Review

Rice hoja blanca: a complex plant–virus–vector pathosystem

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Abstract

Rice hoja blanca (RHB; white leaf) devastated rice (*Oryza sativa*) plantings in tropical America for half a century, before scientists could either identify its causal agent or understand the nature of its cyclical epidemics. The association of the planthopper *Tagosodes orizicolus* with RHB outbreaks, 20 years after its emergence in South America, suggested the existence of a viral pathogen. However, *T. orizicolus* could also cause severe direct feeding damage (hopperburn) to rice in the absence of hoja blanca, and breeders promptly realized that the genetic basis of resistance to these problems was different. Furthermore, it was observed that the causal agent of RHB could only be transmitted by a relatively low proportion of the individuals in any given population of *T. orizicolus* and that the pathogen was transovarially transmitted to the progeny of the planthopper vectors, affecting their normal biology. An international rice germplasm screening effort was initiated in the late 1950s to identify sources of resistance against RHB and the direct feeding damage caused by *T. orizicolus*, making better progress in the development of hopperburn-resistant than for hoja-blanca-resistant rice lines. In the 1980s, the identification of a novel virus as the causal agent of RHB, and genetic studies on the interaction of this virus with its planthopper vector, confirmed previous studies on the pathogenic nature of the virus to *T. orizicolus* and helped explain the cyclical nature of RHB epidemics. This disease is best controlled by hybridization of susceptible *indica* and resistant *japonica* rice genotypes and the adoption of integrated disease control practices.

Keywords: Rice hoja blanca (RHB) virus, *Oryza sativa*, *Tagosodes orizicolus*, Sogata, Tenuivirus, *Echinochloa colona*

Review Methodology: We searched CAB Abstracts,CIAT's Rice Program Annual Reports and personal research files.

Introduction

Rice 'hoja blanca' (white leaf) is one of the most complex, puzzling, challenging and, all the same, interesting pathosystems encountered in the field of plant virology. Rice hoja blanca (RHB) gained notoriety in the mid-1950s, when it emerged in the state of Florida, threatening rice production in the southeastern USA [1]. However, RHB was first observed in rice fields of the Cauca Valley AQ1 department of Colombia, around 1935 [2]. Two decades later, RHB was present in the neighbouring countries of Panama, Venezuela and Costa Rica, and had crossed the Caribbean Sea to reach Cuba and the Florida peninsula [3]. Within a decade, RHB was widely distributed throughout tropical and subtropical America, affecting rice plantings in Brazil, Belize, Colombia, Costa Rica, Cuba, Dominican Republic, El Salvador, Ecuador, Guatemala, Guyana, Honduras, Mexico, Nicaragua, Panama, Peru, Puerto Rico, Surinam, USA and Venezuela [4]. Besides its notable spatial dissemination capacity, the main concern of rice growers and agricultural scientists was the significant yield losses (25–75%) that this disease could induce in susceptible rice cultivars, as a result of seedling death, reduced photosynthetic capacity, plant dwarfing and panicle sterility [3, 5, 6].

The broad geographic distribution of RHB and its capacity to breach extensive natural barriers, such as the Caribbean sea, were explained in 1957 when preliminary investigations conducted in Cuba [7, 8] and Venezuela [9] associated the incidence of RHB with the presence of the

planthopper (Homoptera: Delphacidae) *Sogata orizicola* (Muir) in RHB-affected rice fields. Further testing of these suspected vectors led to the conclusion that only *Sogatodes orizicola* was the vector of the causal agent of RHB [10, 11]. In 1993, the taxonomy of this planthopper species was further reviewed to include it in the genus *Tagosodes*, species *orizicolus* [12]. Rice planthoppers are known long-distance fliers, capable of covering distances in excess of 1000 km without alighting [13]. Planthoppers are also proven vectors of different plant viruses affecting plant species of the Gramineae, including rice, which suggested that RHB was caused by a virus [2, 14]. However, the aetiology and epidemiology of RHB had to wait almost half a century to be elucidated.

One of the most puzzling characteristics of the RHB pathosystem is its erratic, 'cyclical' epidemic behaviour. RHB caused significant (25–50%) yield losses in 1957–1964, just to fade away in the following 15 years (1965–1980), and then re-emerge in full force from 1981 until 1984 [1, 3, 15] (Peter R. Jennings, 2010, unpublished data). The last outbreak of RHB in Colombia took place in 1996–1999. Considering that no hoja-blanca-resistant rice varieties were available during those years, it became evident that the cyclical nature of the RHB epidemics was more likely determined by the interaction between the pathogen and the insect vector *Tagosodes orizicolus* [7, 16, 17].

The Plant Host

Rice (*Oryza sativa* L.) is the second most extensive food crop grown in the world (*c.* 159 million ha) after wheat and is the main food crop grown in the tropics. Half of the world's population (over 3 billion people) depends on rice, particularly in Asia, where annual per capita rice consumption may be as high as 170 kg in countries such as Vietnam. Asia produces almost 90% of all the rice cultivated in the world (141 million ha), mainly China and India, where rice domestication and cultivation seems to have started over 5000 years ago. Africa cultivates approximately 9.5 million ha, and the Americas 6.8 million ha of rice. Nevertheless, over half a billion people consume up to 43 kg of rice per year/per capita in Latin America and the Caribbean, which demonstrates the importance of this grain crop in the regional diet [18].

Unlike the complex phytosanitary situation of rice in Asia, where several viruses affect this grain crop, RHB was the only viral disease of rice in the Americas until a new virus, *Rice stripe necrosis virus* (RSNV), emerged in South America in 1991 [19]. However, the economic importance of RSNV has been so far relatively negligible. On the contrary, the pathogenicity and severity of the causal agent of RHB were apparent from the time this disease was originally observed in the Cauca valley of Colombia. Affected rice plants showed the characteristic yellowish, chlorotic stripes that often coalesce to give RHB its name (white leaf); stunting, panicle sterility and/or plant death. Affected plants may show the disease symptoms in some tillers, while other tillers remain symptomless. The panicles of diseased tillers are shorter and malformed, including the lemma and palea of florets that dry out and show a brownish discoloration [20]. Although disease incidence and severity were variable, some affected rice fields in the Cauca Valley had to be ploughed under in 1958. Varieties such as Bluebonnet 50, Century Patna 231, Early Prolific, Fortuna, Guayaquil, Lady Wright, Nato, Rexoro, Santa Maria and Zenith proved highly susceptible, as well as many of the 2200 lines of the world collection of the US Department of Agriculture of the USA evaluated in Colombia, Cuba and Venezuela. A second group of 1725 lines from the USA and FAO genebanks was also screened in Cuba and Colombia. Of these field evaluations, about 400 resistant rice genotypes were identified in these collections, mainly belonging to short-grain *japonica* types from Japan, China, Korea and Taiwan [5, 6]. The main sources of resistance selected from the US collection were Gulfrose and Lacrosse (subtropical *japonicas*). Disease incidence and severity seemed to depend on the time of infection: with early infection (1–3 leaf stage) resulting in higher incidence and disease severity [2, 21]. Based on these field evaluations conducted in 1956 and 1957, a breeding project was initiated in Palmira (Colombia) to transfer the resistance found in the *japonica* grain types to the susceptible long-grain, *indica* varieties preferred in tropical America.

The Insect Vector

T. orizicolus (Muir), commonly referred to as 'sogata', has been consistently shown to be the vector of the causal agent of RHB [7, 9, 10, 11]. A related planthopper species, *T. cubanus*, common in rice fields, can be experimentally forced to transmit the RHB agent from rice to its preferred wild host, *Echinochloa colona* (L.) Link, and from *E. colona* to *E. colona* under natural field conditions [22]. The species *T. orizicolus* and *T. cubanus* (Homoptera: Delphacidae) are restricted to tropical and subtropical America, although *T. cubanus* has been reported from West Africa as well [23]. However, the genus *Tagosodes* appears to be of Asian origin [23, 24], as is the case with its main hosts, rice and *E. colona* [25].

