Lower-Than-Expected Floret Sterility of Rice under Extremely Hot Conditions in a Flood-Irrigated Field in New South Wales, Australia

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Abstract: Rice florets are susceptible to high-temperature damage at anthesis, but rice production remains stable in the Riverina region of Australia even when the air temperature during flowering exceeds 40°C. To identify the mechanism that supports rice production under these conditions, we examined sterility and pollination in relation to microclimate and panicle temperature in an extremely hot paddy field in the Riverina region of New South Wales. In windy > 40°C weather, the panicle temperature was > 38°C at the windward edge of the crop but around 35°C inside the crop, probably because of strong transpirational cooling due to the extremely dry wind (15% RH). Pollen from the windward edge of the crop showed extremely poor germination, yet that from inside the crop showed sufficient germination for fertilization. Moreover, sterility inside the crop was significantly lower than that at windward edge. We concluded that the wind with large vapor pressure deficit enabled stable rice production under the extreme heat during flowering.

Key words: High temperature, Pollen germination, Pollination, Rice, Sterility, Transpirational cooling.

Air temperatures over 35°C induce rice floret sterility both in controlled-environment experiments (Satake and Yoshida, 1978; Kim et al., 1996; Matsui et al., 2001; Jagadish et al., 2007) and in the field (Osada et al., 1973; Tian et al., 2010). Some crop scientists therefore project that global warming will make high-temperature-induced floret sterility (HTIFS) an important problem (Horie et al., 1996; Nakagawa et al., 2004). Nevertheless, no serious yield losses have been reported in Australia, where the daily maximum temperature sometimes exceeds 40°C during the flowering season (Angus, 1997). Stable rice production without heat damage under these conditions casts doubt on the projections of HTIFS, and may imply that factors other than temperature affect HTIFS.

One reason for the stable rice production in Australia might be transpirational cooling caused by a large vapour pressure deficit under the high temperatures and low humidity that typically occur during the flowering season. In a paddy field in the Riverina region of New South Wales, we found that the panicles inside the canopy were 6.8°C cooler than the outside air under dry, windy conditions when the air temperature was around 35°C (Matsui et al., 2007). Although our observation supports the hypothesis that transpirational cooling of the canopy coupled with tolerant cultivars enable stable rice production in the Riverina, at least below 36°C, we were unsure whether these mechanisms would be effective under extreme conditions, such as hot winds at temperatures of over 40°C.

Another reason may be that the cultivars grown in this region are heat tolerant. The cultivar concerned also had long dehiscence at the base of the anthers (Matsui et al., 2007). Basal dehiscences are located just above the stigma and open at the time of floret opening. Moreover, the basal dehiscence is at the bottom of the anther when the anthers stand erect at the time of the floret opening. Therefore, pollen grains in anthers with long basal dehiscences readily drop out of the basal dehiscence onto the stigma. In this way, long basal dehiscence stabilizes pollination (Matsui and Kagata, 2003) even under high temperatures (Matsui et al., 2005). Since insufficient pollination was the main cause of HTIFS in previous studies (Satake and Yoshida, 1978; Matsui et al., 2001), we thought that the cultivar might have been heat tolerant (Matsui et al., 2007).
Here we report observations of the effects of extremely hot winds over 40°C on panicle temperature and pollination under field conditions in 2006. The objectives of the study were to clarify whether transpirational cooling is enough to support stable rice production under such hot winds, and whether the cultivar grown in the fields we examined in the Riverina has long basal dehiscence relating high temperature tolerance. To demonstrate the transpirational cooling effect, we observed the microclimate in the crop and examined the relationships between distance from the windward edge of the canopy, panicle temperature, pollination and sterility in a crop that experienced extremely hot winds at flowering. We also examined the length of basal dehiscence of the anthers as well as pollination and sterility to assess the tolerance of the cultivar.

