RELATIONSHIP BETWEEN INSECT VECTORS ABUNDANCE AND OCCURRENCE OF RICE YELLOW MOTTLE VIRUS IN FARMERS' FIELDS IN KILOMBERO DISTRICT

BONAVENTURE JANUARY

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ABSTRACT

Rice yellow mottle virus (RYMV) endemic to Africa is spread within and between rice fields by several species of Chrysomelid beetles and grasshoppers. In Tanzania and particularly in Kilombero District, the virus is increasingly becoming a serious problem to rice production. The relationships between the insect vectors and RYMV disease incidence and severity were not fully known hence the need for this study. The assessment of both disease incidence and severity of RYMV and population abundance of its insect's vectors were conducted in the three divisions of Mngeta, Ifakara and Mang'ula in Kilombero District, Tanzania in 4m2 quadrat. Insect sampling was conducted using sweep net while RYMD incidence and severity were visually assessed in a 4 m^2 quadrat. Results of the insect identification indicated the presence of two insect vectors of RYMV i.e. (*Chaetocnem*a spp. and *O. hyla*). The population densities of these RYMV vectors were higher at the border parts of the rice fields than at the middle parts. On the other hand, the incidence and severity of RYMV disease increased with the age of the crop. Results of within field distribution also indicated a random distribution of RYMV-affected plants in the rice fields in the agro ecosystem. The field studies of the virus–vector relationship established that RYMV occurrence varied in space and time and crop development stages. The partial correlation analysis showed a positive relationship between insect vector's population density and the incidence and severity of RYMVD. The two insect species were tested for their ability to transmit RYMV and both were able to transmit the virus from RYMV-infected plants to healthy rice seedlings suggesting their potential contribution to RYMVD prevalence in the agro-ecosystem.

DECLARATION

I **BONAVENTURE JANUARY** do hereby declare to the Senate of Sokoine University of Agriculture that this dissertation is my own original work and that it has neither been submitted nor concurrently being submitted for degree award in any other Institution.

Bonaventure January	**Date**
(MSc. Candidate)	

The above declaration is confirmed

Dr. G. M. Rwegasira	**Date**
(Supervisor)	

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DEDICATION

To my father January John and my mother Fausta L. Kimaro who laid the foundation for my education.

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LIST OF ABBREVIATIONS AND SYMBOLS

< - Less than

> - Greater than

AAF - Acquisition access feeding

ACMV - African cassava mosaic virus

ANOVA - Analysis of variance

A.M - Ant meridian

DF - Degrees of freedom

E - East

GFLV - Grape vine fern leaf virus

GRV - Groundnut rosette virus

IAF - Inoculation access feeding

IPM - Integrated pest management

IRRI - International Rice Research Institute

KATRIN - Kilombero Agricultural Training and Research Institute

MAFSC - Ministry of Agriculture Food Security and Cooperatives

m.a.s.l - Meters above sea level

MSV - Maize streak virus

MS - Mean square

N - North

RNA - Ribo nucleic acid

RYMV - Rice yellow mottle virus

RYMVD - Rice Yellow Mottle Virus Disease

S - South

SE - Standard error

SV - Source of variation

SS - Sum of square

SUA - Sokoine University of Agriculture

ToYLCV - Tomato yellow leaf curl virus

WARDA - West Africa Rice Development Association

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background Information

Rice (*Oryza sativa* L.) is one of the most important cereals in the world. It is the primary staple food for more than 51% of the world's population (Nguyen and Tran, 1998). In Tanzania, rice is a key staple food but its production is affected by many diseases, the most important of which is the Rice Yellow Mottle Virus Disease (RYMVD) caused by *Rice Yellow Mottle virus* (RYMV) (Abo *et al*., 2000). The disease, which is endemic to Africa, was first reported in Kenya in 1966 (Bakker, 1974). It is now known to occur in almost all irrigated and rain fed (flooded) rice producing agro-ecologies in Africa (Hull and Fargette, 2005).

Rice yellow mottle virus is a highly infectious virus consisting of a single-stranded-positive RNA genome that specifically infects rice and is mechanically transmitted in the field by insect vectors, vertebrates, wind mediated means and irrigation water (Sarra, 2005; Nwilene *et al*., 2008). Population densities of insect vectors of RYMV can influence the incidence and severity of RYMV disease. Banwo *et al*. (2001) reported a close positive relationship between RYMV-vectors population density and disease incidence and severity in Tanzania. Reckhaus and Andriamasintseheno (1997) reported the wide distribution and abundance of *Chaetocnema* spp. in the RYMV endemic areas in Ivory Coast and suggested that *Chaetocnema* spp were the most important vectors responsible for the occurrence and widespread distribution of the virus. Other insect vectors of RYMV have been identified. They include *Trichispa sericea* Guerin, *Oxya hyla* Stål, *Snootriba similis* Mulsant, *Conocephalus longipennis* de Haan and *Locris rubra* Fabricius *(*Moury *et al*., 2007).

1.1.1 RYMV transmission

Virus transmission by insects is a common way for viruses to spread between different host plants and this is possibly as a result of a protein that plant viruses attach to as they hitch to an insect ride between plants (Bebelliure *et al*., 2008; Uzest *et al*., 2007). Understanding the transmission process and the intimate relationship between a virus and its vector can facilitate the development of novel opportunities for designing control strategies against plant viruses, including the genetic manipulation of vectors and the expression of recombinant proteins in transgenic plants to neutralize the transmission process (Bebelliure *et al*., 2008; WARDA, 2000). Given the limitations of the current control strategies against viruses, there is a need for efficient and environmentally sound alternatives for sustainable agricultural production (Voinnet, 2007).

1.1.2 RYMVD control

Rice yellow mottle virus is the most stable and difficult virus to control in sub-Saharan agriculture today (Nwilene *et al*., 2009). The virus is capable of surviving in particularly harsh weather conditions. Unlike the other major viruses of the region, such as African Cassava Mosaic Virus (ACMV), Maize Streak Virus (MSV), Groundnut Rosette Virus (GRV) and Tomato Yellow Leaf curl Virus (ToYLV), all specifically transmitted by insect vectors, RYMV is transmitted by several means (Fargette and Konaté, 2004; Inoue and Sakurai, 2006). Control strategies against viruses are usually designed to mitigate the considerable losses viruses can cause by reducing the sources of infection and limiting the spread by vectors (Lecoq *et al.*, 2006).

1.1.2.1 Sanitation and controlling of vectors

Roguing and removal of infected plants can control viral diseases but seldom achieve a complete control of virus diseases by interfering with vectors' activity (Woin *et al*., 2007).

Measures against vector activity are among the most successful approaches used to suppress virus epidemics (Raviv and Antignus, 2008). Control measures against vectors and vector activities can be grouped into three classes: (1) killing the vectors with insecticides, (2) reducing the virus sources and (3) interference with vector landing on the crop (Kumar and Poehling, 2006).

1.1.2.2 Use of insecticides

Despite the wide range of the available insecticides, their use to prevent vector activity is not a preferred solution due to the reason that many viruses are introduced into crops by visiting insects that inoculate during their first probing activities. Vectors for non persistent (and partly semi persistent) viruses need relatively short inoculation times, much shorter than the time needed for insecticides to kill (Ritzenthaler, 2009). In addition, insecticides can induce restlessness in insects, with the result that they make more inoculation attempts than do calm insects. Exceptions are vectors that colonize the crop and transmit circulative viruses, for which insecticide control may result in reduced spread of virus (Raviv and Antignus, 2008). Chemical control of vectors can reduce the spread of plant viruses but their effectiveness against vectors is highly variable and there may be adverse biological and environmental consequences related to their use (Perring *et al.*, 2009). In light of the situation described above, integrated pest management (IPM) would be the best strategies in combating the virus spread (Malstron *et al*., 2006).

1.1.2.3 Reducing virus sources

The use of virus-free seeds and/or vegetative propagative materials can results in minimal primary infection. This can be complemented by removal of sources of infection in and around the crop, removal of plant remains from the previous season and, if necessary,

creation of a time gap between crops and/or space gap between plots. These operations will reduce the numbers of viruliferous insects that reach the crop (Lecoq *et al.*, 2006).

1.1.2.4 Vectors interference

Interference with vector landing on crops is achieved by altering the attraction of insects to colours. Insects like aphids are repelled from reflective surfaces (Ng and Falk, 2006). This effect led to the use of metallic reflective surfaces, straw mulches or kaolin particle films. Landing can be prevented by the use of physical barriers. Insect-proof nets greatly reduced virus incidence and the need for insecticide applications against the vectors. Camouflaging nets greatly reduce insect landing and also virus infection (Raviv and Antignus, 2008).

1.2 Problem Statement and Justification

One of the main constraints to rice production in Tanzania as elsewhere in Sub-Saharan countries is the increasing incidence and severity of RYMV (Luzi-Kihupi *et al.*, 2000). Although there have been much efforts to identify and develop RYMV resistant rice varieties, the role of vectors in the epidemiology of RYMV has not given due attention to the extent that vectors continue to be a hindrance as their control is uncertain. Over twelve RYMV insect vectors including beetles and grasshoppers have been reported from different countries in Africa. Among these, eight are found in East Africa (Nwilene *et al.*, 2008). These are *Trichispa sericea, Chaetocnema pulla* Chapius, *Dactylispa bayoni* Gest (Hispinae), *Dactylispa viricyanea* Kraatz (Hispinae), *Dactylispa gestroi* Chapius (Hispinae), *Oxya* spp, *Sesselia pussilla* (Galerucinae) and *Conocephalus merumontanus* Sjostedt *(*Nwilene *et al.,* 2008*).*

Yield losses due to RYMV have been estimated at 58-100% in West African countries of Mali and Niger (WARDA, 2000). A better understanding of RYMV requires information

on the insects that serve as vectors and alternative hosts that act as reservoirs (Matsuura and Hoshino, 2009). One important aspect towards understanding the incidence and severity of RYMV disease is the study of its vectors population abundance on hosts. Banwo *et al.* (2004) reported that severe RYMV infections in the rice at Sakassou in Ivory Coast were associated with high *Trichispa sericea* populations. In Tanzania, the relationship between RYMV vectors population and the severity and incidence of RYMV disease has never been established hence the need for this study. Several other means of RYMV transmission may contribute to the severity and incidence of the disease. The ability of the insect vectors to move long distances probably plays more significant role in the epidemiology of the virus than the other means of transmission because this may lead to carrier of new viral strains which are more virulent than the former.