T. orizicolus was originally described by Muir [26] as *Sogata orizicola* in 1926 from specimens collected in British Guiana. The name *Sogata brasilensis* or *brazilensis* has also appeared in the early literature. In 1963, Fennah [27] changed the genus to *Sogatodes*, and Ishihara and Nasu [28] changed the spelling of the species name from *orizicola* to *oryzicola*. Finally, as mentioned above, the name of this planthopper species was changed to *T. orizicolus* in the 1990s [12, 29]. *T. cubanus* was first described by Crawford [30] as *Dicranotropis cubanus* from Cuban specimens. Other names used in the past to describe this species

were: *Megamelus flavolineatus*, *Delphacodes pallividitta* and *Chloriona* (Sogatella) *panda* [23]. *T. orizicolus* and *T. cubanus* are similar in size (2–5 mm long), but *T. cubanus* tends to be smaller. Females are characteristically larger and more robust than males, and their body colour varies from dark to light brown, with males being darker than females. Both alatae and brachypterous individuals are found in any given population of *Tagosodes* spp., with brachyptery being more frequent in *T. orizicolus* and females of both species. *T. orizicolus* requires moderately high temperatures and high humidity (about 27°C and >80% RH, respectively) for normal development. Eggs are laid in a cluster in the midrib of the rice leaf blade or in the leaf sheath, usually in multiples of seven. Eggs are about 0.7 mm long, white, and are very sensitive to desiccation. Incubation periods vary according to temperature with a minimum of a week at 27°C and a maximum of two weeks at 18°C. Each nymphal stadium takes 3–7 days and 2–3 weeks for complete nymphal development. The first instar nymph (0.5 mm long) emerges on the adaxial surface of the leaf and starts feeding within 24 h. Second instar nymphs are 1 mm long and subsequent inmatures belonging to the third, fourth and fifth instars grow up to an average size of 2.5 mm. Adult females are larger than males and live longer than males within the 24–36 longevity range described for *T. orizicolus*. Mating may start two days after the final moult, and mated females begin oviposition three to five days later, laying about 10 eggs/day for a total average of 160 eggs. Virgin females may lay fewer eggs (about half as many), but parthenogenesis has not been reported for these species [14].

T. orizicolus is also a direct pest of rice, being capable of causing significant feeding damage (hopperburn) and yield losses in rice varieties that attract and support large populations of this planthopper species. Genetic resistance to the direct damage of sogata is available and widely used in rice improvement programmes, but the genetic basis of this resistance is different from the genetics of resistance to RHB virus (RHBV) [31].

The RHB Pathogen

Although, following the demonstration in 1957 that a planthopper (sogata) was associated with RHB [7, 9] and the observation that RHB symptoms were similar to those of rice stripe in Japan [32], the causal agent was assumed to be a virus, its isolation and characterization took almost 50 years from the original report of RHB in Colombia, in 1935 [4, 33]. The reasons for this long period of fruitless research on the aetiology of RHB were multiple: the difficulty of working with viruses at a time when plant virology was still developing as a science; the lack of the advanced molecular techniques we have today at our disposal; and the fact that RHBV belonged to a group of plant viruses not yet known to plant virologists prior to 1980. The first attempts to isolate the causal virus were made at an advanced virology laboratory (Instituto Venezolano de Investigaciones Cientificas, IVIC), in Caracas, Venezuela. The RHB investigation conducted at about 1968 resulted in the isolation of isometric (round-like) particles (42 nm in diameter) from RHB-affected rice plants [34]. However, electron microscopy studies conducted in Japan a year later [35] revealed the presence of filamentous particles (8–10 mm in diameter and about 2000 nm in length) in the cells of infected rice plants and sogata vectors, similar to those reported for closteroviruses (e.g. *Citrus tristeza virus*). A Brazilian study yielded similar results upon examination of the infected cells of the weed *E. colona* [36], which often shows hoja blanca symptoms in RHB-affected rice fields. Adding to the discrepancy of these previous reports, a new investigation on the aetiology of RHB, conducted in Cuba [37], concluded that the causal agent was not a virus, but a phytoplasma (bacteria-like micro-organism of the class Mollicutes).

The final attempt at isolating and characterizing the RHB pathogen began in the early 1980s, taking advantage of new disease outbreaks in CICA 8 and IR 22 rice fields located in the department of Tolima, Colombia. The infected plant extracts were processed following new purification protocols based on self-forming caesium chloride and caesium sulphate density gradients. Light-scattering bands were formed in both caesium salts when diseased rice plant extracts were centrifuged, but not when extracts from healthy rice plants were used. The electron microscopy of the recovered bands obtained after 19 h of centrifugation revealed the presence of very fine filamentous particles, approximately 3 nm in diameter and of variable length, with a tightly spiralled configuration. These particles had the characteristic ultraviolet absorption spectrum of a nucleo-protein when assayed by UV spectrophotometry ($A_{260/280\,nm}$ ratio of 1.4 ± 0.02). The purified RHB preparations produced a predominant protein of 34×10^3 Da and a minor protein of 17.5×10^3 Da. An antiserum prepared with these purified suspensions specifically detected the causal agent of RHB in diseased rice plants, but did not react with extracts from healthy rice plants [33]. These findings led to the conclusion that the RHB causal agent belonged to a new group of plant viruses not yet recognized by the International Committee on Taxonomy of Viruses (ICTV), together with two other new viruses affecting rice in Asia (*Rice stripe virus*, RSV), and maize in North America (*Maize stripe virus*, MspV) [38, 39]. These viruses were accepted and classified by the ICTV in 1995 and finally placed in the new *Tenuivirus* genus [40].

Tenuiviruses have a thin (3–8 nm in diameter) filamentous shape and variable lengths determined by the size of the RNA molecules they contain, and the different structural forms they adopt (spiral, circular or branched particles). These particles are nucleoproteins without an envelope. Purified tenuivirus particles can be separated into four or five components by sucrose density gradient

centrifugation, but form only one component when centrifuged in caesium salts, to a buoyant density of 1.28 g/cm^3. The ssRNA genome of tenuiviruses consists of four or more segments. The RHBV genome has four visible ssRNA species [41]. The ~9 kb RNA-1 is generally of negative polarity, whereas the remaining RNAs 2–4 have an ambisense translation strategy. The nucleocapsid protein of RHBV has a size of 34 kDa [33] and is encoded by the 5′-proximal region of the virion-complementary sense strand of RNA-3. The virion-sense RNA-4 molecule encodes a major non-structural protein (NCP) of 17.5 kDa, which accumulates in RHB-infected rice plants [33, 41]. There is no evidence of significant pathogenic variability or development of more pathogenic RHBV strains in Latin America. For instance, a comparative study of Colombian and Costa Rican isolates of RHBV yielded nucleotide sequence homologies between 91 and 98.9% for the coding and non-coding RNA-3 and RNA-4 components of the selected isolates [42].

Relationships to other *Tenuiviruses*

The genus *Tenuivirus* includes two additional viruses of rice: RSV and *Rice grassy stunt virus* (RGSV) in Asia; MspV, of worldwide distribution; and two neotropical viruses of weeds, related to RHBV: *Echinochloa hoja blanca virus* (EHBV) and *Urochloa hoja blanca virus* (UHBV). Tentative tenuiviruses (not yet accepted by the ICTV) include: another rice virus, namely Rice wilted stunt virus, Maize yellow stripe virus and at least four tenuiviruses isolated from wheat (ICTV). At the molecular level, RHBV is more closely related to EHBV and UHBV than to RSV, RGSV or MSpV [43]. RHBV is not serologically related to the Asian rice tenuiviruses RSV or RGSV.

