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Accepted Version

Lukac, M. ORCID: <https://orcid.org/0000-0002-8535-6334>, Gooding, M., Griffiths, S. and Jones, H. (2012) Asynchronous flowering and within-plant flowering diversity in wheat and the implications for crop resilience to heat. *Annals of Botany*, 109 (4). pp. 843-850. ISSN 1095-8290 doi: 10.1093/aob/mcr308 Available at <https://centaur.reading.ac.uk/24627/>

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To link to this article DOI: <http://dx.doi.org/10.1093/aob/mcr308>

Publisher: Oxford University Press

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**Asynchronous flowering and within-plant flowering diversity in wheat and the implications for crop resilience to heat.**

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## **Abstract**

Self-pollination dominates in wheat , with a small level of out-crossing due to flowering asynchrony and male sterility. However, the timing and synchrony of male and female flowering in wheat is a crucial determinant of seed set and may be an important factor affecting gene flow and resilience to climate change. Here, a methodology is presented for assessing the timing and synchrony of flowering in wheat. From the onset of flowering until the end of anthesis, the anther and stigma activity of each floret was assessed on the first five developing ears in potted plants grown under ambient conditions and originating from cv Paragon or cvs Spark-Rialto backgrounds. At harvest maturity, seed presence, size and weight was recorded for each floret scored. The synchrony between pollen dehiscence and stigma collapse within a flower was dependent on its relative position in a spike and within a floret. Determined on the basis of synchrony within each flower, the level of pollination by pollen originating from other flowers reached approximately 30% and did not change throughout the duration of flowering. A modelling exercise parameterised by flowering observations indicated that the temporal and spatial variability of anther activity within and between spikes may influence the relative resilience of wheat to sudden, extreme climatic events which has direct relevance to predicted future climate scenarios in the UK.

## Introduction

The time of flowering in cereals is usually stated as when 50% of ears are in flower, which is adequate for differentiating gross differences in flowering time between genotypes, such as for photoperiod sensitivity (Marcello and Single, 1971), or between environments (Griffiths et al., 2009). This type of characterisation, however, ignores differences in the temporal and spatial variation in flowering that occurs between spikes, amongst spikelets, and within florets of a single spikelet (Percival, 1921). Further fluctuations in timing may include diurnal effects, as demonstrated in rice to avoid high midday light intensities and temperature (Virmani and Edwards, 1983), but also in wheat (De Vries, 1974). Hence, florets of a single cereal genotype do not flower simultaneously. This variability in the temporal and spatial pattern of flowering has potential implications for (i) gene flow between plant populations (Willenborg et al., 2009, Demotes-Mainard et al., 1996); (ii) plant breeding; and (iii) physiological escape of those florets which are active before or after a major abiotic stress event (Saini and Aspinall, 1982, Zhao et al., 2010).

Wheat flowering biology was first studied in detail by Russian and Hungarian scientists when hybrid wheat production came to the fore (see Pickett et al.(1993) for review). The stages of floral development which have been identified as sensitive to climatic stress include pollen formation (Lalonde et al., 1997, Saini and Aspinall, 1982, Craufurd and Peacock, 1993), anthesis (Bidinger et al., 1987, Ferris et al., 1998, Zhao et al., 2010) and seed set (Tashiro and Wardlaw, 1990). Experimentally, such vulnerable stages have been identified through applying extended durations of heat stress, usually lasting for a number of days. High temperature stress seems increasingly relevant in UK conditions, maximum daily temperatures in major wheat growing areas of the UK reached between 28°C and 30°C on two days in 2010 (23 May and 4 June), shortly before and during flowering. At these growth stages, prolonged exposure to such temperatures can be associated with significant reductions in seed set in UK wheat (Ferris et al., 1998). These heat stress events commonly only last for a few hours around midday and in the early afternoon for consecutive days. For this reason, it is the number of florets at vulnerable stages during each of these successive, short duration stress events that is likely to be directly relevant to the success or otherwise of seed set of the crop.

In bread wheat, out-crossing is estimated to be around 6% in the absence of stress (Hucl, 1996). However, most estimates of cross pollination in wheat have been obtained during a total loss of pollen from one vulnerable genotype. For example, cross pollination in field conditions was found to reach 32% in cold and dark-sensitive Moulin, compared to 7% in Aubaine - a less sensitive cultivar (Demotes-Mainard et al., 1996). Hence there is little understanding of *within* plant pollen movement, and of the potential scope of individual genotypes buffering the loss of pollen in one floret by pollination with pollen from another floret on the same spike or between spikes of the same or different plants. In this paper, the terminology *same floret* pollination will refer to pollination of the stigma by the pollen formed in the same flower, *cross floret* pollination will refer to a stigma

pollinated by the pollen from a different floret (either within the same spikelet, on the same spike or between spikes). Within plant pollen movement cannot be quantified genetically, but accurate analyses of the developmental stages of the stigma and the stamen of each flower can provide an estimation of the diversity of flowering behaviour within single genotype. A quantitative scale for flower development in wheat and barley was created by Waddington et al. (1983). However, the classification only characterises the development of the stigma, with no reference to anther development or pollen release. The intrinsic variability between anther and stigma activity, driven by the flowering phenology of each genotype, interacts with stress factors of differing type, severity and duration, thus limiting our understanding of stress responses at various physiological stages leading to, and following on from anthesis.