1.3 Objectives

1.3.1 Overall objective

To assess the contribution of insect vectors of RYMV on the incidence and severity of RYMV disease with a view to develop strategies for managing the disease.

1.3.2 Specific objectives

i. To identify existing insect vectors of RYMV in Kilombero;
ii. To determine spatial and temporal abundance of insect vectors of RYMV in farmers' fields in Kilombero;
iii. To assess incidence and severity of RYMV in farmers' fields in Kilombero and
iv. To examine the ability of existing vectors to transmit RYMV on a susceptible variety.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 RYMV Transmission by Insect vectors

Rice yellow mottle virus (RYMV) is transmitted in a non-persistent manner by several species of insect vectors. The insect species feed on an infected plant, collect the virus particles and pass them on to the next plant that they feed on (Ali, 2001; Gal-on, 2007; Sere *et al*., 2008). The virus does not undergo any changes within the insect itself, but simply uses it as a vehicle hence the non-persistent mode of transmission (Ali, 2001; Banwo *et al.*, 2001; Maris *et al*., 2007). Insects can infest rice at any growth stages and feed on all parts of the plant. RYMV is transmitted by insects with biting and chewing mouthparts. It is most efficiently transmitted by Chrysomelid beetles and grasshoppers in a semi-persistent manner (Bakker, 1974; Banwo *et al*., 2000).

2.2 Spatial and Temporal Abundance of RYMV Insect Vectors

Banwo *et al*. (2001) reported that the wide distribution and abundance of *Chaetocnema* sp in RYMV endemic areas in Ivory Coast RYMV indicates that the species could be the most important vectors responsible for new infections of RYMV in these areas. Information on RYMV prevalent areas and identity of its insect vectors exist in the literature (Banwo *et al*., 2001; Ali, 2001). However, information on special and temporal distribution in the field and crop growth stages at which the vectors population density is high is sketchy. Several insect species with chewing mouthparts, particularly Chrysomelid beetles, can transmit RYMV to rice crop from wild hosts and weeds (Trao *et al*., 2006). Different host plant growth stages may influence the abundance of the RYMV vectors. Sere *et al*. (2008) reported that the identity of RYMV host species and vector population in relation to the availability of susceptible hosts are key determinants of the disease prevalence in the host community.

2.3 Host Range

According to Awoderu (2001), the host range of RYMV is narrow and is mainly restricted to the grass family, which includes both the wild and cultivated rice tribes and grass weeds. The grass weeds are *Echnochloa crusigalis, Echnochloa colona, Eleusine indica, Digitaria* spp, *Imperata cylindrical* and *Cynodon dactylon* while the wild rice tribes include *Oryza longistaminata, Oryza barthi, Oryza punctata* and *Oryza rufipogon*. Both the grassy weeds and wild rice tribes serve as alternate hosts or inoculum reservoirs for the virus (Banwo *et al.,* 2004; Traore and Traore, 2000).

2.4 Transmission of RYMV

Transmission is an important step in the biological cycle of viruses because it ensures their maintenance and survival (Jeger *et al.,* 2009; Fereres and Moreno, 2009). Most plant viruses are transmitted by vectors from one host to another, although they are efficiently disseminated by human activities such as vegetative plant propagation, grafting, global exchange of infected material, changes in cropping systems, and the introduction of novel crops in existing or new agricultural areas (Uzest *et al*., 2007). Vector-virus transmission consists of several successive steps: acquisition of virions from an infected source, stable retention of acquired virus particles at specific sites through binding of virions to ligands, release of virions from the retention sites upon salivation or regurgitation and delivery of virions to a site of infection in a viable plant cell (Gergerich, 2001; Chen and Gibetson, 2008). Each step of this sequence is needed for transmission to be successful (Andret-Link and Fuchs, 2006). The two types of RYMV's transmission now clearly established are: insect-borne transmission and transmission by artificial mechanical inoculation.

2.4.1 Insect-borne transmission

Banwo *et al*. (2001) has reported two Chrysomelid beetles namely *Chaetocnema* sp and *Dactylispa* sp which are capable of transmitting the RYMV in Tanzania. Several other leaf beetle species with the potential to transmit the virus namely *Sesselia pussilla* (Galerucinae), *Chaetocnema pulla* Chapius (*Halticinae*) and *C. dicladispa* (*Chrysispa*) Kraatz (*Hispinae*) has been reported by Bakker (1974) in Africa. Grasshopper species *Dactylispa bayoni* Gest (*Hispinae*), *Trichispa sericea* Guerin (*Hispinae*), *Oxya hyla*, *Conocephalus* sp, *Zonocerus variegatus*, *Euscyrtus* sp. and *Parattetix* sp. and leaf bugs *Cofana spectra*, *Cofana unimaculata*, *Locris rurba* were reported by Nwilene *et al.* (2009) as vectors of RYMV in Madagascar. However, the role of these vectors in the epidemiology of rice yellow mottle disease in Kilombero District has never been determined.

2.4.2 Transmission specificity of plant viruses by vectors

The transmission of a virus by a vector is often characterized by some degree of specificity. Transmission specificity can be broad or narrow but it is a prominent feature for numerous viruses and vectors (Ng and Perry, 2008). Specificity of transmission is defined as the specific relationship between a plant virus and one or a few vector species but not others (Andret-Link and Fuchs, 2006). For instance, a virus transmitted by aphids is not transmitted by nematodes or by any other vectors. A virus transmitted by leafhoppers is not transmitted by beetles. An extreme case of transmission specificity is exclusivity, when a vector transmits one virus or one serologically distinct virus strain and this virus or virus strain has a single vector (Kanani *et al.*, 2006; Hodge and Powell, 2008). As examples of the different degrees of specificity is that of grapevine fern leaf virus (GFLV) which is naturally transmitted by a single nematode species, *Xiphinema index* (Andret-Link *et al.*, 2009), while some polyviruses are transmitted by more than 30 aphid

species (Jeger *et al.*, 2009). Also, the whitefly *Bemisia tabaci* transmits numerous viruses from various genera and families while *Chaetocnema* spp transmits only RYMV. In contrast, only some viruses are transmitted by more than one vector. For instance closteroviruses, which are transmitted by aphids, mealy bugs or whiteflies (Moury *et al*., 2007). The specificity of transmission is explained by several characteristics including a recognition event between the virion, or a viral protein motif and a site of retention in the vector (Brown and Weischer, 1998).

2.4.3 Diversity of plant virus vectors

Vectors of plant viruses are taxonomically very diverse and can be found among arthropods, nematodes, fungi, and plasmodiophorids (Froissart *et al.*, 2005; Hull, 2008). Arthropod vectors that transmit most plant viruses are aphids, whiteflies, leafhoppers, thrips, beetles, mealy bugs, mirids, and mites (Spence, 2008). RYMV so far is transmitted by beetles (Coleoptera), sucking bugs (Homoptera) and grasshoppers (Orthoptera). About 55% of the vector transmitted virus species are transmitted by these three groups of insects (Andret-Link and Fuchs, 2006).

2.4.4 Modes of virus transmission

Different modes of virus transmission have been characterized depending on the retention time, sites of retention and internalization of virions by vectors. Non persistent viruses are retained by their vectors for less than few hours where as semi persistent viruses are retained for days, weeks or even years (Uzest *et al.*, 2007). Viruses in these two categories are acquired from infected plants and inoculated within seconds or minutes to recipient plants (Seddas and Boissinot, 2006). In addition they do not require a latent period, that is time interval between acquisition and transmission and do not replicate in the vector (Andret-Link and Fuchis, 2006). Non persistent and semi persistent viruses are

specifically associated with the epicuticle that lines the stylets (mouthparts) or the foreguts of their arthropod vectors respectively or the cuticle lining of the feeding apparatus of their nematode vectors. Since the cuticle including the lining of the mouth parts and fore gut is shed during moulting, acquired viruses are lost at each moult (Froissart *et al*., 2010). Collectively the non persistent and semi persistent viruses are referred to as non circulative because they are not internalized by vectors. In other words, they do not enter the haemocoel (vector body cavity) or cross any vector cell membrane (Gray and Banerjee, 2009).

Persistent viruses, once acquired from infected plants are associated with the vector for the remainder of their life time. They require long acquisition times (hours to days) and long latent periods (one day to several weeks). Successful transmission of persistent viruses requires an internalization of the ingested viruses that are actively transported across several cell membranes. Thus they are found in the haemocoel of vectors and retained by vectors after moulting. Ultimately they must associate with the vector salivary system to be transmitted into a new host. Persistent viruses are referred to as circulative. They can be further divided into propagative that is viruses that replicate in their arthropod vectors in addition to their plant hosts and non propagative viruses that is viruses that replicate only in their plant hosts but not in their vectors (Gray and Banerjee, 2009). A single mode of transmission is characteristic of most viruses (Bault *et al.*, 2010). Features of the different modes of virus transmission are important for transmission specificity (Raccah and Fereres, 2009). RYMV are transmitted in semi persistent manner by their vectors because they are not internalized by their vectors (Sere *et al.,* 2008).

