the infected plants. In the high plant density experiment 12 out of the 20 healthy plants became infected within ten to twenty days after transplanting.

In the experiment using a plant density according to farmers' practice, one plant became infected in basin 1 and two plants in basin 2 next to the mechanically inoculated plant (Table 5). The infected plants in both basins stood next to the inoculated plants. Since the soil was not disturbed and leaf contact was prevented between the inoculated and the infected plants, the infection should have resulted from a spread of the virus via soil. Most of the border plants that were trimmed with contaminated scissors were infected two weeks after trimming (Table 5). The last seven trimmed plants remained healthy. An evident gradient could not be observed in the series of the first 17 trimmed plants among which the infected plants occurred. The plants trimmed with clean scissors became infected in the period between the second and third week after trimming (Table 5). These results show that plants became infected by trimming with infected scissors, and by virus spread from the roots of infected plant to those of healthy plants.

Table 4. The average RYMV incidence (%) in 'BG 90-2' stubble plots after mowing four healthy plots with sickles contaminated by mowing infected plots first.

Days after	Distances from the starting point of mowing (m)						Check
mowing	0.5	1.0	1.5	2.0	2.5	3.0	
15	5.0	0.8	0.4	0.0	0.0	0.0	0.0
30	11.9	4.8	3.8	0.0	0.0	0.0	0.0
45	11.9	4.8	5.8	2.9	0.0	0.0	0.0

Table 5. Development of the *Rice yellow mottle virus* infection after inoculating a plant in each of the two basins. The border plants were trimmed after flowering with contaminated scissors and the rest of the plants with clean scissors. The inoculated plants were caged 5 days later to prevent leaf contact.

Treatment of the plants in the basins	Number of plants	
	Basin 1	Basin 2
Seedlings transplanted (Apr. 07)	49	49
Inoculation of the central plants (Apr. 20) and caging them at April 25	1	1
Infection observed on plants between May 12 and July 30	2	1
Border plants trimmed with scissors after cutting the infected plants (July 30)	24	24
Infection on plants trimmed with infected scissors (13, 20, 27 Aug monitored).	7, 1, 0	7, 2, 4
Trimming rest of the plants with a clean scissors (30 July)	22	23
Infection on plants trimmed with clean scissors (13, 20, 27 Aug. monitored).	2, 6, 1	3, 5, 2
Number of plants that remained healthy	30	25

Infection of seedlings by immersing roots in a virus suspension

The data presented here above showed that plants became infected when leaf contact between healthy and infected plants was prevented (Tables 2, 3 and 5). These plants may become infected through wounds inflicted by abrasive contact between soil particles and roots or just by infection of undamaged roots by virus released from infected roots. Infection by the latter route was confirmed by incubating ten-day-old seedlings, which germinated in water, in an extract from plants infected with three isolates differing in aggressiveness. Out of 30 ‘BG 90-2’ plants, 7, 24 and 13 seedlings became infected with the LA, MA and HA isolates, respectively (Table 6). In case of ‘Kogoni 91-1’ 8, 20 and 15 seedlings became, respectively, infected with these isolates. While the infection rate did not differ when the seedlings were exposed to the same virus isolate, the cultivars were significantly different in their susceptibility for the used virus isolates.

Infection of plants through infected guttation water

Infectivity of the guttation water from RYMV infected plants has been reported in the past (Bakker, 1974), although quantitative data were not given. We confirm here that this water is highly infectious as shown in two series plants inoculated with a wetted hand. In one series 108 plants out of 120 and in another 212 plants out 300 became infected. These results show that guttation water can be a potential virus source by direct leaf contact, but may also contaminate the water in seedbeds and fields.

Table 6. *Rice yellow mottle virus* infection of 'BG 90-2' and 'Kogoni 91-1' plants after exposing the roots of each seedling individually to extracts from plants infected with the LA, MA or HA isolates.

RYMV isolate	‘BG 90-2’	‘Kogoni 91-1’
Low aggressive (LA)	7/30	8/30
Moderately aggressive (2004)	24/3*0	20/30
Highly aggressive (2002)	13/30	15/30

*Number of infected plants/number of exposed plants

DISCUSSION

Spread of some plant viruses has been occasionally attributed to farmers' operations (Matthews, 1991; Hull, 2002). The data presented in this paper demonstrate that several practises commonly applied in rice cultivation indeed enhance the incidence of RYMV infected plants, when inoculum sources are present in the seedbed or in the crop. A limited RYMV infection in seedbeds resulted in a considerable increase of infected plants after transplantation (Fig. 1 and 2).

Our experiments demonstrate that the increase of infected plants in the first two or three weeks after transplantation can mainly be ascribed to leaf contact between healthy and infected plants during transplantation. The leaves make abrasive contacts when the plants are uprooted, transported and planted. Leaf contact between seedlings is also a real possibility when two or three seedlings are planted at the same spot. Besides mutual leaf contact between the plants, the hands of the transplanter will also become contaminated during the transplanting seedlings and may thus form a second way to infect plants during the transplantation process. Guttation water from infected seedlings proved to be highly infectious and might thus mediate virus spread by direct leaf contact or by transplantation manipulations. Furthermore, the seedbed surface water will also become contaminated and may act as inoculum.

A steep increase of infection was observed during the second month after transplantation. This revived spread of virus has to be attributed to other factors than transplanting. Abrasive contact of the leaves by wind will be one of the factors by which the virus will then spread (Chapter 4). Spread by beetles can not completely be excluded as two plants became infected in the seedling plots from the healthy 'BG 90-2' seedbeds.

Since the transplanted plots were not treaded for weeding, fertilisation or inspecting the plants, the spread observed four weeks after transplantation is likely due to wind-mediated leaf contact between infected and healthy plants (Chapter 4). Evidence for spread by treading in an infected crop has been obtained in field experiments in which plants became infected by placing footsteps between rows with infected and healthy plants, while leaf contact was prevented by plastic screens positioned between the rows. The infectivity rate was considerably higher in a treatment in which the inoculated and healthy plants were not separated by screens, and could, thus, mainly be attributed to wind-mediated leaf contact between plants. This contact between plants has recently been shown to have a large impact on the spread of RYMV (Chapter 4).

Infection of healthy plants via the root system can occur by several processes. Wounding the roots by placing footsteps between healthy and infected rice plants may stimulate virus release from infected plants and infection of healthy plants. Plants can also become infected without root damaging as shown in experiments in which virus spread was prevented from making leaf contact (Tables 2, 3 and 5) demonstrating that virus, released from infected plants, infects healthy plants. Infection of seedlings immersed in a virus suspension for 24 h (Table 7), show that infection of healthy plants may occur without creating any wound. The release of virus in large quantities in a nutrient solution supports the idea that roots can readily release virus by a normal

excretion process (Chapter 6).

Also mowing may lead to further spread of RYMV. A decreasing gradient of infection was observed in the direction of mowing from the point where the first plants were mowed to the position where the last infected plant was found. Infection of plants trimmed with virus-contaminated scissors confirms that RYMV is indeed spread by cutting plants. Spread of *Cynasurus mottle virus,* also a beetle-transmitted sobemovirus, has been noticed after mowing lawns (Huth and Paul, 1977). This spread also occurred in the direction of mowing.

Mowing infected rice crops at harvest is the first process by which the infection will spread in stubble fields. The infection will further spread in harvested fields in the contra-season by cattle and donkeys grazing on the regrowth, on germinating grains, and on developing wild rice species and other grasses (Chapter 5). Rats, which will move from the dikes and levees to water drained stubble fields, will also contribute to this spread. Spread after harvest will result in an enhanced virus reservoir in the stubble field. This reservoir will likely form a source by which seedbeds and the next crops will be infected. Mammals, which accidentally feed on infected stubble and infected plants along the roads and levees, might spread the virus to seedbeds and crops. These infections may also result from virus released from infected plants plowed down in stubble fields. In addition, infection may also be introduced in the seedbeds and crops by dropping infected feces, or result from infections caused by farmers removing weeds while planting seedlings.

The incidence of the disease is lower in 'Kogoni 91-1' crops than in 'BG 90-2' crops as experienced by farmers. This greater tolerance is supported by the present results demonstrating that RYMV spreads more readily in 'BG 90-2' during handling the crops, e.g. transplanting and treading, than in 'Kogoni 91-1'. The greater increase of infected 'BG 90-2' plants after transplanting shows that this cultivar is also more prone to spread by other factors such as treading and wind-mediated leaf contact between healthy and infected plants.

CHAPTER 4

WIND-MEDIATED SPREAD OF *RICE YELLOW MOTTLE VIRUS* (RYMV) IN IRRIGATED RICE CROPS

A study was carried out to demonstrate that *Rice yellow mottle virus* (RYMV), a virus known to be transmitted by beetles, can spread between rice plants by direct leaf contact caused by wind. Almost all healthy plants surrounding an infected plant became infected when exposed to a fan blowing for 15 min at a distance of 50 cm. Spread of RYMV by plant contact, mediated by wind, was also demonstrated in field experiments; the extent of spread depending on plant density. Infection was almost ten times higher in plots with a density of 33 plants/m^2 than in plots with 16 plants/m^2. Little spread was observed in plots protected by 1.5 m high windscreens. It is suggested that wind-mediated spread of RYMV may result from abrasive contacts between the leaves of plants.

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INTRODUCTION

Rice yellow mottle virus (RYMV), primarily known as a beetle-transmitted virus, is also efficiently transmitted mechanically (Bakker, 1974). The virus is endemic in Africa, south of the Sahara, and occurs mainly in irrigated rice ecosystems. RYMV induces a variety of symptoms depending on the cultivar, including green, yellow or orange leaf mottling, reduced tillering, stunting of the plants and sterility of flowers. Infections affect yield considerably when plants are infected at an early stage of development.

RYMV infections cannot be discerned in most crops in the 'Office du Niger' region of Mali (Peters *et al*., 1999). However, different patterns of disease spread can be observed in crops with infections. Completely or almost completely infected fields occasionally occur. These fields may border fields in which little or no infection can be observed. Large or small distinct patches with infected plants can be found scattered throughout affected fields. The number of such patches varies from one to several or many. All plants in such patches are infected. Infected plants can also be found in clearly defined strips varying in length from a few plants to 10 or 20 m along levees and road sides, or at the corners of parcels with rice (Chapter 4; Sarra and Peters, 2003). The different patterns observed suggest that the virus may spread by different mechanisms and can originate from different sources.

A gradient in incidence or severity or both is often observed from the centre to the border of small spots. Such gradients can also be found in the outermost zones of larger spots. Gradients in infected spots are often the result of virus transmission from initial foci of infection (Thresh, 1976). However, the complete or nearly complete absence of beetle vectors in the rice fields in the region of the 'Office du Niger' suggests that the formation of such gradients in RYMV-infected spots must be the result of processes other than beetle-mediated transmission. As RYMV is readily transmitted mechanically, leaf contact between infected and healthy plants may be an alternative mean of spread.

Wind is often considered to be an important factor in the dispersal of plant viruses via transport of infected vectors. Wind-mediated dispersal of vectors generally occurs over short distances, but evidence exists that some plant viruses are spread over long distances by their insect vectors (Thresh, 1983; Hull, 2002). No evidence has, thus far, been reported for the dispersal of RYMV by beetles in this way. Wind-mediated transmission by direct leaf contact between healthy and infected plants may be an alternative means of spread for this virus. Transmission by leaf contact of some mechanically transmissible plant virus has been the subject of some studies. More potato plants became infected with *Potato virus X* when leaf contact between healthy and infected plants was increased by a fan (Loughnane and Murphy, 1938). Evidence was also obtained for the spread of *Barley stripe mosaic virus* by leaf contact in barley. Infections occurred in plants adjacent to infected plants, whereas no infections were found in more distant plants (Chiko, 1973; Slack *et al.*, 1975). Wind-mediated spread of *Turnip crinkle virus*, a tombusvirus, was demonstrated experimentally (Broadbent and Heathcote, 1958). The high density of rice seedlings suggests that infection by leaf contact might occur more frequently in seedbeds than in the field. However, infection by

leaf contact might proportionally increase with the size of the plants in the field during the growing season. Such leaf contacts between healthy and infected plants will result in a repetitive process of infection forming gradients of symptom severity in small spots and on the edge of larger spots.

Showers occur frequently in the rainy rice-growing season in the 'Office du Niger'. These showers are almost always preceded and accompanied by blasts and squalls leading to fierce contact between leaves. Sand and dust are also transported by these winds, especially at the beginning of the season or after a couple of dry days during the season. This sand and dust could act as an abrasive during leaf contact and may potentially enhance the inoculating effect of wind. In a phenological study on the seasonal occurrence of RYMV no significant correlation between rain and RYMV incidence was found (Heinrichs *et al*., 1997). However, this conclusion was not validated by experimental studies.

This chapter describes the results of screenhouse, glasshouse and field experiments demonstrating that RYMV can spread by leaf contact between healthy and infected plants.

MATERIALS AND METHODS

Inoculation of plants by experimentally created wind

Experiments were conducted to demonstrate spread of RYMV by artificially created wind, first in a screenhouse at the Institut d'Économie Rurale Research Station in Niono (Mali) and later in greenhouses at the Wageningen Agricultural University (The Netherlands).

In the Niono experiments, seedlings of the highly susceptible rice cv. BG 90-2 were planted in buckets, 30 cm in diameter. One seedling, placed in the centre and surrounded by five, seven or 10 other seedlings, was inoculated with RYMV using an extract of 1 g infected leaf material in 10 ml phosphate buffer, pH 7.2. The distance of each test plant to the source was at least 12 cm. Two weeks after inoculation, the 6 six-week-old plants were exposed to a full-speed oscillating fan placed at a distance of 50 cm from of the bucket for 15 min, either in the morning or in the afternoon. The treatments with five and 10 plants per bucket were repeated three times and those with seven plants six times (Table 1). Infection was monitored visually 2, 3 and 4 weeks after exposure to wind and confirmed by ELISA after the last inspection. The control consisted of a similar series of buckets with a virus source and healthy plants that were not exposed to the fan. The results were analysed statistically using the Mann Whitney *U* two sample test ($P = 0.10$).

In the Wageningen experiments, RYMV-inoculated young plants of the cv. BG 90-2 were planted in 50 ml plastic tubes to prevent infection of healthy plants with virus released from roots. One infected source was placed in the centre of a bucket, 15-cm in diameter, with five or 10 healthy plants. To prevent leaf contact between the source and test plants, the source plants were sheathed with transparent foil before exposure to a full-speed oscillating fan producing a wind current of 70 m/min at the plant. The fan was placed 30 cm in front of the bucket for 16 min and the buckets were rotated 90^{o} every

minute. After exposure, the source plants were immediately removed from the buckets. There were six treatments (Table 2). Plants were (i) kept dry during exposure, (ii) kept dry and dusted with carborundum, (iii) kept dry but not exposed (control), (iv) wetted during wind exposure to imitate wind with rain, (v) wetted before exposure to wind, and (vi) wetted without exposure to wind (control). Each treatment was replicated five times (Table 2). The infection was monitored as described above. The source plants in the controls were removed after exposing the plants to wind. The results were statistically analysed using Newman and Keuls' sample test (P = 0.05).

Sweeping infected plants over healthy plants

The effect of wind was imitated by sweeping a bunch of five freshly cut RYMV-infected rice plants six times along and through a group of five and 10 healthy plants (Table 2).

Inoculation of plants by natural wind

The contribution of wind to the spread of RYMV in the field was studied at Niono in a trial during the rice-growing season of 2000. The experimental field consisted of two blocks each with nine plots of 9 m^2 and was situated in the open (Fig. 1). A 1 m wide strip was left between each two rows of the plots and a 50 cm wide strip between each two columns (Fig. 1). The experiments were conducted with the highly susceptible rice cv. BG 90-2 at plant spacings 25 x 25 cm (16 plants/m^2), 20 x 15 cm (33.4 plants/m^2), or according to farmers' practice (32.6 plants/m^2). A rice plant in the middle of each plot was inoculated mechanically 10 days after transplanting using extracts of 1 g of infected leaf material ground in 10 ml water. Carborundum was used as an abrasive. A screen of 1.5 m high plastic foil surrounded each plot in the wind-protection block. Weeds were controlled 10 days after transplanting by applying Ronstar PL consisting of 80 g oxadiazinon and 300 g propanil/l in a dose of 5 l/ha.

The plots were monitored for infection 15, 30 and 45 days after inoculation (without entering the plots) and at harvest (110 days after inoculation). The plants were not monitored for infection in the period between 45 days after inoculation and harvest to avoid spread by treading and trampling of the plots (Ferris *et al*., 1996; Chapter 5; Sarra and Peters, 2003). The position of healthy and infected plants was plotted on a pattern of concentric circles around the source (Fig. 2). Wind effect was calculated by analysing the number of plants showing symptoms in protected and unprotected plots. The results were analysed statistically using the ANOVA calculation of STATITCF and means were separated according to Newman and Keuls' test at a 5% level.

Virus detection by enzyme-linked immunosorbent assay (ELISA)

RYMV infection in the test plants was analyzed by ELISA using leaf disks, 5 mm in diameter, cut with a one-hole perforator. Each disk was ground in 150 µl phosphate buffered saline containing 0.05% Tween-20, pH 7.2. Polyclonal antibodies, raised in rabbits against purified RYMV were used at a concentration of 1 µg/ml.

Table 1. Number of plants infected with *Rice yellow mottle virus* after exposing five, seven or ten plants with an infected plant in a bucket to a blowing fan for 15 min at a wind speed of 70 m/min in three or six treatments**.**

Treatment	Number of plants per bucket		
	5 (N* = 3)	7 (N = 6)	10 (N = 3)
Plants exposed to wind early in the morning	15/15a	41/42a	22/30a
Plants exposed to wind in the afternoon	13/15a	42/42a	28/30a
Plants not exposed to wind (control)	0/15b	0/42b	0/30b

*N = number of buckets per treatment; common letters in a column indicate statistically similar results

Table 2. Average percentage of *Rice yellow mottle virus*-infected plants after exposing five or 10 plants in a bucket with an infected source to a blowing fan for 16 min at a wind speed of 70 m/min in five different treatments, or sweeping infected plants over healthy plants. N = the number of buckets used per treatment. Data followed by the same letter in a row are statistically not different using Newman and Keuls' test at $P > 0.05$.

Treatments	Number of plants per bucket	
	5 (N = 5)	10 (N = 5)
Dry plants exposed to wind	92.0 ± 4.9a	70.0 ± 15.2a
Dry plants and carborundum exposed to wind	92.0 ± 4.9a	90.0 ± 4.4a
Dry plants not exposed to wind (control)	0.0 ± 0.0b	0.0 ± 0.0b
Plants exposed to wind and rain	68.0 ± 10.2c	56.0 ± 6.0c
Plants wetted before exposure to wind	96.0 ± 4.0a	74.0 ± 9.4a
Plants wetted without exposure to wind (control)	0.0 ± 0.0b	0.0 ± 0.0c
Infected plants swept through healthy ones	92.0 ± 4.9a	92.0 ± 3.7a
Healthy plants swept through healthy ones (control)	0.0 ± 0.0b	0.0 ± 0.0b

RESULTS

Inoculation of plants by artificially created wind

Most of the plants were infected within 4 weeks after exposure to the fan in the Niono experiments. No differences in the number of infected plants were found between those exposed in the morning and those exposed in the afternoon (Table 1). These plants were either guttation-water wet (morning) or dry (afternoon), respectively. The number of plants, which became infected was unaffected by plant density. No infection occurred in the controls, demonstrating that infection via any other natural means of spread could be rejected.