RHBV is most closely related to EHBV, to the extent that their capsid and non-capsid proteins are antigenically related and very similar. These two viruses occupy the same agro-ecological zones and usually co-exist in most rice fields where RHB is a problem. However, RHBV and EHBV have different vectors (*T. orizicolus* and *T. cubanus*, respectively), even though *T. cubanus* can be forced to transmit RHBV under experimental conditions [21], which explains why RHBV and EHBV are consistently found to cause single infections in either rice or *E. colona*, respectively. At the molecular level, EHBV has a smaller RNA-4 than RHBV, and their nucleotide sequence identity only amounts to 80–85% and 46–53% in the coding and non-coding regions, respectively [44, 45]. Consequently, RHBV and EHBV are currently considered as distinct species of the genus *Tenuivirus* [43, 46].

Epidemiology of RHBV

The geographic distribution of RHBV is determined by the environmental conditions that favour the reproduction and survival of its insect vector, the planthopper *T. orizicolus*. This planthopper is currently distributed throughout tropical America, and it has been able to survive in some subtropical regions of the Americas, such as southern USA [14] and northeast Argentina [47]. However, RHBV is not currently found in these subtropical areas. *T. orizicolus* requires high humidity and optimum average temperatures in the 24–28°C range. The eggs are extremely sensitive to low relative humidity conditions, but the conditions found in irrigated rice fields or rain-fed rice fields in the tropics are quite favourable for the reproduction of *T. orizicolus*. Under these conditions, a new cycle from oviposition to the adult stage can be completed in less than a month [14]. *T. orizicolus* survives well on certain rice varieties, such as Bluebonnet 50, CICA-8, Metica-1, Oryzica-1, Arkrose, Nato and Gulfrose, but not on all rice genotypes (e.g. Nilo 1, Zayas Bayan and Dima); as well as on some related species, such as *O. perennis*, but not on *O. barthii, O. glaberrima, O. grandiglumis* and *O. latifolia*. Several cultivated and wild grasses have been reported to be experimental or suspected hosts of both the virus and the planthopper vector. Wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.), oats (*Avena sativa* L.) and rye (*Secale cereale* (L.) Bieb.) have been shown to be natural or experimental hosts of RHBV [48–50]. However, these alternate hosts do not seem to act as virus reservoirs for transmission to occur back to rice [22], even though some of these species are highly susceptible to RHBV [15]. Many wild grasses have been observed to show hoja blanca symptoms in RHB-affected regions. *E. colona* seems to be the most frequent weed present in rice fields affected by RHBV, and may have been the original source of RHBV [3], but this weed has been shown to be a dead-end host of the virus in experimental RHBV transmission assays [22]. Other weeds reported to exhibit hoja blanca symptoms are *Brachiaria plantaginea*, *Echinochloa crus-galli*, *E. walteri*, *Panicum fasciculatum*, *P. capillare*, *Paspalum* sp. and *Rottboellia exaltata* [11, 48, 51], but the causal agents have not been shown to be RHBV.

Nymphs, females and young males of *T. orizicolus* are usually located on the lower portion of the plant, whereas adult males may be found higher up on the infested rice plants. Hence, trapping sogata with nets may not reveal the real composition of the planthopper population. In Latin America and the Caribbean, where rice is grown all year round, *T. orizicolus* does not seem to have the need to migrate long distances [52], as rice planthoppers in temperate regions of Asia do [53]. Therefore, short distance dispersal into rice fields and within-field dispersal of sogata populations may be the norm soon after seedling emergence, and as long as the plant remains in its vegetative stage. Upon crop flowering, the winged individuals tend to leave the plants in search of younger rice plantings. With a life cycle of about a month, 2–3 generations may develop in a rice field. Double cropping allows a significant build up of the sogata vector in rice fields [14].

RHBV is a circulative, propagative and transovarially transmitted virus in its insect vector, *T. orizicolus*. These terms mean that a potential planthopper vector requires up to 12 h to acquire the virus from a systemically infected rice plant, even though some individuals can acquire the virus in less than an hour, with an optimum acquisition feeding time of 8 h [16], and then requires a minimum of a week to a month to complete its incubation period inside the vector. During these acquisition and incubation periods, the virus circulates from the sucking mouth parts of the insect to its midgut, where it penetrates into the haemolymph, to finally reach the salivary glands in order to be transmitted back into a healthy rice plant upon feeding by nymphs, female and male adults for 3–7 h to infect at least 90% of the test plants [16, 21]. Considering the short life span of the latter stage, adult males usually acquire the virus through the egg. Transovarial transmission was first demonstrated by Acuña *et al.* [7]. Female adults can acquire the virus during the nymphal stage because of their longer life span [3, 7, 9, 10, 11, 16, 35]. The ability of RHBV to reproduce in its insect vector means that it does it at the expense of the bio-synthetic capacity of the insect (as in a susceptible plant) and, consequently, it can infect and induce deleterious effects in its insect vector. This fact was demonstrated by Jennings and Pineda [17], who showed that *T. orizicolus* vectors laid one-third as many eggs and hatched fewer nymphs than did virus-free individuals. The life expectancy of nymphs and adults was also significantly reduced. The authors associated the observation that only 5–15% of the individuals in a given sogata population can transmit the virus in nature [50], with the deleterious effect of RHBV in virus vectors, and the subsequent decline in the number of RHBV vectors at the end of a disease cycle.

The above hypothesis was investigated by Zeigler and Morales [54], who studied the inheritance of the ability of *T. orizicolus* to support replication of RHBV by following the segregation of progeny derived from crosses between insects of known pedigree and virus transmission capacity. Evidence of RHBV replication inside the sogata individuals allowed access to a virus source was investigated using the immuno-enzymatic enzyme-linked immuno-sorbent assay (ELISA) method [55] adapted for this purpose. A post-acquisition period of 12 days was required before RHBV could be detected by ELISA in the sogata vectors, showing that this virus replicates in *T. orizicolus*. A minimum of 20 days was then required for the virus to be transmitted to susceptible rice plants (Bluebonnet 50), that is, to complete the virus incubation period. In these experiments, individuals who supported RHBV replication, as determined by ELISA, but who could not transmit RHBV before the incubation period (20–25 days) was completed, were considered 'potential' vectors. Non-vectors were identified by their inability to transmit the virus at the end of its incubation period, and by their negative ELISA reaction. 'Active' vectors were those individuals who could transmit the virus before the incubation period was completed. Progenies from non-vector parents from lineages including at least one vector, segregated in a fashion consistent with the presence of a single recessive gene (*r*) controlling the ability to support RHBV replication in *T. orizicolus*. A strong maternal influence on the ability of the planthopper to support virus replication was observed in this investigation. Active female vectors transmitted RHBV transovarially to their progeny regardless of the male parent, and these could transmit the virus to the susceptible test plants. However, these individuals had a lower virus titre than those obtained from ELISA-positive parents, and they could lose their ability to transmit RHBV to susceptible plants.

The above investigation and the low percentage of RHBV vectors in any given population of *T. orizicolus*, even during an epidemic phase of the virus, show that the ability of an individual sogata to support virus replication is under genetic control. As mentioned before, the ability of the RHBV to replicate in the planthopper and its deleterious effects on its fecundity mean that RHBV is also a pathogen of *T. orizicolus*. Thus, the dominant allele (*R*) that does not support multiplication of the virus protects the planthopper from the ill effects of the virus and prevents RHBV transmission in rice fields. At this stage, the proportion of vectors to non-vectors further diminishes, bringing about a reduction in disease incidence and, hence, the end of an epidemic cycle. However, the recessive (*r*) gene that supports virus replication is maintained in homozygous or heterozygous form in the *T. orizicolus* population.