2.4.5 Other means of RYMV transmission

Apart from insect vectors transmission, RYMVD is also transmitted by several other means. Abiotic transmission of RYMV was strongly suspected but is yet to be demonstrated. According to Reckhaus and Andriamasintseheno (1995) transplanting rice into a soil containing cow dung and poorly decomposed crop residues could be responsible for the mechanical transmission of RYMV. Similar types of transmission could be observed when rice is transplanted into a soil on which infected rice re-growths and roots that have been manipulated during ploughing operations (Sy, 1994; Abo, 1998). RYMVD might also be mechanically transmitted by contact of the gutation liquid and irrigation water with rice crop (Bakker, 1974).

2.4.6 Mechanical transmission of RYMV

Apart from insect vectors transmission, RYMV is highly transmitted mechanically. Reckhaus and Andriamasintseheno (1995) reported the possibility of RYMV transmission by man during cropping operations such as transplanting, fertilizer application, irrigation and harvesting. The virus can also move between healthy and diseased plants under the effect of wind (Sarra *et al*., 2004) or by animals such as cow, donkey and grass rats (WARDA, 1994); Sarra, 2003). Infection of rice farms from nurseries infected through contact between diseased and healthy plantlets or between contaminated hands and rice plants was suspected as far back as 1974 by Bakker, but he was not able to demonstrate it.

2.5 Identification of RYMV Vectors

Fourteen insect species which include coleopterans and orthopterans have been reported as vectors of RYMV in Africa (Abo *et al*., 2001; Banwo *et al*., 2001). Insect vectors capable of transmitting RYMV from wild rice (diseased *Oryza longisteiminata*) to other alternative host plants and vice versa have been identified. Nwilene *et al*. (2009) reported

RYMV infection in insects found in wild hosts under natural conditions. The insects were the leaf feeding beetles (*Chaetocnema* spp*, Dactylispa* spp*, Chelomenes linata, Trichispa sericea* and *Snootriba similis*), the leaf feeding grasshoppers (*Oxya hyla, Conocephalus* spp*, Zonocerus variegatus, Euscyrtus* spp. and *Parattetix* spp.) and the sucking bugs *(Cofana spectra, Sessilia pusilla, Cofana unimaculata, Locris rurba*). These insects play important role in transmitting the virus from rice to the alternative host plants and from alternative hosts to the rice crop (Bakker, 1970; Abo *et al.*, 2000b). Of the fourteen RYMV insect vectors only two insect species (*Chaetocnema* spp and *Dactylispa* spp) have been reported in Tanzania (Banwo *et al.*, 2001). Also the importance of grasshoppers in the transmission of RYMV in the field has not been ascertained (Bakker, 1974). Grasshoppers are generally thought to be of secondary importance because of their feeding behavior and the type of feeding damage they cause to rice plants (Woin *et al.* (2007).

Table 1: Insect vectors of RYMV reported in Africa

Country	Order: Family	Species
Ivory Coast	Coleoptera: Chrysomelidae	*Chaetocnema* spp
	Coleoptera: Chrysomelidae	*Trichispa sericea*
	Coleoptera: Coccinelidae	*Epilachna similis*
	Orthoptera: Tettigonidae	*Conocephalus longipennis*
	Orthoptera: Acrididae	*Zonocerus variegatus*
Kenya	Coleoptera: Chrysomelidae	*Chaetocnema* spp
	Coleoptera: Chrysomelidae	*Dactylispa bayoni*
	Coleoptera: Chrysomelidae	*Dicladispa viridicynea*
	Coleoptera: Chrysomelidae	*Sessilia pusilla*
	Coleoptera: Chrysomelidae	*Trichispa sericea*
	Orthoptera: Tettigonidae	*Conocephalus merumontanus*
Madagascar	Coleoptera: Chrysomelidae	*Chaetocnema* spp
	Coleoptera: Chrysomelidae	*Dicladispa gestroi*
	Orthoptera: Acrididae	*Oxya* spp
	Coleoptera: Chrysomelidae	*Chaetocnema* spp
Niger	Coleoptera: Chrysomelidae	*Aulocophora africana*
	Coleoptera: Chrysomelidae	*Trichispa sericea*
	Coleoptera: Chrysomelidae	*Snootriba similis*
	Orthoptera: Acrididae	*Euscyrtus* spp
Nigeria	Coleoptera: Chrysomelidae	*Aulocophora africana*
	Coleoptera: Chrysomelidae	*Chaetocnema* spp
	Coleoptera: Chrysomelidae	*Chelomenes linata*
Tanzania	Coleoptera: Chrysomelidae	*Chaetocnema* spp
	Coleoptera: Chrysomelidae	*Dactylispa* spp

Sources: Abo *et al.* (2001); Banwo *et al.* (2001).

2.6 Economic Importance of RYMV in Tanzania

Rice yellow mottle virus poses a major threat to food security in Tanzania where about 60% of the population depends on rice as a staple food or as a source of income (WARDA, 2000). This problem is complicated by the lack of effective control strategies

that could help limit spread of the virus. Tanzania, one of producers and consumers of rice in sub-Saharan Africa, is severely affected by RYMV (Banwo *et al*., 2004).

2.7 Incidence and Severity of RYMV

High virus incidence has been observed in nearly all rice producing areas, but the major rice producing regions (Morogoro, Mbeya, Shinyanga and Mwanza) are the most seriously affected (Yamamoto *et al*., 1995). Abubakar *et al.* (2006) revealed an exceptionally high diversity of RYMV in Tanzania. The Eastern Arc Mountain biodiversity hot spot harbours most of the RYMV strains found in East African, including the most variable strain, S6 (Fargette and Konaté, 2004). Subsequently, it has been postulated that this area is the centre of origin of RYMV in Africa. From the Eastern Arc Mountains, the virus could have dispersed and differentiated gradually to Central and West Africa along an East to West transect (Traoré *et al*., 2006). The information on the number of isolates and varietal reactions to RYMV already exist but field incidence and severity of the disease is not well documented (Abubakar *et al.,* 2006).

2.8 Distribution of RYMV

There are seven strains of RYMV which have been identified in Africa. However only three (S4, S5 and S6) are found in Tanzania (Kanyeka *et al*., 2007). Assessment of the distribution of the three RYMV strains revealed that strain S4 occurs predominantly in Kyela district and in the three districts of Mvomero, Kilombero and Ulanga in Morogoro region. In contrast, strain S5 is restricted to a few sites only in Kilombero district while strain S6 is widely spread in East Africa and occurs predominantly in all the three districts of Morogoro and in Same District, Kilimanjaro (Ali, 2001; Hull and Fargette, 2005). Identification and distribution of RYMV strains in rice growing areas in Tanzania is known but little information exists of the nature of distribution of the disease in the fields (Kanyeka *et al*., 2007).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Description of the Study Area

The study was conducted in Kilombero Valley of Kilombero District, Morogoro, from September 2010 to May 2011. Kilombero District is located in the lowlands of the Eastern Arc Mountains, approximately 150 km South of Morogoro town at Longitude: 37° 07' 33.09''E and Latitude: 8° 04' 0 3.76''S. Three sites that were more or less100 km apart was sampled for assessing RYMV incidence, severity and population density of RYMV insect vectors. The sites chosen were (1) Mngeta: 8°21'47.19''S, 36°24'56''E and 832 m a.s.l; Ifakara: 10°50'12''N, 14°56'37''E and 305 m a.s.l; and Mang'ula: 7°46'17''S, 36°31'52''E and 602 m a.s.l. From each site, five experimental fields sized 0.25 ha each and l.1 km apart was selected for the study.

3.2 Field Trials

Three commonly grown rice varieties namely Kalamata, Supa and Saro-5 were selected in the three divisions (sites) of Mngeta, Ifakara and Mang'ula for the study respectively. From each site, a total of five fields each grown with the same rice variety were randomly selected for the trial. A split plot experimental design was adopted for assessing both RYMVD incidence and severity and population density of RYMV insect vectors. Variables recorded from each rice field were: RYMV insect vectors population and incidence and severity of RYMVD. Disease severity was recorded according to IRRI severity scale (IRRI, 1988, 1996) while disease incidence was recorded as described by Nwilene *et al*. (2008).

3.3 Identification of RYMV Insect Vectors Present in the Study Area

The experimental fields were divided into four equal parts. A four squared metre quadrant (4 m^2) was used as sampling unit for collecting RYMV insect vectors from each of the four field parts. Sampling was done once at each crop growth stages (i.e. seedling stage, vegetative stage, reproduction stage and ripening stage). Rice yellow mottle virus vectors population were sampled using sweep-net in 4 m^2 quadrat (Banwo *et al*.,2001). Five random sweeps were made per sampling unit. The numbers of different insect species collected were recorded in a specially designed record sheet (Appendix 5). Representative specimens of each vector species collected during the sampling including orthopterans and coleopterans were sent to Kilombero Agricultural Training and Research Institute (KATRIN) Entomology Laboratory for identification and/or confirmation of their identity. The insects were placed in a plastic bottle labelled with their name, date and location collected and name of collector and then refrigerated at 4°C. Sorting was carried out in the laboratory under stereoscopic binocular microscope, and then transferred into 80% alcohol pending identification.

3.4 Spatial and Temporal Abundance of RYMV Insect Vectors

Population of RYMV insect vectors were determined in each of the five rice fields from each site and at each crop growth stages during the morning hours (from 8:00-10:00 A.M), when most of vector species are assumed to land on the crop. The targeted insects during each sampling were based on already known RYMV vectors in Africa (Table 1). In each field, sampling was done shortly before planting (before land clearing), four weeks after sowing (seedling stage), and eight weeks after sowing (vegetative stage), during panicle initiation and differentiation (reproduction stage) and at the ripening stage. Each field was divided into three equal parts i.e. two border parts and middle parts in which the sampling were made. Insect sampling was done as described in section 3.3 above. One 4 m^2

quadrant was set as sampling unit in every field part. The number of different insect species collected was recorded in a designed record sheet (Appendix 6). The representative samples of insect collected were kept in screw-caped bottles with perforated lids and transported to the Entomology Laboratory at KATRIN for further analysis.