Infection of the plants was also unaffected by plant density in the Wageningen experiments (Table 2). No differences were found when the plants were kept dry or were dusted with carborundum. A similar number of plants became infected when they were wetted prior to exposure to wind, whereas fewer plants became infected when they were wetted during exposure to wind. No infection was observed on plants in the control treatments.

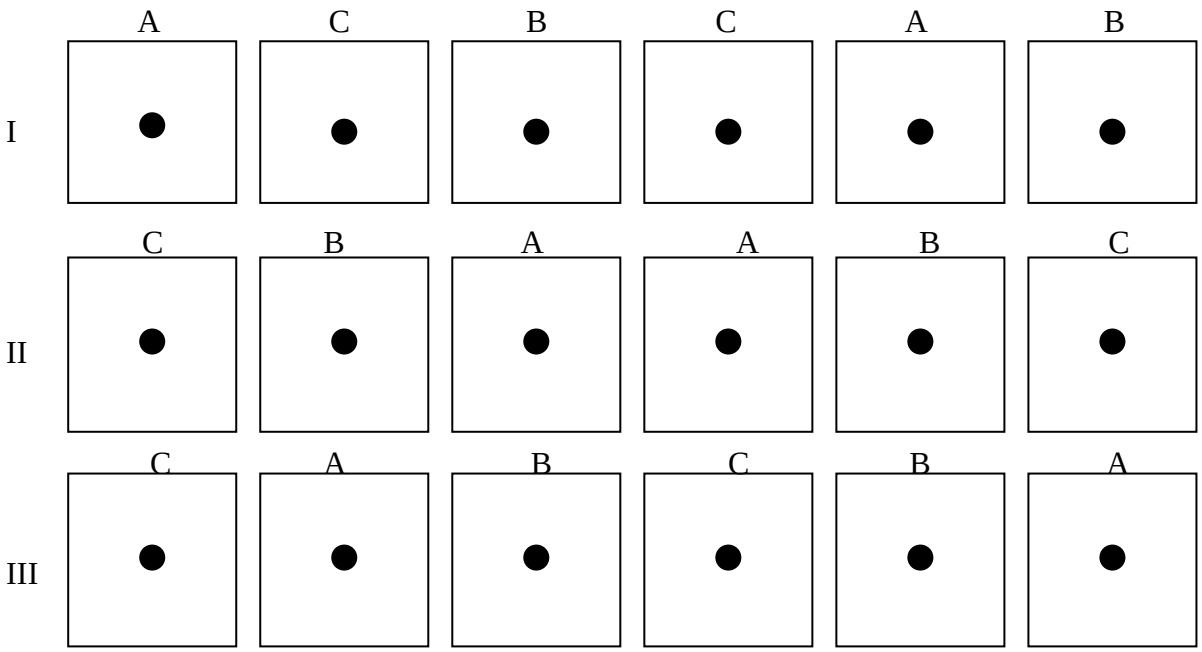

Figure 1. Layout of a field experiment in which wind effects on the spread of *Rice yellow mottle virus* were studied. Open squares: plots not surrounded by screens; shaded square: sub-plots surrounded with screens. I, II and III are repeats, A, B and C: plots with 16.0, 33.4 and 32.6 plants/m^2, respectively. ● = infected source plant. Square size is 9 m^2.

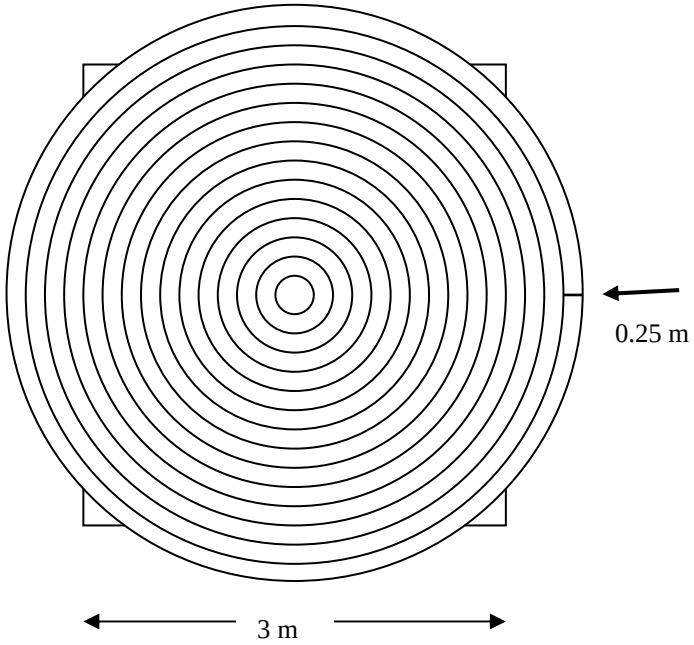

Figure 2. Pattern used to determine the distance of wind-mediated virus spread from the central *Rice yellow mottle virus* source plant in a sub-plot (3 x 3 m^2). Diameters of concentric rings are multiples of 0.25 m.

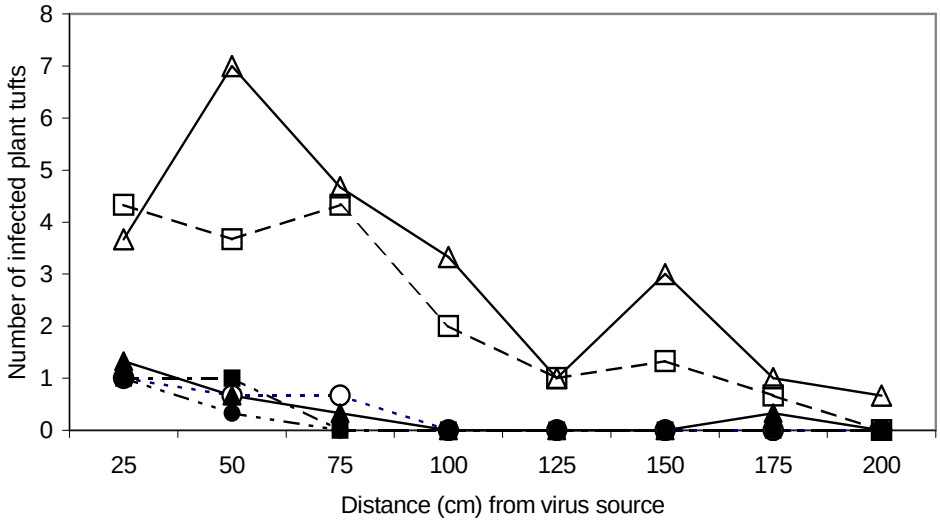

Figure 3 Spread of *Rice yellow mottle virus* in rice plants of the cv BG 90-2 in wind protected sub-plots with 16 plants/m^2: --■--, 33.4 plants/m^2: —▲—, and 32.6 plants/m^2: --●--; and in unprotected sub-plots with 16 plants/m^2: --○-- 33.4 plants/m^2: —Δ—, and 32.6 plants/m^2: --□ --.

Infection by natural wind

The effect of wind on the spread of RYMV was also studied in the field in two blocks, in one of which the plants were protected from wind. No significant spread occurred in the first 15 days after inoculation (Table 3). Only one plant became infected in the first 15 days after inoculation in an unprotected plot with a plant density of 33.4 plants/m^2.

Table 3. Average number of plants infected with *Rice yellow mottle virus* around a virus source in wind protected and -unprotected plots with different plant densities 15, 30, 45 and 110 (at harvest) days post-infection, and the average distance of virus spread at harvest time.

Plant density and plot treatment	Days after infection				Average. distance of virus spread at harvest time (cm)
	15	30	45	110[a]	
32.6 plants/m^2[b]					
Unprotected plots	0.3 (0.3)[c]	2.7 (1.2)	5.3 (1.9)	7.3 (3.0)a[d]	158a
Protected plots	0.3 (0.3)	0.3 (0.3)	0.7 (0.3)	2.0 (3.9)b	33b
33.4 plants/m^2					
Unprotected plots	0.7 (0.3)	2.9 (1.0)	5.3 (2.0)	24.7 (2.9)a	183b
Protected plots	0.0 (0.0)	1.0 (0.6)	1.0 (0.6)	2.3 (1.3)b	42b
16.0 plants/m^2					
Unprotected plots	0.3 (0.3)	0.7 (0.3)	0.7 (0.3)	2.3 (1.3)b	50b
Protected plots	0.0 (0.0)	0.3 (0.3)	0.7 (0.3)	1.3 (1.9)b	33b

[a] Harvest; [b] Plant density resulting from farmers planting practices
[c] Figures in brackets indicate standard error
[d] Numbers followed by the same alphabetical letter in a column are not statistically different

Creating leaf contact by sweeping a bunch of RYMV-infected plants through and along groups of healthy plants also resulted in spread of virus (Table 2).

Some spread was observed 30 days after inoculation in the unprotected plots with densities of 33.4 and 32.6 plants/m^2 (Table 3). Differences between the average number of infected plants in protected and unprotected plots were statistically not significant.

Statistical significant differences between the average number of infected plants in protected and unprotected plots were observed 45 days after inoculation. The average percentages of infected plants at densities of 16.0, 33.4 and 32.6 (farmers' practice) plants/m^2 were 1.6, 8.2 and 5.9, respectively, in unprotected plots and 0.3, 1 and 1, respectively, in protected plots. The spread was thus similar in unprotected plots with densities of 33.4 and 32.6 plants/m^2 (farmers' planting practice), and evidently greater than in unprotected sub-plots with 16.0 plants/m^2 (Table 3).

Virus spread was analysed at harvest by the number of infected plants and the distance of infected plants from the virus source. The virus spread over a distance of 1.75 to 2.0 m in unprotected plots with densities of 33.4 and 32.6 plants/m^2 (Fig. 3). However, the number of infected plants in plots with 33.4 plants/m^2 was significantly higher than in plots with 32.6 plants/m^2. In the latter case the more irregular stand, planted according to farmers' practices, may explain the lower number of infected plants. No significant differences were found between virus spread in protected and unprotected plots with a plant density of 16.0 plants/m^2 and in the protected sub-plots. The distance over which the virus spread did not exceed 75 cm in any protected plot.

The virus infections were always concentrated in the centre of the experimental plots around the inoculated plant. The percentage of RYMV-infected plants decreased from the middle toward the border in all sub-plots (Fig. 3), and varied from 9.1 to 25% in protected plots and from 25 to 50% in unprotected treatments in the area covered by the first ring around the inoculated plant.

Infected patches with a radius of 1 m or more often had an irregular shape. A gradient of decreasing in symptom severity was observed from the centre towards the border in these patches. The existence of such gradients can be explained by a sequential infection, as plants infected early are more severely affected than those infected later.

DISCUSSION

This study shows that RYMV can be spread by wind under experimental conditions in a screenhouse or glasshouse, and under natural conditions. The majority of the plants exposed to wind in screenhouse and glasshouse experiments became infected. The infection rate was not affected by plant density. The absence of a plant-density effect in these experiments can be explained by the high densities used, by which density effects could not be explored.

The significant differences found between the number of infected plants in protected and unprotected plots indicate that RYMV is indeed spread by wind in the field. This spread depended on the plant density. Greater distances between plants in the field result in less leaf contact between leaves early in the season than in higher-density

stands. With the growth of the plants, leaf contacts between the plants will occur more frequently and, as a result, infection will progress continuously in the field.

Disease spread by wind-mediated leaf contact can be explained by infection through wounds, caused by abrasive contact between the leaves of healthy and infected plants. The wounds will release virus from infected plants and form virus entry points on healthy plants. The idea that leaf contact indeed causes the spread of the virus is supported by the higher infection rates found in higher plant density stands in the field and by the imitation of wind-mediated spread by sweeping a bunch of infected plants through healthy plants in the glasshouse. Dust and small sand grains may promote the formation of wounds. However, this could not be confirmed in our glasshouse experiments in this study.

Guttation water of RYMV-infected plants is highly infectious (Bakker, 1974; Chapter 3). Exposure of guttation water-wet plants to wind might have increased the number of infected plants. However, the presence of this water on the infected plants in the morning did not affect the number of infected plants as compared to dry plants exposed to artificial wind in the afternoon (Table 1). Guttation water droplets may fall down as soon as the plants are exposed to wind preventing virus in the guttation water of an infected plant from infecting a healthy neighbour.

Limited spread, restricted to one or two plants next to the inoculated source plant, was observed in all protected plots. On average these infected plants always occurred within 50 cm of the source (Table 3). However, one infected plant was found at 75 cm from the source in a wind-protected plot with a plant density of 33.4 plants m^{-2}. Although all these plots were protected against wind, this limited spread can be explained by the occurrence of wind turbulence during the strong storms, which occur occasionally. These plants may also have become infected by other mechanisms by which sobemoviruses can spread. Infection of plants by irrigation water contaminated with guttation water from infected plants has been suggested (Bakker, 1970). Dissemination of sobemoviruses by animals treading or grazing in the field has been suggested for *Cocksfoot mottle virus* (Upstone, 1969; Benigno and A'Brook, 1972), and has been demonstrated for *Subterranean clover mottle virus* (Ferris *et al*., 1996) and for RYMV (Chapter 5). As animals, including cattle, donkeys, goats, rats and sheep could not reach the experimental fields in the present study, infection by treading or grazing mammals was excluded.

Wind-mediated spread of *Turnip crinkle virus* was demonstrated experimentally in the glasshouse (Broadbent and Heathcote, 1958). Although suggesting that spread by wind contact between plants might also occur in the field, this idea was not confirmed experimentally. These authors speculated that the plants in the field might be more resistant than in the glasshouse. As rice plants continuously produce young leaf material it cannot be expected that mature resistance will play a role in this type of spread.

This study demonstrated RYMV can be spread by leaf contact. It can be expected that highly infectious and mechanically transmissible viruses – such as all other beetle-transmitted viruses, the tombusviruses, the tobamoviruses, etc. will also spread by wind contact between infected and healthy plants. However, no reports exist claiming that

these viruses can spread by wind-mediated plant contact in the field.

Spread by wind contact in the field depended greatly on plant density. An average of five- to eightfold increase was found in the unprotected plots with plant densities of 33.4 and 32.6 plants/m^2, whereas a limited spread wad noticed in these plots 16 plants/m^2 in plots. The spread in unprotected plots with 16 plants/m^2 was comparable to the spread in all protected plots irrespective of their density. The plant density in a rice seedbed is at least 50 - 100 times larger than in the field. As the seedbeds are not protected, the seedlings will also be disturbed by wind. Therefore any infection, introduced in a seedbed will spread by wind, probably at a higher rate in the seedbed than in the field.

CHAPTER 5

RICE YELLOW MOTTLE VIRUS IS TRANSMITTED BY COWS, DONKEYS AND GRASS RATS IN IRRIGATED RICE CROPS

Rice yellow mottle virus (RYMV), endemic in Africa, is believed to be spread by chrysomelid beetles, although the epidemiology often can not be explained by the prevailing number of beetles. Here it is shown that grass rats (*Arvicanthis niloticus*), domestic cows and donkeys are potent and efficient transmitters of RYMV in rice fields. Spread of RYMV by rats was demonstrated in cage experiments and was subsequently confirmed in a field experiment. Virus spread by donkeys and cows was demonstrated in experiments in which they first grazed on partially infected plots and then on healthy plots. A high percentage of seedlings became infected in a seedbed by a cow after consuming infected rice plants. Saliva of cows and donkeys was serologically positive and infectious for some hours after consuming infected rice plants. In these studies donkeys proved to be more efficient transmitters than cows. Transmission was also observed when cows were allowed to graze on stubble of infected fields. These results suggest that grazing cattle will enhance the virus load in the contra season in the field and, hence, the chance that the next crop in a field with an infection history in the previous crop will become infected.

This chapter is an extended version of Soungalo Sarra and Dick Peters, 2003. *Rice yellow mottle virus* is transmitted by cows, donkeys and grass rats in irrigated rice crops. *Plant Disease* 87: 804-808. The extension presents results on the persistency of RYMV in saliva of cows and donkeys, and on serial transmission of RYMV to rice seedlings by cows and donkeys.

INTRODUCTION

Rice yellow mottle virus (RYMV), a member of the family *Sobemoviridae*, was first noticed in 1966 in Kenya (Bakker, 1970). This virus is endemic in Africa, south of the Sahara, and occurs mainly in irrigated rice cultivation systems (Abo *et al*., 1991). RYMV has been reported from several East, Central and West African countries, including the islands of Zanzibar and Madagascar (Bakker, 1970; Banwo *et al*., 2001b; Reckhaus *et al*., 1997; Rossel *et al*., 1982a,b). The virus infects most, if not all, wild rice species (*Oryza* spp.) and some other grass species (Awoderu, 1991; Bakker, 1974; Fauquet and Thouvenel, 1977). The wild rice species *O. longistaminata* is believed to form a natural virus source in the Soudano-sahelian zone. The virus may also be spread from infected *O. barthii* and the grass species *Echinochloa colona*, *Panicum repens,* and *Ischemia rugosum* (Konaté *et al*., 1997).

RYMV induces a variety of symptoms on rice plants. They include mottling, yellow or orange leaf discoloration, reduced tillering, stunting of plants and sterility of flowers (Bakker, 1974; Attere and Fatokun, 1983). This sterility affects crop yield considerably when the plants become infected early in their development. Occasionally, severe crop yield losses, up to 84 to 97%, have been recorded in different crops (Fomba, 1990, Heinrichs *et al*., 1997; IRRI, 1988; Taylor *et al*., 1990). Most, if not all, commercial rice cultivars are susceptible to the virus, although the susceptibility may vary considerably (Fomba, 1988).

Like all sobemoviruses, RYMV is readily transmitted mechanically. Moreover, several beetle species belonging to the family *Chrysomelidae* have been shown to transmit this virus (Bakker, 1974; Banwo, 2002; Reckhaus *et al*., 1997). Of these beetles, *Trichispa sericea* is the most frequently encountered beetle in rice crops and is known as a serious pest on rice in Africa. However, no studies have been made on the significance of this beetle or of any other beetle species in the spread and epidemics of RYMV in the field.

The low number of beetles actually caught in fields in the Soudano-sahelian region and the different patterns of virus spread observed in various fields, strongly suggest that RYMV is not exclusively spread by beetles (John *et al.*, 1985; Oevering, 1996, Peters *et al*., 1999; Sarra *et al*., 1998). In the region of “Office du Niger”, the largest rice-cropping area in Africa, completely infected and healthy fields may occur side by side. In most affected crops, infection varies from a single plant to numerous patches of infected plants; these patches vary in size from 1 to 20 m in diameter, or more, in a field. In addition to these patches, short or long strips of infected plants can occur in rice fileds along levees and roadsides, and frequently at corners of a parcel where the soil is drained. The clustered appearance of the infected plants in these strips and at the corners does not support beetle-mediated spread of the virus as a more scattered spread is expected when beetles are the sole transmitters. These observations and the occurrence of completely infected fields next to fields without any infected plant suggest that RYMV is not solely spread by beetles. During the growing season, numerous rats of the species *Arvicanthis niloticus* live in burrows on the levees and roadsides surrounding the paddy rice fields. The association of feeding damage to plants and the

presence of rat roads and droppings with the occurrence of infected plants suggest that rats can cause infections along the levees and roadsides, and at corners of fields. In this article we present evidence that RYMV is spread by this rat species.

Cattle and donkeys feed on rice stubble in harvested fields in the Soudano-sahelian zone, and occasionally in rice nurseries and in crops along roadsides. Since sheep have been shown to transmit the sobemovirus *Subterranean mottle virus* in subterranean clover by grazing and trampling (Ferris *et al*., 1996; McKirdy *et al*., 1998), here, we have studied the potential of cattle and donkeys to spread RYMV. The significance of the transmission of RYMV by these mammals will be discussed in relation to both spread and survival of RYMV in the field.