**Materials and Methods**

1. **Field and plant material**

   We conducted our experiment on Pevensey Farm (144°34′05″E, 34°35′52″S, 80 m a.s.l.; grey clay), 8 km west of Hay, in the western Riverina, during the 2005 – 2006 growing season. The total area of flood-irrigated rice field in this farm was about 400 ha (2 km × 2 km). We used the southern margin of the 20-ha south-western bay (500 m east – west × 400 m north – south). Before sowing, urea and triple superphosphate with zinc (Zn) were applied as a basal dressing at 103 kg nitrogen (N), 25 kg phosphorous (P) and 36.6 kg Zn ha⁻¹. Pre-germinated seeds of the medium-grain cultivar ‘Amaroo’ were aerially sown on 2 October 2005 at 160 kg ha⁻¹. At panicle initiation stage, urea was applied at 46 kg N ha⁻¹. The water depth was kept at 5 cm from sowing to panicle initiation stage, at 10 cm to pollen microspore stage, at 25 cm to flowering stage, and at 5 cm again after flowering until draining at maturity. The heading date for this crop was 30 January, and maximum plant height was around 85 cm.

   The daily maximum and minimum temperatures around the flowering period were recorded at the Australian Bureau of Meteorology’s weather station in Hay (144°51′16″E, 34°32′58″S, 93 m a.s.l.).

2. **Measurements**

   The site’s microclimate was measured on 1, 2 and 3 February 2006, which corresponded to the full heading stage of the crop. Air temperature, relative humidity (RH), wind speed and solar radiation were measured at the southern (windward) edge of the rice field. There were no paddy fields further south, and thus the measurements represent typical surface conditions in this region. A temperature and humidity sensor (HMP45D, Vaisala Inc., Helsinki, Finland) was installed just on the edge of the canopy, 135 cm above the soil surface with a radiation shield, surmounted by an anemometer and wind vane (Model 03001-5 Wind Sentry, R. M. Young Co., Traverse City, MI, USA) at 170 cm. The variables were measured every 10 s, and 10-min averages were recorded in a data logger (CR10X, Campbell Scientific Inc., Logan, UT, USA). Panicle temperatures \( T_p \) were measured 4 times a day during daytime, including the times of the daily maximum temperature and flowering peak, at 1, 6, 11, 16 and 21 m from the southern edge of the canopy, with infrared thermometers (Model TA-0510F, Konica-Minolta Co. Ltd., Tokyo, Japan) with a fixed emissivity factor of 1.0. To avoid biases due to the directions of the sun and wind, we measured the temperatures of panicles located north, east, south and west of each point 3 times, which took about 45 s to complete. The distance from the thermometer to the panicles was about 1 m. We worked simultaneously from 1 m from the canopy edge to 21 m from the edge and then from 21 m towards 1 m along a parallel transects 10 m apart. The \( T_p \) at each point was based on the 12 readings.

   For observation of pollination, we collected florets on the primary rachis branches that had just completed anthesis at 1, 6, 11 and 21 m from the southern edge of the canopy. Nine florets each on three parallel transects 5 m apart were sampled, giving 27 florets at each distance. The stigmata were detached from the florets and immediately stained with cotton blue. Then the total pollen grains and germinated on each stigma were counted under an optical microscope at 100 × magnification. Pollen grains with tubes and empty grains were judged germinated. Since ≥ 10 germinated pollen grains are required for successful fertilization in rice (Satake and Yoshida, 1978), we
calculated the percentage of florets having < 10 germinated pollen grains on the stigma as an index of failed pollination and fertilization.

To observe dehiscence of the thecae, we collected florets on the primary rachis branches that had completed pollination at the same points as above and measured the length of dehiscence at the base of the anthers (Fig. 1). Nine florets at each distance were used (3 florets × 3 replicates). First, we counted indehiscent thecae and thecae whose stomium had dehisced completely from the apex to the base. Selecting thecae whose stomium had dehisced separately at apex and base, we measured the length of dehiscence at the base under a digital microscope (VH-5000, Keyence Corporation, Osaka, Japan) at 100 to 150 × magnification.