Analyses of temporal and spatial diversity of developmental stages of both male and female flower parts can indicate the scope for individual plants of a single genotype to buffer loss of pollen from a proportion of florets at any one time. Making use of several near isogenic lines (NILs) from Paragon and Spark-Rialto backgrounds, the objectives of this study were (i) to develop a methodology to quantify phenological variation between and within wheat backgrounds; (ii) to determine the timing of flowering and level of synchrony within individual flowers; and (iii) to estimate the effects of stress on same floret pollination.

## **Materials and methods**

### *Plant material*

Plants were grown in 12.5 cm diameter pots filled with a 4:4:2:1 mixture of steam-sterilized 6mm gravel, medium vermiculite, 3mm sharp sand and peat based posting compost. To supplement plant nutrition, 2kg of Osmocote Pro 3-4 months (Scotts, UK) were added per cubic metre of planting mixture. Pots filled with planting medium were soaked with water for a minimum of 5 hours before being planted with single seeds to a depth of 2 - 2.5 cm. In total, 6 Paragon and 9 Spark-Rialto NILs were used in this experiment (Table 1). All pots were placed in a cold room at 4°C for 42 days, at which point the majority of coleoptiles had emerged. Pots were then transferred outside to a fenced off area and raised to a height of approximately 10 cm on bricks to allow free drainage. Plants were irrigated automatically through a drip system and treated against powdery mildew once on the 25<sup>th</sup> June with Flexity (300 g/litre (25.2% w/w) metrafenone; BASF Plc, UK in 0.5 l/ha). From stem extension to maturity, the whole area was protected by bird proof netting.

### *Flowering and yield assessment*

A total of 43 plants from Paragon (Bentley et al., 2011) and Spark-Rialto (currently being developed at JIC, Norwich, UK) NILs were used to assess the progress of flowering in both male and female

parts. All inflorescences to develop on the first five ear-bearing tillers (main stem = tiller 1) were observed every day from the initial signs of anthesis until the cessation of flowering activity. The spikelets of an ear were numbered from the collar upwards, the lowest being '1' and the subsequent numbers alternating between sides such that one side of the ear is 'odd' and the other 'even'. All spikelets were assessed in Paragon NILs, while only odd numbered spikelets were assessed in Spark-Rialto. The lowest potential grain site frequently lacked both stigma and anthers, but this was consistently labelled as spikelet 1. Floret labelling followed the scheme of Kirby and Appleyard (1984), with the first floret from the lower glume labelled as 'A', subsequent florets up the spikelet alternated between sides such that floret 'B' and 'D' were on the same side. Assessments continued until all flowering stopped on tiller 5.

Observations started at 10 am and were consistently completed within 2.5 hours. Emerging ears were first inspected at growth stage (GS) 57 (Zadoks et al., 1974). Scoring of a spikelet was initiated when the lower glume could be opened easily with a thumbnail. In the early stages of flowering, usually during days 1 to 3 after first inflorescence on an ear, not all glumes could be opened as some flowers were lagging in development. Care was taken not to force such flowers, but to observe them on the subsequent day. Subsequent florets within the same spikelet were observed by easing open each lemma. Each floret which could be opened was scored for the developmental stage of both male and female parts according to the scheme outlined in Table 2. Male flowers were assessed on a 4 point developmental scale, while female flowers were assigned 3 stages. Anthers were defined as 'active' when showing signs of pollen dehiscence (stage 3) whereas stigmas were considered receptive to pollination when graded as F (fluffy). Active stigma duration was defined as the number of consecutive days for which a stigma was graded as F. Flowering synchrony was defined as the day of pollen dehiscence minus the day of observed stigma collapse within an individual flower. Negative flowering synchrony values indicate pollen release before stigma collapse. All plants were left to mature outside and were harvested to assess grain yield. Weight of each individual seed was established, together with the position within an ear.

Temporal development of flowering activity was analysed by fitting a Gaussian distribution to the aggregate numbers of active flowers in all five tillers under observation and by testing for differences in the timing of peak flowering. Linear regressions were fitted to flowering synchrony and active stigma duration data, the slope of the regression was then used to assess the significance of temporal and spatial trends. Seed weight and number data were compared by a t-test. A reverse cumulative Gaussian function was used to model the number of surviving male flowers (Figure 6) and a Gaussian distribution to model anther flowering activity (Figure 6). All analyses were performed in Statistica 8.0 (Statsoft Inc.) considering individual plants as true replicates, any effects were deemed significant at  $P < 0.05$ .

