Long-term survival of blast pathogen in infected rice residues as major source of primary inoculum in high altitude upland ecology

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Magnaporthe oryzae is the fungal plant pathogen that causes rice blast. The sources of primary inoculum and overwintering mode of the fungus remain largely unknown. The effect of rice residues on the onset of blast epidemics and the potential for survival of \textit{M. oryzae} in the residues were studied in upland conditions in Madagascar. Blast disease was observed in a 3-year field experiment in three treatments: with either infected or uninfected rice residues on the soil surface, or without rice residues. Leaf blast incidence was significantly higher in the treatment with infected rice residues than in the two other treatments at the early stages of the epidemic. In a second set of trials, the survival of \textit{M. oryzae} on rice residues was monitored. Infected rice stems were placed by lots in three places: on the mulch of rice residues, under the mulch, and buried at a depth of 10 cm in the soil. Each month, samples were taken from the field and tested for sporulation. The survival of the blast fungus decreased rapidly on the stems buried in the soil but remained high for the other conditions. Sporulation of the fungus was observed on stems left on the mulch for up to 18 months. It is concluded that under field conditions, the presence of infected rice residues could initiate an epidemic of blast. The results of this study may help in designing effective management strategies for rice residues infected by \textit{M. oryzae}.

\textbf{Keywords: Magnaporthe oryzae, primary inoculum, rice blast, rice straws, survival}

\textbf{Introduction}

Rice blast, caused by the fungal pathogen \textit{Magnaporthe oryzae}, is a devastating disease of rice worldwide (Pennisi, 2010) and a model for the study of plant–pathogen interactions (Valent, 1990; Dean et al., 2012). The disease is currently managed by the use of resistant cultivars (Tanweer et al., 2015; Raboin et al., 2016), appropriate cultural practices (Pooja & Katoch, 2014; Bregaglio et al., 2017) and treatment with fungicides when possible (Kunova et al., 2014). Alone, none of these methods can completely control blast disease (Guerber & TeBeest, 2006).

Blast is a polycyclic disease propagated by asexual spores (conidia) that infect the aboveground parts of the rice plant in the field. Upon artificial inoculation in controlled conditions, \textit{M. oryzae} is also able to colonize roots by forming hypophydat (Sesma & Osbourn, 2004). In the tropics, airborne conidia are presumably present throughout the year (Ou, 1985), allowing permanent epidemics without overwintering issues. This assumption is unlikely to be valid in high altitude tropical regions, like the Highlands of Madagascar (1000 to 2000 m a.s.l.), and in temperate regions where rice is absent for several months each year. According to some authors (Ou, 1985; Guerber & TeBeest, 2006), the pathogen can survive in infected seeds and residues, which are probably the main primary sources of inoculum in temperate regions. Previous studies have already reported the presence of the blast pathogen in infected rice seeds using PCR (Chadha & Gopalakrishna, 2006) and by visual inspection (Mew & Misra, 1994; Mew & Gonzales, 2002). Puri et al. (2007) found a significant correlation between the panicle infection and survival of \textit{M. oryzae} in seed, and the level of pathogen survival varied on different cultivars tested. The infection process from the contaminated germinating seed to the seedling was investigated by cytological observations by Fairev-Rampant et al. (2013). Several studies have demonstrated the potential role of infected rice seeds in outbreaks of rice blast under field conditions (Manandhar et al., 1998; Long et al., 2001; Guerber & TeBeest, 2006). These studies clearly established that \textit{M. oryzae} is a seedborne pathogen. However, to date, there has been no convincing demonstration that the pathogen can also survive in rice residues in the field and that these residues can serve as primary inoculum (review in Ou, 1985). Harman & Latin (2005) reported that \textit{M. oryzae} from ryegrass can survive on infected residues of its host plant, but that the proportion of surviving inoculum was insufficient to trigger an epidemic.

Rice residues are used in different ways in different countries. In Madagascar, especially in the central...
highlands, most of the rice residues are used to feed livestock during the dry season and, in some regions, some of the rice residues are used as bedding for livestock. After harvest, the residues are dried and stored in the upland rice fields near the farm homestead. In conservation agriculture cropping systems in the Madagascar Highlands, some of the rice residues are left in the field as mulch to ensure the environmental and agronomic sustainability of upland crops (Scopel et al., 2013). The principles of conservation agriculture cropping systems are: (i) no-till or reduced tillage; (ii) permanent soil cover through intercropping or by leaving crop residues in the field; and (iii) the use of crop rotations (FAO, 2016). Due to the permanent presence of rice residues on the soil surface in conservation agriculture cropping systems, and the drying and storage practices of rice residues in upland rice plots, a study was proposed on the role of leftover residues in the field on blast disease development in rice.