At crop maturity, we randomly gathered 30 panicles at 1, 6, 11, 16, and 21 m from the south edge of the canopy on parallel transects 1 to 2 m east of where the pollen samples were gathered, and calculated the percentage of sterile florets. Sterility was examined by finger press.

3. Data analysis

We adopted a randomized block method for statistical analysis, regarding each set of distances from the edge on a track as a block. Using two-way analysis of variance (ANOVA), we tested the effects of the date (or time and date for $T_p$) of measurements and the distance from the windward edge on $T_p$, the length of dehiscence at the base of the anther, the total number of pollen grains and the percentage of germinated pollen grains on the stigma, and the percentage of florets with < 10 germinated pollen grains. We also tested the effect of distance on the percentage sterility. When significant effects of the factors were found, we conducted Tukey’s HSD test. Binomial data such as the percentage of germinated pollen grains on the stigma and the percentage sterility were analyzed after logit conversion. The percentage of florets with < 10 germinated pollen grains was arcsine transformed because the data contained zeros.

Results

1. Temperature around the flowering period

The crop flowered between 27 January and 4 February (Fig. 2). The weather during this period was normal for the region, but the means of the daily maximum and minimum temperatures were higher than the average of the past 50 years by 5.5 and 4ºC, respectively. The daily maximum temperature in Hay exceeded 40ºC from 26 to 29 January and reached 43ºC on 1 February. The daily minimum temperature was 11.7ºC on 25 January but it exceeded 16ºC (and frequently 20ºC) from 26 January.

2. Meteorology in the paddy field and panicle temperature

The daily maximum temperature reached 42.1ºC at 1700 on 1 February, 33.8ºC at 1750 on 2 February and 35.8ºC at 1650 on 3 February (Fig. 3). At the same time, the RH was 14.7%, 24.6% and 10.0%, respectively. The vapour pressure deficit reached 70 hPa on 1 February around the time of daily maximum temperature. The times of flowering peak were 1300, 1340 and 1430, when the air temperature was 37.0, 31.5 and 34.6ºC and the RH was 24.4%, 30.4% and 9.3%, respectively. The wind velocity was around 2 to 3 m s$^{-1}$ at flowering on 1 February and was around 4 m s$^{-1}$ at maximum temperature. The velocity was 4 to 8 m s$^{-1}$ for almost the rest of 3 days (Fig. 3).

The effects of time and date of observation and distance from the windward edge on $T_p$ were significant ($P < 0.0001$), but their interaction was not ($P = 0.23$). $T_p$ was always lower than air temperature ($T_a$) during those 3 days (Fig. 4). On 1 February, $T_p$ was 38.2ºC around the time of daily maximum temperature ($T_a = 42.0ºC$), and 35.2ºC at the time of flowering peak ($T_a = 39.5ºC$), at 1 m from the
windward edge of the canopy: these values of $T_p$ were 4°C lower than $T_a$. $T_p$ decreased steeply from 1 to 6 m from the edge, but did not decrease further to 21 m (Fig. 5). The values of $T_p$ inside the canopy were lower than $T_a$ by 7.5°C. The difference between $T_a$ and $T_p$ increased as $T_a$ rose (Fig. 5).

The differences in panicle temperatures between the edge and the inner part of the crop were larger in the afternoon than in the morning.

### 3. Anther dehiscence

The percentages of indehisced and longitudinally dehisced anthers were very low (0.12% and 2%, respectively). The effects of distance ($P = 0.025$) and date ($P = 0.009$) on the length of dehiscence at the base of the anther were significant, but their interaction was not ($P = 0.88$). The length of dehiscence ranged from 357 to 522
pollen grains was around 50% at 1 m, but the values at the other points were almost 0% (Table 4).

At 1 m from the edge, the total number of pollen grains deposited on the stigma ranged from 167 to 255 in florets with < 10 germinated pollen grains (Table 5). However, the percentages of germinated pollen grains on the stigma were ≤ 2% in the same florets.
5. Seed set

The effect of distance from the edge of the canopy on sterility was significant ($P = 0.039$). Sterility reached 25% at 1 m from the edge, but only 14% at 21 m (Table 6). The percentage decreased linearly with increasing distance from the edge.