MATERIALS AND METHODS

Transmission and spread of RYMV by the grass rat *A. niloticus*

Four male and two female rats were caught after covering their burrows by a net and pouring water into the entrances. The caught rats were individually placed with 15 healthy plants of the highly susceptible rice cv. BG 90-2 interspersed with five RYMV-infected plants in 2 by 2 m cages for 9 h. The plants in a seventh cage without a rat, but with the same number of healthy and infected plants served to monitor any other potential mode of natural virus spread (e.g. by beetles). To prevent any unwanted spread the rats were killed after the experiment. All cages were enclosed within a screenhouse and sprayed weekly to control insects. The plants were visually inspected for infection 3 weeks later and tested by enzyme-linked immunosorbent assay (ELISA).

Spread of RYMV by grass rats in the field

Spread in the field was studied in an experiment in which rats had free access to rice plants at only one side of a levee. This levee, 40 cm wide, separated two fields (A and B) both with the rice cv. BG 90-2. The soil sloped slightly upwards to the levee at side A. This slope gave the rats access to the plants depending on the water level. The soil at side B did not slope so that the plants grew in a water layer varying between 15 and 20 cm deep, by which the rats had no access to the plants. The presence of several rat burrows, as judged by the presence of fresh entrances showed that the rats were living along the 90 m long levee separating the fields, and on the 100 cm wide dike on the drain side of both fields. A few infected specimens of susceptible grass species and *O. longistaminata* (a natural host of RYMV) grew on this levee. Virus incidence in both fields was recorded in three blocks 4 m long and 0.7 m wide. These blocks had their center at 15, 45 and 75 m of the 90 m long levee.

Competence of cows and donkeys to transmit RYMV

Cattle-mediated spread of RYMV was studied during the growing season of 2000. Seeds of the rice cv. BG 90-2 were sown directly at a distance of 25 by 25 cm in two plots of 11 m^2, hence each plot counted 162 plants. Fifteen days after sowing, 10 seedlings were inoculated mechanically in the first plot (A) using an extract of 1 g of infected plant material in 10 ml of 0.01 M phosphate buffer, pH 7.2. The inoculated

plants were distributed randomly in the plot and their position marked. No plants were inoculated in the second plot (B). When symptoms (15 days after inoculation) appeared, a 1-year-old calf grazed plot A for 1 h and was then transferred to plot B for 4 h to test whether the virus could be spread to a plot after grazing a plot with infected plants. Disease incidence was monitored visually and by ELISA 3 and 6 weeks post roaming and grazing. After the last monitoring, the calf was given free access to both plots for 10 h. The plants infected were counted 3 and 6 weeks after the second visit. An adjacent plot (C) with 112 plants was used to monitor any natural spread.

Donkey-mediated transmission was studied in a similar experiment. Plots A, B and C counted 191, 143, and 60 plants, respectively. The donkey finished plot A in 25 min and was then transferred to plot B, which was grazed in 30 min. This donkey again was given access to both plots following monitoring the infection six weeks after the first visit.

The efficiency, by which RYMV is spread by a calf and a donkey, was studied by offering an infected rice plant first and then a series of 49 healthy plants. These plants were individually planted in 15 l pots, placed in a row and offered in the order of this row. The number of infected plants was visually monitored.

Persistence of infectivity of RYMV in the saliva was studied by feeding a donkey and a cow an infected plant. Saliva was collected from their mouths at various intervals and inoculated on five ‘BG 90-2’ plants. After collecting the saliva, the donkey and the cow were allowed to eat five transplanted BG 90-2 plants.

Cow-mediated transmission of RYMV to a seedbed

To study the probability that cattle can infect seedlings in a seedbed, each of two seedbeds were sown with approximately 1200 seeds of the rice cv. BG90-2 and twenty seeds in four buckets. The seedlings of two buckets were inoculated with RYMV. Three weeks later, the non-inoculated seedlings were fed to a cow, which then was allowed to graze one of the seedbeds. A day later this cow was allowed to graze the second seedbed after ingesting the infected seedlings. One thousand seedlings were transplanted from each seedbed in the field two weeks after being grazed. Disease incidence was visually monitored every two days starting six days after transplanting.

Spread of RYMV by cows in stubble fields

To study the spread of the virus to rice stubble, a field experiment was designed after the harvest in two partially infected rice crops. In the field (field 1), 25 plots of 1 m^2, scattered over the field were selected randomly. The total number of plants in these plots ranged between 12 and 31, of which the number of infected plants ranged from 1 to 6. In the second field (field 2) 72 plots, each with 10 to 30 plants, were selected among which 20 plots were infected. The number of infected plants varied from 1 to 9 plants in the 20 plots with infected plants. The location of the plots was mapped and the infected plants labelled. Seven cows roamed and grazed for nine h in field 1 and fifteen cows for four h in field 2. RYMV infection and rat damage was checked in the plots of both fields every 2 weeks by counting the number of stubble with infected regrowth

without entering the plots. Ten 1 m^2 plots were surrounded by a barbed wire fence and served as controls in each field.

Insect infestation

The insect population was monitored in the stubble fields in which the cattle-mediated virus spread was studied. One hundred catches were made with a butterfly net at fifteen, thirty and forty-five days post grazing to trap insects in each field. Insects trapped were brought for identification to Mr. Y. Jongsma at the Laboratory of Entomology, Wageningen University, Wageningen.

Enzyme-linked immunosorbent assay (ELISA)

The infected plants of the cv. BG 90-2 produce pronounced symptoms, therefore most surveys were visually done on leaves or on young developing sprouts. ELISA was used to demonstrate infection in those cases when doubtful symptoms appeared on the leaves or sprouts and when small numbers of plants were used. The ELISA procedure originally described by Clark and Adams (1977) was essentially followed. The wells of the plates were incubated with 0.1 ml of immunoglobulin G (1 µg/ml), sample, conjugate (1 µg/ml) or substrate (1 mg/ml) in the respective incubation steps for two hours at room temperature. The antiserum used was produced against a virus preparation purified from infected BG 90-2 plants according to a procedure described by Fauquet and Thouvenel (1977). Samples were prepared by grinding leaf material in a weight:volume ratio of 1:10 with sample buffer, containing 0.05% Tween 20.

RESULTS

Rat-mediated transmission of RYMV

Potential virus spread by grass rats was first studied in cage experiments, where 15 healthy and 5 RYMV-infected rice plants where exposed to individual rats. In the cages containing male rats, stems and leaves of most of the rice plants were felled by gnawing. Some of the felled stems were even cut in large pieces. This damage was similar to damage found in the fields at spots along levees and roadsides and at corners where infections occurred and the soil sloped upward. In the cages with males, 10 to 14 out of 15 healthy plants became infected (Table 1). Disease symptoms were observed within 20 days post exposure to the rats and did not increase in the following 25 days. Four plants damaged by the males did not develop disease symptoms and reacted negatively in ELISA. No infected plants were found in the control cage and in the cages containing females, although one plant was damaged. Instead of feeding on the offered plants, the female rats were trying to escape from the cages or were sitting in the darkest corner. The absence of any spread in the cages with female rats and the control cage also demonstrates that spread of the virus by beetles or any other alternative vector or mechanism did not occur in this experiment.

Table 1. Transmission of *Rice yellow mottle virus* to ´BG 90-2' plants by individual grass rats of the species *Arvicanthis niloticus* placed in cages with 15 healthy and 5 infected plants being exposed for 9 h.

	Number of plants		
Rat	damaged/cage	damaged but not infected	infected
Male 1	19	1	14
Male 2	20	1	13
Male 3	17	3	10
Male 4	20	0	14
Female 1	0	0	0
Female 2	1	1	0
Control	0	0	0

Table 2. Transmission of *Rice yellow mottle virus* by the grass rat *Arvicanthis niloticus* in two fields along a levee[a].

	Number of tufts sampled		Number of infected tufts		Incidence (%)	
Block	Side 'A'	Side 'B'	Side 'A'	Side 'B'	Side 'A'	Side 'B'
1	526	560	334	0	63.5	0
2	546	743	360	0	65.9	0
3	883	689	678	0	76.8	0

[a]The rats had access to the plants at side A of the levee, but not to plants on side B of the levee. Plants were sampled for infection in three blocks, 4 m long and 0.7 m wide at 15, 45 and 75 m of the 90 m long levee.

Spread of RYMV by rats in the field

Next, rat-mediated spread was studied in the field. The first infected plants were observed three weeks after transplanting the seedlings at the levee side (A), where the rats had access to the plants (Table 2). This spread coincided with the presence of rat roads on the soil, truncated plants and felled plant remains. The felled leaves and stems occasionally formed a bridge by which the rats could move from plant to plant. The initial infections were noticed in the first row of plants along the levee and extended to the second and third row and occasionally to the fourth row during the growing season. Almost all plants in the first row were infected over the whole length of the levee. The infection rate varied from 64 to 77% in the plots sampled at levee side A (Table 2). No virus-infected rice plants could be discerned at the other side of the levee (B), where the water level prevented rats from feeding on the plants (Table 2). The poaceous flora, consisting of *Oryza longistaminata* plants and grass species on this levee, appeared partially to be infected by RYMV as shown by ELISA (data not shown).

Transmission of RYMV by cattle and donkeys

As it is common use in Mali that cattle and, to a lesser degree, donkeys graze on stubble fields, it was investigated whether these animals are also involved in virus spread. Giving a calf excess to a partially infected plot (A) and next to a healthy plot (B), all plants were almost completely eaten during the first grazing round. In plot A, 46

Table 3. Number of *Rice yellow mottle virus* (RYMV) infected plants by either a calf or a donkey grazing first in plot (A) with infected plants and then in another plot (B) with only healthy plants and 6 weeks later in both plots.

	Number of plants infected with RYMV after being grazed by a					
	Calf			Donkey		
Plot	A	B	C[a]	A	B	C[a]
Number of plants in plot	162	162	112	191	143	60
Before grazing	10	0	0	10	0	0
After first round of grazing	46 (29)[b]	16 (10)	0	99 (55)	51 (36)	0
After second round of grazing	89 (55)	39 (24)	0	78 (43)	77 (54)	0

[a]C = control; [b]Number in parenthesis = percent of infected plants

plants (29%) and 16 plants (10%) in plot B became infected (Table 3). The infected plants on plot A were almost randomly distributed over the whole plot, whereas most of the plants in plot B became infected in that part where the first plants were consumed. These results show that cattle are able to transmit RYMV by grazing and that the virus can substantially spread from an infected plot to a healthy one. In the second round of grazing, another 43 plants became infected in plot A and 23 in plot B (Table 3).

In a similar experiment a donkey was also shown to be a potent virus transmitter. This animal was first released in an infected plot (A) and then in a non-infected plot (B). The donkey, remarkably, ate the plants row by row. The first infected plant was the fourth in the first row grazed. The next consumed plant in this row became the first inoculated one. During the first grazing the virus was transmitted to 99 plants in plot A and 51 plants in plot B, and an additional 78 plants became infected in plot A and 26 in plot B in the second round of grazing six weeks later (Table 3). In both control plots (C) all plants remained healthy, indicating that neither beetles nor any other alternative vector were responsible for the RYMV transmission in the grazed plots.

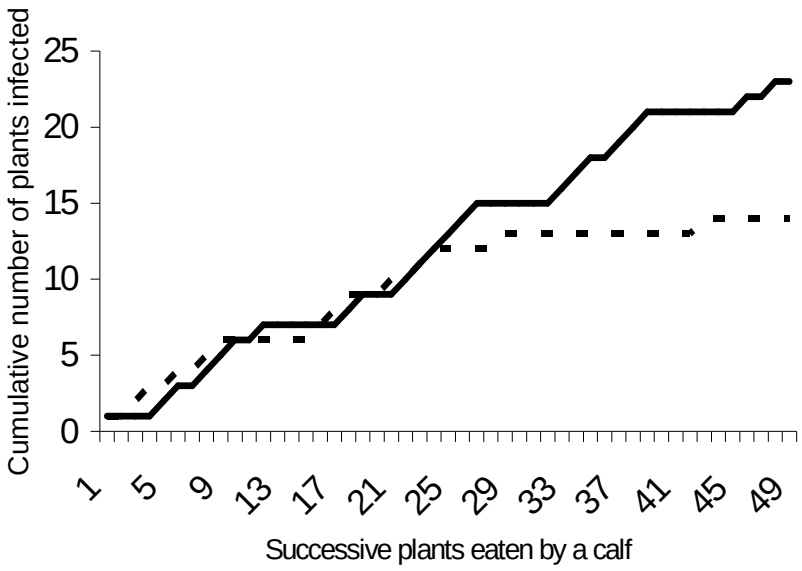

Figure 1. Efficiency by which a calf (– –) and a donkey (—) transmit *Rice yellow mottle virus* to rice plants in a row after acquisition of the virus by ingesting an infected plant.

Table 4. Persistence of *Rice yellow mottle virus* in the saliva of a cow and a donkey after ingesting an

infected plant. Infectivity of saliva samples was tested by inoculating five BG 90-2 plants, and the ability of these animals to transmit by grazing five BG 90-2 plants.

Time between virus ingesting and inoculation (h)	Donkey		Cow	
	Plants infected by			
	saliva sampled	grazing	saliva sampled	grazing
0	5/5[a]	5/5	5/5	5/5
0.25	5/5	3/5	5/5	3/5
0.5	5/5	2/5	5/5	2/5
1	4/5	2/5	3/5	3/5
2	3/5	1/5	2/5	0/5
4	1/5	0/5	1/5	0/5

[a] number of infected plants/number of test plants used

The donkey transmitted RYMV with a higher efficiency than the calf (Table 3). This was confirmed when both were first offered an infected plant and then 49 healthy rice plants in a row (Fig. 1). RYMV was transmitted by the calf and the donkey on the first 25 plants equally efficiently, thereafter the donkey maintained this efficiency but the calf not.

The saliva of both the donkey and the cow proved to be infectious for at least 4 h after ingesting an infected rice plant (Table 4). None of the healthy plants became infected four h after that the donkey had ingested the infected plant. The cow failed to infect a plant after one h and the donkey after 2 h (Table 4).

Seedbed infection by cattle

Of 1000 seedlings transplanted from a seedbed, grazed by a cow after consuming ten RYMV-infected seedlings, 43 percent became infected (Fig. 2). None of the 1000 seedlings of a seedbed grazed by a cow after consuming 10 non-inoculated seedlings became infected.

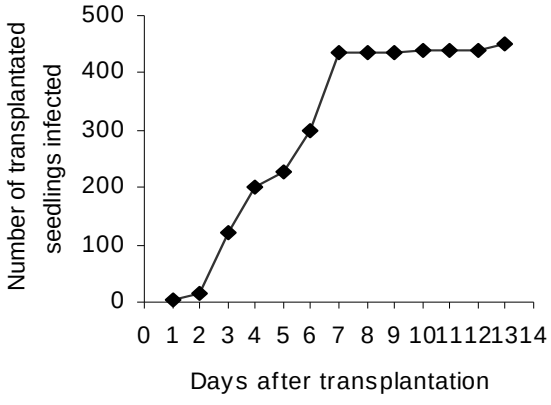

Figure 2. Number of seedlings out of 1000 transplanted ones that were infected after grazing a seedbed with 1200 plants by a cow that had been fed first with ten *Rice yellow mottle virus* infected plants.

Table 5. Increase of the mean number of RYMV-infected plants in stubble of two partially infected fields after being grazed by cattle.

	Field 1		Field 2	
Mean # of plants/plot	24	10	19	10
Treatment	grazed	not grazed	grazed	not grazed
# infected plants before grazing	9	8	4	4
# infected plants 15 days after grazing	20	8	6	4
# infected plants 30 days after grazing	29	8	14	4
# infected plants 45 days after grazing	36	9	21	4

Table 6. The number of insects trapped in two stubble fields 15, 30 and 45 days post grazing by cattle.

	Number of insects trapped	
Insect family	Field 1	Field 2
Heteroptera	1	5
Diptera	69	82
Hymenoptera	1	0
Coleoptera	4	14
Homoptera	7	20
Orthoptera	1	5
Odonate	0	2
Arachnidae (spiders)	7	13

Cattle-mediated spread of RYMV in the field

Spread of RYMV by cattle was confirmed in stubble fields that were partially infected. Before the cattle roamed these fields, the average disease incidence in the selected plots was 9% in field 1 and 4% in field 2 (Table 5). Fifteen days after grazing, the percentage of infected plants increased to 20% (field 1) and 6% (field 2) (Table 5). Thirty and 45 days after the cattle had visited these fields, the infection increased to an average of 29 and 36 % in field 1, and of 14 and 21 % in field 2, respectively. The number of infected plots increased from 20 to 63 in field 2. This increase has to be explained by transmission of the virus by the cattle from infected plants present in the direct surroundings of the healthy plots. Transmission by rats could be excluded as no damage was observed in the plots. The incidence of RYMV infection did not increase in control plots, as cattle had no access to them.

Insect population

The prevailing insect population in the stubble fields, wherein cattle had grazed, was determined 15, 30 and 45 days post grazing. The number of insects caught was extremely low and consisted of *Diptera*, *Coleoptera*, and *Homoptera*. A few members of the *Hymenoptera*, *Heteroptera*, *Odonate*, *Orthoptera* and *Arachnidae* (spiders) were found in the catches made in fields 1 and 2 (Table 6). Beetle species, reported as possible RYMV vector, or any other chrysomelid beetle species were not encountered.

DISCUSSION

Most plant viruses are transmitted from plant to plant by phytophagous invertebrates. Vertebrates can also transmit plant viruses during their foraging as shown in this study on RYMV. The grass rat *A. niloticus* efficiently transmits RYMV to healthy rice plants after gnawing and foraging on infected plants. While in cage studies this is true for male rats, females appeared not to spread virus, probably due their behaviour in captivity. Indeed, they showed clear signs of stress, searching for ways to escape from the cages, or remaining inert in the outermost corner. As this rat species lives, preferably, on cultivated rice plants, it is likely that under field conditions, females, like males, will also be involved in the spread of RYMV.

A few plants remained healthy after being damaged by the male rats. Possibly these plants were damaged before infected ones were gnawed or, alternatively, the saliva may have lost its infectivity. It can also not be ruled out that transmission is a matter of probability as shown for the calf and the donkey. After consuming an infected plant a probability of 0.56 was found within the first 25 plants offered (Fig. 2). The calf then had almost lost its capacity to infect, whereas the donkey could proceed at the same rate.

Spread of RYMV by rats was confirmed in the field. Infected plants were detected only at that side of a levee where rats had access to plants (Table 2). Where the rats could not feed on plants because access was prevented by water, no infection was observed.

Slopes, which give rats access to plants, are often found along the levees and road sides around a field. Plants at such positions often are infected and usually occur in a closed formation of plants. The frequent presence of infected plants at corners of the rice fields can be explained by drainage of the soil at corners. Inspection of infected spots along levees and at corners showed that they often coincided with the presence of rat roads on soil, plants of which the stems and leaves are felled, and plant remains and debris of the felled stem and leaves. Rat burrows were not always found at or close to these spots with infected plants, but were readily detected in the neighbourhood.

The first plants infected by rats were usually the result of a new introduction of virus in a crop. The source has to be sought in the natural vegetation on the levees and roadsides. Plants of the wild rice species O. *longistaminata* growing on the levees are probably the main source of infection. Plants of this species maintain themselves through rootstocks and will, when infected, continuously produce new infected sprouts. Control of this species and other susceptible grasses should be one of the main objectives in the control of RYMV.

In the rice-growing season, rats live on the levees, roadsides, and other dry places. In the contra season, however, the rats live in the harvested fields where they have access not only to spilled grains but also to green sprouts of the stubble. The damage observed on the green sprouts makes it likely that transmission will occur between the sprouts of infected and healthy stubble. No studies were undertaken to quantify this spread.