## Results

The majority of flowering activity takes place in mid-morning (De Vries, 1974), all plants were assessed within a relatively short time window (2-3 hours). In order to increase the number of assessed plants, it was possible to score only one side of an ear (i.e. only odd or only even spikelets). This did not affect the mean flower score of male ( $P=0.335$ ) or female flowers of Paragon NILs ( $P=0.620$ , data not shown).

No differences were observed in the timing of male and female peak flowering at the plant level, across all tillers in Paragon NILs (see also Supplementary Figures A1-B3) and in the majority of Spark-Rialto NILs, apart from: SR74-2 SR20 ( $P=0.009$ ) and SR75-1 SR13 ( $P=0.006$ ). Comparing Paragon NILs to each other, there was no difference in the timing of peak flowering of male ( $P=0.104$ ) or female parts ( $P=0.210$ ). Peak flowering in Spark-Rialto NILs, however, took place at different times both for male ( $P=0.001$ ) and female flowers ( $P=0.016$ ).

In order to account for observed differences between wheat lines and to allow for an analysis of flowering development in time, the activity of the stigma relative to the anthers was expressed on a relative time scale where time 1 is defined as the day when the first active flower was observed on a plant. This flowering synchrony was calculated as the difference between the day pollen dehiscence was observed and the day stigma showed signs of pollination.

After aligning the flower synchrony data on this time-scale, the synchrony between male and female parts within individual flowers decreased with the temporal progress of flowering both in Paragon (Figure 1a) and in Spark-Rialto NILs (Figure 1b). Negative values of synchrony indicate the pollen release took place before stigma collapse. The majority of flowers which had both active stigma and pollen releasing anthers were concentrated at or close to the 0 line indicating a high likelihood of same-floret self-pollination. Defining the window of opportunity for such self-pollination as synchrony between the male and female parts from -1 to 1 day (De Vries, 1971, Flint and Caldwell, 1984, Saini and Aspinall, 1982), an estimate could be made of the proportion of flowers where same-floret self pollination took place (Figures 2a and 2b). There was no difference between the Paragon and Spark-Rialto backgrounds in the overall level of same-floret self pollination potential (70% in Paragon, 68% in Spark-Rialto,  $P=0.619$ ). Similarly, there was no difference in spatial distribution of the potential self-pollinated flowers between the two backgrounds. The highest proportion of self-pollinated florets was found in the outermost florets in the middle of the ear in both Paragon and Spark-Rialto NILs (see Supplementary Figures B1-B2).

The relationship between the position of a flower and its flowering behaviour was significantly influenced by both the spikelet and floret designation. Paragon NILs were characterised by the longest duration of active stigmas in the middle spikelets, with the average duration significantly decreasing towards the top and the base of the ear. The observed pattern of stigma longevity may be related to the



fact that the most asynchronous flowers are found in the middle of the ear (Figure 2a). In contrast to the Paragon NILs, Spark-Rialto showed no significant difference in the mean length of active stigma duration but a significant trend ( $p=0.0002$ ) was identified in flowering synchrony, which decreased from the base of the ear towards the top (Figure 3b). Floret position significantly influenced active stigma duration and flower synchrony both in Paragon and Spark-Rialto backgrounds. The mean length of time during which a stigma is receptive is the longest in the outermost and the shortest in the innermost florets ( $P<0.001$  both for Paragon and Spark-Rialto NILs). Similarly, the flowering synchrony is the lowest in floret A and the highest in floret E ( $P<0.001$  and  $p=0.001$  for Paragon and Spark-Rialto respectively, Figure 3c and 3d).

Finally, at harvest, the presence or absence of seed in each position was determined within ears which had been manually assessed at anthesis. In Paragon, repeated handling of flowers during scoring significantly reduced seed yield ( $P=0.040$ ), with an apparent affect on seed number ( $P=0.104$ ) per individual ear. In Spark-Rialto, on the other hand, there was no observed effect of scoring on yield ( $P=0.402$ ) or seed number ( $P=0.612$ ).

Figure 4 shows the relationship between individual flower synchrony and successful seed set. There was a significant relationship between flower synchrony and the proportion of flowers with seed (Figure 4;  $P=0.015$  in Paragon and  $P=0.024$  in Spark-Rialto). The probability of successful seed set decreases linearly between positive and negative values of synchrony. There was no difference between the slope of the decrease between the two wheat backgrounds ( $P=0.930$ ). The position of an individual flower within an ear also appears to influence the probability of seed set. Both in Paragon and Spark-Rialto NILs, the relative number of filled grain sites where active stigma had been recorded decreased with increasing spikelet number ( $P<0.0001$  and  $P=0.001$  in Paragon and Spark-Rialto, respectively). The flower position within a spikelet did not influence success of seed set in Paragon ( $P=0.776$ ) or in Spark-Rialto NILs ( $P=0.697$ ).

The timing of peak flowering differed both within and between spikes in Paragon and Spark-Rialto NILs. There was a significant difference between the date of peak flowering on the spike of tiller 1 and tiller 3 ( $P=0.004$ ) and tiller 4 ( $P=0.011$ ) in Paragon, while spikes of tillers 2 and 5 flowered at the same time as tiller 1. Spark-Rialto lines showed a significant difference between peak flowering between tillers 1, 2 and 3 ( $P<0.015$ ).