This study thus had two specific objectives: (i) to determine the role of leftover rice residues on blast disease development; and (ii) to determine how long the blast pathogen can survive on infected rice residues in the field.

Materials and methods

Two experiments were conducted in upland conditions at Andranomanalatra, 16 km north of Antsirabe in the Vakinankaratra region of the Highlands of Madagascar (19°47′S, 47°06′E). The altitude is 1645 m a.s.l. and the climate is tropical with average annual rainfall of 1360 mm. The climate is characterized by two separate seasons: a warm rainy summer from October to April, and a cool dry season from May to September. The mean temperature is 20 °C or below. Generally, the upland rice is sown in this region between mid-October and November, the reproductive phase (from booting to end of grain filling) occurs during February to March, and harvesting is in April.

Rice residue preparation and experimental design

The trials were carried out in the 2012/13, 2013/14 and 2014/15 cropping seasons (hereafter 2013, 2014 and 2015). In 2013 and 2014, two treatments were compared: either no rice residues (bare soil: BS), or infected rice residues (soil with diseased residues: SDR) left on the soil. In the last cropping season in 2015, a third treatment, uninfected rice residues left on the soil surface (soil with uninfected residues: SUR) was also included. In 2013, the infected rice residues of the susceptible cultivar Fofifa 152 (F152) were collected from a previous blast trial conducted in Andranomanalatra during 2012. Infected rice residues from trials of 2013 and 2014 were used for trials of 2014 and 2015, respectively. The uninfected rice residues used in the 2015 trial were collected from the resistant cultivar Fofifa 172 (F172) grown for the trial of 2014. Rice residues were collected at harvest time and kept in the field in straw piles until the next cropping season, and were manually spread on the ground after seedling emergence.

A randomized complete block design with four replications was used for the trials. The individual plots of 3 × 4 m (15 × 20 rows with 300 hills in total) were sown with the susceptible cultivar F152. The plots were separated by a buffer strip planted with the resistant cultivar F172 to limit the spread of the disease among the plots. Six to eight seeds were sown manually in hills spaced 20 × 20 cm apart. Organic fertilizer (cattle manure 5 t ha⁻¹) and mineral fertilizers (NPK: 11% N, 22% P₂O₅, 16% K₂O) 300 kg ha⁻¹, dolomite (CaMg(CO₃)₂) 500 kg ha⁻¹ and two top-dressings of urea (46% N) 50 kg ha⁻¹ each, were applied.

Blast disease assessment

Initially, blast lesions were counted on the leaf at the beginning of the epidemics to evaluate the effect of rice residues on the onset of the disease in the field. Thereafter, leaf and panicle blast severity were measured to assess the progress of the blast epidemic in the trials.

To monitor the onset of the epidemic, the lesions were counted on the leaves of plants growing on the 176 innermost hills in the plot (11 × 16 rows) early in the season to account for the abundance of inoculum. Lesions were counted on 11, 15 and 24 January 2013 and on 16, 21 and 28 January 2014. In 2015, as the disease occurred earlier in the cropping season than in the two previous years, the lesions were counted only on 6 January. Counting was continued until the lesion numbers became numerous (50 in one hill). To evaluate the progress of the blast epidemic in later stages, the severity of leaf and panicle blast were assessed. For leaf blast severity, 10 hills were selected randomly along the diagonal of the plot. The total number of tillers (nbT) and the number of diseased tillers with at least one susceptible symptom (nbDT) were counted on each hill, and the leaf area affected by blast (%DLA) was estimated on the four uppermost leaves of three diseased tillers (when available).

The leaf blast severity of one hill was defined as LBSH = %DLA × nbDT/nbT. The leaf severity of one plot (LBSP) was defined as the mean of the leaf severity on the 10 hills sampled.

To measure panicle blast severity, 10 hills were selected randomly along the diagonal of the plot. The total number of panicles, nbP, and the number of diseased panicles (with at least one susceptible symptom, i.e. a black spikelet and an empty grain), nbDP, were counted on each hill, and the percentage of affected grains (%DG) was estimated visually on five diseased panicles (when available). When the neck nodes are infected by the fungus, the panicle dries and becomes white (counted as 100% of grains affected).