Cows and donkeys appeared to be effective transmitters of RYMV, donkeys being the most efficient of the two (Table 3, Fig 1). Saliva collected from the mouthparts of a

donkey and a cow remained infectious for several hours after ingesting an infected plant. As the virus persists in their mouths for some time donkeys and cows might spread the virus over a considerable distance.

Infection rates increased 5 or 10-fold after a calf or donkey was given access to the plots with the infected plants (Table 3). Having grazed the infected plot, the calf and the donkey infected 16 and 51 % of the plants, respectively, in the second plot, which consisted initially of healthy plants. These results indicate that cattle and donkeys can transmit the virus to plants over some distance and that a substantial number of plants can be consumed before the saliva looses its infectivity. The donkey had consumed at least 138 plants in plot B before infecting the last plant, hence 51 plants out of 138 consumed plants in the second plot became infected with virus acquired in the first plot. Since only ten plants became infected when the cow was released in the second plot during the first grazing, it can be concluded from this experiment that donkeys are more effective transmitters than cows.

Transmission of RYMV by rats, cattle and donkeys might suggest that other mammals can also be transmitters of RYMV. The first mammals that come into mind are sheep and goats as often encountered on the roads in the Soudano-sahelian zone. However, due to the texture of rice plants, they do not prefer to forage on rice. This may indicate that the potential of these mammals in the spread of RYMV will be low or negligible.

Rats, cattle and donkeys infect plants at spots on roadsides where they have access to rice plants. Infection by rats can well be distinguished from those by cattle and donkeys. The latter two progressively decapitate the plant from top to bottom, whereas rats felled the plant and leave the felled stem and leaves.

Trampling plants is considered to be a mechanism by which sheep may spread SCMoV (Ferris *et al*., 1996; McKirdy *et al*., 1998). This type of spread may be the result of creating wounds on the roots, release of virus and subsequent infection of the roots of healthy plants. This mechanism can be excluded in our experiments when the calf and donkey grazed plot B in the first round, but not when they had access to the infected plots (A) in the first grazing round and to both plots (A and B) in the second round. Spread by trampling might have occurred in this experiment, but will be of minor importance.

Our study shows that cows can transmit the virus to seedbeds infecting a considerable number of seedlings. Field surveys made in 2002 confirmed that seedbed infection could result in dramatic effects. Due to the late start of rainfall in that year cattle were in large need for food. The early-sown seedbeds, to which they had freely access, were frequently visited before the regrown seedlings were transplanted. Some crops appeared severely or completely infected when seedlings were used from the seedbeds frequented once or more by cattle during the excessive long dry period.

Results show that cattle also transmit the virus after harvest. This spread is considerable lower in stubble fields than in experimental plots wherein the calf was given access to young plants. This observation indicates that the size of the spread in stubble fields will greatly depend on the development of the stubble.

Cattle herds roam the stubble fields during the off season and enhance the number

of infected plants. The infected stubble forms a source from which the virus may be released after plowing into the soil and into surface water, and may infect the next crop. The size of this inoculum will depend on different factors, such as the number of infected stubble, the time at which the regrowth starts, the development of the regrowth, which depends largely on the rainfall before plowing and the period between plowing and transplanting. Plowing of a well-developed and heavily infected regrowth just before transplanting may result in the release of a large inoculum, which can infect the seedlings when transplanted.

Like cattle, rats will enhance the number of infected plants and the virus load in stubble fields by their feeding activities. However, of more importance may be their return to dryer places to feed on seed grains as soon as the seedbeds are sown and on the new seedlings when they have access to the germinating seedlings.

CHAPTER 6

SURVIVAL OF POTENT *RICE YELLOW MOTTLE VIRUS* SOURCES DURING THE CONTRA-SEASON

A study was made on the survival of *Rice yellow mottle virus* (RYMV) in abiotic and biotic material present during the contra-season in rice fields infected in the previous season. Samples of soil and roots collected from dead plants and of straw left in the field after harvest were found to be infectious. Regrowing rice stubble and *Oryza longistaminata* plants sampled at spots where infected soil, roots and straw were found, appeared also to be infectious. Infectious surface water was, occasionally, found in two fields. RYMV could survive in an artificial mixture of soil and in an extract from RYMV infected rice plants for 45 days. The feces produced by a calf that was fed with infected rice plants contained high amounts of virus. These feces were infectious by inoculating to seedlings, and when seedlings were transplanted into soil fertilized with these feces. Feces collected from a cow, which was fed infected straw for several weeks, appeared also to be infectious. Application of manure produced from dung of cattle, which grazed in fields with abundant *O. longistaminata* plants resulted in severely infected crops. The results obtained showed that abiotic as well as biotic material might be potent sources for initiating RYMV infections in seedbeds and crops of the following cropping season.

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INTRODUCTION

Rice yellow mottle virus (RYMV), a sobemovirus endemic in rice in Africa, was first noticed in Kenya in 1966 (Bakker, 1970), but since then reported in almost all bottom valleys and irrigated rice growing areas in sub-Saharan Africa (Bakker, 1974; Fomba, 1988; Reckhaus and Andriamasinthseheno, 1997; Abo *et al*., 1998; Awoderu *et al*., 1991; Banwo *et al*., 2001c). RYMV can cause severe infections in some crops, occasionally resulting in the loss of a complete harvest. The virus is apparently transmissible by chrysomelid beetles (Bakker, 1974; Banwo, 2001a,c). However, a correlation between density of insect vector populations and incidence of RYMV infections was not found in a study made in Côte d'Ivoire (Heinrichs *et al*., 1997). These authors suggested that other, unknown, vectors might transmit RYMV or that the virus is transmitted by other means than by insects. Recent studies have demonstrated that the virus could be spread by cattle, donkeys and the grass rats foraging in rice seedbeds and crops (Chapter 5). Wind-mediated leaf contact between healthy and infected plants has also been shown to play a role in the spread of RYMV (Chapter 4). The first RYMV infections in a crop may either occur in seedbeds or in freshly transplanted crops. Beetles may introduce the first infections, but rats foraging on germinating seeds and cows and donkeys can also introduce and spread the virus when visiting seedbeds (Chapter 5). Beetles, cows, donkeys and rats will acquire the virus from sources such as infected regrowth, *Oryza longistaminata* plants or other weeds, and transmit it to seedlings. Transplantation of a seedbed with a limited number of infected seedlings can result in a considerable increase of infected plants in the crop (Chapter 3).

Next to living sources, contaminated soil, infected plant debris like straw and dead root material, feces of animals grazing on infected fields and manure are reported to play a role in the infection of plants with highly stable and mechanically transmittable viruses (Broadbent, 1965; Broadbent and Fletcher, 1966; Fletcher, 1969; Koenig, 1986; Pares *et al*., 1996). *Tomato mosaic virus* (ToMV)-contaminated soil infects roots of tomato plants without the involvement of fungi, nematodes or arthropods (Broadbent, 1965). Tomato plants can become infected by ToMV-contaminated soil coming into contact with leaves or soil containing infected tomato debris (Allen, 1981; Broadbent *et al*., 1965). Virus released from decaying greenhouse debris of ToMV-infected tomato plants and of *Cucumber green mottle mosaic virus* (CGMMV)-infected cucumber plants can readily infect tomato and cucumber plants (Pares *et al*., 1996; van Dorst, 1988).

Besides contaminated soil, dung and manure also form a virus source. Occasionally, farmers have experienced that their rice crops on parcels leased out to cattle farmers for stalling their herds during the night in the contra-season could be severely infected with RYMV. Some suggested that manure produced by cows, which had grazed on stubble of infected fields, was also infectious. Studies in the past already demonstrated that plant viruses do not necessarily lose their infectivity in a passage through cows as shown for CGMMV (van Dorst, 1988).

Since other sources than plants could be suspected as a source from where the virus may spread to rice, the survival of RYMV was studied in soil, in straw and roots of dead stubble, in regrowth and *O. longistaminata* plants during the contra-season

between December and June. Additionally, possible infectivity of feces was investigated. The results obtained in this study may be used to develop soil management strategies to control and eradicate RYMV from the field prior to the next cropping season.

MATERIALS AND METHODS

Persistence of infectious RYMV in soil

Survival of virus infectivity has been studied in soil collected around roots of dead stubble, in roots and straw of this dead stubble. The samples were collected in harvested rice fields with severe infections in the zones of Fouabougou, Molodo, N3 and N8 in the “Office du Niger”. In each zone, a field cropped with highly susceptible cv. BG 90-2 and one with the more tolerant cv. Kogoni 91-1 were selected in each of which two spots were identified in which the samples were taken. One of these spots was selected in that part of the field where some regrowth could flourish due to the presence of some water, and the soil at the other spot did not contain sufficient water to support regrowth of the stubble.

The first samples were collected one month (January 21, 1999) after harvest so that living and dead stubble could readily be differentiated. Infectivity of soil, straw and root sample extracts was tested by inoculating carborundum-dusted leaves of two weeks old cv. BG 90-2 seedlings. The extracts were prepared by grinding the samples in a ratio of 1/10 (w/v) in distilled water. The inoculated seedlings were kept in a screenhouse. Infection was monitored by the development of characteristic symptoms and confirmed by ELISA.

Survival of RYMV in soil under experimental conditions

Survival of RYMV in soil was confirmed by mixing soil with a virus extract. Fifty kg of sterilized soil was mixed with 180 g of infected rice plants ground in 1800 ml of distilled water. Ten plastic pots of 30 cm diameter were filled with 5 kg of this soil-extract mixture. Ten three-weeks-old healthy seedlings of ‘BG 90-2’ were transplanted to a pot after being incubated for 0, 15, 30, 45, 60, 75, 90, 105, and 120 days at the prevailing temperature. A pot containing only sterile soil was used as control at each interval.

Survival of the virus in soil was confirmed in a second experiment in which ‘BG 90-2’ and ‘Kogoni 91-1’ seedlings were transplanted in a soil-virus mixture prepared as described above for 0, 20, 40 and 60 days at the prevailing temperature. Infection of the transplanted plants was in both experiments monitored by symptom development and ELISA at intervals of every 15 days and 20 days in the first and second experiments after transplantation.

The severity of symptoms was expressed in a range from 0 to 9 as proposed by the Standard Evaluation System for Rice (IRRI, 1988) in which 0 represents immunity and 9 extreme susceptibility (death of the plants).

Infectivity of feces

To study whether RYMV could survive a passage through the intestinal tract of cattle, a cow was fed only dry straw from infected plants every day for a period of two months. To avoid contamination with other virus sources, the feces were collected once a week collected from the cow's colon using gloves. These feces were thoroughly mixed with PBS in a ratio of 1/5 (w/v) and inoculated to 54 pots with ten four-week-old 'BG 90-2' plants. The youngest leaf of the plants in each pot was analysed for infection by ELISA 45 days after inoculation.

In another experiment, 700 mg of fresh infected 'BG 90-2' plants were fed to a five months old calf, weighing 150 kg, in one meal between her daily meals. During the following 5 days, cow pats were sampled two or three times a day starting 18 h after ingesting the rice plants (Fig. 1). Thirty g of each feces sample was ground with 80 ml phosphate buffered saline (PBS) and centrifuged 10 min at 10.000 rpm. Three aliquots of 0.2 ml of each supernatant were analysed by ELISA and the infectivity of these supernatants by inoculation to 20 'BG 90-1' seedlings. The infectivity of the feces was also tested by transplanting 50 two-week old seedlings and 50 five-week old seedlings in two trays each with 125 g of a pool of the samples collected 24, 31, 38 and 55 h after feeding the calf. These feces were introduced at a level of 2 cm below the soil surface in the trays, which measured 27 x 40 cm. The amount of feces applied corresponded with 10.000 kg per ha. The virus content of the ELISA positive feces was determined in a three-fold dilution series after thoroughly grinding 1 g feces in sample buffer and titrated against a virus standard. The dry matter of the feces was determined by drying three feces samples in an oven until a constant weight was obtained.

Spread of the virus by manure

Manure, produced from cow pats, was collected in an area of 900 ha in the zone of Macina. This area is abandoned by farmers due the abundant growth of *O. longistaminata* and is now in use by farmers to graze their herds. Manure produced in this area was experimentally applied in a farmer's crop. The seedbeds and fields were respectively fertilized with 5000, 1500 and 0 kg manure/ha. The seedbeds were sown in the first week of July and transplanted in the first week of August.

Release of virus from roots of infected plants

Five one-week old 'BG 90-2 and 'Kogoni 91-1' seedlings were individually incubated in approximately 22 ml Hoagland nutrient medium (Hoagland and Broyer, 1936). This solution medium was in a 20 ml syringe-cylinder (without the plunger), closed at the bottom by a sealed needle. The rubber of the plunger was after drilling a hole inserted at the top of the syringe-cylinder and served as support for the plants. The syringe was covered with black tape. The seedlings, kept in a climate chamber at 25ºC in a daily regime of 16 h light and 8 h dark, were inoculated 2 days later. The nutrient media were collected 7 days after inoculation and then at every three or four days over a period of a month and their volumes determined. Each sample was analysed in triple by ELISA using 0.2 ml nutrient solution per well.

Table 1. Infectivity of soil, root and straw material sampled in the contra season in infected stubble fields in Fouabougou (Fbg), Molodo (Mld), N3 and N8. Each sample was inoculated on ten 15-days-old BG 90-2 seedlings. Sample infectivity was measured by the percentage of plants showing symptoms.

Sampling	Soil				Root				Straw				Average
period	Fbg	Mld	N3	N8	Fbg	Mld	N3	N8	Fbg	Mld	N3	N8	(%)
January	0	0	0	0	100	0	50	50	0	0	50	0	21
February	0	0	0	0	75	0	75	100	100	0	0	25	31
March	40	20	20	40	80	60	0	60	60	40	0	60	40
April	75	25	0	40	25	100	0	0	0	0	0	0	22
May	0	0	50	0	25	0	0	0	0	25	25	-	12
June	0	0	50	50	0	0	0	0	50	50	0	-	18
Avg. (%)	19	8	20	22	51	27	19	35	35	19	12	14	24

RESULTS

Persistence of RYMV infectivity in soil, in root material, and decaying stubble

To study whether the virus can survive in stubble fields of an infected crop in the contra season, soil was collected around dead roots, dead root material and straw and tested for their infectivity by inoculating 'BG 90-2' seedlings (Table 1). An average of 24% of the samples appeared to be infectious. Most infectious samples (35%) were found in the Fouabougou fields. Dead root material samples (33%) were more infectious than the soil samples (17%) or straw samples (20%). The period in which the infectious samples were found varied considerably. No infectious soil samples were found in January and February, whereas the highest infectivity was found in soil samples during March and April. The root samples were infectious in the first four months, although the infectivity declined rapidly in March and April from 50 and 25% to 0% in May and June. An almost even distribution of infectivity was found in the straw samples over the sampling period (Table 1). Infective soil and straw samples were found in May and June.

Infected regrowing stubble was found in the first four months of sampling. In January, all specimens sampled in the Fouabougou fields were positive whereas no infectious samples were found later in the contra-season. Infected samples were found in the Molodo and N8 fields in the first four months. No infected regrowth were encountered at the selected spots in the N3 fields.

Specimens of the wild rice species *O. longistaminata* were found in water-soaked spots in the Fouabougou field. Out of the 60 plants sampled, nine were ELISA positive and infectious when mechanically inoculated to 'BG 90-2'.

Infectious surface water was found in the N3 and N8 fields. This water originated presumably by seepage from the neighbour's irrigated field on which rice was produced. This water, collected in April, appeared to be infectious.

Table 2. Infection of 'BG 90-2' rice seedlings after transplanting in a soil-*Rice yellow mottle virus* mixture being incubated for 0, 15, 30, 45, 60, 75, 90, 105 and 120 days.

Storage of the soil-Virus mixture (days)	0	15	30	45	60	75	90	105	120	Control
Infected plants	4/10	8/10	3/10	5/10	0/10	0/10	0/10	0/10	0/10	0/10
Symptom development time (days)	11	11	10	8	–	–	–	–	–	–

Survival of RYMV in experimentally contaminated soil

BG 90-2 seedlings transplanted in soil mixed with an extract from infected plants became infected after storing this soil for 0, 15, 30, and 45 days (Table 2). No infections were obtained when this soil was stored for 60 days or longer. RYMV-characteristic symptoms appeared on the infected plants within 8 to 11 days after transplanting indicating that the plants became infected in the transplantation process. The number of infected plants could not be correlated with an expected decrease of the infectivity during the storage of the soil-virus mixtures. No infections were found on the plants transplanted in virus-free soil.

The rate at which the virus lost its infectivity in this soil-virus mixture was confirmed in a similar trial, in which 'BG 90-2' and 'Kogoni 91-1' seedlings were transplanted in soil containing the same concentration of virus (Table 3). The percentage of infected plants ranged between 80 and 100% for the 'BG 90-2' seedlings transplanted at 0, 20 and 40 days after soil contamination, and between 40 and 80% for the 'Kogoni 91-1' seedlings. No infection was observed in seedlings transplanted 60 days after soil contamination (Table 3).

Table 3. Persistence of *Rice yellow mottle virus* infection in a mixture of sterile soil and a virus extract. The infectivity was tested by transplanting five 'BG 90-2' and five 'Kogoni 91-1' seedlings in this mixture after being incubated at the prevailing temperature.

Interval (days) at which the soil-virus mixture was tested for infectivity	Percentage infected plants	
	BG 90-2	Kogoni 91-1
0	100 (5/5)[a]	80 (4/5)
20	100 (5/5)	60 (3/5)
40	80 (4/5)	40 (2/5)
60	0 (0/5)	0 (0/5)

[a]Number of infected plants/number of transplanted plants.

Table 4. Detection of *Rice yellow mottle virus* by ELISA in 1:5 diluted extracts of feces from a cow, which was fed RYMV infected straw by inoculating fifty four pots each with ten 'BG 90-2' seedlings.

Week of sampling	1	2	3	4	5	6	7	8	9	Total
RYMV infection	6/54*	4/54	8/54	3/54	0/54	0/54	2/54	2/54	0/54	25/486

*Number of ELISA positive pots /number of inoculated pots

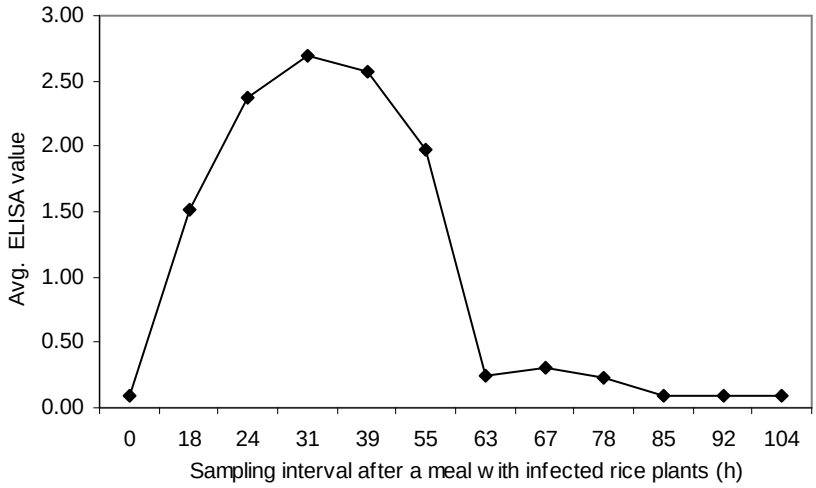

Figure 1. Detection of *Rice yellow mottle virus* (RYMV) of feces from a calf which was fed 700 g of rice plants in one meal. Droppings were collected two or three times a day and analysed by ELISA and by inoculation to 'BG 90-2' seedlings.