The temporal spread of anther activity between the spikes of tillers 1 to 5 and the interaction with a potential sequence of short-term extreme stress events lasting 1 to 8 days was estimated. A conceptual model of pollen loss was constructed to identify the potential number of active anthers (i.e. releasing pollen) for a given stress duration. Figure 5 show decreasing male flower survival with increasing stress event duration in Paragon and Spark-Rialto NILs. The model assumes 100% mortality of active anthers within a floret at the time of heat stress. Overall pollen availability started to decline in

Paragon after *ca* 5 days due to tiller clustering, while in Spark-Rialto later tillers lost very few of their male flowers even after a 7 day stress event (Table 3). Maximum observed stigma duration was 7-8 days in both backgrounds (Figures 1 A and B).

## Discussion

The first signs of anthesis in wheat appear in the middle of an ear and flowering then proceeds in both directions towards the top and bottom spikelets (Waines and Hegde, 2003), Figures A1-A3). Similarly, the outermost florets in a spikelet flower first, followed by the florets found on the inside of a spikelet (Percival, 1921). Furthermore, the spike of tiller 1 flowers before subsequent spikes (De Vries, 1973). The results presented in this paper agree with previous literature, but further insights have been achieved with regard to the relative temporal and spatial activity of the anthers and the stamens based on findings from the novel flower scoring methodology. Wheat is generally considered to be a self-pollinating plant with fertilization likely to happen within florets, between florets at the spike and plant level, but also between plants. There are recorded instances of cross-pollination in wheat (e.g. summary in (Waines and Hegde, 2003), where the highest observed levels reach 10 - 14% and relate mostly to loss of pollen viability due to stress or were recorded in male-sterile plants (Demotes-Mainard et al., 1996, Khan et al., 1973, Virmani and Edwards, 1983). Previous literature may have under-estimated cross-floret pollination because the activity within a spike would remain undetected. In this research, ten percent of active stigmas in both the Paragon and Spark-Rialto NILs had collapsed before pollen extrusion in the same floret. Waines and Hedge (2003) speculate that the large amount of pollen shed by wheat outside the florets (in turn influenced by the relative extrusion of anthers and the angle of floret opening) is indicative of a higher amount of cross-pollination in wheat than has previously been predicted. There remains however, a need to identify the interaction between this cross-floret self-pollination and the cross pollination as defined by previous authors. The timing of stigma receptivity and pollen dehiscence define floret *synchrony*, which may have a direct bearing on the level of self pollination. Timar et al. (1997) found developmental asynchrony of male and female gametes in wheat which increases with ploidy level, thus creating an opportunity for cross-floret pollination. The position of a floret within a spikelet and on a spike significantly affected the level of synchrony in Paragon and Spark-Rialto. Flowers in the middle of the ear and on the outer edges of a spikelet tend to have larger asynchrony between pollen release and stigma collapse. It has also been shown that the stigmas of these flowers remain receptive for longer periods of time, which may result from a delay in the supply of same-floret pollen.

Within a spikelet, the innermost florets D and E were the least likely to open both in Paragon and Spark-Rialto NILs, which agrees with previous findings that the inner florets are frequently cleistogamous (closed), while the outer florets are chasmogamous (open) (Waines and Hegde, 2003).

Florets D and E had the highest level of synchrony between pollen dehiscence and stigma collapse, which supports the hypothesis that closed florets are largely same-floret pollinated compared to the greater chance of cross-floret pollination in positions A, B and C. Florets with longest lasting active stigmas in the middle of the spike, which are also the most asynchronous, would appear to have the greatest probability of being pollinated by pollen from other florets.

Flower fertility was calculated in all wheat lines used in this experiment by comparing the number of flowers where viable stigma was observed with the number of seed. The probability of a viable flower delivering a seed decreased from the bottom of the ear towards the top, but was not affected by the position of the florets within a spikelet. Interestingly, individual flower synchrony may be one of the determinants of successful seed set. Both in Paragon and in Spark-Rialto, the probability of seed set was strongly related to synchrony (Figure 4). The probability of successful seed set was the highest for the most positively asynchronous florets. Since the stigma collapsed several days before same-floret pollen release, these florets had the greatest likelihood of out-pollination. However, further evidence is needed to differentiate the effect of floret asynchrony and assimilate supply {Slafer, 1994 #108}

Wheat pollen has very short viability in open environments, thus decreasing the probability of viable pollen transfer over longer distances. As a result, flowers with high levels of asynchrony (< 1 day) or total absence of viable pollen are likely to receive pollen from elsewhere. This is most likely in florets which are active during peak flowering, when a large amount of pollen is present around the ear. Based on the findings in this paper, the earliest flowers – those in the middle of an ear and in florets A and B – tend to flower during a period of low ‘background’ pollen availability, have the greatest asynchrony and show the greatest potential for out-crossing as a result. A study of wind pollinated hybrids of *Fraxinus excelsior* L. and *F. angustifolia* has confirmed that female flowers receive pollen from more distant individuals when flowering early, rather than during peak flowering (Gerard et al., 2006). Both Paragon and Spark-Rialto NILs exhibit similar patterns of flowering behaviour; the stigmas of early flowers are receptive for longer periods, increasing the chance of out-crossing. Due to the spatial arrangement of florets, and possibly due to their earliness, they tend to produce the largest seeds (data not shown).