It is also possible, as in the case of insects that develop genetic resistance to insecticides or pathogens [56–58], that there is a 'fitness cost' [59] for the RHBV-resistant individuals of *T. orizicolus*. That is, a trade-off in which alleles conferring higher fitness in one environment (e.g. AQ3 presence of RHBV) reduce fitness in an alternative environment (e.g. absence of RHBV), which may eventually lead to an increase in the proportion of *rr* individuals in the absence of the virus once an epidemic comes to an end. Be that as it may, either because of a gradual increase in the number of *rr* individuals derived from heterozygous (*Rr*) resistant individuals or because of a genetic reversion to the original (optimal) fitness state in the absence of the pathogenic virus, the proportion of *rr* individuals capable of supporting RHBV replication eventually increases, thus creating the conditions for a future RHB outbreak. The genetic control of RHBV transmission in *T. orizicolus* is also demonstrated by the observation that highly viruliferous sogata colonies produced by selected crosses between proven RHBV vectors eventually regress to populations containing a reduced number of RHBV vectors, after the directed crosses are terminated [21, 60].

Genetic Resistance

In 1956, a severe RHBV epidemic swept throughout Central America, the Caribbean and tropical South

America. All rice varieties under cultivation at that time were highly virus- and sogata-susceptible and yield losses were up to 100%. Crop losses were exclusively the result of virus infection as farmers had not yet begun using insecticides to control planthopper vectors.

In 1957, the Colombian Ministry of Agriculture requested assistance of the Colombian Agricultural Program of the Rockefeller Foundation to create a national rice research programme. The initial objective of the new breeding programme was to develop rice varieties with hoja blanca resistance. The USDA provided its germplasm collection, totalling a few thousand accessions, for evaluation at the Ministry's Palmira Experiment Station under heavy natural virus pressure from 1957 until the epidemic subsided in 1964 [1]. Natural infection in Palmira revealed the high susceptibility (ratings of 7–9) of all tropical *indicas* and most subtropical *japonicas*. Strong resistance rated as 1–3 was found only in temperate *japonicas*, largely from Taiwan and Japan, and in a few subtropical *japonicas*. In subsequent years, other resistant sources, all *japonicas*, were introduced from West Africa that, in turn, trace back to Taiwanese varieties. It is possible that the absence of RHBV in either northern Asia or West Africa has not given this neotropical pathogen time to adapt to temperate *japonica* rice varieties. Despite the presence of another tenuivirus, RSV, in temperate Asia, RHBV and RSV are antigenically unrelated and their genomes show significant differences in nucleotide and amino acid identities [43] to expect that resistance to RSV could be effective against RHBV. In fact, high levels of resistance to RSV have been identified in *indica* varieties; and sources of resistance to RSV, such as Zenith, are highly susceptible to RHBV [61, 62]. An early study on the varietal reaction of rice to RSV and RHBV had led to the conclusion that rice stripe and RHB are caused by different viruses [63].

Given the significant crop losses caused by RHBV in 1956–1958 in Cuba, Venezuela, Panama, Costa Rica and Colombia, RHBV resistance breeding began in 1958 using several temperature- and photoperiod-insensitive, short-grain *japonicas* from Taiwan as donors in crosses with long-grain (USA) subtropical *japonicas*. Segregating generations were selected under natural RHB incidence, and elite lines were recycled into the crossing programme. RHBV-resistant varieties released in subsequent years, Napal, ICA10, and Colombia 1, were commercially unaccepted because of poor plant types, low yielding capacity, susceptibility to direct sogata damage and bad cooking quality. These defects were not addressed until the introduction of semi-dwarf Asian *indicas* in 1966–1967. Colombia 1 and resistant lines derived from crosses with the new parental materials were used extensively in later years as sources of virus resistance (Peter Jennings, 2010, unpublished data).

Having no acceptable virus-resistant varieties, farmers in the early 1960s resorted to insecticides in a futile attempt to reduce vector populations. The result was the destruction of biological control and a resurgence of sogata causing massive crop damage through hopperburn. However, swollen sogata populations did not extend the hoja blanca epidemic or incite a new one in subsequent years. After the RHBV epidemic ended in about 1964, and the mechanical damage caused by sogata became more important, RHBV resistance breeding ceased in all Latin America programmes except that of Peru, where the Amazonian locality of Bagua remained an intermittent hot spot for field selection of RHBV-resistant parents and segregating populations in the following years [64] (Peter Jennings, 2010, unpublished data). This change in crop improvement priorities shifted the RHB breeding efforts to the search for sources of resistance to hopperburn, which is controlled by different genes [65].

In 1967 the Rockefeller Foundation–Ministry of Agriculture programme, soon to morph into the newly created International Center for Tropical Agriculture (CIAT), began receiving tropical Asian *indica* germplasm from the International Rice Research Institute (IRRI) located in the Philippines. This material was evaluated for insect resistance in greenhouse cages containing a non-viruliferous sogata colony. The basic method, which has hardly changed over time, involved infestation of trays of 15-day-old seedlings, 10 per accession, with roughly ten individuals (males, females and nymphs) per seedling. Test materials were rated for damage when the susceptible check, Bluebonnet 50, died some 8–9 days later. Resistance to feeding, ranging from moderate to immunity, is conferred by differing combinations of antibiosis, antixenosis and tolerance. Insect resistance is common in tropical *indicas*, whereas all Asian temperate *japonicas* are susceptible as are most subtropical *japonicas*. This is the direct opposite of reaction to RHBV wherein all *indicas* are susceptible while many *japonicas* are resistant. CIAT breeders discard both asymptomatic (extreme antibiosis) and highly susceptible materials, preferring only moderately resistant reactions having a scale rating of 3–5. National programmes have released over 300 semi-dwarf varieties in the American tropics during the past 40 years. The great majority are moderately resistant and a few highly antibiotic. There are no confirmed reports of insect resistance 'breakdown' given the emergence of new sogata biotypes, in contrast to the appearance of successive biotypes of the brown planthopper *Nilaparvata lugens* [66] in tropical Asia, presumably caused by the release of monogenic, highly antibiotic resistance.

A high incidence of RHB in the period 1957–1963, in rice nurseries planted at Palmira, Cauca Valley, Colombia, permitted the classification of selected rice varieties into four disease reaction groups: (1) a group of RHB-resistant varieties (Pandhori No. 4, Lacrosse×C253, Colusa, Lacrosse×Zenith-Nira, Gulfrose, Lacrosse and Asahi); (2) a moderately resistant group (Tainan-iku No. 487, Sadri, Missouri R-500 and Arkrose); (3) a moderately susceptible group (Zenith and Century Patna 231); and (4) a group of RHB-susceptible varieties (Toro, Nato, Fortuna, Bluebonnet 50 and Magnolia). These results were fairly

consistent over a 3 year period, and in different geographic locations in Venezuela, Cuba and Central America [49]. In Venezuela, a similar RHB evaluation experiment with 16 rice varieties resulted in the identification of three disease reaction groups: (1) a highly susceptible group (Bluebonnet 50, Starbonnet, NP-125, Nato, Bluebell, Zenith, Fortuna, Caloro, Dulor, Century Patna, Kanto 51 and Saturno); (2) a moderately susceptible group (Var. 501 and IR-8; and (3) a resistant group made up by Lacrosse (Colusa-Blue Rose×Shoemed (R)-Fortuna (R)) and Gulfrose (A Brunimissie selection (R)×Zenith) [67]. These last two rice varieties also behaved as resistant to RHB in Colombia. The previous breeding efforts mentioned above, seeking resistance to RHB, seemed to have paid off when some new lines, namely ICA-3 and ICA-10, proved to be resistant when exposed to field and glasshouse colonies of *T. orizicolus* under experimental conditions at Tibaitata, Cundinamarca, Colombia. However, none of these ICA lines became a variety or was used effectively in subsequent breeding programmes. Other rice genotypes evaluated as resistant to RHB in that study included Nilo 3A, ICA3, ICA10, IR 5, Mudgo and Napal. Only Mudgo and IR5 were resistant to both the virus and the vector (hopperburn). The most susceptible genotype, as in previous experiments, was Bluebonnet 50 [68]. This susceptible cultivar was widely cultivated in Colombia in 1957–1958, when RHB caused up to 100% yield losses in many rice fields.