Infectivity of feces collected from a cow fed RYMV infected plant material

Twenty-five out of 486 pots gave positive reactions in ELISA 45 days post inoculation. These infectious samples were obtained in the first four weeks and the weeks 7 and 8 that the straw was fed to a cow (Table 4). In the second experiment, the first infected feces was sampled 18 h after feeding a calf with infected rice plants and the last infected sample was found after 55 h (Fig. 1). The samples collected 63, 67 and 78 h after feeding contained some virus (Fig. 1). All seedlings inoculated with the highly ELISA-positive samples became infected, whereas no infection was obtained with the other samples. Out of the 50 young seedlings transplanted in the fertilized trays 36 became infected and out of the 50 old day seedlings 6 became infected. Analyses of 1 g feces of the samples collected 18, 24, 31, 38 and 55 h after ingesting revealed that these contained 0.9, 6.5, 6.4 and 9.5 µg virus/g feces, respectively.

Infectivity of manure

The effect of manure produced from cattle dung on the development of RYMV infection in rice was studied by applying 5000, 1500 and 0 kg manure per ha in three parcels. The crop fertilised with 5000 kg of manure became completely and severely infected, the crop fertilised with 1500 kg manure was also completely infected but to a milder extent. Except for a severely infected spot, two m in diameter, no infection occurred in the crop in which no manure was applied.

Table 5. Infectivity of feces collected at various periods after a calf has been fed with 700 g of infected *Rice yellow mottle virus* 'BG-90-2' rice plants.

	Infectivity of feces								
Sampling interval (h)	0	18	24	36	48	60	72	84	96
Infectivity*	0/10	3/10	5/10	6/10	4/10	0/10	0/10	0/10	0/10

* number of infected plants/number of inoculated plants.

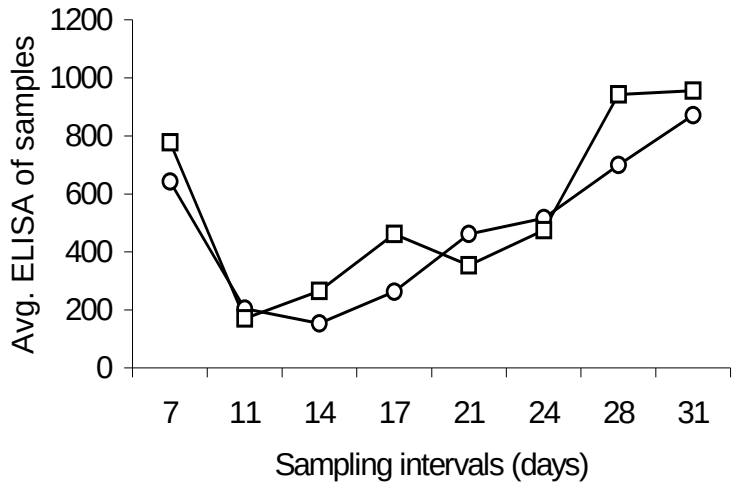

Figure 2. The average ELISA values x 10^3 *Rice yellow mottle virus* released from the roots of six infected BG 90-2 (—□—) and six infected Kogoni 91-1 (—o—) plants in Hoagland nutrient media sampled at intervals of 3 or 4 days.

Release of virus by roots

The first samples to study the release of RYMV from the roots of infected plants in nutrient medium were collected seven days after inoculation, a few days before the appearance of symptoms on the leaves. These samples already contained detectable amounts of virus (Fig. 3). A lower amount of virus was found in the samples collected four days later. The virus titer continuously increased after this delay during the development of the plants. The amount of virus released and the volume of water that evaporated differed considerably between individual plants. The first plants started after five weeks to release virus when they started to degenerate and the oldest leaves necrotized. An average amount of 17 µg virus was released per plant in the sampling period. Each of 24 and 28-day samples appeared to be infectious when inoculated to ten BG 90-2 seedling samples.

DISCUSSION

In this chapter it is shown that *Rice yellow mottle virus* (RYMV) can survive both in soil and plant debris (dry roots and straw) in previously infected rice fields between two cropping seasons. Infectivity was found in 17% of the soil samples collected around roots of dead rice stubble. Extracts of 33% of the root samples and 20% of the straw samples were also infectious. The infectious samples were irregularly distributed over the half-year period that the samples were collected. This irregularity in the detection of the infectious samples has partly to be explained by the random collection of the samples, the distribution of the infected plants in the harvested crops, and the moment at which the stubble resumed growth or the plants died. Survival of RYMV for at least half a year indicates that soil, root and plant debris in infected fields form one of the sources from which the first infections may arise in seedbeds or in the transplanted

crop. It is highly likely that the infectivity of soil, and of root and plant debris shall gradually diminish during the intercropping period. These sources will be more potent to infect the next crop in the short intercropping periods when two crops are produced in a year. Less virus may survive when only one crop is produced in the main cropping period. However, the first rains prior to the main cropping season may enhance the virus reservoir as rice stubble and weeds will resume growth and will form an attractive source of food for cattle, donkeys and rats, by which the reservoir will be enlarged. Virus may be released from the roots of the developing plants and when plowed down just before sowing the seedbeds and transplantation of the seedlings, the leaves will also release virus (Chapter 2). Appreciable amounts of infectious virus are released from roots when incubated in nutrient medium (Chapter 3).

Contra-season cultivation of rice is of minor importance in the 'Office du Niger' and the farmers started until very recently at February 10. This date is considered to be the end of the cold period and the temperatures are expected to become gradually higher. This contra-season crop was usually characterised by low incidences of RYMV infections. However, several farmers started directly with the cultivation of rice in November or December after the harvest of the main 2003 crop. Several of these farmers experienced incidences of 60 % or more RYMV infections in their crops. These severe incidences show that the virus was insufficiently eliminated in the stubble fields when the farmers resumed rice cultivation directly after the harvest of the main crop or after a short intercropping period.

Detection of infectivity by transplantation of seedlings in soil-virus mixtures being stored at the prevailing temperature shows that the virus retains its infectivity in soil for at least 45 days. These infection results were obtained by monitoring the visible symptoms. However, as infection via the soil may be restricted to the root system, more plants might have been infected as shown for *Tobacco mosaic virus* (Pares *et al*., 1996). Plants with such restricted infection will not have the potential to act as foci for secondary spread, but act as an inoculum source by releasing virus. The period that the soil-virus mixture is considerably shorter than found in 'in vitro' longevity experiments. The virus remained infectious for 99 days by incubating extracts from infected plants at room temperature (Fauquet and Thouvenel, 1978). This difference in infectivity of both inocula can not only be explained by the temperatures at which the soil-virus mixtures (30-43℃) and the virus extracts were incubated, but also by the techniques used (transplantation of seedlings in soil versus direct inoculation to plants). The lower virus concentration in the soil-virus mixture has also to be taken into account. In addition, properties like the pH, the presence of various components and bacteria in soil may be other factors, which might be more detrimental to the virus in soil than in plant extracts.

The disease symptoms appeared between 8 and 11 days after transplanting. Similar incubation periods have also been reported by Fauquet and Thouvenel (1978). In other studies incubation periods of 4 to 6 days has been observed (Fomba, 1988). This variation in the incubation period might be the result of virus concentration in the inocula, the susceptibility of the rice variety used, and the prevailing temperature and age of the plant when inoculated.

Studies with *Cucumber green mottle mosaic virus* (CGMMV), a tobamovirus, have

shown that plant viruses can survive a passage through the intestinal tract of cattle being fed with infected plant material (van Dorst, 1988). Extracts of such feces produced in the first two days infected all inoculated plants while only a single plant became infected using extracts from feces collected at the third day after virus ingesting. In the present study RYMV could be detected by ELISA over a period of two days in the feces of a calf after ingesting infected rice plants. Those samples containing large amounts of virus appeared to be infectious RYMV. The infectious samples infected also seedlings transplanted in soil fertilized with 125 g of feces, which corresponded with 10,000 kg dung/ha, became also infected. The results show that the feces were highly infectious and that fresh droppings in rice fields may be inoculum sources for infection in seedbed and fields.

The feces contained 5 to 9 µg virus per 1 g of feces, corresponding with 1 -2 % of the amount of virus present in 1 g of infected plant material. A calf of 150 kg may produce 10 kg of feces a day. Interpolation of our results and the infected feces production shows that a calf may produce sufficient feces to infect 200 plants on 8 m^2.

A considerable lower infectivity rate was found when straw of infected plants was fed to a cow. It is plausible that sun-mediated drying of plants has a detrimental effect on the infectivity of the virus. Alternatively, rice straw might be less digestible than fresh plants.

The use of manure produced from dung of cattle that fed on infected plants appeared also to be a virus source as demonstrated in a field experiment. The occasional, but often severe, infection of parcels rented to cattle farmers on whom their cattle could be stalled during the night might be explained by the infectious feces dropped on these parcels. Since these droppings are not spread over a larger area when the fields are prepared, the concentration of the virus may be rather high at those spots where the feces are dropped and so enhance the probability that plants become infected at spots where the feces are dropped.

The presence of infected *O. longistaminata* confirms earlier observations that RYMV can survive the contra-season in this wild rice species (Bakker, 1974; Awoderu, 1991). This plant species can readily be found in freshwater swamps and in ‘bas fonds’. Its propagation by rootstocks makes this plant an-always-present natural host from which RYMV can radiate into rice.

In conclusion, the current study shows that RYMV can ‘over-season’ in several potent sources from which infections into the next season crop may be initiated. Regrowth of infected stubble will start to abundantly flourish at the start of the cropping season and will probably form the major source infecting the next crops. Grazing mammals will enhance this reservoir. That this reservoir can infect soil by release of RYMV has been demonstrated experimentally and can infect neighbouring plants with or without disturbing soil (Chapter 3). Plowing the fields will enhance the virus reservoir in the soil and may lead to an infection of the developing seedlings and the seedlings when transplanted. When transplanted into contaminated soil, infection of seedlings is the result of direct plant-virus contact, or between contaminated hands of the farmer and seedlings.

CHAPTER 7

ASSESSING SEED TRANSMISSION OF *RICE YELLOW MOTTLE VIRUS* IN RICE

A variety of plant viruses is known to be seed-transmitted. This type of transmission provides an effective mean of introducing virus into a crop at an early stage, giving randomised foci of infection over a crop. For the sobemovirus *Rice yellow mottle virus* (RYMV) several studies have - at one hand - indicated that it is not seed-transmissible while the phenomenology of some reported infections – at the other hand - is suggestive for such a type of transmission. This study shows that rice seeds become infected with RYMV during their development. Seeds of most 21 rice genotypes studied became almost all ELISA-positive upon infection of their parental plants. High virus titres were frequently found in seed coats, whereas a lower percentage of endosperm and embryo's contained RYMV antigens. No seed-borne infections were found when large numbers of seeds from infected plants of the susceptible genotype BG 90-2 or the more tolerant genotype Kogoni 91-1 were tested in greenhouse or field experiments. The results demonstrate that, although mature and dry seeds are ELISA positive, infected seeds do not give rise to RYMV epidemics in rice crops.

Part of this chapter has been published as Konaté, G., Sarra, S. and Traoré, O. 2002. Rice yellow mottle virus is seed-born but not seed transmitted. European Journal of Plant Pathology 107: 361-363.

INTRODUCTION

Among sobemoviruses seed transmission is not a common phenomenon. Only four out of the 17 known species in this genus are known to be seed-transmitted, i.e. *Southern bean mosaic virus* (SBMV), *Southern cowpea mosaic virus* (SCPMV), *Sowbane mosaic virus* (SMV), and *Subterranean clover mottle virus* (SCMoV) (Tamm & Truve, 2000). Up to 80 % of the progeny of SMV-infected *Chenopodium* sp plants were shown to be infected (Kado, 1971; Teakle, 1996). Reports on SBMV showed that approximately 5% of the seeds of infected bean plants were infective after storage for seven months (Cheo, 1955; Zaumeyer and Harter, 1943), whereas higher infection rates were found in non-mature seeds (Tremaine and Hamilton, 1983). Reported infectivity of seeds from SCPMV-infected cowpea plants varied from 3 to 40% (Shepherd and Fulton, 1962; Tremaine and Hamilton, 1983).

The introduction in the 1960s of Asian rice varieties in sub-Saharan Africa and the islands of Zanzibar and Madagascar resulted in the emergence of *Rice yellow mottle virus* (RYMV) (Abo *et al*., 1998) causing a highly infectious disease in most rice growing regions with epidemic proportions in irrigated and lowland cultivated rice in the 1990s.

RYMV has been reported to be transmissible by beetles (Bakker, 1970, 1974; John *et al*., 1985; Banwo *et al*., 2001) and by mechanical inoculation. Recently, transmission by cattle, donkeys and grass rats has been demonstrated under experimental and field conditions and by wind-mediated leaf contacts (Chapter 4; Sarra *et al*., 2004). Transmission of RYMV through seeds has thus far not been demonstrated for this virus (Bakker, 1975; Fauquet *et al*., 1977; Sarra, 1998; Konaté *et al*., 2001). Yet, the occasional development of unexpected infections, like the assumed introduction of RYMV in the Mopti region with seeds from the Niono region (both in Mali), has been attributed to seed-born transmission.

The argument that the virus is seed transmitted is frequently heard when farmers buy seedlings to supplement their own seedling shortage from other farmers, whose seedlings appear to be infected. Infections in non-inoculated plots in resistance field trials were considered to be the result of using infected seeds (Awoderu, 1991). Due to these and other claims, the idea that RYMV is seed transmitted is widespread among farmers.

In the light of the importance to demonstrate or rule out that RYMV is seed-transmissible, greenhouse and field studies have been performed using seeds from infected rice plants of different genotypes. The results obtained demonstrate that, although both freshly harvested and mature seeds from infected plants may be seropositive for RYMV, the rate of actual seed transmission is virtually zero.

MATERIALS AND METHODS

RYMV detection in seeds produced by infected rice plants

The indirect double antibody sandwich (IDAS)-ELISA was used to study the presence of RYMV in mature and dry seeds from infected plants. The buffer systems and ELISA format used were as described previously (Konaté *et al.*, 1997). The seeds

were ground in 0.3 ml sample buffer, and the homogenates were divided over three wells, each pre-coated with 0.2 ml polyclonal rabbit antibody (1 mg/ml). After a two-h incubation, biotinylated gamma globulins and streptavidin-alkaline phosphatase conjugate were added to the wells. Absorbance values at 405 nm were recorded 1 h of incubation of substrate, using a Metertech Σ960 microplate reader. Extracts of seeds collected from healthy plants were incubated in triplicate as negative control. The mean absorbance value plus three times the standard deviation of the negative control was taken as a negative-positive threshold.

Dectection of RYMV in seed coats from infected 'BG 90-2' and 'Kogoni 91-1' seeds

The presence of RYMV in seed coats from five months old seeds from healthy and from infected 'BG 90-2' and 'Kogoni 91-1 plants were analysed by ELISA. These cultivars are considered to be highly susceptibility to RYMV and rather tolerant to RYMV, respectively (Coulibaly, 1999). Hundred and twenty seeds of both varieties were divided into three groups. Forty seeds of each variety were left intact. The seed coats of forty seeds were chopped up in a few pieces and the seed coats of the last forty seeds were ground in sample buffer. All samples were incubated in sample buffer for two hours.

Production of infected seeds and their analysis for infection

Seeds of 21 cultivars (16 cultivars of *Oryza glaberrima* and 5 cultivars of *O. sativa*), differing in susceptibility and resistance (Coulibaly, 1999), were produced on plants grown in 10 l buckets and kept in an insect-proofed greenhouse. Half of the approximately 34 plants of each cultivar were inoculated with a pathogroup A virus isolate and the other half with a pathogroup B virus isolate. These isolates were discovered in West Africa (Konaté *et al*., 1997). Pathogroup B isolates have the capacity to break down the high resistance of cv. TOG 5675. The rice seedlings were mechanically inoculated four weeks post-germination. The mature seeds were collected from infected plants and stored for 4 months at 30-35℃. To monitor the presence of virus 92 mature and dry seeds of each variety were individually assayed by ELISA.

Analysis of seed parts for infection

Hundred and forty seeds from ten varieties (Table 3) differing in RYMV susceptibility (as listed in Table 2) were dissected into the seed coat, the embryo and the endosperm four months after harvest and divided over samples with twenty specimen. Each sample was ground and assayed by ELISA.

Infectivity of seeds from infected plants

Thousand seeds of each variety listed in Table 2 were collected from plants infected with the pathogroup A isolate and 1000 seeds from plants infected with the pathogroup B isolate, and tested for their infectivity in a greenhouse. The seeds were sown in a 50 x 50 cm wide and 25 cm deep trays, and maintained in an insect-proof greenhouse for at 25-30°C and a relative humidity of 80-90%. Half each lot was visually monitored for symptoms over a period of 3 weeks. The other half of the seedlings was tested for the

presence of virus using indirect double-antibody sandwich ELISA by pooling the youngest leaves of 20 seedlings in one sample six weeks after sowing. The seed coats recovered from the soil in which the seedlings grow, were combined in a sample and tested by ELISA and by inoculating 6 pots with 5 seedlings. The infectivity of the seed coats was tested by inoculating 6 pots with 5 BG 90-2 seedlings. The seedlings obtained were tested in the same way 6 week after sowing.

In a second experiment, 300 seeds from infected BG 90-2', 'Kogoni 91-1', 'Seberang MR 77' and 'Gambiaka Kokum' plants were sown in a tray and incubated at 25° C. After emerging, the seedlings were visually inspected for symptom development every two days. The young leaves of ten seedlings were pooled to a sample, which was subjected to DAS-ELISA three weeks after sowing.

Infectivity of infected seed coats

A sample with twenty pooled seed coats from infected 'BG 90-2' and from Kogoni 91-1' plants were ground in 1 ml 1 x PBS (w/v 1:10) and mechanically inoculated to three–week-old seedlings of the same varieties to demonstrate the presence of infectious virus in the seed coats. The inoculated seedlings were monitored visually and by ELISA for infection 15 and 30 days after inoculation.

Seed-borne transmission in the field

Dry and mature seeds harvested from infected and healthy 'BG 90-2' and of 'Kogoni 91-1' plants in the previous season were sown in 4 m^2 large plots in four replicates placed in a Latin square design (Kawanchai and Gomez *et al*., 1983) to study the seed transmission of RYMV in field conditions. To avoid virus transmission by transplantation, the seeds were individually sown at a distance of 20 x 20 cm. A 1.5 m wide strip consisting of ten rows and ten columns with plants of the immune rice variety Gigante was placed around each plot. A 5 m wide zone with Gigante plants was laid down around the experimental plot as buffer to prevent infection from outside. Weeds were controlled once a month after sowing by spraying Calriz consisting of 360 g Propanil/l and 72 g Triclopyr/l as ingredients at a rate of 5 l/ha. The plants were visually monitored for virus infection without entering the plots 21, 42 and 72 days after sowing. Any plant suspected to be infected was analysed by ELISA.

Table 1. Percentages of ELISA-positive ($A_{405\ nm}$) of intact, chopped up and macerated seed coats collected from RYMV-infected BG 90-2 and Kogoni 91-1 plants, and their average ELISA values.

Variety	Number of seeds tested	Intact seed coats infected		Infected seed coats chopped up		Infected seed coats macereated	
		%	$A_{405\ nm}$	%	$A_{405\ nm}$	%	$A_{405\ nm}$
BG 90-2	40	18	0.49	27	0.40	97	0.49
Control	40	0	0.04	0	0.06	0	0.08
Kogoni 91-1	40	55	0.47	57	0.82	90	1.33
Control	40	0	0.03	0	0.07	0	0.07

Table 2. Presence of *Rice yellow mottle virus* (RYMV) in seeds collected from different *Oryza sativa*[#] and *O. glaberrima* cultivars infected with a pathogroup A or B isolate as determined by ELISA.