Increased ambient temperature at mid-anthesis has been shown to induce sterility in barley by anther degeneration (Sakata and Higashitani, 2008) and low pollen dehiscence in rice (Matsui and Omasa, 2002). In addition, wheat pollen remains viable for an estimated 30 minutes (De Vries, 1971), with significant decreases in viability during heat events (Saini and Aspinall, 1982), and with high sunlight intensity (Flint and Caldwell, 1984). Even if viable pollen successfully evades unfavourable environmental conditions and reaches the stigma, there are indications that high temperature at anthesis limits pollen germination and pollen tube elongation (Salem et al., 2007). Based on these

findings, a period of one day either side of stigma pollination as the time window favourable to self-pollination was selected for the conceptual model. Stigma activity was not considered in the modelling exercise, since the female flower is much less sensitive to heat stress at anthesis in cereals (Cárcova and Otegui, 2001, Sakata and Higashitani, 2008). This distinction is based on the frequency of anthesis assessments in this research, a higher resolution could be achieved with multiple assessments per day with concomitant evaluation of ultra violet levels, temperature and humidity. The model provides an indication of potential anther activity, and therefore volume of viable pollen on the assumption that anther activity is the determinant of pollen number for each wheat NIL. Joppa (1968) demonstrated that the relative volume of pollen could be estimated for different varieties by assessing the number of pollen grains per anther, and the number of extruding anthers.

The model produced in this paper was developed to predict the interaction between the number of active anthers (and therefore the volume of viable pollen) and the effect of a sudden, extreme stress events which caused a total loss of viability at the specific time of the stress event. Lalonde (1997) identified sporogenesis as one of the most vulnerable stages in pollen formation, if a heat stress event occurs at e.g. over 30°C for one day, a scenario increasingly probable in the UK (UKCP09, 2009), the pollen at the most vulnerable stage of development will be killed on that day. If the heat stress occurs on three successive days, pollen viability is likely to be lost for three days running. Based on the flowering model, a difference in effects of heat stress on potential pollen viability was identified between the Paragon and the Spark-Rialto NILs for a given stress duration (Table 3). The genotypes with a wider spread of anthesis lost a smaller proportion of pollen for a given stress duration, compared to a rapid, short duration genotype. Furthermore, the model can be used to hypothesise that crops with a higher number of tillers per plant flower longer and therefore are more resilient to a short and intensive heat stress event. Significant variation was observed in the duration and the timing of flowering activity within Paragon and Spark-Rialto NILs, hinting at the existence of temporal and spatial flowering diversity in Northern European wheat varieties. Further work is needed to understand the interaction between flowering phenology and predicted climate variability to understand how physiological escape from effects of abiotic stress can be exploited, and indeed be a component of increasing the relative stability of yield in UK wheat.

## Conclusions

This paper demonstrates that the presented methodology is sufficiently sensitive to study the timing and the synchrony of individual flowers. The level of synchrony and consequent potential for out-crossing is determined by the position of the flower within an ear. The first florets to flower in a wheat ear have the largest negative asynchrony and their stigmas are receptive for the longest period of time. Comparing several wheat NILs originating from Paragon and Spark-Rialto backgrounds,

significant differences were identified in the timing and the distribution of flowering even among plants with the same background. These findings indicate that there is a significant potential for out-crossing, especially in the early flowers. Climate change, and the consequent plant stress, is likely to have a major impact on flower phenology, underlying the need to create wheat varieties with diverse flowering habits.

## **Acknowledgements**

This work was funded by a research grant from the Biotechnology and Biological Sciences Research Council (BBSRC) as part of project (BB/H012176/1). We would like to thank Caroline Hadley and Laurence Hansen for their excellent technical support, and Frederick Steinmeyer and Rajneet Uppal for their help in data collection and the scoring of flowering. We thank Luzie U. Wingen for commenting on the manuscript.

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**Table 1.** Details of Paragon and Spark-Rialto lines used for flowering and seed assessment.