A number of CICA genotypes possessing high levels of resistance to sogata feeding damage were developed in the 1970s. CICA 4 had slight RHBV and good sogata resistance [69]. Unfortunately, following its characteristic cyclical pattern, new outbreaks of RHB in 1981 and 1982 in Colombia severely affected the CICA lines, particularly CICA 8, widely planted in the eastern plains of Colombia in 1981 [70]. The main cultivars affected at that time by RHB were IR 22 and CICA 8. The latter genotype is highly resistant to the direct feeding damage caused by *T. orizicolus*, thus demonstrating the need to conduct simultaneous efforts to breed for multiple disease and pest resistance, including RHB, planthopper damage and other diseases of economic importance, such as rice blast. RHB also affected rice fields in Ecuador, Peru and Venezuela in the early 1980s, particularly rice cultivars such as CICA 8, IR 6, INTI and Araure 1. In response to these RHB outbreaks, a special nursery was planted in these countries to select resistant genotypes. A total of 74 breeding lines developed from crosses with selected sources of resistance, namely: Colombia 1, CICA 4, CICA 7, and Pelita 1, two RHB resistant controls (ICA 10 and CICA 7) and three susceptible checks, were included and evaluated in this international nursery. RHB incidence was high in Ecuador (Guayas) and Peru (Bagua); moderate in Venezuela (Calabozo); and low in Colombia (La Libertad). The high disease pressure in Ecuador and Peru affected all of the breeding lines and even some of the 'resistant' controls, particularly CICA 7. However, ICA 10 expressed its high level of resistance to RHBV in all of these locations [64].

Crop losses to hopperburn and RHB in Cuba, once appreciable, were greatly reduced after the economic embargo restricted insecticide importations. Rational use of insecticides in most Latin American countries has gradually diminished feeding damage and RHBV incidence associated with high sogata populations. These observations suggest that pesticide abuse affects the parasitoids and predators of *T. orizicolus* in rice fields, and that rational use of insecticides permits beneficial organisms to effectively reduce sogata populations. At present, breeders place little emphasis on sogata resistance, as most commercial varieties and parental materials have acceptable levels of hopper burn resistance. Only elite, advanced lines are screened for hopper burn resistance to eliminate susceptible genotypes. CICA 8, an improved variety released in 1978 through a joint effort of CIAT, the Colombian Agricultural Institute (ICA) and the Colombian National Rice Growers' Federation (FEDEARROZ), had to be taken out of cultivation during the 1980–1982 RHBV epidemic, despite its high yielding capacity and moderate resistance to hopper burn [70]. Fedearroz 50, another widely cultivated variety having strong antibiotic resistance to direct sogata damage, recently suffered severe yield losses caused by RHBV in Calabozo, Venezuela. The relevant fact is that resistance to the feeding damage caused by sogata does not adequately protect against hoja blanca attack in virus-susceptible varieties, but it seems to reduce RHBV incidence and moderate virus outbreaks under natural field conditions.

Experiments conducted in the early 1990s [65] showed that the resistance of rice plants to RHBV was not the result of differences in vector feeding behaviour. Vectors reared for eight generations on resistant plants showed no increased ability to transmit to resistant lines or decreased ability to transmit to susceptible ones. The longevity of vectors was similar when reared on virus-resistant or susceptible plants. The incubation period of the virus in resistant plants was significantly longer than in susceptible plants. Resistance increased with plant age in both resistant and susceptible cultivars. Increased virus dosage, as determined by an increase in the number of viruliferous vectors per inoculated plant, caused an increase in transmission to both resistant and susceptible cultivars. However, the ranking of resistant and susceptible genotypes remained the same across the experimental range of dosage and plant age. It was concluded that the resistance studied is to virus infection and there is little risk of 'breakdown' occurring as a result of genetic or behavioural changes in the vector population. The authors suggested that these findings would permit the application of economic thresholds to planthopper feeding damage with little risk of RHBV outbreaks.

To study the genetics of resistance to planthopper feeding damage, the more frequently used sources of

resistance to hopper burn in Latin America were characterized as resistant or susceptible based on free-choice and no-choice tests and based on the survival and oviposition of the insect. Two groups resulted: (1) Mudgo, Amistad 82, IRAT 120, IRAT 124 and Makalioka as resistant materials and (2) Chianan 8, Colombia 1, Bluebonnet 50, IR 8 (ICA), IR 8 (IRRI), Tetep and CICA 8 as susceptible materials. It was found that the damage caused by the insect to the materials was associated with insect survival, oviposition and egg hatching. To determine heritability, two parents were selected: Makalioka and Mudgo, which were crossed with IR 8. The F_1 and F_3 populations were also evaluated based on a free-choice test. Finally, based on the crosses' reactions to the insect damage and comparing them with the parents, a genetic model of resistance was proposed for Mudgo and Makalioka, consisting of a single dominant gene in homozygous form (AA), accompanied by a modifier gene that interferes to a greater or lesser degree with the resistance expression (bb>Bb>BB) depending on the material and on the exposure time of the plant to the insect. For Mudgo, the recessive homozygous form (bb) accelerates the expression of the susceptible phenotype, and for Makalioka the homozygous dominant form (BB) delays the expression of the susceptible phenotype [71]. Lack of proper hopperburn evaluation and classification of some of the test genotypes used in this study suggest that further studies on the genetic nature of hopper burn resistance are necessary.

From the 1970s to the 1990s, RHB appeared erratically throughout the American tropics, although not with the severity or broad geographic distribution of the 1956–1964 epidemic. By then, many new *indica* varieties resistant to direct feeding damage but virus susceptible had replaced the handful of subtropical *japonica* varieties highly susceptible to both. Some areas such as Bagua in Peru and Calabozo in Venezuela became hot spots with moderate to high RHB incidence annually. These localized epidemics, likely perpetuated by excessive insecticide applications, facilitated some degree of localized field selection against virus susceptibility by breeders. In recent decades, no appreciable RHB appeared on the CIAT station, possibly influenced by strict pesticide control. Consequently, CIAT breeders were limited to small-scale evaluation of virus resistance in greenhouse tubes and cages, unsatisfactory for a high-volume hoja blanca breeding programme.

Given the slow progress in RHBV resistance breeding in the late 1980s, CIAT made a renewed effort based on induced RHB field epidemics to allow the selection of segregating populations. By then, prior greenhouse evaluations had identified several virus-resistant parents, all derived from Colombia 1 and related lines that received their resistance from Taiwanese *japonicas* in crosses made in the late 1950s. Oryzica-1 and Metica-1 were two of the new varieties possessing 'tolerance' to RHBV, derived from Colombia 1 in the early 1980s [72]. In addition, a few virus-resistant West African varieties, also products of crosses with the same Taiwan *japonicas*, had been incorporated into the breeding programme, resulting in additional materials carrying RHBV resistance. All these new parents had been transformed into *indica*, semi-dwarf, long grain, tropical backgrounds.

The renewed CIAT approach featured the massive greenhouse multiplication of a highly efficient RHBV vector colony for release and infestation of specialized field nurseries including several thousand rice parents, lines and segregating populations. Vector colony multiplication in large screened greenhouse cages involved the following key procedures:

- 50–60 proven female vectors were mated singly with known vector males, establishing 50–60 sub-colonies.
- About 30 nymphs from each mating were individually evaluated for virus transmission on individual healthy Bluebonnet 50 seedlings.
- Surviving insects from sub-colonies having about 90% transmission were transferred to a large cage containing 40–60-day-old diseased Bluebonnet 50 plants grown in pots.
- Two insect generations later, a sampling of individual insects was tested for virus transmission on healthy seedlings to estimate the percent colony vectors.
- Given the labour-intensive crossing and nymph evaluation in colony formation, the method was recently modified whereby some 2000 field collected insects are tested individually for virus transmission. Proven vectors are transferred to a large cage for three generations of multiplication on diseased rice plants. Percent colony transmission is estimated from a sample of 100 individuals.