3.5 Incidences and Severity of RYMVD

The incidence and severity of RYMVD were assessed at four different rice growth stages (viz., seedling, vegetative, reproduction and ripening) in each field from each site. Fields were divided into four equal parts (two borders and middle parts). Quadrant of 4 m^2 was used as sampling unit in assessment of RYMVD indices in each field part. Disease incidence was determined using the formula described by Nwilene *et al*. (2008) as follows:

$$\text{Disease incidence (\%)} = \frac{\text{Number of plant hills with RYMV symptoms}}{\text{Total number of hills}} \times 100 \quad \text{.....(1)}$$

Disease severity was determined using IRRI Standard Evaluation System (SES) 1-9 scale (Table 2) as described by Kanyeka *et al*. (2007), where 1= No disease, 3 < 5% severity, 5= 6-25% severity, 7= 25-75% severity and 9 > 75% severity. Data collection sheet is presented (Appendix 7).

Table 2: Description of RYMVD severity assessment scale used in the study

Severity scale	Description/Symptoms
1	No symptom
3	Leaves green but with spare dots or streak and less than 5% of height reduction.
5	Leaves green or pale green with mottling, 6 to 25% of height reduction and flowering slightly delayed.
7	Leaves pale yellow or yellow, 26 to75% of height reduction and flowering is delayed
9	Leaves turn yellow or orange, more than 75% of height reduction, and flowering or some plants dead.

Source: IRRI (1996).

3.6 Transmission Studies

Ten adult insects of each of the two vectors species *O. hyla* and *Chaetocnema* spp were collected for virus-vector transmission studies. Insects of each vector species were reared in five plastic containers (four vector species per container) containing three rice seedlings closed by perforated lid with a closable opening. All twenty insect species were starved for 24 hours prior to acquisition access feeding (AAF). The starved insects were thereafter allowed to feed on RYMV-infected 40 days old potted rice plants of the variety Saro rice plants inoculated with RYMV three weeks earlier for 24 hours in a cage (Plate1) to acquire inoculum (AAF). Four potted healthy Saro-5 rice seedlings previously established on sterile soils were used for transmission test. The test plants were raised in 12x12x13 cm plastic pots (i.e. four plants per pot) filled with 4kg heat sterilized soil (Plate 2) ready for inoculation access feeding (IAF). Three insects of the same species were transferred from RYMV affected plants to each pot containing heath rice seedlings of 14 days old for IAF.

A mouth operated aspirator, hand picking and camel hairbrush was used to collect and transfer the insects from plant to plant and from cage to plant as used by Banwo *et al*. (2001). Only adult insects were used in the transmission tests since they are responsible for the dissemination of vectored viruses under most field conditions (Andret-Link and Fuchs, 2006). An enclosed net measuring 30 cm x 60 cm x 30 cm (Plate 3) was used to retain viruliferous insects on test plants for 24 hours, 48 hours and 72 hours for each vector species tested. Timing for IAF to 14 days old plants was based on Bakker (1974) findings that rice plants are more susceptible to RYMVD at seedling stage. Test plants were kept in screen house for three weeks to allow symptom development and subsequent observation. Plants that developed RYMV symptoms were scored with a positive sign (+) and those on which RYMV symptoms were not exhibited were scored with a negative sign (-). The RYMVD symptom assessments were repeated three times, at three, six and nine weeks after inoculation.

Plate 1: Acquisition access feeding

Plate 2: Fourteen days old disease free potted rice seedlings

Plate 3: Insect inoculation access feeding

3.7 Data Analysis

Data for insect counts were log-transformed (log x+1) whereas that of disease incidence were arcsine-transformed prior to statistical analyses to assume the normal distribution of the data using the GenStat 13th Edition statistical software. Analysis of variance (ANOVA)

was used to determine whether there was significant difference among the disease incidences, severity and insect population density at different plant growth stages. Mean separation test was done at 0.05 confidence interval. Pearson's linear correlation was carried out to establish the relationship between population density of insect vectors and RYMVD incidence and severity using the GenStat 13th Edition statistical software. Data were converted back to the original value after analysis as described by Pitocchel (2001).

CHAPTER FOUR

4.0 RESULTS

4.1 RYMV Insect Vectors Present in the Study Area

By using sweep net on quadrant and in situ counting, many insects were collected. After carefully sorting based on already known RYMV vectors two insect species (Oxya hyla (Plate 4) and Chaetocnema spp (Plate 5) were found to exist in the study area. Rice yellow mottle virus insect vector species, *Chaetocnema* spp and *O. hyla* are wide spread and most abundant in all the sampled rice fields. In each field, population density of these RYMV vectors was higher at the borders (field margin) than at the middle parts of the fields throughout the crop growth stages (Fig. 1). The population of *Chaetocnema* spp was higher than that of *O. hyla* in all parts of the field (Table 3). Analysis of variance results (Appendix 1 and 2) and mean separation tests (Tables 4) indicates significant variations between RYMV insect vector counts in parts of the fields sampled. A slight variation was recorded in *O. hyla* whereby the number of insects at border 2 did not differ significantly with that of the middle part of the field.

Plate 4: *Oxya hyla* sampled in one of the rice field at Mngeta

Plate 5: *Chaetocnema* spp sampled in one of the rice field at Mang'ula

4.2 Spatial and Temporal Abundance of RYMV Insect Vectors

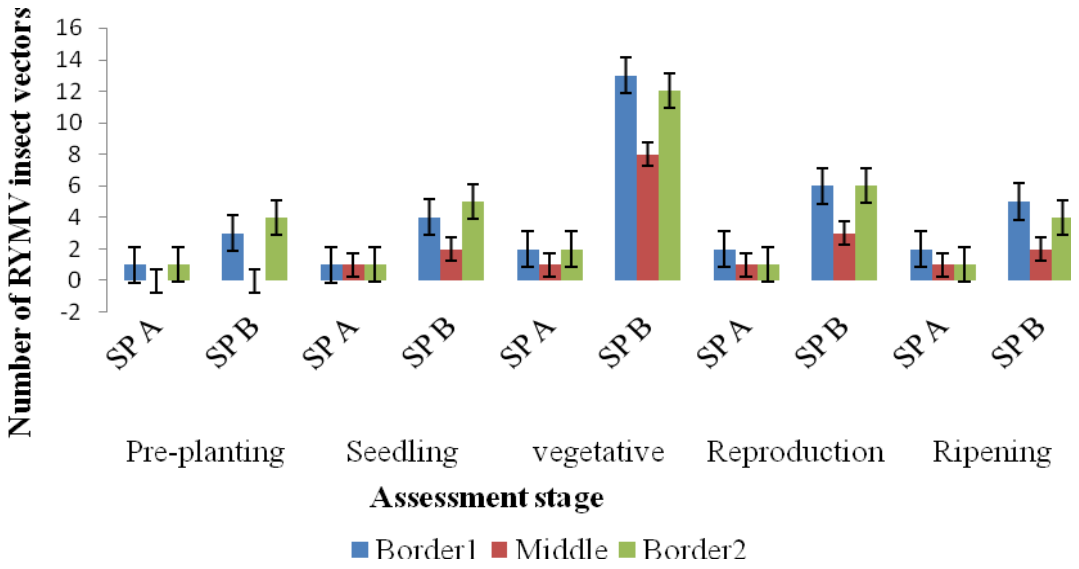

Figure1: RYMV insect vectors abundance in three rice field parts at five different crop assessment stages. SP A, indicates *Oxya hyla* and SP B, indicates *Chaetocnema* spp. Error bars were established based on the computed standard error for each of the parameter.

Assessment with respect to plant growth stages revealed that the number of *Chaetocnema* spp was higher than that of *O. hyla* (Table 5). Population density of these insect vectors was lowest at pre-planting (3 insects per 4 m^2 quadrant) but increased with crop growth stage and attained the highest level (13 insects per $4m^2$ quadrant) at the vegetative stage. The population declined during the reproductive stages and further at the ripening stage with a density of 6 and 5 insects per 4 m^2 quadrant respectively. Mean separation tests (Table 6) indicate a significant variation of insect population of both vector species with respect to plant growth stages.

Table 3: Relative species abundance of RYMV vectors in field parts

Field part	Total number of vectors per 4 m^2	Proportion of O. *hyla*	Proportion of *Chaetocnema* spp
Border 1	8	2 (25.0%)	6 (75.0%)
Middle	4	1 (25.0%)	3 (75.0%)
Border 2	7	1(14.3%)	6 (87.7%)

Table 4: Spatial and temporal distribution of RYMV insect vectors in rice fields

Field Part sampled	*Oxya hyla* (Insects /4 m^2) Mean ± SE	*Chaetocnema* spp (Insects/4 m^2) Mean ± SE
Border1	2 ± 0.14a*	6 ± 0.52a*
Middle	1 ± 0.12b	3 ± 0.38b
Border2	1 ± 0.14b	6 ± 0.47a
	LSD = 0.55	LSD = 1.17

*Values followed by different letters in a column were significantly different ($P \leq 0.05$).

Table 5: Proportional of RYMV vectors abundance with respect to crop growth stages

Growth stage	Total no of vectors/4 m^2	Proportional of *O. hyla* per 4 m^2	Proportional spp per 4 m^2 of *Chaetocnema*
Pre-planting	3	1 (33.33%)	2 (66.7%)
Seedling	5	1 (20.00%)	4 (80.0%)
Vegetative	13	2 (15.38%)	11 (84.6%)
Reproduction	6	1(16.66%)	5 (83.3%)
Ripening	5	1 (20.00%)	4 (80.0%)

Table 6: Spatial and temporal distribution of RYMV vectors with respect to crop growth stages

	Oxya hyla (Insects/4 m^2)	*Chaetocnema* spp (Insects/4 m^2)
Growth stage	**Mean ± SE**	**Mean ± SE**
Pre-planting	1 ± 0.12a*	2 ± 0.35a*
Seedling	1 ± 0.13b	4 ± 0.34b
Vegetative	2 ± 0.19c	11 ± 0.65c
Reproduction	1 ± 0.18ab	5 ± 0.37b
Ripening	1 ± 0.16ab	4 ± 0.32b
	LSD = 0.52	LSD = 0.93

*Values followed by different letters in a column were significantly different ($P \leq 0.05$).