Rice variety	Resistance scoring*	Percentage of infected seeds	
		Pathogroup A	Pathogroup B
BG 90-2[#]	S	98	65
Bouaké 189[#]	S	99	99
Kogoni 91-1[#]	S	98	91
Metica	S	78	76
TOG 7214	S	92	95
TOG 7217	S	91	92
TOS 3554	S	65	65
TOS 16101	S	96	98
VL 17	S	66	75
VL 166	S	70	82
Wita 7	S	91	89
Wita 8	S	97	98
Wita 9	S	51	48
FKR-33[#]	R	94	79
IRAT 104[#]	R	100	100
Morobérékan	R	100	100
TOG 5681	HR	0	0
TOG 5675	HR	0	4
VL 6	HR	96	99
VL 123	HR	75	70
TOG 5672	I	0	0

*S = Susceptible, R = Resistant, HR = Highly resistant, I = Immune, [#] = *Oryza sativa* cv.

Table 3. Distribution of *Rice yellow mottle virus* over seed coat, endosperm and embryo in seeds from plants cultivars infected with pathotype A or B differing in their ability to infect cv TOG 5681. Virus was detected using ELISA.

Rice variety	Seed coat		Embryo		Endosperm	
	A	B	A	B	A	B
Bouaké 189	7/7*	7/7	1/7	6/7	1/7	6/7
IRAT 104	7/7	7/7	4/7	2/7	1/7	2/7
Kogoni 91-1	7/7	7/7	7/7	7/7	4/7	3/7
Metica	7/7	7/7	6/7	7/7	0/7	0/7
Morobérékan	7/7	7/7	7/7	7/7	7/7	7/7
TOG 5675	7/7	7/7	0/7	2/7	0/7	0/7
TOG 7214	7/7	7/7	4/7	1/7	1/7	0/7
TOS 3554	7/7	7/7	6/7	6/7	2/7	4/7
TOS 16101	7/7	7/7	3/7	7/7	2/7	2/7
VL 123	7/7	7/7	2/7	4/7	1/7	4/7

*Teller infected plants/numerator used plants

RESULTS

Presence of RYMV in seeds from infected rice plants

First studies were focussed on seeds collected from infected plants of the rice cultivars ‘BG 90-2’ and ‘Kogoni 91-1’, which are considered to be highly susceptibility and rather tolerant to RYMV, respectively (Coulibaly, 1999). Antigens to RYMV were

detected in 18 and 55% of the non-macerated seeds produced on ‘BG 90-2’ and ‘Kogoni 91-1’ plants (Table 1). When seed coats were chopped up 27% of 'BG 90-2' and 57% of 'Kogoni 91-1' seeds were positive in ELISA, while 97 and 90% of the ‘BG 90-2’ and of ‘Kogoni 91-1’ seeds, respectively, were positive when the seeds coats were macerated. These results show that the extent by which virus can be detected in seed coats depends on the integrity of the seed coats (Table 1).

Seed infection of 21 rice varieties differing in virus susceptibility

Next the presence of RYMV could be demonstrated in seeds of both susceptible and resistant varieties infected with either a pathogroup A or a B isolate of RYMV (Konaté *et al*., 1997) (Table 2). Using the student’s t-test, no significant differences were observed between seeds infected with a pathogroup A or B virus isolate ($t = 0.122$, $P>0.90$). Five of the used varieties considered being resistant or highly resistant to RYMV did not differ notably in the percentage of infected seeds from the susceptible varieties. No virus could be detected in seeds of the varieties TOG 5681 and TOG 5672, whereas a small proportion of TOG 5675 seeds (4 out of 92) from plants infected with the pathogroup B isolate became infected. Surprisingly, a high percentage of seeds of the varieties the highly resistant VL6 and VL23 varieties (Coulibaly, 1999) appeared to be infected. No virus was detected in seeds of the immune variety TOG 5672 (Coulibaly, 1999).

Distribution of RYMV over different seed parts of selected rice varieties

The distribution of RYMV antigens over the seed parts, viz seed coat, embryo and endosperm was studied for seeds from a selected set of rice varieties infected with either pathogen A or B isolate (Table 3). While all seed coat samples were positive in ELISA, 63 % of the embryo and 42 % of the endosperm samples gave a positive reaction (Table 3). The infection rate of these seed parts differed significantly ($\chi^2 = 1.375$, $P < 0.001$), whereas this rate did not differ for a seed part infected by either pathotype A or B ($\chi^2 = 1.463$, $P = 0.48$).

Infectivity of RYMV-positive seeds in greenhouse tests

Seeds of the varieties listed in Table 3 and collected from plants infected with either pathogroup A or B isolate were tested whether they would develop into virus infected seedlings. None of the obtained 1000 seedlings showed any virus symptom or gave a positive ELISA reaction 3 or 6 weeks after sowing. The samples with the recovered seed coats gave a positive reaction in ELISA, but were not infectious as shown by inoculation to BG 90-2 test plants (data not shown).

Table 4. Average ELISA values of BG 90-2 and Kogoni 91-1 seeds harvested from freshly infected plants exposed 0, 3 and 6 weeks to water while the seeds were germinating.

Cultivar	Exposure to water (weeks)				
	0		3		6
	Seeds ground	Incubate	Seeds ground	Incubate	Seeds ground
BG 90-2	1.44±0.20	2.14±0.15	2.09±0.100	2.30±0.08	2. 10±0.10
Kogoni 91-1	1.97±0.14	1.96±0.28	1.77±0.184	2.33±0.3 1	2.16±0.55

In a second experiment, seeds from infected plants of the susceptible variety BG 90-2, and the tolerant varieties Kogoni 91-1, Seberang 77MR and Gambiaka Kokum were also tested for their competence to transmit RYMV. Again none of the seedlings germinated showed virus symptoms three weeks after sowing or gave positive reactions when the samples of the 20 pooled seedlings were monitored by ELISA.

Infectivity of seeds coats

High concentrations of virus antigen could still be detected in seed coats recovered from the growing plants three or six weeks after sowing. However, these seed coats appeared not infectious to BG 90-2 test seedlings.

Seeds from BG 90-2 and Kogoni 91-1 seeds freshly harvested from infected plants proved to be positive in ELISA when incubated in water for three and six weeks (Table 4). This incubation water gave also positive reactions in ELISA after three or six week of incubation of the seeds.

Seed-borne infection in the field

The seeds collected from the infected plants showed a poor germination rate. Only 48 and 32% of seeds collected from BG 90-2 and Kogoni 91-1 plants, respectively, germinated and produced seedlings. None of the seedlings grown from seeds of infected 'BG 90-2' and 'Kogoni 91-1' plants developed RYMV characteristic symptoms (Table 5). ELISA carried out on plants with suspected symptoms were all negative.

DISCUSSION

Despite the presence of ELISA-detectable amounts of RYMV in embryo's, endosperm and notably in seed coats, the results presented demonstrate, that this virus is not transmitted through mature rice seeds. In tests with mature seeds from 21 different rice genotypes, varying in virus susceptibility, not a single case of seed-mediated transmission was observed, when the plants were infected with either pathogroup A or B isolate. The failure to detect seed transmission of RYMV confirms earlier results obtained by Bakker (1974) and Fauquet and Thouvenel (1977), but contradict the frequent statements of farmers unexpectedly suffering from RYMV infection in their rice crops in Mali.

Table 5. Incidence of infected plants grown from seeds from infected 'BG 90-2' and 'Kogoni 91-1'.

Origin of the seeds	Number of seeds/plot	Avg. # of germinated plants per plot	Number of infected plants
Infected BG 90-2	100	48	0
Healthy BG 90-2	100	88	0
Infected Kogoni 91-1	100	32	0
Healthy Kogoni 91-1	100	73	0

Konaté *et al*., 2001 demonstrated that unripe seeds from infected plants were infectious when inoculated after maceration to BG 90-2 seedlings. In the present study mature and dry seeds, harvested in the previous season, were used. These results indicate that storage and drying of these seeds for some weeks suffice to inactivate the virus.

It was shown in our experiments that seed coats remain ELISA positive, even after having been stored for half a year. Freshly harvested seeds were still positive in ELISA when stored in water for three to six weeks. This observation indicates that the virus is not completely released by the seed coat while exposed to water, or that the virus antigen did not loose its capacity to react serologically.

RYMV did not only infect seeds of all susceptible varieties, but also seeds of several reportedly resistant varieties FKR-33, IRAT 104 and Morobérékan or the highly resistant varieties VL 6 and VL 123. They appeared to be infected to the same extent as seeds of the susceptible varieties. Since the resistant varieties are classified on the basis of visually foliar symptoms and not by absence or presence of virus (Coulibaly, 1999), it is likely that the “resistant” varieties are tolerant rather than resistant (Walked, 1985). No virus was detected in the seeds of the varieties TOG 5681 and TOG 5675, confirming that these cultivars are highly resistant as proposed (TOG 5681) or even immune (TOG 5672) (Coulibaly, 1999).

RYMV antigens were readily detected in the seed coats of both varieties BG 90-2 and Kogoni 91-1. The number of positive coats found depended on the form in which they were incubated. A low number was positive when intact coats were incubated, more became positive when chopped up, and most samples were positive when the coats were ground. Grinding the seed coats shows that most if not all seeds of the susceptible varieties become infected. The high proportion of infected seed coats compared to that of the endosperm and embryo show that the virus may invade the seed coat more efficiently than other parts of the seed. Differential invasion of seeds has also been demonstrated for *Turnip yellow mosaic virus* (TYMV) and *Tobacco mosaic virus* (TMV) infecting *Arabidopsis thaliana* plants. It should be realised though, that the values obtained for the different seed parts reflect only the reactive antigen level at a certain stage in the maturation of the seeds. A more accurate picture of virus levels and distribution over the seed parts may be obtained in time course studies during the seed maturation process.

Desiccated seeds lost their infectivity from 100% to virtual zero within one month after flowering (Konaté *et al*., 2001). The high infectivity of freshly collected seeds may indicate that non-mature seeds spilled early in the field or at the spot where the plants are threshed may produce infected seedlings.

Lack of infectivity of virus-containing seeds is not restricted to RYMV-infected rice seeds. Embryo invasion is a common feature of seed-transmitted viruses but finding the virus in the embryo does not always lead to seed transmission (de Assis Filho and Sherwood, 2000; Matthews, 1991; Maule and Wang, 1996). A large proportion of seeds from *Southern bean mosaic virus* (SBMV)-infected bean plants produces infected plants directly after harvest, but looses its infectivity almost within two months after blooming (Cheo, 1955). This fits with reports demonstrating that SBMV-infected seeds do not

develop into infected plants seven months after storage (Zaumeyer and Harter, 1943).

The rapid loss of infectivity is not always associated with a decrease of antigen titre in seeds as shown for *Alfalfa mosaic virus*, an Ilarvirus, infected lucerne seeds. The virus titre declines only slowly during the seed maturation process (Baillis and Offei, 1990).

The germination of seeds from RYMV-infected plants appeared to be significantly lower than that of seeds from healthy plants (Table 5), suggesting that seed infection affects its viability. A delay in germination of seed from *Turnip yellow mosaic virus* (TYMV)-infected *Arabidopsis thaliana* plants has been reported, whereas *Tobacco mosaic virus* (TMV) has no effect on the germination rate of infected seeds (de Assis de Filho and Sherwood, 2002). Affecting germination might be related to invasion of the embryo by the virus, since RYMV and TYMV invade the embryo, whereas TMV does not (this study; de Assis de Filho and Sherwood, 2002).

The present study unequivocally indicates that seed invasion, even when the embryo is invaded, does not lead to seed transmission of RYMV in rice, showing that ELISA-positive seed parts are not a useful indicator for predicting seed transmission. Despite this conclusion, farmers have rather to refrain from using seeds from infected plants as seed from infected plants germinates poorly.

CHAPTER 8

SUMMARY AND CONCLUDING REMARKS

Since the discovery of *Rice yellow mottle virus* (RYMV) in Kenya more than fifteen beetle species, able to transmit this virus, have been reported and to date it is widely accepted that beetles are the main transmitters of this virus. The actual role of beetles in the spread and epidemiology did not gain much attention, though, while the observed patterns of RYMV incidence in rice fields are not readily explained by only insect-mediated spread (Fig. 1).

While some patterns of virus spread, such as the occurrence of single infected plants or small spots with infected plants, are suggestive for beetle-mediated transmission, the occurrence of large, occasionally field-wide spots with infected plants is more difficult to explain by such transmission (Fig. 1). These large spots consist of a large centre with severely infected plants, which are apparently infected at the same moment, while a gradient in decreasing severity occurs at the border of these spots. Small strips with a closed stand of infected plants occurring along dikes, levees or road sides, and the frequently encountered infections at corners must also be the result of other transmission factors. Finally, the occasional occurrence of completely infected crops side by side with apparently healthy crops excludes the involvement of virus spread by beetles. Besides that the various infection patterns can not easily be explained by beetle activity, also the occurrence of completely infected crops in areas, like the 'Office du Niger' in Mali, where the beetle population is extremely low, indicates the existence of alternative transmission strategies. On the contrary in regions, where beetles occur more abundantly, no evident correlation can be detected between the incidence of virus infection and beetle populations (Reckhaus and Andriamasintseheno, 1997; Oral reports of staff members of the Périmetrè de Sélingue). In the absence of beetles severely infected crops can occur in this périmetrè. All these observations suggest that other mechanisms play a role in the spread of RYMV, and that the impact of beetle transmission has probably been greatly over-emphasized.

The study presented in this thesis aimed to elucidate and to analyze other native mechanisms by which RYMV can spread in irrigated rice crops. The outcome is that RYMV - due to its high stability, its high titres in infected plants and its property to be mechanically transmitted - is actually spread

- by farmers' operations in rice crops (Chapter 3),
- by wind-mediated leaf contact (Chapter 4),
- by mammals grazing on rice (Chapter 5),
- by virus released into soil (Chapter 6),
- by application of manure prepared from infected feces and material (Chapter 6), and
- release of guttation water from infected plants (Chapter 3).

These various mechanisms will be evaluated while some control measures to avoid RYMV epidemics will be suggested.

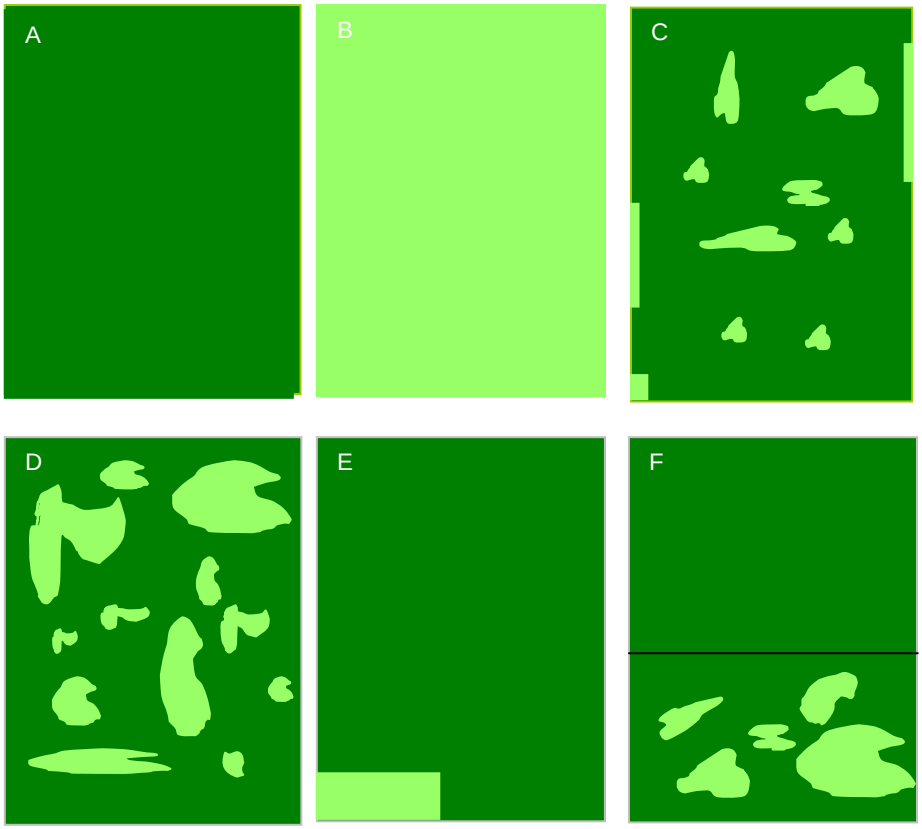

Figure 1. Various patterns of *Rice yellow mottle virus* spread in the field. A: completely healthy field; B: completely infected field; C: small strips with infected plants frequently found along dikes, levees and road sides and a spot at a corner of a rice field; D: large patches with virus infected plants scattered throughout the whole field; E: infection at the corner of the field due to the use of seedlings obtained from other farmers to supplement the shortage in the farmer's own supply of seedlings; F: infections due to dung produced by cows housed during the nights in the contra-season in a part of a field. Light grey: infected field and spots, dark grey: areas with healthy plants.

Farmers' operations

Handling seedlings. Infection of rice crops with RYMV through contact either between diseased and healthy plants or between contaminated hands and rice plants was already suspected by Bakker (1974). The observation that the incidence is often higher in transplanted crops than in directly sown crops tends to confirm this hypothesis (Sy, 1994).

In Chapter 3, it is demonstrated that several farmers' practices indeed enhance the incidence of RYMV diseased plants. It is shown that a limited seedbed infection already results in a significant virus incidence in the transplanted crop. It is argued in Chapter 3 that the increase during transplantation is caused by mutually abrasive contact between leaves, and between leaves and hands of the transplanter. A second increase observed after transplantation can be explained by wind contact between leaves of infected and healthy plants (Chapter 4), and by inoculation through soil-borne virus released from infected plants (Chapter 6). The large increase of infected plants over the number of infected seedlings suggests that infection in a seedbed is likely the most important source from which the virus can spread into irrigated rice crops.

Treading the crop. Evidence for spread of virus by farmers' operations by programmed

treading was also demonstrated by pacing out partially infected plots (Chapter 3). Treading may create wounds from which the virus can be released in soil and through which other plants are infected. Alternatively, virus released from roots can penetrate roots without wounding as shown in *in vitro* experiments when roots are exposed to a virus suspension and in greenhouse experiments when leaf contact between plants was prevented (Chapter 6).

Mowing. Spread of RYMV was also observed when non-infected plots were mowed with a contaminated sickle. A decreasing gradient of infected plants were noticed between the point where the first strokes were made and the last infected plants were found. The small distance over which the infected plants were found shows that sickles rapidly lose their infectivity. Spread of infection by cutting plants was confirmed in a greenhouse experiment when infected plants were decapitated with scissors. Spread of the beetle-transmitted sobemovirus, *Cynasurus mottle virus*, was also observed after mowing lawns (Huth and Paul, 1977) and occurred in the direction of mowing.

While transplanting seedlings and treading crops will infect the present crop, mowing at harvest will enhance incidence in regrowing stubble. Cattle, donkeys, rats and possible also other animals grazing on the stubble will spread the virus to stubble and to germinating ratoon, *Oryza longistaminata* plants, and other susceptible grass species, which develop in stubble fields during the contra-season (Chapter 6). Since cattle, donkeys, and grass rats remain infectious for some time (Chapter 5), the infection can also be spread to, thus far, healthy stubble fields. Increase of the infection in stubble field results in an enhanced virus reservoir from where infection can spread to seedbeds and the next crop by beetles and mammals, by farmers removing weed while planting seedlings and by virus released in soil. This will especially be the case when regrowth can develop after the first rains before sowing and planting the next crop.