Discussion

1. How the rice canopy mitigated the heat damage to panicles

Our data show how the rice canopy mitigated the effects of extremely hot winds on panicle temperature and pollination. On the first study day, wind of over 40°C entered the paddy field with velocity around 5 m s$^{-1}$, and the panicle temperature reached over 38°C at 1 m from the edge, but was around 35°C at 6 m or more from the edge. On the following 2 days, the panicle temperatures were below 34°C. We thus considered that the negative effects of high temperature on pollination during those 2 days were much smaller than on the first day. Florets without sufficient germinated pollen grains were found only at 1 m during the 3 days (Table 4), where panicle temperatures were highest (Fig. 4), showing the mitigation of the negative effects of high temperature on pollination further inside the crop. We previously observed a similar decrease of $T_p$ with increasing distance from the windward edge of the canopy under hot dry winds below 36°C. We showed that simple heat budget model for estimation of $T_p$ in the rice canopy (Yoshimoto et al., 2005) could explain the steep $T_p$ gradient; the $T_p$ gradient from edge was formed by the continuous cooling of hot and dry air entering the community. Using the heat budget model, we also predicted that strong transpirational cooling could contribute to stable pollination under extremely hot winds of over 40°C (Matsui et al., 2007). Present results confirm this prediction. Julia and Dingkuhn (2013) also suggest the importance of microclimate in the crop and panicle temperature for prediction of HIFS on basis of the four paddy fields with much different environments.

Information about threshold panicle temperatures that induce floret sterility is limited. A panicle temperature of 33.7°C for 1 hr at flowering was enough to induce floret sterility in a growth cabinet (Jagadish et al., 2007). An average daily maximum panicle temperature of 35 to 37°C for 3 days during flowering induced about 20% sterility in a temperature gradient chamber (Maruyama and Ohba, 1996). In our crop on 1 February, $T_p$ at flowering was about 33°C at 1 m and below 35°C inside the field, $T_p$ at the time of the daily maximum temperature was > 38°C at 1 m and about 35°C inside. These temperatures were above the reported threshold $T_p$ at 1 m and slightly lower than it inside the crop. Our high rates of poor germination at 1 m were consistent with these previous reports of threshold panicle temperature.

The significantly lower sterility inside the crop than at 1 m from the edge (Table 6) supports the notion that

| Distance (m) | Date | Floret sterility (%) |
|-------------|------|----------------------|
|             | 1 Feb | 2 Feb | 3 Feb | Mean |
| 1           | 48.1  | 66.7  | 48.1  | 54.3 a |
| 6           | 0.0   | 0.0   | 0.0   | 0.0 b  |
| 11          | 0.0   | 0.0   | 0.0   | 0.0 b  |
| 21          | 0.0   | 3.7   | 0.0   | 1.2 b  |

Means with the same letters within the column are not significantly different by Tukey’s HSD test ($P = 0.05$). The effects of distance and the interaction between distance and date were not significant by 2-way ANOVA ($P = 0.05$).

| Distance (m) | Florets with < 10 germinated pollen grains | Florets with ≥ 10 germinated pollen grains |
|-------------|--------------------------------------------|-------------------------------------------|
|             | No. of pollen grains | % germinated pollen grains | No. of pollen grains | % germinated pollen grains |
| 1 Feb       | 183.9 ± 24.8         | 1.31 ± 0.47                  | 142.1 ± 36.9         | 22.98 ± 2.22               |
| 2 Feb       | 167.1 ± 49.2         | 0.68 ± 0.49                  | 442.8 ± 48.4         | 9.41 ± 3.14                |
| 3 Feb       | 254.8 ± 79.7         | 2.02 ± 0.68                  | 411.3 ± 30.5         | 9.31 ± 0.46                |