4.3 Distribution of RYMVD Affected Plants in Rice Fields

The incidences and severities of RYMV were dependent on the crop growth stage (Fig. 2 and 3). The ANOVA results (Appendix 3 and 4) and the Duncan's mean separation tests (Table 7) indicated significant influences of crop growth stage on the disease indices. RYMV damage was low at the seedling stage (0.94% incidence and severity score of 2.383) but was highest at the ripening stage (44.6% incidence and severity score of 6.5) and remained so in all the four field parts. On the other hand, no significant variations were observed in disease severities or disease incidences between the middle and the border parts of the field (Table 8).

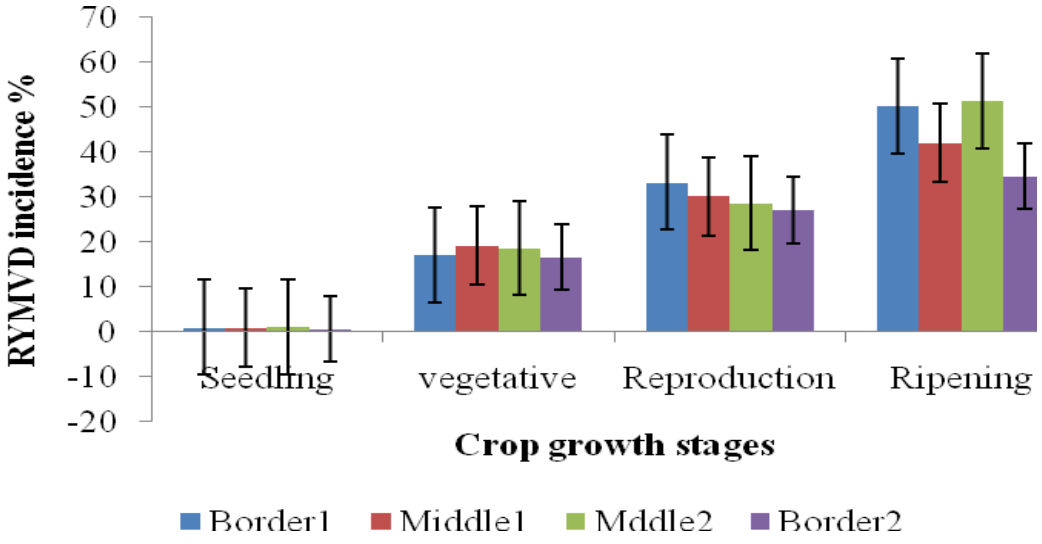

Figure 2: Distribution of RYMV incidence in rice fields at four major rice growth stages. Error bars were established based on the computed standard error for each of the parameter.

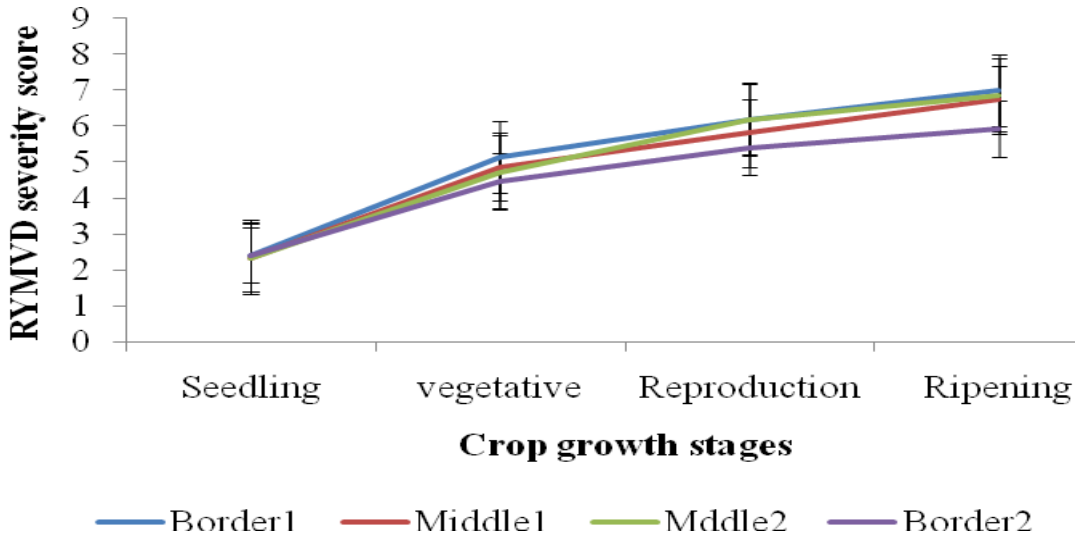

Figure 3: Disease progress curves for RYMVD in the rice fields. Error bars were established based on the computed standard error for each of the parameter.

Table 7: RYMVD incidence and severity assessed at different crop growth stages

Crop growth stage	RYMVD incidence (%) Mean ± SE	RYMVD severity Mean ± SE
Seedling	0.94 ± 1.15a*	2.38 ± 0.14a*
Vegetative	17.95 ± 1.18b	4.80 ± 0.17b
Reproduction	29.81 ± 2.13c	6.00 ± 0.27c
Ripening	44.56 ± 4.01d	6.50 ± 0.42c
	LSD = 6.03	LSD = 0.57

*Values followed by different letters in a column were significantly different (P≤ 0.05).

Table 8: RYMVD incidence and severity within assessed field part

Field part	RYMVD incidence (%) Mean ± SE	RYMVD severity Mean ± SE
Border1	19.78 ± 5.80a*	4.57 ± 0.53a*
Middle1	23.08 ± 4.90a	4.93 ± 0.52a
Middle 2	24.98 ± 5.60a	5.03 ± 0.55a
Border 2	25.42 ± 5.00a	5.15 ± 0.59a
	LSD = 8.27	LSD = 1.11

*Values followed by different letters in a column were significantly different (P≤ 0.05).

4.4 RYMVD Incidence and Severity as per Experiment Fields

The incidence and severity of RYMVD in five experimental rice fields at Mngeta, Ifakara and Mang'ula are shown in Figures 4 and 5. Generally, high incidence and severity of RYMVD was observed at Mang'ula than at Ifakara and Mngeta in all the experimental fields. Highest incidence of RYMVD was observed in field 5 at Mang'ula (47%) compared to Mngeta and Mang'ula, which were 31% and 38%, respectively. The lowest incidence (29.2%) was observed at Mngeta in field 2 followed by Mang'ula and Ifakara, whose incidences were 29.4% and 25.5% respectively. RYMD Severity was higher in Mang'ula followed by Mngeta and Ifakara. Highest RYMVD score of 6 was recorded at field five of Mang'ula against the RYMVD infected fields in Mngeta and Ifakara, both of which recorded a score of 5. The lowest RYMVD score (3.9) was recorded at Mngeta while at Mang'ula and Ifakara the RYMVD scores were 5.2 and 4.6 respectively.

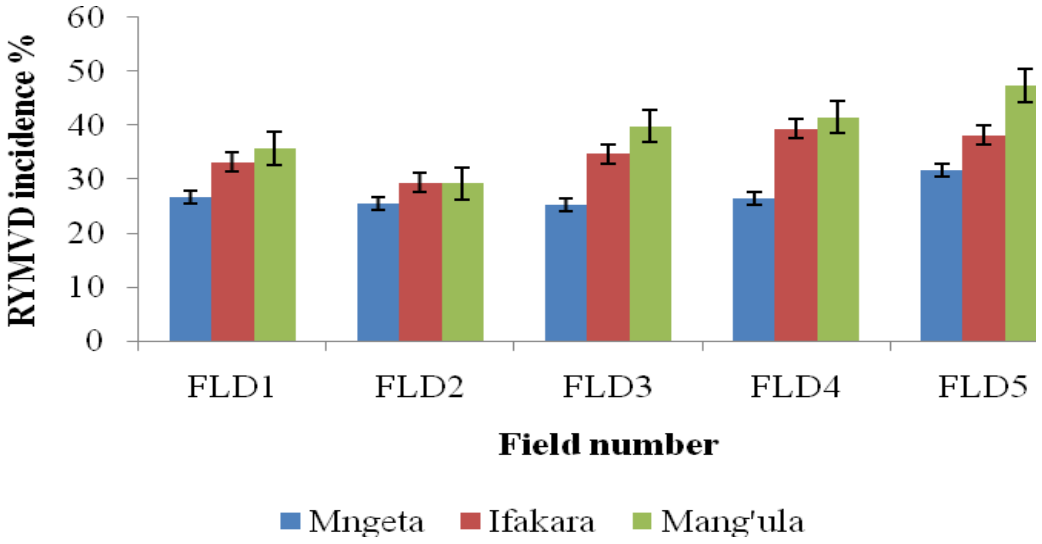

Figure 4: Field incidence of RYMVD at Mngeta, Ifakara and Mang'ula. Error bars were established based on the computed standard error for each of the parameter.