These modified planthopper colonies typically contain about 50–60% RHBV vectors at the time of field release. Two colonies are produced each year for two seasonal field screenings. Following the second field trial, remnant insect stocks are destroyed and the process begins anew with insects collected from rice farms. Specially prepared fields contain 50-cm-long rows of plants, separated by 10 cm in raised beds 1.1 m wide. Irrigation water is held in 30-cm-wide canals between beds. RHBV-resistant and -susceptible controls are also included in a randomized fashion throughout the beds.

The RHBV resistance derived from Colombia 1 is first expressed when seedlings are about 17 days old. Thus, the evaluation plots are infested with the vector colony 18 days after germination. Potted infested plants from the multiplication cages are gently shaken above the beds until all nymphs and adults are transferred to the rows of test seedlings. Vector dosage, difficult to calculate, depends on percent transmission within the colony and an estimate of colony vigour. Generally, an average of 1.5–2.0 insects (0.75–1.0 vectors in a 50% transmitting colony) is released per seedling. After 3 days of infestation, insecticide

is applied to halt further transmission. Rice lines are evaluated 28–30 days later, adequate time for virus incubation and symptom expression.

On a scale of 1–9 (R–S), the resistant genotype Colombia 1 is usually scored 3–5, occasionally 7. Having no insect resistance, Colombia 1 is a preferred planthopper host, which contributes to variability in hoja blanca scores depending on sogata population dynamics, that is, the number of viruliferous *T. orizicolus* individuals that feed on a Colombia 1 plant, plot or field, mainly at the seedling stage. Thus, antixenosis (non-preference) and, to a lesser extent, antibiosis traits in rice genotypes may influence RHBV incidence in check varieties and test materials. The highly resistant check, Fedearroz 2000, invariably rates 1–3, while highly susceptible Bluebonnet 50 consistently receives a score of 9. As ratings of some checks are typically variable, all selected materials are evaluated each generation from the F_3 to the F_6. Materials equal to or better than Colombia 1 are selected, using the nearest checks as the basis of comparison. The F_1, produced from some 400–500 triple crosses a year, each having a minimum of two resistant parents, is handled distinctly. About 20–22 days after infestation, seedlings of each F_1 family are pulled and sorted, discarding all plants with any hoja blanca symptoms. Symptomless plants transplanted in the permanent field are destroyed if RHB symptoms later appear.

Despite the cost and difficulties inherent in the production of highly viruliferous vector colonies and inconsistent disease ratings in the seedling beds, RHB evaluations have been regularly conducted twice yearly since the early 1990s. Superior hoja-blanca-resistant plant types with good quality traits are recycled as parents in continuing high volume crossing. Most of the current RHBV-resistant rice genotypes selected so far have Colombia 1 in their distant pedigrees, tracing back to Takao-iku 18, a Taiwan *japonica*, from the 1950s crosses. Other resistant lines trace to West African IRAT subtropical *japonicas* that also arose from Taiwanese *japonicas*, notably Chainan 8. Some lines have both resistance sources in their parentage. All these parents are now typical long-grain, semi-dwarf *indicas*.

These RHB screening methodologies could be complemented with more precise biological transmission assays based on the probability of infection (p) or no infection (q) of a selected rice genotype by one or more active vectors of *T. orizicolus*. This biological assay is based on a simple binomial formula [$p+q=1$] and can be performed under controlled conditions for promising, advanced lines [73]. Should it be necessary to use more than one active vector per plant to infect an RHBV-resistant genotype, as is often the case with rice genotypes possessing above average levels of RHBV resistance, the probability of infection of that genotype under field conditions can be predicted with acceptable accuracy. For instance, in previous tests, the probability of infection of one plant of the susceptible variety Bluebonnet 50 by one active vector of *T. orizicolus* was $p(1)=0.75$. This result means that it only takes one viruliferous sogata per plant to infect 75% of the Bluebonnet 50 plants in a field. However, when this assay is performed on Colombia 1, a source of RHBV resistance, it took six viruliferous sogatas per plant to induce 75% RHB incidence. This experiment illustrates various issues treated in this review. First, Colombia 1 is neither immune nor highly resistant to RHBV, because if one increases the number of viruliferous sogatas per plant, disease incidence above 75% would likely occur in Colombia 1. However, even for 75% disease incidence, each plant of Colombia 1 would have to be visited by at least 60 individuals of *T. orizicolus* at an early seedling stage and within a short period of time to induce this level of RHBV incidence in the field, taking into account that only about 10% of the sogatas in a given population can transmit the virus. This is an unlikely case, which explains why the Colombia 1 type of resistance is effective under field conditions. More important, varieties such as Fedearroz 2000 or some IRAT lines, such as IRAT 124, would theoretically require over 200 sogata individuals visiting every plant just to reach a threshold of about 20 viruliferous individuals per plant to cause a RHBV incidence of 50% in these genotypes. This is the kind of information derived from controlled virus transmission tests under glasshouse and individual insect cage conditions that provides rice breeders with an experimental but fairly reliable vision of varietal behaviour under different hypothetical RHBV incidence conditions in the field.

Numerous studies on the inheritance of resistance to RHBV have been conducted in the field during epidemics, and in the greenhouse under controlled conditions. The early crossing of RHBV-resistant and -susceptible rice genotypes suggested that resistance was inherited simply, probably controlled by a single gene, with resistance dominant over susceptibility [74]. In glasshouse experiments, reaction to the virus in the F_1 of IR8×Mudgo and Bluebonnet 50×ICA-10 showed that resistance was dominant over susceptibility. Segregation in the F_2 indicated that resistance in ICA-10 was governed by two pairs of dominant major genes with complementary action [75]. Conclusions from different studies vary from the control by one to two genes, with or without QTLs, and from dominant to partially dominant to recessive action [65, 74–77]. This confusion is attributable to several experimental weaknesses including:

- Variation in virus readings during natural epidemics likely because of differing levels of sogata individuals in the field, or presence in the host of antixenosis and/or antibiosis against the insect vector.
- Greenhouse cage studies of parents, F_1 and segregating generations are likewise skewed by these components, principally antixenosis, of resistance to the vector. Study of individual plants confined in tubes might attenuate this variable.

- Virus dosage effects are an intractable problem in RHBV transmission and rice germplasm screening studies. Colonies of vectors differ in their ability to transmit the virus, and host reaction varies according to the number of viruliferous vectors that feed on a rice plant.
- Seedling age affects disease incidence, with greater susceptibility manifesting in rice genotypes inoculated in juvenile stages.
- Host reaction is usually expressed as disease incidence (number of plants expressing symptoms over total number of plants exposed to vectors) without regard to disease severity or the existence of possible tolerance traits in the sensitive host. Greenhouse plants with traces of infection are considered to be as susceptible as killed plants. Such plants in the field would be rated as resistant.

The nature of RHBV resistance remains uncertain. Greenhouse and field screening methods conducted at CIAT in the early 1990s identified a total of ten possible sources of resistance to RHBV. Genetic analysis indicated that resistance to RHBV was dominant and controlled by one gene in cultivar Blue Rose and incompletely dominant in Takao Iku 18 and PI215936. Resistance was controlled by one gene in Takao Iku 18 and by two independent genes in PI215936 [78]. As mentioned above, the F_1 of IR8×Mudgo and Bluebonnet 50×ICA-10 showed that resistance was dominant over susceptibility. Segregation in the F_2 indicated that resistance in ICA-10 was governed by two pairs of dominant major genes with complementary action [75].

Despite these confounding factors in genetic studies, breeders consider RHB resistance to be basically a dominant trait. Yet, it is a difficult breeding objective because of the inherent variability of the resistance sources available in response to the number of successful virus inoculation events (virus dose) per test plant, and inconsistencies of the screening methods used. Marker-assisted selection is suggested by some breeders as the best solution to the expensive and variable field screening.