Values are means ± SE ($n = 3$).
transpirational cooling of the canopy mitigates heat damage. The sterility, however, decreased gradually with increasing distance from the edge, which is somewhat inconsistent with the dramatic improvement in pollen germination (Table 4) and panicle temperature at 6 m from the edge (Fig. 4). Our crop had experienced high temperatures for 3 days at the start of flowering before our 3 days’ observation (Fig. 2). The relationship between the occurrence of sterility and distance from the windward edge mainly depends on balance among the air temperature, humidity, wind, sun radiation, and cooling ability of canopy. If the transpirational cooling of the rice canopy is not enough, heat damage can intrude into the crop. The gradual decrease in sterility might show the insufficient adaptation of the rice crop to several days of the high temperatures at the start of flowering.

2. Effect of panicle temperature on pollination and seed set

At 1 m from the edge of the crop, about 50% of the florets had < 10 germinated pollen grains. These florets would be sterile. Around 200 pollen grains were deposited on the stigmata of these florets, considerably fewer than the mean of 300 in the experiment, but not enough to effect fertilization. However, the percentage of germination was ≤ 2%.

In past studies of high-temperature-induced floret sterility in controlled environments, the main cause of sterility was poor pollination (Matsui et al., 2001), especially in intolerant cultivars (Satake and Yoshida, 1978). The main cause of HTIFS in field conditions in China was poor pollination (Tian et al., 2010). The sterility we observed in the Riverina seems somewhat different in the mechanism of HTIFS from those previous observations. Nabeshima et al. (1988) reported damage from a one-time high temperature of 40°C for 2 hr at flowering time. The main causes of sterility were poor pollination of florets exposed to high temperature at the time of flowering and poor pollen germination on the following day. The extremely high temperature decreased germination of pollen grains on the following day rather than on the high temperature day (Nabeshima et al., 1988). Our results of a dramatic decrease of pollen viability on days following high temperature are consistent with that report.

3. Effect of long basal dehiscence of anthers on pollination

The mean length of basal dehiscence of anthers of ‘Amaroo’ was 430 µm, as long as that of moderately tolerant cultivars (Matsui et al., 2005). Dehiscence was longer on 2 and 3 February than on 1 February. Although the number of pollen grains on 2 and 3 February was greater than on 1 February, in parallel with the length of dehiscence, we could not find any effect on the percentage of florets with < 10 germinated pollen grains. When germination is ≤ 2%, more than 500 pollen grains are required to achieve the 10 germinated pollen grains. In the case of HTIFS due to extremely low pollen germination, the effect of long dehiscence seems limited.

4. Diurnal change in air temperature

The diurnal range of $T_c$ was very large (Fig. 2), and the $T_c$ peaked late in the day (Fig. 3), probably owing to the flow of heat from the adjacent bare land to the paddy field. Although the time of flowering peak was also late, the duration from flowering peak to daily maximum temperature appeared somewhat longer than that in monsoonal Asia. Since flowering stage is the most sensitive to high temperatures (Satake and Yoshida, 1978), the stable production under extreme heat might be at least partly due to the time lag between flowering peak and maximum temperature.

Conclusion

Our observations exemplify the cooling function of paddy field that works enough to mitigate HTIFS against > 40°C wind. Even when the field was subjected to the extremely hot dry wind, the panicle temperature was lower inside the canopy and thus the panicle escaped damage. Since severe poor germination that directly induced floret sterility has been observed on the windward edge of crop, we consider cooling to be the most important factor behind stable rice production in the extremely hot environment in the Riverina region. However, cooling depends on strong transpirational cooling, and thus on the extreme dryness of the wind. We therefore cannot expect an equal cooling effect under humid, low-wind conditions.

The hot, dry wind induced floret sterility on the windward edge of the field. The main direct cause of sterility was the low percentage germination of pollen grains on the stigma. The advantage of long dehiscence of the anther base for pollen release would be limited in such HTIFS on account of low pollen germination.

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