Plowing of infected stubble fields. Stubble fields will form a reservoir from which virus will be released from plants being plowed down before sowing the seedbeds and preparing the field before transplanting. Since the seedbed plots to be sown and the fields to be cropped are usually just plowed before sowing and transplanting, an extra virus release might occur when the regrowing stubble is plowed down. The size of this inoculum will depend on different factors such as the number of infected plants, the time at which the regrowth starts, the development of the regrowth, which depends largely on the rainfall before sowing, and the period between plowing and transplanting. Plowing of a well developed and heavily infected regrowth just before transplanting may result in the release of a large inoculum, through which seedlings can become infected when transplanted.

Spread by wind-mediated leaf contact

Spread of plant viruses by wind-mediated leaf contact, has not gained much attention in the literature. Infection of barley plants with *Barley stripe mosaic virus* might occur by wind-mediated leaf contact. This suggestion was based on the observation that plants adjacent to infected plants became infected, whereas no infection occurred

in more distantly located plants (Chiko, 1973; Slack *et al*., 1975). Wind-mediated spread of *Turnip crinkle virus*, a tombusvirus, was experimentally demonstrated by exposing a small group of healthy and infected to a fan (Broadbent and Heathcote, 1958). Our studies show that RYMV can be spread by wind-mediated leaf contact under artificial as well as natural conditions (Chapter 4). The significant differences found in the number of infected plants in plots, protected and non-protected for wind, shows that RYMV will indeed spread by wind-mediated abrasive leaf contact between plants in virus-infected crops. Spread of the virus without applying carborundum to the leaves suggested that the virus could even infect healthy plants when the wind does not carry dust or sand.

Transmission by vertebrates

Most plant viruses are transmitted from plant to plant by invertebrates. Vertebrates appeared also to be able to transmit RYMV both by foraging behaviour and consumption of rice plants as shown in this thesis (Chapter 5). The grass rat *Arvicanthis niloticus* efficiently transmits RYMV to healthy rice plants after gnawing and feeding on infected plants. Out of the 60 plants exposed for 9 h to four rats in cages, 51 plants became infected. These results were confirmed in a field experiment, in which infected plants were detected at that side of a levee where rats had access to plants. No plants were infected at the other side of the levee, where the rats had no access to the plants due to the absence of any soil sloping along the levee. Soil sloping along levees and roadsides bordering fields gives rats access to plants and, hence, the opportunity to infect plants. The frequent presence of infected plants at corners of rice fields has also been explained by virus transmission by rats as they have often access to plants due to drainage of soil at corners of a field. Inspecting strips and corners with infected plants showed that they often coincided with the presence of rat roads on the soil, plants with felled stems and leaves, and plant remains and debris of felled stem and leaves. The first plants infected by rats in a crop were usually, though not always, the result of a new virus introduction. The source has to be sought in the natural vegetation on the levees and roadsides. Plants of the wild rice species *O*. *longistaminata* growing on the levees are probably the main source of this spread, as they constitute an always present virus source.

A 5 or 10-fold increase of infected plants was obtained when a calf or a donkey was given access to plots with the infected plants (Chapter 5). Moreover, having grazed in the infected plots, the calf and the donkey infected 10 and 36 % of the plants, respectively, in a healthy second plot. These results indicate that cattle and donkeys can transfer the virus to plants over some distance and that a substantial number of healthy plants can be consumed before the saliva has lost its infectivity. Saliva of a cow and a donkey appeared to be infectious for some hours after having consumed an infected plant. Trampling of fields by sheep has also been considered to be a mechanism by which *Subterranean clover mottle virus* might be spread in infected subterranean clover fields (Ferris *et al*., 1996; McKirdy *et al*., 1998). Spread by trampling can probably not be excluded in our experiments when the calf and donkey were given access to the plots with infected plants. The transfer of virus to healthy plots indicated that trampling might be of minor importance.

Observations made during the 2002 season showed that grazing of seedbeds by

cattle had dramatic effects on the spread of RYMV. Due to the late start of the rainfall in that year, cattle in search for food had occasionally free access to unprotected early-sown seedbeds along roads. Some seedbeds were frequented two or three times by cattle. Several crops appeared to be severely or completely infected when seedlings were used from these seedbeds.

The observed transmission by cattle, donkeys and rats suggests that other mammals will also transmit RYMV. Sheep and goats, for instance, do hardly graze on rice and will play therefore a minor role in the spread of RYMV.

Spread of RYMV through soil and plant debris

Soil might also play a role in the transmission of RYMV from one to the next cropping period. Soil collected during the contra season in infected fields appeared to be ELISA-positive, whereas an artificial mixture of soil and virus, kept at prevailing temperatures, lost its infectivity as shown by transplanting seedlings into this soil virus mixture (Chapter 6). Naturally, the virus may be set free in soil by decomposing root material. Virus is also actively released by infected root systems. Infectious virus is released for at least a month from roots of infected plants grown on nutrient media. Plants became infected in experiments when healthy and infected plants were prevented to make leaf contact. These infections result evidently from either root contact between healthy and infected plants or by the inoculation of healthy plants with virus released by the roots of infected plants. These results suggest that virus will also be released continuously in fields with growing plants and even more virus might be released from plants damaged by plowing infected fields, hence, becoming an inoculum infecting seedlings in seedbeds and after transplanting.

Besides soil, roots and straw left in the field might also be a source for virus infection. These sources appeared to be ELISA positive. Fomba (1988) reported the survival of RYMV in dry material for several months after the harvest. However, to which extent the virus liberated from this material is infectious has not been analysed.

Seed transmission

Several plant viruses are transmitted by seeds produced on infected plants. This type of transmission provides an effective mean of introducing infectious virus into a crop, resulting in randomised infected foci. Some earlier studies have indicated that RYMV is not seed-transmissible (Bakker, 1974; Fauquet and Thouvenel, 1977; Sarra, 1999), although the phenomenology of some infections was occasionally assumed to be the result of seed transmission. In Chapter 7 convincing evidence has been obtained that rice seeds become infected. Most seeds of 21 rice genotypes studied were still ELISA-positive four months after harvesting. Analysis of the coat, endosperm and embryo of dissected seeds showed that all seed coats and only a part of the endosperm and the embryos gave a positive reaction in ELISA, even after maturation of the seeds. No infected plants were found in greenhouse or in field experiments when mature and dry seeds from infected cv BG 90-2 or Kogoni 91-1 plants were tested (Chapter 7). These results confirmed earlier experiments by Bakker (1974), Fauquet and Thouvenel (1977), and Sarra (1999), but contradict the frequent statements of Malian farmers

whose crops became, unexpectedly, infected with RYMV.

Invasion of seed is a rather common feature among plant viruses, but does not always lead to transmission (Matthews, 1991; Maule and Wang, 1996). The virus is often rapidly inactivated during the maturation process as has been shown for *Tobacco mosaic virus* in *Arabidopsis thaliana* seeds (de Assis Filho and Sherwood, 2000) and for *Southern bean mosaic virus* (SBMV) in bean (Cheo, 1955). Nearly all seeds of SBMV-infected bean plants are infectious at harvest whereas the embryo's lose their infectivity within two months after blooming (Cheo, 1955). This observation endorses the results of an older report demonstrating that SBMV-infected seeds completely lost their infectivity within seven months of storage (Zaumeyer and Harter, 1943). A rapid decline of infectivity of *Alfalfa mosaic virus*, a bromovirus, occurs also in infected lucerne seeds, whereas the antigen titre decreases only slowly during the seed maturation process (Bailiss and Offei, 1990).

A rapid loss of infectivity has also been demonstrated for RYMV infected rice seeds during the development and maturation of the seeds (Konaté *et al*., 2001). Their results explain the failure of Bakker (1974) to demonstrate seed transmission of RYMV as the seeds were tested six weeks after harvesting. However, it can not be excluded that developing and immature rice seeds may give infected seedlings when they are spilled in the field or at spots where the rice is threshed as has occasionally been observed.

The rapid loss of RYMV infectivity in seeds is not associated with the loss of antigen detection. Positive reactions were found in four months old seeds, of which the seed coats gave higher ELISA-readings than the embryo's and endosperm (Chapter 7). A complete loss of the antigen was even not observed when the seeds were incubated for at least six weeks in water.

The use of contaminated manure and feces

In Chapter 6, it has been established that feces collected from a calf, which was fed infected rice plants in one meal, or from a cow, which has been fed RYMV-infected rice straw is infectious and contains ELISA-detectable amounts of virus. Freshly produced feces will form an inoculum in the spread of the virus, but infectivity is lost within two months when cow dung is sun-dried (Sarra, 1998). Consequently, sun-dried cow dung does not seem to be a favorable environment for RYMV preservation.

For some time, farmers considered manure to be a source of the virus. This suspicion has now been confirmed in a field experiment. The use of 5000 and 1500 kg manure/ha resulted in a completely and severely infected crop, and in a completely but less severely infected crop, respectively (Chapter 6). The manure was produced from feces of cows grazing in an area in the Macina périmetrè with a high density of *O. longistaminata* plants (ON, 1990). No infection was observed in a third crop in which no manure was applied.

Usually, large spots with severe infections are found in rice crops on fields which were rented to cattle farmers in the contra-season to house cattle during the night. The dung produced forms a source, which infects the soil and the subsequent crop. Similar

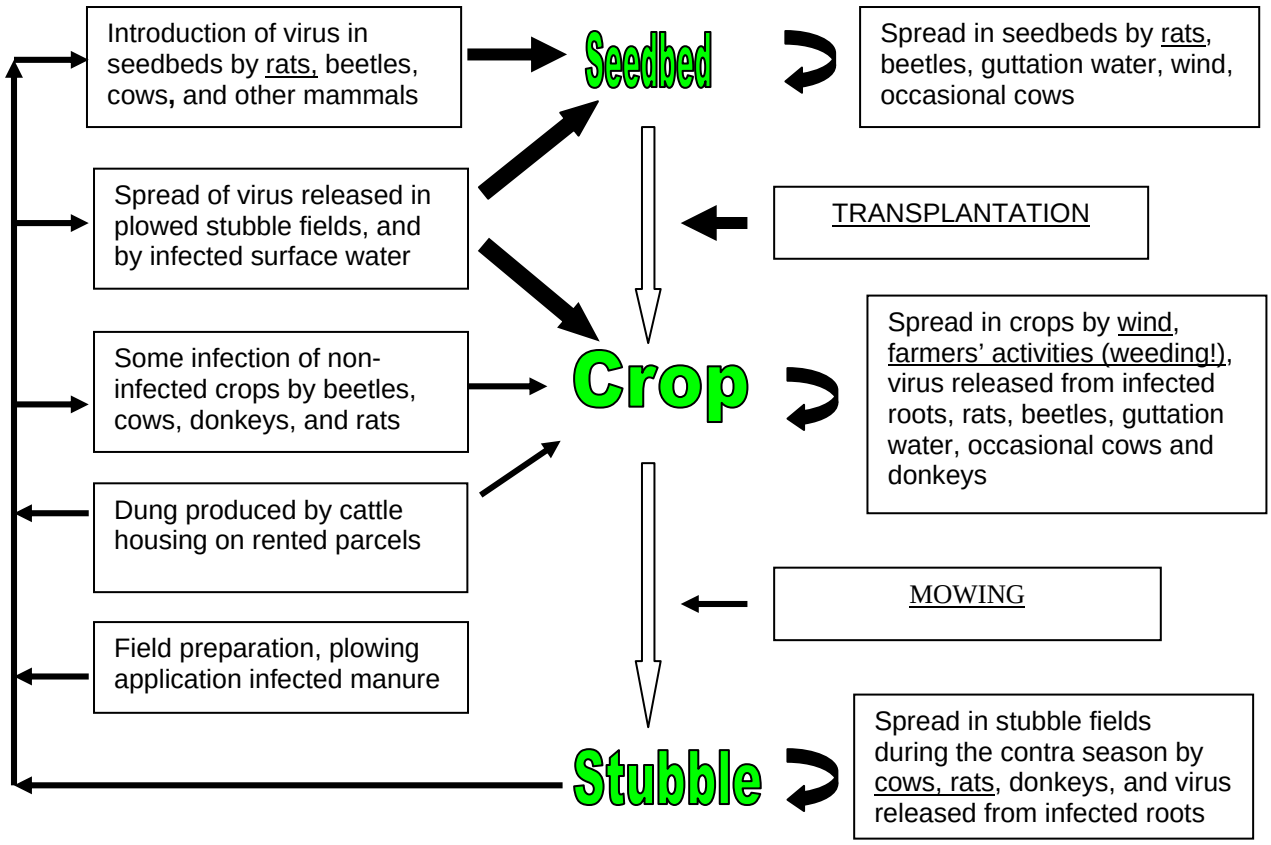

Figure 2. A descriptive model of *Rice yellow mottle virus* (RYMV) spread to and in seedbeds, to and in crops, and in stubble fields during the contra-season. The content of the boxes summarizes the agencies and factors spreading RYMV. The agencies and factors probably playing the most important role in the spread of RYMV are underlined.

observation were also made by Reckhaus and Andriamasintseheno (1995). These authors reported that infected plants were obtained by transplanting rice into a soil containing cow dung and poorly decomposed crop residues.

An epidemiological model of RYMV in irrigated crops

Relevant information on RYMV epidemiology is scanty. Using the collected information and interpreting the obtained experimental results, a model can be developed to describe the spread of RYMV in rice. This epidemiological model is based on several factors such as vector transmission, existence of natural sources, effective preservation of this highly stable virus in various substrates as well as the numerous transmission mechanisms elucidated in this study (Fig. 2). This model is not only valid for the 'Office du Niger', but is basically also valid for other areas where rice is cropped under irrigated conditions. The latter areas will ecologically differ from the ecology in the 'Office du Niger', in which the beetle population will probably be lower than in the other areas.

Seedbed infection. The first infected plants may occur in seedbeds (Fig. 2). In this stage infections can be introduced in different ways. Beetles, cattle, donkeys and rats can

introduce the virus into the seedbed from infected volunteer plants including regrowth, from *O. longistaminata*, a perennial that survives by rootstocks and is seed propagated, or from early infected *O. barthii* and susceptible grass species. The virus may also be introduced in the seedbed by contaminated manure or feces dropped by cows before plowing the seedbed plot. Alternatively, virus released from plowed-down infected regrowth, ratoon and weed may infect seedlings. Spread in the seedbed can occur by rats as long as they have access to or can live in the seedbed, by cattle as long as the seedbeds are not irrigated, and by wind-mediated leaf contact. Spread by infected guttation water can not be excluded. This water will contaminate the seedbed water.

Crop infection. Crops can become infected substantially when seedlings from even limitedly or partially infected nurseries are uprooted, transported, and planted (Chapter 3, Fig. 2). This spread is mainly the result of abrasive leaf contact between healthy and infected plants, and of hand-and-plant contact. Infected guttation water-wetted hands will aggravate this man-made increase. The transplanted crop can, even when the seedbed was not infected, become further infected by virus released in soil from infected plant remains, by infected manure and by beetles, or mammals (cattle, donkeys and rats). Infection by these mammals will mainly be restricted to the borders and corners of a field. In whatever way a crop becomes infected, treading fields for weeding and applying fertilizer, leaf contact created by wind, and transmission by beetles will promote further infection. Finally, infection may expand around an infected plant by virus released from the roots of this plant.

Survival of the virus in the field during the intercropping period. Harvesting an infected rice crop will result in an increase of the infection in the stubble. Infection of healthy fields may also occur when sickles are not cleaned after mowing an infected field. Cattle, donkeys and rats will spread the infection in harvested fields by grazing on stubble, spilled grain (rats), straw, volunteer plants, regrowth and germinating of spilled grain. These vertebrates may also spread the virus to healthy stubble fields after grazing on infected stubble. The infected stubble fields will form a large infection source for the next season. The small interval between the contra-season crop and the season crop can explain the higher incidence in the latter crops, whereas the lower incidence in the contra-season crop is due to the longer interval between the season and contra-season. The high incidence of the disease in the 2004 contra-season crop was positively correlated with early sowing of seedbeds, i.e. in November/December 2003 or in January 2004, instead of sowing the seedbed after the traditional date of February 10.

The virus sources surviving the intercropping season will be amplified in the stubble fields as soon as the rains preceding the main season crop will stimulate regrowth of rice stubble and other plants. Cattle and donkeys attracted by the regrowth and rats living in these stubble fields will further spread the virus to healthy plants. This amplification will also result in an enhancement of the infectious virus pool in the soil.

Beetle-mediated transmission versus other forms of RYMV transmission

In this study, virus was deliberately introduced by inoculation, by transplanting infected plants or in contaminated substrate in the experimental plots. Control plots were included in all experiments. Out of the numerous plants used in the controls only two plants in one control plot became infected after transplanting the healthy seedlings (Chapter 3). The extremely low infection in the controls demonstrates that all plants infected in the experimental plots became infected by the transmission mechanism studied. This conclusion indicates that beetle-mediated transmission could be excluded in the field experiments performed. As consequence the conclusion can be drawn that natural spread by beetles is of minor importance in the spread of RYMV.

Elimination and control of RYMV

Control through the use of resistant cultivars is the most effective and, probably, the cheapest way of combatting this virus disease. The seed infection studies revealed that several cultivars supposed to be resistant were indeed tolerant (Chapter 7). The use of tolerant cultivars must be discouraged as these cultivars may harbour large amounts of virus that may spread unnoticed in the crop and as a consequence a larger inoculum may be built up in the environment.

To control the spread of RYMV, farmers are advised to eliminate the virus from their infected fields. Completely infected fields have to be burnt directly after harvest, and subsequently inundated. After a few weeks the field has to be re-plowed and again inundated. This plowing and inundation might be repeated in order to promote the decomposition of potentially infectious root and plant material, and to prevent an early development of regrowth and vegetation in the harvested fields. Smaller spots with infected plants have to be rogued as soon as the infection becomes visible and the first two circles of plants around the spot have to be rogued also. The rogued material should not be left in the field but should be destroyed, so that mammals can not consume the infected plants.

Seedbeds have to be sown at spots where no infections have been observed in the previous crops and the developing regrowth has to be controlled as extensively as possible in a period some months before sowing. The seedbeds should preferentially not be located along roads used by cattle on the way to the foraging grounds. The seedbeds should be inundated slightly as soon as possible after sowing to prevent rats from entering the seedbeds and to chase them away from the seedbeds. Control of rats requires continuous attention.

Cleaning the levees, dikes and roadsides should be a matter of continuous concern. This cleaning has to be done before the plants start to bloom. Special attention should go to the elimination of *O. longistaminata*. Elimination of this weed should include removal (digging up) of the rootstock.

Weeding of the rice crop is an essential practice in the rice production. The rice fields are usually weeded two times. This is, in most cases, done when the plants start to bloom or have already set seeds. A more frequent weeding might result in fewer weeds as the seed production will be lower. A more frequent weeding will also form fewer constraints to the farmers as the plants to be removed are smaller.

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RÉSUMÉ

De nouvelles visions dans la transmission du virus de la panachure jaune du riz en riziculture irriguée

La panachure jaune du riz communément appelée virose, dont l'agent étiologique a été caractérisé par Bakker en 1970 au Kenya et qui l'a nommé *'Rice yellow mottle virus'* (RYMV) est transmissible par des coléoptères et facilement par voie mécanique. Ce virus est endémique en Afrique et se manifeste presque dans tous les périmètres de riziculture irriguée situés au sud du Sahara. Son incidence était négligeable jusqu'au début des années 1990. Depuis lors, des pertes de rendement de 25-97% ont été notées.