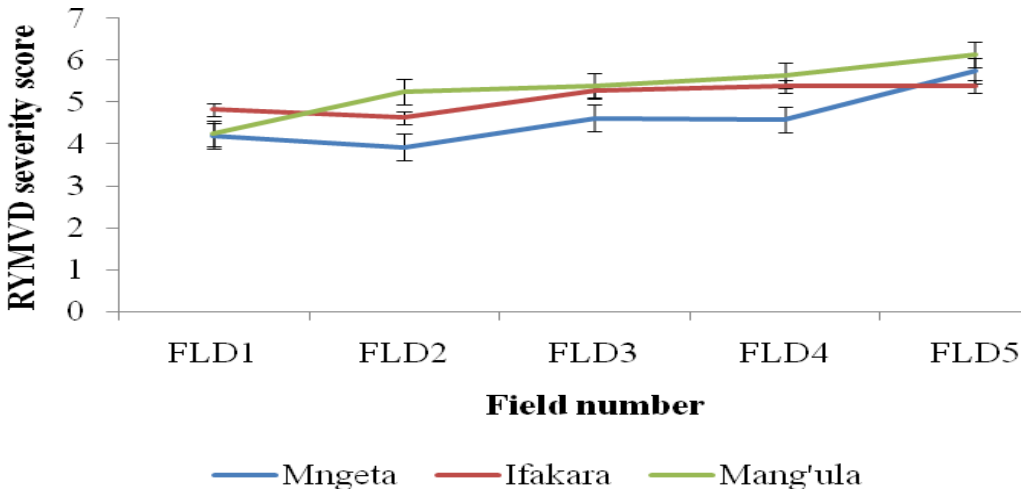

Figure 5: RYMVD progress curves in five fields at Mngeta, Ifakara and Mang'ula. Error bars were established based on the computed standard error for each of the parameter.

4.5 RYMVD Incidence and Severity Assessed per Rice Varieties

The incidences of RYMVD as assessed per rice variety in the three study locations were as shown in Figure 6. The incidence of RYMVD on the three rice varieties: Kalamata, Supa and Saro-5 increased with the crop growth stage. Saro-5 exhibited high incidence of

RYMV at all crop growth stages, followed by Supa and Kalamata in that order. The disease progress curves for the rice varieties under field condition at each crop growth stages were as presented in Figure 7. All varieties develop severe foliar RYMV symptoms under field conditions. Disease severity increased with the crop growth stage (Plates 6 - 9).

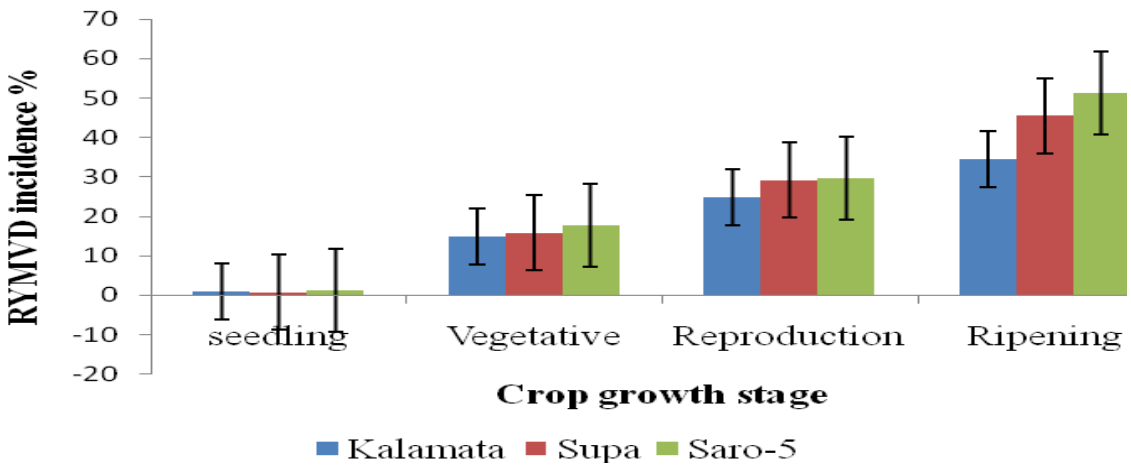

Figure 6: RYMVD incidence trend at different rice crop growth stages for three rice varieties; Kalamata, Supa and Saro-5. Error bars were established based on the computed standard error for each of the parameter.

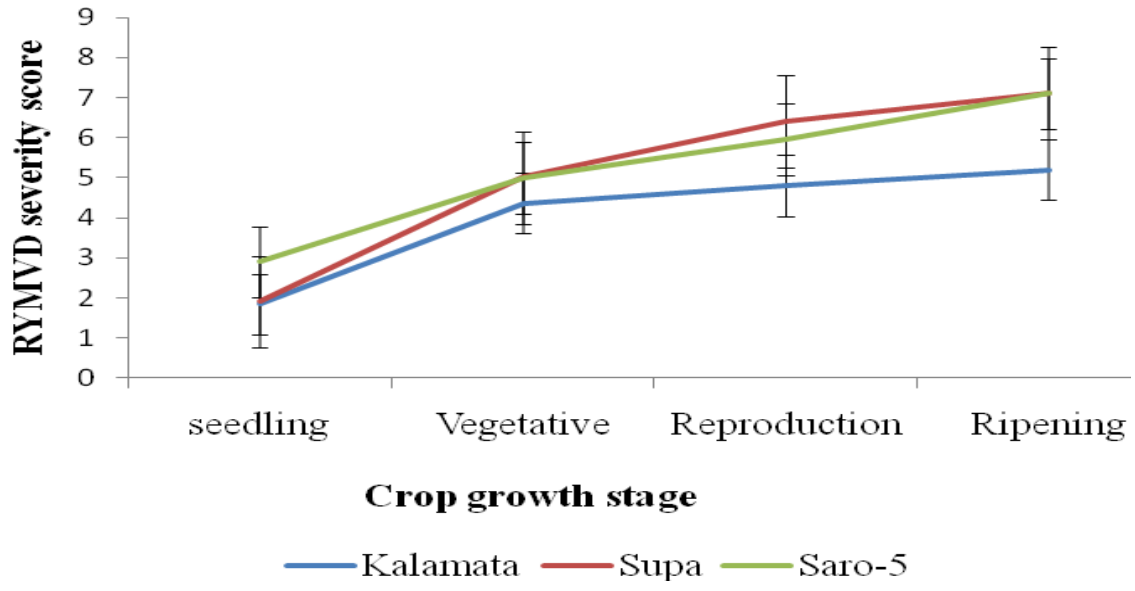

Figure 7: RYMD progress curves of three rice varieties., Kalamata, Supa and Saro-5 at different rice crop growth stages in three experimental sites. Error bars were established based on the computed standard error for each of the parameter.

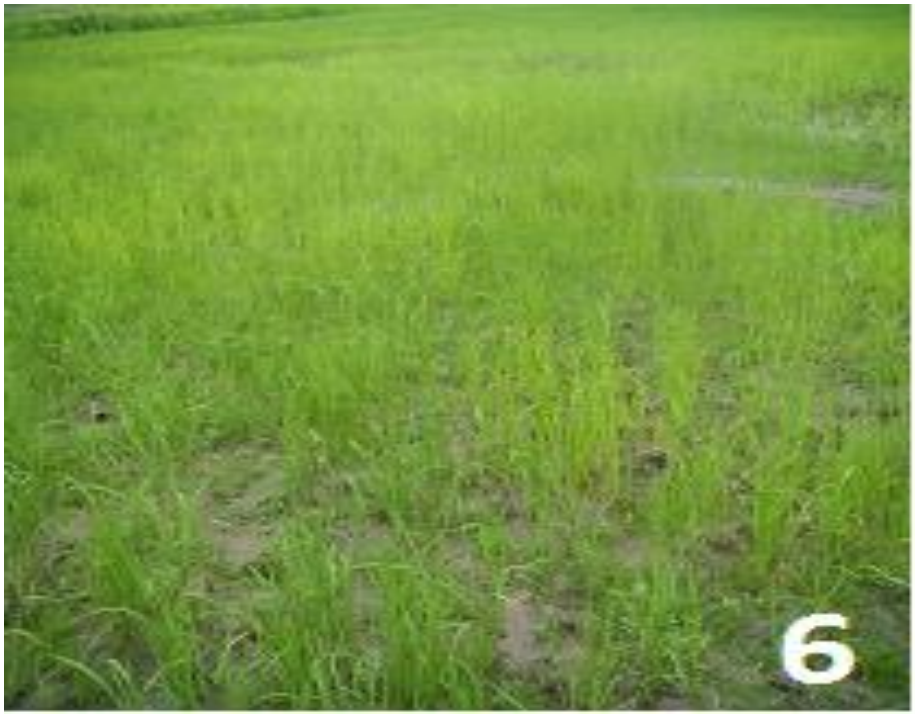

Plate 6: RYMVD severity of Saro-5 rice variety at seedling stage

Plate 7: RYMVD severity of Saro-5 rice variety at vegetative stage

Plate 8: RYMVD severity of Saro-5 rice variety at reproduction stage

Plate 9: RYMVD severity of Saro-5 rice variety at ripening stage

4.6 Population Density of RYMV Vectors per Site in Relation to RYMVD Incidence and Severity

The number of RYMV vectors in relation to RYMVD incidence and severity per sampled area in each of the three study sites were as shown in Table 9. Highest numbers of insect vectors were observed in Mang'ula followed by Mngeta and Ifakara.

4.7 Relationship between Vectors Population and RYMVD Incidence and Severity

Partial correlation analyses between the insect vectors and disease indices were as shown in Table 10. The correlation suggests positive relationship between number of RYMV insect vectors and RYMVD incidence and severities across the study sites. *Chaetocnema* spp was found to be highly correlated with RYMVD incidence (r = 0.62) and severity (r= 0.801) while *O. hyla* was less correlated with RYMVD incidence (r = 0.160) and severity (r= 0.157). On the other hand, the correlation between *Chaetocnema* spp and O. *hyla* were not significant (r = -0.039).