<b>Paragon</b>			<b>Spark-Rialto</b>		
<i>Line</i>	<i>Region of interest</i>	<i>Number of scored ears</i>	<i>Line</i>	<i>Region of interest</i>	<i>Number of scored ears</i>
Paragon [GS-100 Ppd-A1a]	2A	10	SR13-418-2	3B	5
			SR20-388-4	3B	15
Paragon [Sonora 64 Ppd-D1a]	2D	10	SR20-387-1	3B	15
			SR13-417-3	3B	20
Paragon [Sonora 64 Ppd-B1a]	2B	10	SR13-418-2	3B	15
			SR23-441-1	1D	25
Paragon [Chinese Spring Ppd-B1a]	2B	10	SR23-413-2	1D	20
			SR9-413-2	1D	25
Paragon	n.a.	5	SR9-411-2	1D	20



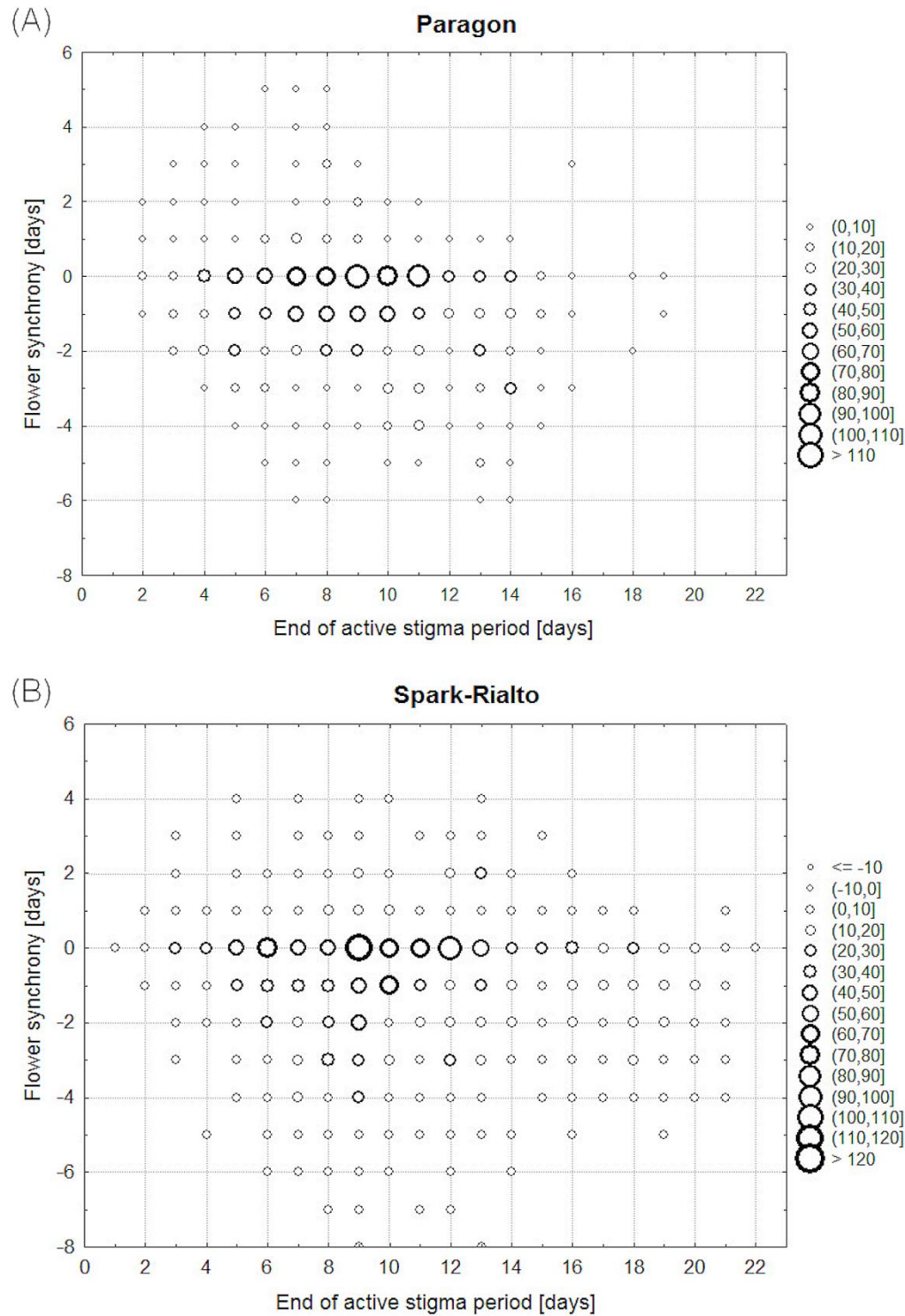
**Table 2.** Definition of scoring terminology for male and female parts of a wheat ear. The female scoring system is compared to the equivalent scale developed by Waddington et al (1983).

Score	Flowering stage description	Waddington <i>et al</i> (1983)
<i>Female</i>		
HF	Styles and stigmatic branches erect or spreading.	9.25 - 9.5
F	Stigmatic branches spread widely, stigma curved and fully spread.	10
PF	Stigmatic branches curled or collapsed. Stigma showing partial or total collapse.	n.a.
<i>Male</i>		
1	Immature anthers green and alongside immature styles.	n.a.
2	Anthers pale green to pale yellow and filaments extending. Anthers no longer parallel with style.	n.a.
3	Pollen extrusion, anthers bright yellow. Anthers inside or outside the floret lemma.	n.a.
4	Anthers pale yellow, collapsed or missing. Anthers inside or outside the floret lemma.	n.a.