The Latin American Fund for Irrigated Rice (FLAR), located at CIAT, began breeding in 1995, making extensive use of RHBV-resistant parents produced by CIAT. These FLAR crosses resulted in some 30 new varieties released internationally in several tropical countries in 2008–2009. All are rated as resistant to the virus and to vector feeding. The RHBV resistance generally is equal to or slightly better than that of Colombia 1. One new variety, Fedearroz 2000, has high virus resistance, first manifested in 5-day-old seedlings while moderate resistance first appears in seedlings after about 17 days. Fedearroz 2000 gives consistent field ratings of 1 with an occasional 3. An indication of the value of this material comes from Calabozo, Venezuela, a hot spot with an endemic hoja blanca situation. The new FLAR varieties and lines maintain their resistance in this environment.

Cultural Practices

The *T. orizicolus*–RHBV complex is a rice production problem in most neotropical environments where these planthopper and virus species find warm temperatures, adequate humidity and suitable plant hosts for most of the year. In tropical America, people preferentially consume long grain, *indica* rice varieties, which are very susceptible to RHBV. While significant temperature fluctuations may occur in some of these tropical environments, average monthly and annual temperatures are always high enough to meet *T. orizicolus* temperature requirements. Under these general climatic and agricultural conditions, control measures directed to the avoidance of the causal agent and the RHB disease are of little value. As mentioned before, RHBV is already widely established in tropical America, mainly due to the high mobility of its planthopper vector and the propagative nature and vertical transmission of the virus in *T. orizicolus*. The active international movement of rice germplasm does not play any role in the epidemiology of RHB, other than contributing to genetic homogeneity in the region, because RHBV is not seed-borne [9].

RHBV probably has more alternate hosts than those cultivated and wild grasses mentioned above, which might act as sources of inoculum. However, some of the cultivated cereals, such as barley, oats and rye, reported as hosts of RHBV, occupy different ecosystems isolated from the traditional rice-growing areas of Latin America, and the wild grasses suspected of harbouring the virus have not been shown to be of epidemiological importance. Thus, the eradication of grasses suspected of acting as RHBV reservoirs is not recommended given the long distance dispersal capacity of *T. orizicolus*, but it would be interesting to confirm the role of the many grasses that have been implicated as possible RHBV reservoirs. On the contrary, the elimination of volunteer or rattoon rice plants in rice fields prior to sowing or transplanting of the rice crop is highly recommended.

Rice needs water to produce, either from rainfall or irrigation sources, which satisfies the humidity requirements of *T. orizicolus*. It is been reported that heavy rainfall depresses sogata populations [79], but farmers and technicians alike have considerable difficulty in evaluating population dynamics based on climatic parameters, such as the effect of rainfall, because insect population dynamics seldom show synchrony with most climatic phenomena. Theoretically, planting rice at the onset of the rainy season or under irrigation, following a prolonged dry season without rice or alternate host plantings, should lower the direct or indirect impact of sogata in newly planted rice fields. However, studies conducted in Colombia [20] show that sogata populations are still relatively high prior (January–February) to the onset of the first rainy season in Colombia (March–April), when upland rice is planted. This is probably a consequence of the residual populations that move from end-of-the-year

plantings (October rainy season), and the rather short dry seasons that characterize the bimodal distribution of rains in Colombia, as opposed to the prolonged dry seasons that take place (November–April) in Central America and the Caribbean. On the other hand, it must be borne in mind that *T. orizicolus* is very sensitive to dry environments because of the deleterious effect that low relative humidity can have on egg viability [14].

Cropping systems have a major influence on the incidence of RHBV. The continuous planting of rice throughout the year in some rice-growing regions creates a permanent source of food and inoculum for *T. orizicolus* to thrive and act as a virus vector. This situation is further aggravated by the presence of a genetically homogeneous crop, usually represented by only one or few cultivars grown over a large agro-ecosystem in most rice production regions of Latin America and the Caribbean.

Biological Control

Most pest problems in agriculture are man-made as a result of the abuse of pesticides. These agrochemicals often have the most negative impact on non-target beneficial organisms normally feeding on insect pests, such as sogata. The elimination of beneficial bio-control organisms (parasitoids, predators or entomopathogens) favours the increase of more resistant or mobile insect pest population in crops receiving high doses of insecticides or other noxious agro-chemicals. Pesticide abuse is a widespread problem in the tropics, where farmers have little technical assistance, other than the frequent visits of pesticide salespeople, who have an easy task convincing farmers that pests, such as *T. orizicolus*, can result in economically important crop losses if not chemically controlled. The main problem with the concept of biological control is that it does not work effectively in highly disturbed (chemically contaminated) environments. It takes a concerted effort on the part of farmers in a given agricultural area or community to stop abusing pesticides at the same time and for all the crops present. Once a rational programme of pesticide application has been implemented in an agricultural area for some time, biological control agents can have a chance to control important pests, such as sogata. This process is best achieved by rapidly changing non-selective insecticides by new chemistries (e.g. neonicotinoids and growth regulators) possessing systemic and selective action against sucking insects. Among the bio-control agents known to act against *T. orizicolus*, there are **parasitoids**: *Haplogonatopus* sp. (Hymenoptera), *Atrichopogum* spp. (Diptera) and *Elenchus* sp. (Strepsiptera); and **predators**: *Coleomegilla* sp. and *Cicloneda* sp. (Coccinellidae), *Zellus* sp. (Hemiptera) and several species of spiders. Entomo-pathogens, such as *Beauveria bassiana* and *Metarrhizium anisopliae* (fungi), have also been used to control sogata, but they require high humidity and low radiation conditions [79]. The case of Cuba, an island affected by an embargo that makes the importation and use of chemical pesticides difficult and infrequent, demonstrates the fact that this situation is likely responsible for the notable reduction in RHBV incidence and sogata damage in Cuba's rice fields. On this island, the parasitoid *Paranagrus perforator* (Hymenoptera) and the predator *Tytthus parviceps* (Hemiptera) effect a regular biological control of *T. orizicolus* [80].

Chemical Control

As mentioned above, chemical control has been one of the most widely used methods to control the transmission of RHBV and hopperburn, despite disappointing results in RHBV control with most products used in the past [9]. The extensive and intensive use of non-selective, contact insecticides, mainly organo-phosphates and pyrethroids, for sogata control in past decades has been one of the main factors responsible for the outbreaks of RHBV and sogata as a pest. However, the RHBV–sogata complex is one of the few cases of biological virus transmission for which a selective systemic insecticide could be effectively used to reduce RHBV incidence, given the circulative and propagative nature of RHBV inside the sogata vector, which takes long enough for a systemic insecticide to kill potential vectors. Even active vectors take a few hours to transmit the virus, long enough for a systemic insecticide to kill many viruliferous individuals before they can transmit the virus. The use of selective, systemic insecticides is particularly indicated against viruses transmitted in a persistent manner and from within-crop sources, so that the insect vectors die before the virus completes its latency period [81]. Should it be necessary to use a systemic insecticide to control RHBV in highly disturbed agro-ecosystems, it is important to apply the systemic insecticide at sowing time and then every 15 days depending on the degree of RHBV susceptibility of the selected cultivar up to the first month of vegetative growth. Whereas the cost of these new chemistries is relatively high, some of their active ingredients (e.g. imidacloprid) are already available as 'generic' products at a lower cost. But, again, the use of chemicals is a last resource to control sogata when large populations of this planthopper threaten the rice crop as vectors of RHBV or as direct pests, because even the most selective insecticides available at present have a deleterious effect on some important bio-control organisms [82].