Table 9: Number of vectors per site in relation to RYMVD incidence and severity

Site	*Chaetocnema* spp (Insects/4 m^2) Mean± SE	*Oxya hyla* (Insects/4 m^2) Mean ± SE	RYMV incidence (%) in 4 m^2 Mean ± SE	RYMV severity in 4 m^2 Mean ± SE
Mngeta	3 ± 0.48	1 ± 0.80	32.05 ± 4.10	5.050 ± 0.44
Ifakara	5 ± 0.84	2 ± 0.33	32.79 ± 4.05	5.550 ± 0.47
Mang'ula	6 ± 0.48	2 ± 0.80	35.97 ± 4.90	6.025 ± 0.39

Table 10: Partial correlation between RYMVD incidence and severity, and mean number of *Chaetocnema* spp and *O. hyla*

	RYMVD Incidence (%)	RYMVD Severity	*Chaetocnema* spp	*O. hyla*
RYMVD incidence (%)	1.00			
RYMVD severity	0.548**	1.00		
Chaetocnema spp	0.620**	0.801**	1.00	
O. hyla	0.160*	0.157*	-0.039NS	1.00

** Highly significant, * Significant, NS none significant. Correlation coefficients *'r'* tested (P< 0.05).

4.8 Transmission Studies

Transmission studies results were as shown Tables 10 and 12. Both *Chaetocnema* spp and *O. hyla* were able to transmit RYMV from infected rice plants to health rice plants. Symptoms expression on the inoculated rice plants were similar to those observed in the RMVD-affected fields at the study sites. RYMVD symptoms were observed earlier (3 weeks after inoculation) on plants inoculated by *Chaetocnema* spp than that which were inoculated by *O. hyla*. In three weeks after inoculation, nine test plants out of twelve plants inoculated with *Chaetocnema* sp for 24, 48 and 72 hours shows RYMVD symptoms but when inoculated with *O. hyla*, only four plants out of twelve shows RYMVD symptoms (Table 11). In six weeks after inoculation all twelve plants inoculated with *Chaetocnema* spp for 24, 48 and 72 hours shows RYMVD symptoms but only 6 plants out of 12 plants shows RYMV symptoms when inoculated with *O. hyla* (Table 12). In nine weeks after inoculation, all test plants inoculated with *Chaetocnema* spp were severely affected by RYMV but only 8 plants out of 12 plants inoculated with *O. hyla* were severely affected with RYMV (Table 13).

Table 11: Transmission test outcome at three weeks after inoculation

Inoculation Access time	***Chaetocnema* spp**					***Oxya hyla***				
Hrs	P1	P2	P3	P4	AT	P1	P2	P3	P4	AT
24	+	+	-	-	2/4	-	-	-	-	0
48	-	+	+	+	3/4	+	-	-	-	1/4
72	+	+	+	+	4/4	+	+	-	+	3/4

Key: P = Plant number, AT = Average transmission, (+) = RYMV symptoms observed and (-) = No RYMV symptoms observed.

Table 12: Transmission test outcome at six weeks after inoculation

Inoculation Access time	***Chaetocnema* spp**									***Oxya hyla***
Hrs	P1	P2	P3	P4	AT	P1	P2	P3	P4	AT
24	+	+	+	+	4/4	-	-	-	-	0
48	+	+	+	+	4/4	+	+	-	+	3/4
72	+	+	+	+	4/4	+	+	-	+	3/4

Key: P = Plant number, AT = Average transmission, (+) = RYMV symptoms observed and (-) = No RYMV symptoms observed.

Table 13: Transmission test outcome at nine weeks after inoculation

Inoculation Access time	***Chaetocnema* spp**					***Oxya hyla***				
Hrs	P1	P2	P3	P4	AT	P1	P2	P3	P4	AT
24	+	+	+	+	4/4	-	-	-	-	0
48	+	+	+	+	4/4	+	+	+	+	4/4
72	+	+	+	+	4/4	+	+	+	+	4/4

Key: P = Plant number, AT = Average transmission, (+) = RYMV symptoms observed and (-) = No RYMV symptoms observed.

CHAPTER FIVE

5.0 DISCUSSION

Results from this study confirmed that insect vectors contribute to the transmission of RYMV. *Chaetocnema* spp and *O. hyla* were found to be the only vectors of RYMV in Kilombero District. Previuos studies (Banwo *et al.,* 2001) only reported the occurrence of *Dactylispa* sp and *Chaetocnema* spp as vectors of RYMV in Tanzania. Therefore, this is the first report of *O. hyla* as vector of RYMV in Tanzania. The *O. hyla* was first reported as vector of RYMV in Ivory Coast by Abo and Sy (1998) but had never been reported in Tanzania. These findings suggest that either the number of important vector species on rice might have increased over the years or the previous studies were restricted to few locations (limited coverage) which comprised of a few insect samples. Several insect species have been reported as vectors of RYMV in Ivory Coast (Nwilene *et al*., 2008), in Kenya (Bakker, 1970), Madagascar (Abo *et al*., 2000b) and Nigeria (Abo, 1998). The proportional abundance of *Chaetocnema* spp found in the study area was much higher with average proportion of 85% compared to that of O. *hyla* with average proportion of 15%. This indicates that beetles were the most abundant insect species than grasshoppers in the study area. AS such *Chaetocnema* spp might either be highly competitive or favored by existing environment when compared to *O. hyla*.

Since *Chaetocnema* spp was the most abundant and widely distributed in RYMV prevalent areas, it is therefore considered as an important vector of the disease causing virus in farmers' fields in Kilombero District. This observation also concurs with the report of Abo (1998), who associated the fast spread of RYMV in rice fields by *Chaetocnema pulla* in Cote d'Ivoire due to its agile behavior. The spatial and temporal distribution assessment of the vectors indicated that, the population density of both *O. hyla*

and *Chaetocnema* spp decreased with the distance from the borders to the middle parts of the field. Both vector species were more abundant at the borders than at the middle. This is possibly because field borders were closer to the surrounding bushy vegetations where most of the alternative host plants of the insect species are believed to survive (Nwilene *et al*., 2009). Thus, RYMV vectors survive more in alternative host plants outside the fields than within the fields. These observations suggest that there could be many alternative host plants particularly of graminaceae family in which insect vectors survive during off season or after the rice crop has been harvested. Future work should target at identifying the alternative host plants of O. *hyla* and *Chaetocnema* spp in order to widen the knowledge on alternative options available hence the increased options for the control of the vectors that would ultimately reduce incidences of RYMVD in rice fields.

The current study also established that that population density of RYMV vectors were dependent on crop growth stages. The number of vectors increased with increase in crop age. At each crop growth stage, number of *Chaetocnema* spp was higher than that of O. *hyla*. The average insect population density in all crop growth stages for *Chaetocnema* spp was 6 insects/4 m^2 and that of *O*. *hyla* was 2 insects/4 m^2 which translates to proportional of 79.0% and 21.0% respectively. Vector numbers was lowest at pre-planting (3 insects/4 m^2) and attained peaks at vegetative stage (13 insects/4 m^2) and there after started to decline during reproductive stages and further at ripening stage with 6 and 5 insects per 4 m^2 quadrant respectively. The causes of such variation may be due to the fact that insects prefer tender leaves which are always available for them during seedling and vegetative stage. During reproduction and ripening stage the crop is at its limited growth stage where no more tender leaves are produced, which cause insects to migrate to new locations in search of fresh tender leaves.

The incidence and severity of the disease under this study was observed to increase with the age of the crop. The RYMVD incidence and severity was therefore observed to be higher at reproductive and ripening stages than at the seedling and vegetative stages. This observation suggests that following infection, virus tend to multiply and translocate slowly and gradually from site of infection to uninfected cells to cover the whole plant (Hogle, 2008). Since rice crop is severely affected at reproduction and ripening stage, this affects seed formation and results into empty spikiletes and consequently reduces crop yield.

The incidence and severity of RYMVD in the study area ranged from 0.9% to 51.2% and 1 to 7 respectively. The severity levels were dependent on rice variety, rice field assessed (location) and crop growth stages. Saro-5 variety had the highest RYMVD incidence at all crop growth stages as compared to Kalamata and Supa rice variety. High RYMVD incidence was observed in all 5 experimental fields grown with Saro variety at Mang'ula than fields grown with Kalmata and India variety at Mngeta and Ifakara respectively. The reason for this difference could be due to the fact that Saro-5 variety more susceptible to RYMVD than Kalamata and Supa varieties. Kanyeka *et al*. (2007) reported Saro-5 (TXD 306) as susceptible to RYMVD hence often used as reservoir of RYMV strains for transmission studies in screen houses. On the other hand there might be higher RYMVD inoculums present at Mang'ula than Mgeta and Ifakara. High incidence and severity of the RYMVD could also be attributed to the cultivation of exotic varieties that are less adapted to the environment of Kilombero. Abo *et al*. (2000) reported that new varieties are inherently more vulnerable to pests and diseases than the traditional landraces they have replaced. Most of the rice growers at Mang'ula grow Saro variety which is exotic variety throughout the year due to availability of water for irrigation. Although this variety is highly susceptible to RYMV, they prefer it mostly because it is high yielding and it has good aroma. The availability of water which allows continued

production through irrigation ensures suitable environment for vectors and RYMV hence the continued spread of the disease and subsequent yield losses as observed. The possibility of available water for irrigation permitting continuous production of rice and consequently the incidence and severity of the RYMVD is further supported by the findings of Bakker (1970) who reported that the area originally affected with RYMV in Kenya was a part of irrigation project which had led to an increase in rice cultivation due to availability of water for substantial planting through the year.Rice yellow mottle virus was observed to spread fast from plant to plant eventually covering all neighbouring plants. Sampling results showed that RYMV-affected plants were randomly distributed in the fields. This type of distribution of RYMV-infected plants suggests that vectors might have largely contributed to the spread. Similar observations were reported by Sarra (2005) in irrigated rice fields in Niger. The study findings indicated the distribution of infection by RYMV in rice fields as random and across the same region and fields. The random distribution of RYMV infected plants within and between fields was presumably caused by insect vectors as they move from plants to plant and between fields. Thus, apart from the vector-based transmission of RYMV, there could be many other mechanisms through which the virus is spread within and between rice fields.