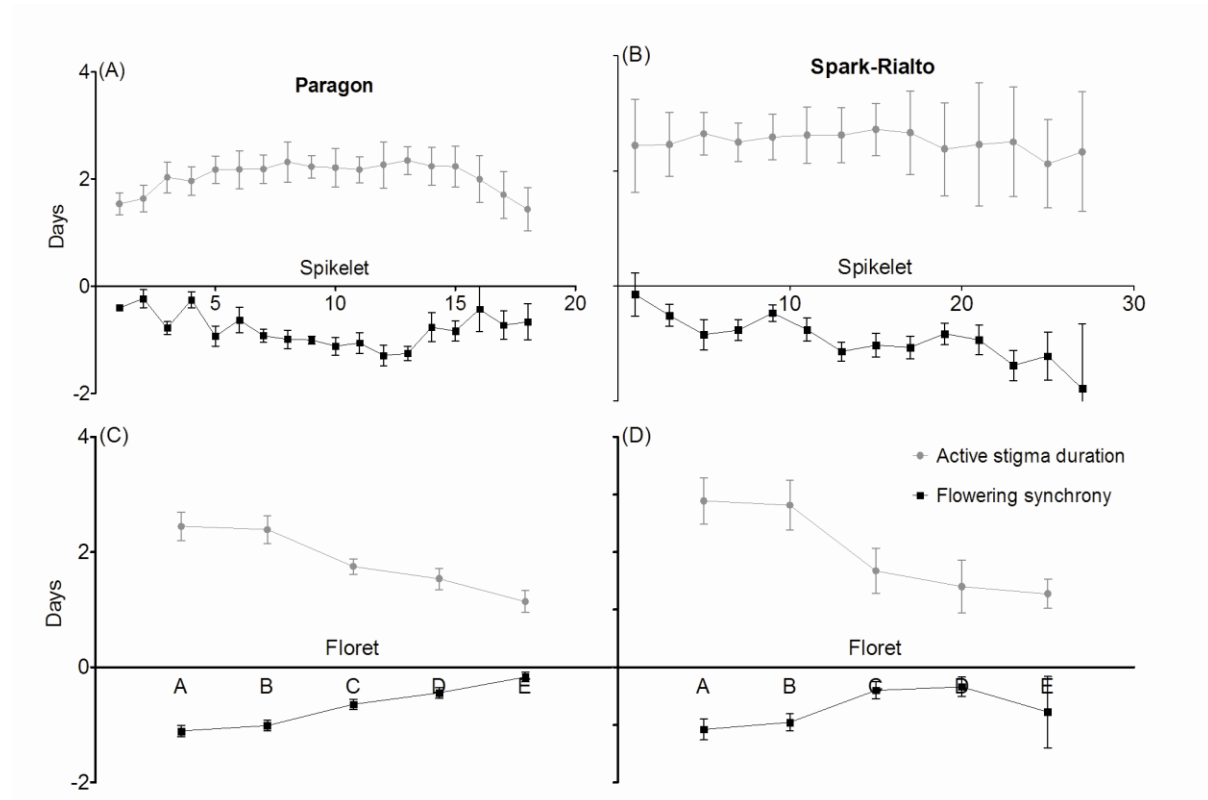
**Table 3.** Interactive effect of heat stress duration (in days) and number of tillers on the percentage of killed pollen in Paragon and Spark-Rialto.

Heat duration	Tillers	1	1-2	1-3	1-4	1-5	1	1-2	1-3	1-4	1-5
		Paragon					Spark-Rialto				
1		0%	0%	0%	0%	0%	8%	5%	3%	3%	2%
2		3%	2%	1%	1%	1%	23%	14%	10%	8%	7%
3		16%	9%	6%	4%	4%	45%	28%	21%	16%	14%
4		46%	27%	17%	14%	13%	67%	45%	33%	26%	22%
5		78%	53%	34%	29%	28%	84%	64%	47%	37%	32%
6		95%	76%	52%	46%	47%	92%	81%	62%	49%	42%
7		99%	91%	69%	65%	67%	95%	92%	75%	60%	51%