Conclusions

Undoubtedly, the use of RHBV-resistant rice varieties is the best strategy to manage RHB, but the expression of RHBV resistance remains 'dose-dependent'. This means that the reaction of an improved rice genotype to the virus depends on the number of viruliferous vectors that

feed or come into contact with rice plants. Moreover, RHBV-resistant genotypes may be suffer significant yield losses if inoculated with RHBV at a very early seedling stage (e.g. 1–3 leaf stage), as was shown for the moderately resistant rice cultivars Arkrose, Gulfrose and Pandhori No. 4 [83]. RHB is not a unique case in the field of plant virology, regarding the lack of immunity to a virus within the plant species. For instance, this is also the case of common bean (*Phaseolus vulgaris* L.) and whitefly-transmitted viruses (begomoviruses) affecting this crop, such as *Bean golden mosaic* and *Bean golden yellow mosaic viruses*, against which no immune common bean genotype has ever been identified [84]. Nevertheless, acceptable levels of virus resistance can be achieved under natural field conditions in common bean production regions affected by these whitefly-transmitted viruses. Similarly, the RHBV screening methods described above permit the discrimination and identification of different levels of RHBV resistance and subsequent selection of the most resistant lines to be released as improved rice cultivars possessing adequate levels of field resistance to RHBV.

The development of rice genotypes possessing high and stable levels of RHBV resistance is possible, as observed in the case of Fedearroz 2000, a genotype that shows an unexpectedly high level of RHBV resistance. Crosses between Fedearroz 2000 and the susceptible line WC 366 showed a segregation of resistant, intermediate resistant and susceptible genotypes. The possible genetics of these observations was a two-gene model with one dominant and one recessive gene or a three-gene model with one dominant and two recessive genes controlling RHBV resistance in Fedearroz 2000 [85]. In a biological transmission experiment using two viruliferous sogata individuals per (15-day-old) seedling, Fedearroz 2000 showed only an 8.3% RHBV incidence, as compared with 58% for Colombia 1 (a widely used source of RHBV resistance) and 100% for the susceptible control Bluebonnet 50. In this experiment, the female parent of Fedearroz 2000, P 3084/P 3844 showed an RHBV incidence of 66.6%, and the male parent, CT 8154, an intermediate 33.3% virus incidence. The latter genotype also showed considerable plant vigour, and the few infected plants only exhibited mild RHB symptoms [73]. A molecular study is currently under way to further investigate the genetic traits responsible for the high level of RHBV resistance observed in Fedearroz 2000. Although this investigation might disclose the existence of new genes effective against the virus, the senior author believes that this genotype also possesses desirable physiological traits derived from CT 8154, which might help Fedearroz 2000 escape infection, a known mechanism of virus avoidance in plants [84]. The fact that RHBV incidence in rice genotypes depends to a large extent on the age of seedling inoculation shows that the plant possesses mechanisms to escape infection. These mechanisms may not necessarily be virus-specific, but would allow inoculated rice plants to 'outgrow' the virus so that it cannot reach the meristematic tissue, where it can rapidly replicate and then systemically infect the plant. Alternatively, Fedearroz 2000 may have inherited genetic traits that slow down the cell-to-cell or phloem movement of the virus inside infected plants or restrain virus multiplication in the plant.

RHB can be used as a model to study complex virus pathosystems. In this system, the traditional 'disease triangle' of plant pathology: the plant host, the pathogen and the environment, has to be converted into a 'disease pentagon' to include the vector and the environmental conditions that affect its population dynamics. The plant host, rice, continues to be a major staple in the diets of millions of people in tropical America, and will increase its area to meet future demands. Low-yielding upland rice will continue to be replaced by more profitable irrigated rice, particularly in Central America, northern Colombia, Bolivia and tropical Brazil. Some traditional irrigated areas will decline as water becomes less available, resulting in a shift to new production areas having abundant water. Whereas the environmental consequences of changes in rice production systems in Latin America are rather unpredictable, they might not drastically modify the current RHB pathosystem, but they will surely alter the epidemiology of RHBV. The pathogen itself, RHBV, has not mutated so far, probably because of the fact that the few sources of resistance used to date do not confer immunity, so that the pathogen does not need to mutate to survive. This situation could change if new, and as yet unknown, genes for RHBV resistance are found and used by rice breeders. The environmental conditions in tropical America will continue to be the same, perhaps somewhat warmer, which might permit *T. orizicolus* to expand its geographic range back into the subtropics. This is occurring already in the case of northeastern Argentina, where *T. orizicolus* is not only present, but it has been shown to transmit a different plant virus of maize (*Mal del Rio Cuarto Virus*), fortunately under experimental conditions [86]. According to this report, *T. orizicolus* survives in cultivated grasses such as wheat, barley and triticale, which are more widely grown in subtropical and temperate regions. While this adaptation to new hosts may not be of epidemiological importance in tropical America, where those cereals are grown mostly in the highlands, it does pose the possibility that *T. orizicolus* could develop physiological races better adapted to other grasses different than rice. Moreover, these cereals (i.e. wheat and barley) have been shown to be RHBV reservoirs [4, 48]. On a positive note, the indiscriminate and irrational use of insecticides against sogata is expected to diminish considerably as rice growers become better informed about the negative effects of agro-chemicals on the environment, their health and, more important, on biological control organisms. This factor, together with the recent incorporation of stable RHBV resistance after so many decades of effort, will likely reduce the incidence of RHB in tropical America.

RHBV and other tenuiviruses transmitted by planthoppers in the Americas (e.g. MspV), Asia (e.g. RSV) and other continents of the world [87] belong to the same taxonomic genus (*Tenuivirus*) but to different phylogenetic clusters [43]. RHBV is more closely related to EHBV and UHBV, two exotic weed tenuiviruses frequently found in RHB-affected rice fields. These weed tenuiviruses are believed to have evolved from an ancestral form of RHBV [88]. Considering that the above-mentioned tenuiviruses are not seed-borne in their respective plant hosts [40] and that these grass species are not vegetatively propagated, it is possible that the ancestral tenuivirus was disseminated to different species of Gramineae by infected planthopper vectors. It is also possible that the ancestral tenuivirus could have been an insect virus that eventually adapted to one of the insect's plant hosts. This hypothesis has been considered in relation to the molecular evolution of plant RNA viruses [89, 90], which would help explain the complex interaction between these plant viruses, their vectors and plant hosts. Additionally, it would explain the genetic variability of tenuiviruses and their broad geographic distribution in the absence of seed transmission. The recent reports of *T. orizicolus* as a new pest of maize in Argentina [47, 86] demonstrate the capacity of this planthopper to adapt to new hosts and the concern about the potential role of *T. orizicolus* as a vector of plant viruses.

Summary

RHB remains an important rice production problem in tropical America, despite over 70 years of continuous research on this disease since it emerged in Colombia in 1935. The main obstacle in the implementation of an integrated RHB management programme has been the scarcity of sources of resistance to RHBV, in the cultivated rice species *O. sativa*, particularly in the *indica* grain types preferred in tropical Latin America. Fortunately, partial resistance to RHBV has been detected in *japonica* rice genotypes of Asian origin, which have been used as parental materials to develop commercial rice varieties possessing moderate levels of RHBV resistance for the Latin American market. The level of RHBV resistance achieved so far has been adequate for most planting seasons when virus pressure is moderate, but significant yield losses can occur during the periodic, 'cyclical' epidemics of RHBV that take place in most rice-producing countries of tropical America. Recently, higher levels of RHBV resistance have been observed in some new commercial rice varieties produced in Colombia, but the genetic basis of this virus resistance is not yet understood. Understanding the genetic interaction between the insect vector *T. orizicolus* and RHBV has helped explain the cyclical nature of RHBV epidemics, to the extent that the virus is also able to infect susceptible individuals of this planthopper species, reducing its reproductive capacity and life span. Further studies on the molecular basis of RHB resistance should result in the selection of rice cultivars possessing higher levels of virus resistance. In the meantime, the implementation of an integrated RHB management scheme, including: rational use of selective insecticides; biological control; better cultural practices; and use of RHBV-resistant rice varieties, is highly recommended.

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