La panachure jaune du riz se manifeste par un jaunissement et/ou une coloration orange des feuilles, des nécroses, une réduction du tallage, un rabougrissement des plants et une stérilité des fleurs. Les premiers symptômes apparaissent sous forme de mosaïque vert clair sur les jeunes feuilles. Les rizières infectées présentent un aspect général jaune orange. Lorsque l'infection est précoce (avant deux semaines après semis), les plantules des cultivars sensibles meurent.

L'infection de la parcelle peut se présenter sous plusieurs formes. Des parcelles avec un taux d'infection élevé ou complètement infectées peuvent être contiguës á des parcelles sans infection apparente (Fig. 1 Chapter 8). De larges et/ou petites taches en formes irrégulières ou circulaires apparaissent dans certaines rizières, alors que ce sont de petites taches allongées qui sont occasionnellement observées le long des digues, des pistes et aux angles de la parcelle. Ces différentes formes de contamination des parcelles indiquent une propagation de la maladie par plusieurs mécanismes et non pas seulement par les coléoptères qui sont en très faible densité. Les études entreprises dans cette thèse ont pour but d'élucider et d'analyser les autres mécanismes par lesquels le RYMV peut s'introduire et se propager dans les rizières en riziculture irriguée. Les résultats ont montré que le plant de riz peut être infecté et que la maladie se propagerait dans les rizières par

- certaines pratiques paysannes (Chapter 3),
- le contact entre les feuilles de riz sous l'action du vent (Chapter 4),
- des mammifères (Chapter 5),
- la diffusion du virus dans sol (Chapter 6),
- l'utilisation de fumier préparé à partir de matériel végétal infecté (Chapter 6), et
- la contamination de l'eau d'irrigation par l'eau de guttation des plants infectés (Chapter 3).

Dans le cadre de la compréhension de ces différents mécanismes de transmission du virus de la panachure jaune du riz, une nouvelle enzym-linked immunosorbent assay (ELISA) basée sur la diffusion du virus des disques de la feuille a été développée (Chapter 2). Aucune différence statistiquement significative n'a été observée entre les valeurs des densités optiques enregistrées en utilisant les disques ou les extraits de

feuille infectée. Cette technique est moins contraignante, permet d'économiser du temps et d'éviter la contamination des échantillons par les mortiers et les pilons utilisés pour le broyage des feuilles sclérotiques. La propagation du RYMV dans les rizières les contacts entre les plants infectés et les plants sains ou entre les mains contaminées des travailleurs et les plants sains avait déjà été soupçonnée par Bakker en 1970. Ici, nous rapportons que certaines opérations culturales peuvent propager la panachure jaune du riz (RYMV) dans les rizières (Chapter 3). Il a ainsi été démontré que le repiquage de plantules issues d'une pépinière contaminée entraîne des sévères incidences 93 à 98% et 5 à 17% respectivement pour 'BG 90-2' et 'Kogoni 91-1' de la maladie dans la rizière. L'infection de la pépinière semble donc être la plus importante source de propagation de la maladie en riziculture irriguée avec repiquage. La propagation de l'infection du RYMV par l'action de marcher dans les rizières contaminées et le fauchage du riz a également été démontrée. Marcher entre des plants infectés et des plants sains a entraîné des incidences de 26 à 38% et de 14 à 17% du RYMV respectivement dans les parcelles de 'BG 90-2' et 'Kogoni 91-1'. Un gradient d'incidence de la maladie a été notée du premier au dernier plant de riz fauché avec une faucille préalablement utilisée pour récolter des parcelles infectées (Chapter 3).

Dans le Chapter 4, nos études montrent que le vent peut propager la panachure jaune du riz en conditions artificielles aussi bien qu'en conditions naturelles. Les différences considérables entre le nombre de plants infectés dans les parcelles protégées et les parcelles sans protection contre le vent indiquent que le RYMV se propagerait par contact entre plants sains et plants malades sous l'action abrasive du vent.

La plupart des virus des plantes sont transmis par les invertébrés au cours de leur prise de nourriture. Les vertébrés peuvent aussi transmettre certains de ces virus en se nourrissant des plantes comme démontré pour le RYMV dans cette étude (Chapter 5). Nous avons démontré en conditions contrôlées que le rat *Arvicantis niloticus*, les bovins et les ânes transmettent le RYMV au riz sain en rongeant ou en broutant des plants infectés. Ce résultat a été confirmé en plein champ où la source du virus doit être recherchée dans la végétation naturelle comme l'espèce de riz sauvage *Oryza longistaminata* qui végète sur les digues et le long des routes. La transmission du RYMV par les rats, les bovins et les ânes, démontre que d'autres mammifères peuvent également le transmettre.

La survie du RYMV dans le sol a été étudiée en mélangeant du sol non contaminé avec des broyats de plants de riz infectés (Chapter 6). La persistance de la virulence du virus dans le sol a aussi été démontrée après la récolte dans les rizières contaminées. Le sol est resté infectieux pour au moins 7 mois après la récolte. Les racines et la paille de riz abandonnées dans le champ après le battage et la rhizosphère des repousses sont restées infectieuses principalement pendant les trois premiers mois (janvier, février et mars) après la récolte. La persistance de la virulence du virus dans l'eau d'irrigation a été notifiée jusqu'au mois d'avril.

Il a été établi que lorsqu'un bovin est nourri avec la paille infectée de riz, le virus infectieux peut être détecté dans les excréments frais (Chapter 6). Cependant, lorsque

ces bouses sont séchées au soleil, le virus est vite dégradé et perd sa virulence en l'espace de six semaines. Par conséquent, la bouse de vache semble ne pas être un milieu favorable pour la conservation du RYMV. L'introduction du RYMV dans les rizières paysannes par le fumier contaminé a été démontrée en apportant 1500 et 5000 kg/ha de fumier à base de bouse de vaches pâturant dans une zone fortement infestée d'*O. longistaminata* infecté. Cette expérimentation s'est soldée par une sévère incidence de la maladie dans les parcelles ayant reçu le fumier contaminé.

Beaucoup de phytovirus sont transmissibles par les graines produites par des plants infectés. Concernant RYMV, nous avons démontré que les graines de riz acquièrent le virus pendant leur développement. Presque toutes les graines de la plupart des 21 génotypes de riz étudiés ont réagit positivement à l'ELISA. Cependant, aucune infection des plantules via les graines n'a été notée ni dans les essais conduits en serre ni dans les expérimentations en plein champ. Il a également été établi que la perte rapide de la virulence du RYMV dans les graines n'est pas associée à celle de l'antigène qui reste détectable dans les glumelles, l'embryon et l'endosperme quatre mois après la récolte et même lorsque les graines ont été incubées dans l'eau pour au moins six semaines (Chapter 7).

Un modèle descriptif de la propagation du RYMV dans les rizières a été développé à partir de nos résultats expérimentaux. Ce modèle épidémiologique est basé sur plusieurs facteurs tels que la transmission par vecteur, la présence de sources naturelles, la stabilité du virus dans les différentes sources ainsi que sur les mécanismes de transmission élucidés dans cette thèse (Fig. 2, Chapter 8). Ce modèle est valable pour l'Office du Niger ainsi que pour toutes les régions où la riziculture irriguée est pratiquée.

A l'égard des résultats obtenus au cours de ces études, certaines mesures et recommandations aidant à éviter ou à minimiser les épidémies dévastatrices ont été proposées. La résistance variétale est certes la méthode de lutte la plus efficace et peut être la moins coûteuse pour contrôler ce virus. Cependant, les études de transmission du RYMV par les graines ont montré que certaines variétés de riz qualifiées de résistantes étaient plutôt tolérantes. L'utilisation de ces variétés tolérantes doit être déconseillée, car elles peuvent contribuer à rehausser le niveau de l'inoculum du virus dans le milieu et accroître ainsi les risques d'épidémies. Pour éviter infection de la culture, les pépinières doivent être installées dans les parties non contaminées de la parcelle. Elles ne doivent pas être localisées le long des routes permettant aux animaux d'avoir accès aux plantules. Les repousses de riz de la culture précédente doivent être éliminées plusieurs mois avant l'installation des pépinières. Le maintien d'une lame d'eau constante dans les pépinières est recommandé pour éviter les rats d'avoir accès aux plantules. Il est conseillé d'éviter l'apport du fumier contaminé mal décomposé provenant du matériel végétal infecté dans les pépinières aussi bien que dans les rizières. Le nettoyage du réseau d'irrigation (digues, arroseurs, drains) doit être un travail permanent. Une attention particulière doit porter sur l'élimination de *O. longistaminata* qui est le principal hôte naturel du virus. La maîtrise de cette espèce et d'autres espèces d'adventice susceptibles d'héberger le virus doit être un des principaux objectifs de programme de lutte contre cette maladie.

En cas d’infection, il est conseillé d’éliminer le virus de la parcelle par la destruction de tous les plants infectés. Lorsque toute la parcelle est infectée, il est recommandé de brûler tous les résidus de récolte et de procéder immédiatement à un labour suivi d’inondation de la parcelle. Ce cycle de labour et d’inondation doit se poursuivre jusqu’à la décomposition totale de tout le matériel végétal infecté. Un labour de début de cycle n’est pas conseillé juste avant l’installation de la culture suivante, car la probabilité que la culture soit fortement infectée pourrait augmenter avec la diffusion du virus contenu dans les racines contaminées découpées par la pratique de ce labour. Lorsque l’infection se présente sous forme de petites taches, les plants infectés ainsi que les plants sains des deux premières couches qui les entourent doivent être arrachés et détruits pour éviter que la maladie se propage dans la parcelle. Le paysan doit éviter de marcher dans les parties infectées du champ.

SAMENVATTING

Het “*Rice yellow mottle virus”* (RYMV) is tot op heden het enige virus waarvan bekend is dat het grote schade kan veroorzaken in de geïrrigeerde rijstteelt in Afrika, ten zuiden van de Sahara. De ziekte, die dit virus veroorzaakt, is in 1966 voor het eerst in Kenia waargenomen maar wordt heden ten dage in nagenoeg alle gebieden gevonden, waar deze rijstteelt wordt uitgeoefend. RYMV-infectie kenmerkt zich door een gele of oranje verkleuring van de rijstbladeren, groeiremming en verminderde uitstoeling van de plant. Deze verschijnselen leiden tot een lagere opbrengst, maar de voornaamste schade wordt geleden door het optreden van steriliteit, indien het gewas in een jong stadium wordt geïnfecteerd.

Reeds in de jaren 70 van de vorige eeuw is aangetoond dat verschillende keversoorten uit de familie Chrysomelidae het virus kunnen overbrengen. Deze kennis heeft er toe geleid dat men de verspreiding van het virus in het veld steeds aan deze insecten toeschrijft. Echter in het veld worden diverse infectiepatronen waargenomen, die moeilijk verklaard kunnen worden door verspreiding via kevers. Zo kunnen compleet geïnfecteerde velden naast geheel gezonde velden liggen (zie Fig. 1 in Summary and Concluding Remarks). In zwaar besmette velden komen kleine infectiehaarden naast grote, soms perceel-wijde, haarden voor. Ook is de infectie in rijstvelden vaak beperkt tot hoeken of tot smalle stroken (van slechts 1 à 2 rijen planten) langs wegen, paden en waterkeringsdijkjes. Deze, en andere waarnemingen duiden erop dat RYMV zich mogelijk ook via andere mechanismen kan verspreiden. De doelstelling van het in dit proefschrift beschreven onderzoek was dan ook om meer inzicht te verkrijgen in de daadwerkelijke mechanismen waarmee RYMV zich in geïrrigeerde rijstvelden verspreiden kan. Daarbij werd uitgegaan van het gegeven dat RYMV een heel stabiel virus is dat zich eenvoudig mechanisch laat verspreiden. Het vermoeden was dan ook dat mechanische overdracht zoals dat kan optreden tijdens handmatige bewerking van het gewas en door vee dat tijdens het contra-seizoen in de velden wordt toegelaten, wel eens een grotere rol kon spelen dan overdracht door kevers. Het beschreven onderzoek vond plaats in de “Office du Niger” in Mali.

Om het onderzoek te vergemakkelijken is, gebaseerd op de waarneming dat guttatie-water van met RYMV geïnfecteerde rijstplanten veel virus bevat, een ELISA techniek ontwikkeld, waarbij het virus uit bladschijfjes kan lekken in plaats van gebruik te maken van extracten uit het moeilijk te homogeniseren rijstblad. De waarden die aldus met bladschijfjes werden verkregen waren gelijkwaardig aan die verkregen m.b.v. bladextracten (Chapter 2). Nader onderzoek leerde dat meer virus uit bladschijfjes lekt dan in de beschadigde cellen aan de snijrand aanwezig is. Dit duidt er op dat het virus ookop een andere wijze dan slechts door uitspoelen van de beschadigde cellen uit de schijfjes vrijkomt. De nieuw ontwikkelde techniek blijkt ook toegepast te kunnen worden op andere door kevers overdraagbare plantenvirussen.

Vervolgens is nagegaan in hoeverre de handmatige bewerking door rijsttelers kan leiden tot mechanische verspreiding van RYMV. Bij het uitpoten van zaailingen maakt de rijstteler immers verschillende malen contact met de planten en tevens maken de

planten onderling bladcontact. In Chapter 3 is aangetoond dat een lichte besmetting van zaaibedden door het handmatige uitpoten kan leiden tot een aanzienlijke uitbreiding van de infectie. Enige weken na het uitpoten breidt deze infectie zich in het rijstveld opnieuw aanzienlijk uit. In Chapter 4 wordt aannemelijk gemaakt dat dit te wijten is aan enerzijds door de wind gestimuleerd onderling bladcontact en anderzijds door infectie van gezonde planten met virus dat uit wortels van geïnfecteerde planten wordt afgescheiden. RYMV-infectie kan zich vervolgens verder uitbreiden tijdens het maaien van het gewas. Deze verspreiding leidt tot een aanzienlijke verhoging van het inoculum in het veld voor aanvang van het volgende groeiseizoen.

In Chapter 5 is nader onderzocht in hoeverre grazend vee maar ook in het wild levende grasratten die rijstvelden vaak koloniseren, het RYMV via hun fourageergedrag kunnen verspreiden. Aldus werd aangetoond dat ezels, koeien maar ook de grasratten, het virus efficiënt kunnen overbrengen wanneer zij in de gelegenheid zijn rijstplanten te consumeren (Chapter 5). Dit doet zich in de praktijk voor bij ratten zodra de grond langs wegen en dijkjes droog valt, en wanneer ezels en koeien aan de rand van percelen wat planten kunnen eten. In de onderzochte regio in Mali blijken de onbeschermde zaaibedden langs wegen frequent bezocht te worden door koeien op weg naar hun voedselgebieden. In het contra-seizoen kan het virus zich handhaven en zelfs verder uitbreiden in uitlopende rijststoppels, kiemende rijstplantjes en eventueel aanwezige vatbare grassen door in stoppelvelden grazende koeien en daarin levende ratten. Het virus kan daarbij over grotere afstanden verspreid worden aangezien het speeksel van ezels en koeien enige uren na het consumeren van een zieke plant nog besmet is (Chapter 5).

Behalve dat het virus gedurende het contra-seizoen kan overblijven in levend plantmateriaal, kan het ook in grond, stro en andere dode plantenresten nog lange tijd infectieus blijven (Chapter 6). Een mengsel van grond en geïnfecteerd plantenextract bleek na twee maanden bewaren nog infectieus. In fecaliën van koeien kan infectieus virus aangetoond worden voor een periode tot 60 uur na het nuttigen van besmette planten.

Fecaliën, mest en compost kunnen bronnen van infectie zijn. Zwaar besmette rijstgewassen kan men vinden op percelen waarop koeien, die gedurende het contra-seizoen op de stoppelvelden in de Zone du Niger polders grazen, de nacht hebben doorgebracht. Infecties worden ook gevonden op percelen waarop mest en compost zijn toegepast, waarin fecaliën zijn verwerkt van koeien die besmette planten hebben gegeten (Chapter 6).

Weliswaar hebben diverse onderzoekers in het verleden gerapporteerd dat RYMV niet met het zaad van besmette planten overgaat; hieraan wordt echter door rijsttelers veelvuldig getwijfeld. Derhalve is in het kader van dit promotieonderzoek dit aspect, met de huidige technieken, nogmaals onderzocht. In verschillende experimenten werd geen enkele zaadoverdracht gevonden (Chapter 7), waarmee de vroegere conclusies bevestigd konden worden. Een complicatie bij het interpreteren van de gegevens kan echter zijn dat zaad afkomstig van besmette planten wel RYMV kan bevatten. Nader onderzoek leerde dat vooral de zaadhuid een positieve reactie in ELISA geeft, terwijl endosperm en embryo minder vaak besmet zijn. Desondanks kan uit de getoonde

resultaten geconcludeerd worden dat geen overdracht van het virus door zaad plaatsvindt.

In de diverse uitgevoerde experimenten zijn veel planten gebruikt. In een enkel experiment werden slechts twee virus-geïnfecteerde controleplanten aangetroffen, terwijl veel planten, die aan de veronderstelde overdrachtsmechanismen blootgesteld werden, ziek zijn geworden. Dit wijst er op dat een natuurlijke verspreiding van RYMV door kevers - vanuit de omgeving naar de planten in het experiment, of vanuit de aanwezige geïnfecteerde planten van het experiment zelf - geen of nauwelijks een rol heeft gespeeld. Deze constatering duidt eens te meer op een geringe betekenis van insectentransmissie in de verspreiding en epidemiologie van RYMV.

Relevante informatie over de epidemiologie van RYMV is nauwelijks beschikbaar. Gebruikmakend van de verkregen resultaten is een beschrijvend model voor de verspreiding van RYMV in geïrrigeerde rijstteelt uitgewerkt en gepresenteerd in een figuur (zie Fig. 2 in Summary and Concluding remarks).

EPILOGUE

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CURRICULUM VITAE

Soungalo Sarra was born in 1957 at N'Togonasso, a small village of 1000 inhabitants situated at 40 km east of Koutiala, Mali. He was registered at the primary school of Kouniana under threat of confinement of his father by the local administrator in 1965. In 1979, he obtained his diploma of technician in agriculture of the Farming Polytechnic Institute of Katibougou (Institut Polytechnique Rural (IPR) de Katibougou). From 1980 to 1989, he headed the Point d'Appui de Recherche of Kita, a substation of the Rural Economy Institute (IER), the national agricultural research institute of Mali. His work consisted of research on peanut, sorghum, pearl millet, maize and cotton. After admission to the IPR in 1989, he obtained his Bachelor degree in Agriculture (Ingénieur d'Agriculture et du Génie rural) in June, 1993. Benefiting from a fellowship offered by the Embassy of the Netherlands in Mali within the framework of the Projet Riz Irriguée at Niono, he received his Master degree (Diplôme d'Etudes Approfondies) at the University of Ouagadougou, Burkina Faso, in 1998 on a thesis entitled '*Some ecological aspects of the Rice yellow mottle virus (RYMV) in the soudano-sahelian zone'* under the supervision of Dr Gnissa Konaté. He worked successively as weed scientist and as head of the crop protection section in the IER irrigated rice research program in Niono from 1993 onto this day. In 2000, he was offered a WOTRO fellowship for a PhD sandwich study at the Laboratory of Virology, Wageningen Agricultural University, the Netherlands, on the epidemiology of *Rice yellow mottle virus* in irrigated rice under the supervision of Dr. Dick Peters and Prof. Rob Goldbach.

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LEGENDS FRONT AND BACK COVER

Picture front cover: Loading of rice seedlings from a heap onto a donkey cart.

Pictures back cover: A) cows grazing in stubble field; B) seedbed grazed by cows; C) a single infected plant; D) a few infected plants at a corner of a field; E) a completely infected field (left) and an entirely healthy field separated by a levee with weeds; F) an advanced infection in an early infected spot.