Panicle blast severity of one hill was defined as PBSH = %DG × nbDP/nbP. The panicle severity of a plot (PBSP) was defined as the mean of panicle severity on the 10 hills sampled (Dusserre et al., 2017).

Leaf blast severity was assessed on one date in 2013 (12 February) and 2014 (4 February), and two dates in 2015 (13 and 20 January). Panicle blast severity was assessed on one date each year, on 18 March in 2013 and 2014, and 17 March in 2015.

Monitoring M. oryzae survival in infected rice residues

The infected panicles used in this study were collected from upland cultivars naturally infected with rice blast fungus in the area surrounding the experimental trials at rice harvest time. The trials were carried out to monitor the survival of M. oryzae in infected rice residues in 2011 and 2013. In 2011, infected residues placed in two positions (on top of the mulch of rice residues and under this mulch) were compared by using infected stems of upland cultivar Fofifa 161 (F161) (3840 stems of
panicle to prepare 192 batches of 20 stems wrapped in nylon mesh). In 2013, infected residues placed in three positions (on top of the mulch, under the mulch and at a depth of 10 cm in the soil) were compared by using infected stems of upland cultivar F152 (3760 stems of panicle to prepare 288 batches of 20 stems). These batches were placed in each position in experimental plots in no-tillage cropping systems and with four replications. The trials were initiated on 27 May 2011 and on 23 May 2013. In these two trials, the monitoring was planned for 2 years. For the first trial in 2011, the periods from May 2011 to April 2012 and May 2012 to April 2013 are called hereafter the first and second year, respectively. For the second trial in 2013, the periods from May 2013 to April 2014 and May 2014 to April 2015 are also called hereafter the first and second year, respectively.

Four batches of panicle stems were removed from each position in the field each month to assess survival of M. oryzae. The stems were placed in Petri dishes with dampened filter paper to stimulate sporulation of M. oryzae and observed under a binocular microscope to check for the production of spores. Evaluation was based on the presence or absence of typical spores of M. oryzae. The percentage of remaining stems was calculated as the number of stems counted after the samples were collected, divided by the initial number of stems per batch (20). The percentage of stems with living M. oryzae was calculated as the number of stems with visible sporulation of the pathogen divided by the number of stems remaining in the batch. Average percentages and standard errors were calculated for the four replicates.

For the climate data, daily temperature and rainfall data were collected by on-site automatic meteorological stations (ENERCO 404 series, Cimel, France) at Andranomanelatra site for the time period 1 May 2011 to 30 April 2015.

Analysis

Analysis of variance was worked out with data on blast severity. To obtain homoscedasticity, the number of lesions per hill and the leaf and panicle blast severity values were transformed using the logarithm and arcsine of the square root transformation, respectively.

The percentages of remaining stems and of stems with living M. oryzae were transformed with the arcsine of the square root. After transformation, changes over time in the rate of remaining stems and in the rate of stems showing sporulation of M. oryzae were analysed with a linear model.

All analyses were conducted using R v. 3.2.5 (R Core Team, 2013).

Results

Effect of infected rice residues on the onset of blast epidemics in the field

The number of lesions on the leaf per hill was counted to assess the influence of rice residues on the onset of blast in the field. Examples of the occurrence and distribution of lesions on the 176 hills for 2013 are shown in Figure 1. At the first count, leaf blast was found on a few hills in plots with no rice residues (bare soil, BS), and in almost every hill in plots with infected rice residues (soil with diseased residues, SDR). After 2 weeks (lesion count on 24 January 2013), the number of lesions per hill in the BS treatment ranged from 0 to 4, and in the SDR treatment ranged from 0 to 46. The number of lesions per hill in BS plots was significantly lower than that in SDR plots at each sampling date, except the last sampling date in 2014 (28 January; Fig. 2). The quantitative evaluation of 2013, 2014 and 2015 campaigns indicate that overall, the development of leaf blast differed in the two treatments, with blast remaining at a lower level in BS than in SDR (Fig. 2). In 2015, no significant difference was found in the number of lesions per hill between the BS treatments and the treatment with healthy rice residues (soil with uninfected residues, SUR). However, the SDR treatment presented significantly more lesions than the SUR treatment. The average number of lesions per hill was 25.6 for SDR and only 2.8 for SUR on the only sampling date in 2015.