Although other factors such as environmental factors (Banwo *et al*., 2004), the strain of the virus (Kanyeka *et al*., 2007) and the time of infection (Traoré *et al*., 2009) might have influenced the prevalence of the disease, the trend of RYMVD incidence under this study varied from one field to the other. This observation suggests that RYMVD problems may vary from one area to the other in view of the diverse and immediate environment under which rice crop is grown (Ng *et al.*, 2006). This suggestion is further reinforced by Bakker (1974) observations that expression of the virus symptoms is strongly influenced by different field conditions, crop cultivars grown and growth stage of the crop.

The numbers of RYMV vectors observed from the study sites were more abundant at Mang'ula than Mngeta and Ifakara with an average density of 8, 7 and 4 insects per 4 m^2 respectively. The disease incidence assessment results in these three sites were also found to be higher in Mang'ula (35.97%) than that of Mngeta and Ifakara which were respectively 32.05% and 32.79%. It is also Mang'ula site which had severe RYMVD in assessed fields than the other two. Thus Mang'ula had more favorable conditions for RYMVD and the virus vectors. Although Saro-5 rice variety which is grown at Mang'ula is highly susceptible to RYMVD the prevalence of RYMV vectors might have contributed largely to the high incidence and severity of the disease that was recorded in the study sites. Wherever insect numbers were observed to be high, incidences and severity of RYMVD were also high and vice versa.

The partial correlation analysis results show that there is positive influence of insect's population density on the prevalence of RYMV disease. Positive correlation between insect vector's population and RYMVD incidence and severity was recorded. Bakker (1970) and Reckhaus and Andriamasintseheno (1997) reports the same observation in which they established positive relationship between RYMV vector number and RYMV infections but it differs from Abo *et al.* (2001) report made in Nigeria who found no link that exists between population densities of the insect vectors and RYMV disease incidences in rice fields. The positive correlation between RYMV vectors and RYMVD in this study therefore re-affirms the potentiality of these vectors in transmission of RYMV.

The transmission test of *Chaetocnema* spp and *O. hyla* in this study revealed quick transmission of RYMV by *Chaetocnema* spp than *O. hyla*. This was observed in the transmission test experiment between two vector species where *Chaetocnema* spp were able to transmit RYMV from infected rice plants to health rice seedlings as it was retained shortly (between 24 to 72 hours) on test plants as compared to *O. hyla* which were able to

transmit RYMV when it was retained for long time (between 48-72 hours). Thus, *Chaetocnema* spp required shorter acquisition access and inoculation access period than *O. hyla* making it an efficient vector. This might be due to agile behavior of *Chaetocnema* spp as compared to O. *hyla.* The fast transmission of RYMV by *Chaetocnema* spp was also reported by Abo (1998) who tested the transmission ability of *Trichispa sericea* and *Chaectonema pulla*, and established that T. *sericea* was able to transmit RYMV when it stayed on rice crop for 8 days where as *C. pulla* were able to transmit RYMV after it stayed on the rice crop for only one day which was suggested that, because of the agile behavior of *C. pulla*, it must be responsible for fast transmission of RYMV in rice field. The poor transmission ability of *O. hyla* compared to *Chaetocnema* spp is also implicated in the report by Banwo *et al*. (2001) and Bakker, (1974) who indicated that the importance of grasshoppers in the transmission of RYMV has not been ascertained. In their studies grasshoppers were generally thought to be of secondary importance because of their feeding behavior and type of feeding damage they cause to the rice plants.

In order to minimize the incidence and severity of RYMVD in the study area, control of RYMV insect vectors such as those found in the study area is vital. It should be noted that it is difficult to eliminate viruses from infected plants directly and thus the best approach of controlling RYMV is prevention of vector perpetuation, mobility and alternative sources of inoculum. No single plant virus disease control method is effective for elimination of the RYMVD (Malstron *et al*., 2006), thus the best approach could be achieved through Integrated Pest Management (IPM) approaches such as utilization of virus free stock and seedlings, field sanitation, cultural practices and control of virus vectors.

CHAPTER SIX

6.0 CONCLUSION AND RECOMMENDATIONS

6.1 Conclusion

Rice yellow mottle virus incidence and severity is influenced by presence of insect vectors (*O. hyla* and *Chaetocnema* spp), susceptibility of the grown rice variety as well as crop growth stages within agro ecosystems of Kilombero. The population density of *Chaetocnema* sp and *O. hyla*, were variable with respect to the growth stage of the rice crop and in turn influenced RYMVD incidence. High population were recorded during the reproductive stage and gradually decreased with crop maturity. The more mature the crop was the lesser the number of the two respective vectors. Therefore, the occurrence of RYMVD in Kilombero basin is influenced by the RYMV vectors that are endemic and omnipresent wherever rice is grown. Most of the grown varieties are susceptible to RYMVD thus yield losses associated with the disease could be very high. The need for appropriate strategies to manage the disease should be over emphasized.

6.2 Recommendations

In view of the findings from the current study, the following are recommended:

i. Appropriate education should be given to farmers on RYMVD, its causal agent and spreading mechanisms to help reduce the inoculum pressure and the associated yield losses caused by the disease;

ii. Farmers should be educated on the identification and management of RYMV vectors in rice fields;

iii. Weeds within and around rice fields which act as alternative hosts to RYMV vectors during offseason should be removed timely as habitat based management option for RYMVD and RYMV vectors and

iv. Researchers should intensify their efforts to develop varieties which are resistant to RYMVD but with farmers preferable.

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APPENDICES

Appendix 1: Analysis of variance table for spatial and temporal abundance of *O. hyla*

S. V	D. F	S. S	M. S	F pr.
Replication	2	10.347	5.173	
Field no.	4	4.827	1.207	0.328
Field part sampled	2	16.187	8.093	0.072
Stage of assessment	4	41.716	10.429	<.001
Residual	28	33.11	2.619	
Field part. Growth stage	8	19.591	2.449	0.069
Field part. Field no.	8	8.84	1.06	0.42
Field no. Growth stage	16	12.596	0.787	0.724
Field part. Growth stage. Field no.	32	26.631	0.832	0.756
Residual	120	123.867	1.032	
Total	224	297.36		

Appendix 2: Analysis of variance table for spatial and temporal abundance of *Chaetocnema* spp

S. V	D. F	S. S	M. S	F pr.
Replication	2	141.36	70.68	
Field no.	4	118.329	29.582	<.001
Field part sampled	2	480.667	240.333	0.003
Stage of assessment	4	2262.018	565.504	<.001
Residual	28	122.373	10.643	0.147
Field part. Growth stage	8	54.622	6.828	0.694
Field part. Field no.	8	32.444	4.056	0.837
Field no. Growth stage	16	60.516	3.782	0.999
Field part.Growth stage.Field no.	32	71.378	2.231	
Residual	120	698.933	5.824	
Total	224	4042.64		

Appendix 3: Analysis of variance table for distribution of RYMVD incidence at different crop growth stages

S. V	D. F	S. S	M. S	F pr.
Replication	2	510.54	255.27	
Field part.	3	237.25	79.08	0.586
Residual	6	678.81	113.13	
Growth stage	3	12272.14	4090.71	<.001
Field part. Growth stage	9	397.36	44.15	0.477
Residual	24	1075.38	44.81	
Total	47	151.49		

Appendix 4: Analysis of variance table for distribution of RYMVD severity at different crop growth stages

S. V	D. F	S. S	M. S	F pr.
Replication	2	13.5117	6.7558	
Field part.	3	2.2892	0.7631	0.652
Residual	6	7.9483	1.3247	
Growth stage	3	121.3425	40.4475	<.001
Field part. Growth stage	9	3.6542	0.406	0.541
Residual	24	10.8333		
Total	47	159.5792		

Appendix 5: Insect collection recording sheet 1

Division:
Owner:
Field no:
Field size:
Date:

Assessment stage	**Quadrant no.**	**Collected insects**	**No. of insects known of RYMV**	**Name and number of spp**	
				Name	Number
Seedling stage	1				
	2				
	3				
	4				
Subtotal					
Vegetative stage	1				
	2				
	3				
	4				
Subtotal					
	1				
	2				
	3				
	4				
Sub total					
Reproduction	1				
	2				
	3				
	4				
Sub total					
	1				
	2				
Ripening stage	3				
	4				
Sub total					
	1				
	2				
	3				
	4				
Sub total					

Appendix 6: Insect collection recording sheet 2

Division:
Owner:
Field no:
Field size:
Date:

Assessment stage	**Field Part assessed**	**Insect spp**	**Stage of insect**	**Number**	**Host plant**	**Part of plant colonized**
Pre-planting	Border1					
	Middle					
	Border2					
Subtotal						
seedling stage	Border1					
	Middle					
	Border2					
subtotal						
vegetative stage	Border1					
	Middle					
	Border2					
Sub total						
Reproduction stage	Border1					
	Middle					
	Border2					
Subtotal						
Ripening stage	Border1					
	Middle					
	Border2					
Sub total						

Appendix 7: Disease incidence and severity scoring sheet

Field no:
Owner:
Source of water (Irrigation/rainfall):
Method of planting:
Rice variety:

Qrt no.	**No. of plants**	**S.S**			**V.S**			**Re. S**			**Ri.S**		
		plants with virus	Inc%	Sc	plants with virus	Inc%	Sc	plants with virus	Inc%	Sc	plants with virus	Inc%	Sc

The terms used as short hand in table infers to; ‘S.S’ for seedling stage, ‘V.S’ for vegetative stage, ‘Re.S’ for reproduction stage, ‘Ri.S’ for ripening stage, ‘Inc%’ for RYMVD incidence, and ‘Sc’ for RYMVD severity score.