After the onset of the epidemic, progress of the blast epidemic in the trials was assessed by a late evaluation of leaf blast severity and by measuring panicle blast severity. In 2013 and 2014, no significant difference for leaf blast severity was observed between treatments (Fig. 2, right panel). In 2015, significantly more severe leaf blast was found in the treatment with infected residues than in the two other treatments. There was no difference in panicle blast severity in the three treatments, whatever the season (Fig. S1).

Survival of M. oryzae on infected rice residues

The rate of degradation of the stems was monitored by calculating the ratio of stems remaining in each batch that originally contained 20 stems. During the two trials, the number of remaining stems decreased over time (Fig. 3a,b). The stems of F161 persisted longer in the field than the stems of F152: F161 stems were recovered over a period of 19 months (from May 2011 to December 2012; Fig. 3a) whereas F152 stems were only recovered for 10 months (from May 2013 to March 2014; Fig. 3b). On each sampling occasion, with a few exceptions, the number of remaining stems was the same in all three positions (on top of the mulch, under the mulch and at a depth of 10 cm in the soil). However, in August, November and December 2013 and in March 2014, there was a significant difference between the number of stems of F152 remaining in the soil at a depth of 10 cm and the number remaining on top of and under the mulch (Fig. 3b).

Initially, in May of 2011 and 2013, 100% of stems were found to produce spores of M. oryzae. In 2011, rates of stems with pathogen sporulation were high and remained stable from June 2011 to November 2011 on stems placed on top of and under the mulch. In December 2011, the rate of sporulating stems started to decrease significantly, two months after the beginning of the rainy season (Fig. 4a). The rate of sporulating stems placed on top of the mulch was significantly higher compared to stems placed under the mulch from December 2011 to October 2012. The last sporulation of M. oryzae was observed 16 months (September 2012) after the
beginning of the trial for stems placed under the mulch and 18 months (November 2012) for stems on top of the mulch.

In the second trial, with cultivar F152, the rate of sporulating stems when buried in the soil decreased significantly after the first observation and no more sporulation was found after November 2013, 5 months after burial. The number of sporulating stems both on top of and under the mulch was similar over a period of 10 months (May 2013 to March 2014; Fig. 4b).

Overall, a decrease in the rate of sporulating stems over time was observed, but with some variation - in January 2012 for the first trial and in June, July and August 2013 for the second trial.

Environmental data for Andranomanelatra site

Monthly rainfall and average temperature from May 2011 to April 2015 is presented in Figure 5. For the first trial in 2011, the recorded rainfall was 1278 and 1089 mm for the first and second years, respectively, and for the second trial in 2013, the recorded rainfall was 1290 and 1549 mm for first and second years, respectively. Monthly average temperature was similar for all years in both trials.

**Discussion**

Understanding the origin of inoculum is key to developing integrated management strategies against plant pathogens. The ecology of the blast pathogen in respect to overwintering mode, particularly in high altitude upland, is not clearly characterized yet. The main primary sources of blast inoculum previously reported were infected rice seeds and rice residues (Ou, 1985). The role of rice seeds as a primary source of inoculum for rice blast has been investigated under field conditions and clearly demonstrated (Manandhar et al., 1998; Long...
In contrast, survival on rice residues has rarely been studied and reported. To the authors’ knowledge, the present work is the first to quantify the potential role of infected rice residues in the onset of rice blast and to evaluate survival of *M. oryzae* on rice residues in high altitude upland field conditions. Repeated field experiments including treatments with no rice residues, with healthy rice residues, and with infected rice residues left on the soil surface showed that the presence of infected rice residues on the soil surface had a positive effect on the onset of blast in the field during the first stages of the epidemics. These results demonstrate the role of infected rice residues in the onset of these epidemics.

![Figure 2](image-url)
Monitoring the survival of Pyrenophora tritici-repentis (Summerell & Burgess, 1989) and of Gibberella zeae (Pereyra et al., 2004) on wheat residues showed that these fungi survived when infected wheat residues were left on the soil surface, whereas incorporating the wheat residues into the soil reduced the viability of the fungi. The presence of resting structures and of ascospores of these two fungi in the field is known, which explains their adaptation under different field conditions. However, resting structures and sexual stage have not been reported for M. oryzae in the field so far. The results here suggest that the blast pathogen needs infected rice residues to survive for longer periods under field conditions. Results revealed that on cultivar F161, the blast pathogen was able to produce spores after 18 months and on cultivar F152, after 10 months when the infected residues were left on top of or under the mulch in the field. Long-term conservation on straw or

Figure 3 Variation over time of the percentage of panicle stems remaining in the field for different positions (on top of the mulch, under the mulch, in the soil) and rainfall per month (grey bars), (a) with cv. F161 in the 2011 trial and (b) with cv. F152 in the 2013 trial. * show a significant (P < 0.05) effect of position. Vertical bars are standard errors of the means.

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artificial media has already been demonstrated under controlled conditions, particularly when conditions are dry (Latterell & Rossi, 1986). The observations here show for the first time that the rice blast pathogen can survive on rice residues in the field between at least two cropping seasons. However, recovery of viable \textit{M. oryzae} declined rapidly at the first observation on residues buried at a depth of 10 cm in the soil. This result supports the hypothesis that \textit{M. oryzae} survives poorly in the soil, probably because of competition with other microorganisms. \textit{Magnaporthe oryzae} is considered to be a poor saprophytic competitor (Ou, 1985). Filippi & Prabhu (1997) and Manandhar \textit{et al.} (1998) found that blast lesions were rare when infected seeds were completely covered by soil. These observations and the present results of infected rice residues buried in the soil suggest that rice residues that are ploughed under just after harvest may not be a source of primary inoculum.

\textbf{Figure 4} Variation over time of the percentage of panicle stems left in the field in different positions (on top of the mulch, under the mulch, in the soil) producing \textit{Magnaporthe oryzae} spores, and rainfall per month (grey bars). (a) with cv. F161 in the 2011 trial and (b) with cv. F152 in the 2013 trial. *show a significant ($P < 0.05$) effect of position. Vertical bars are standard errors of the means.
Environmental conditions (temperature, relative humidity, precipitation and leaf wetness) have a strong influence on the rice blast epidemic in the field (Suzuki, 1975; Greer & Webster, 2001). Increased cumulated rainfall was expected to influence the survival of \textit{M. oryzae} on rice residues through maintenance of wet conditions in the field. During the survival monitoring, a decrease in the rate of sporulating stems on the top of and under the mulch was observed during both trials, in December 2011 and in November 2013. The cumulated rainfall before these periods reached 220 mm in October and November 2011, and 200 mm in October 2013. Moisture on the soil surface exhibits more variation than in the soil (Montzka \textit{et al.}, 2014), and so the rapid decrease in \textit{M. oryzae} survival and residue degradation at a depth of 10 cm could be due to the constant moisture, with the expected higher activity of microorganisms in the soil.

The rate of sporulating stems would normally decrease over time. However, for the first trial, a higher rate of sporulating stems was observed in February 2012 than in the previous month. No satisfactory biological explanation could be found, and an isolated technical problem cannot be excluded. The decrease in sporulating stems found in June, July and August 2013 for the second trial could be explained by difficulties with sporulation of \textit{M. oryzae} in some stems because of the cold conditions in the laboratory where sporulation was induced.

Several reasons could explain the differences observed in the degradation rate of stems between F152 and F161. In August 2013, an early slight decrease in the number of F152 stems could be due to the lower temperature during that period. The degradation of residues in the second trial was accelerated compared to first trial, which implies lower resistance of the residues. This could be due to differences between the F152 and F161 stem characteristics (Raboin \textit{et al.}, 2014) or to the impact on rice stems of different environmental conditions during the rice growing seasons.

In the no-tillage cropping systems recommended to halt declining soil fertility and to limit erosion in hillside fields in the Madagascan highlands, rice residues are left on the ground (Douzet \textit{et al.}, 2010). In a previous study, it was shown that blast severity was significantly lower in the conservation agriculture cropping system than in the conventional tillage system (Sester \textit{et al.}, 2014). The reduced blast epidemics observed in conservation agriculture were explained by reduced plant development, reduced nitrogen uptake and progressive assimilation of nitrogen (Sester \textit{et al.}, 2014; Dusserre \textit{et al.}, 2017). The results concerning the persistence of \textit{M. oryzae} on infected rice residues and its role in the onset of blast epidemics suggests that the management of rice residues and the practice of crop rotations should be reviewed.

These findings on the survival of blast pathogen on infected rice residues and its role in the onset of rice blast in high altitude upland field conditions should help to fill the gap in understanding epidemiological factors that favour the initiation of blast epidemics in the field, and should improve the management of this disease.

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References


Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher’s web-site.

Figure S1. Panicle blast severity in plots with different residue treatments: soil with no rice residues (bare soil, BS), soil with diseased residues (SDR) in 2013, 2014 and 2015, and soil with uninfected rice residues (SUR) in 2015. Vertical bars are standard errors of the mean.