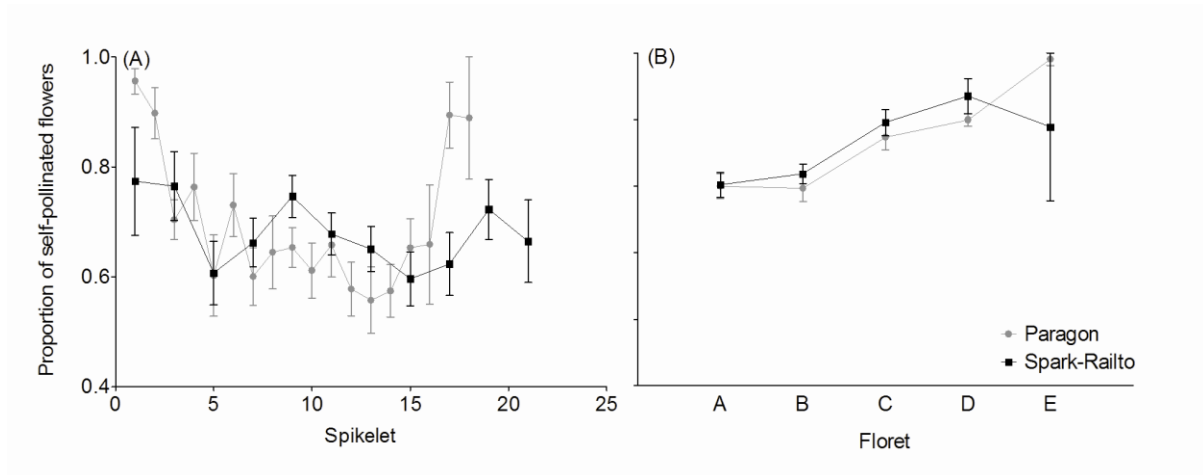
**Figure 1 a and b** – Frequency scatterplots of flowering synchrony calculated as the day of pollen dehiscence minus day of stigma collapse for Paragon (a) and Spark-Rialto lines (b). Negative synchrony values imply dehiscence before stigma collapse, zero on x-axis indicates the first day of flowering in a plant (N = 11 for Paragon and N = 32 for Spark-Rialto).



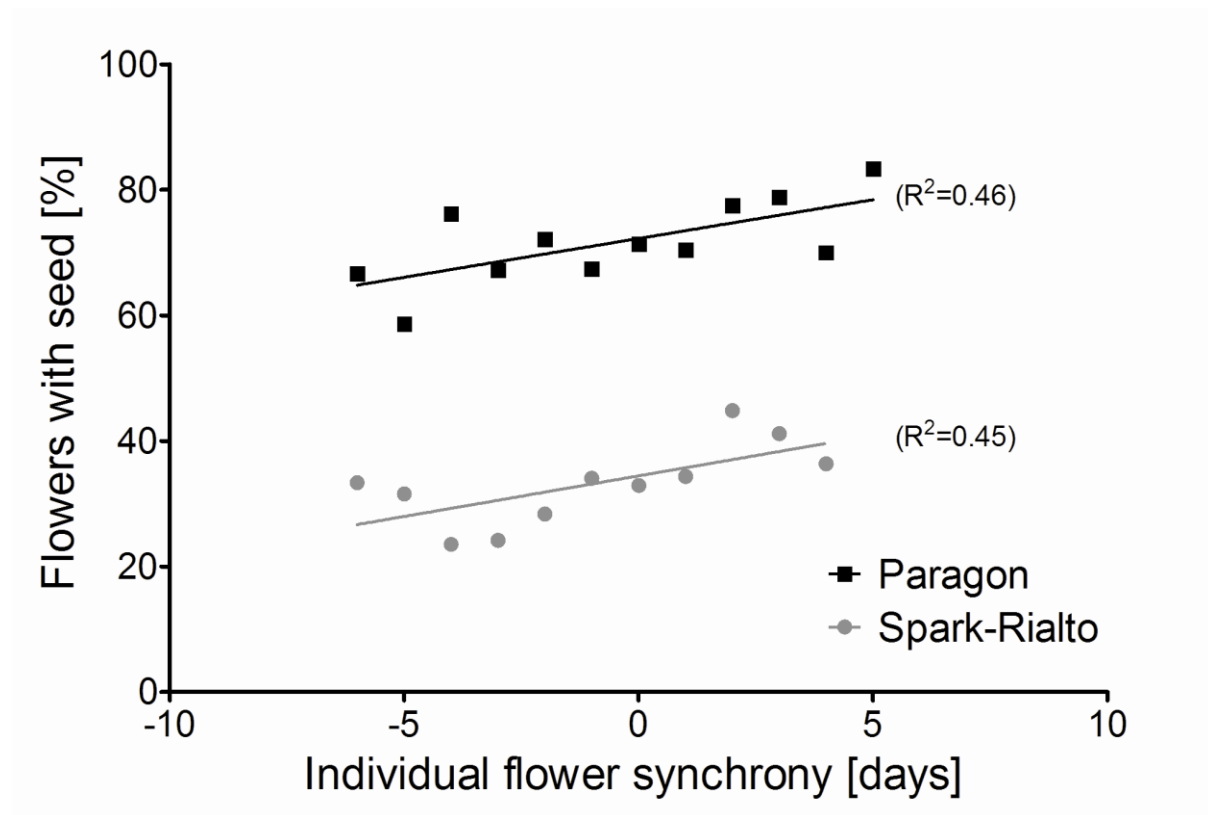
**Figure 2 a-d** – Average duration of active stigmas (grey circles) and mean flowering synchrony between male and female flowers of Paragon (N=11) and Spark-Rialto (N=26) NILs (black squares). Figures show duration and synchrony within spikelets (Figure 1A and B) and florets (Figure 1C and D, mean  $\pm$  SEM).



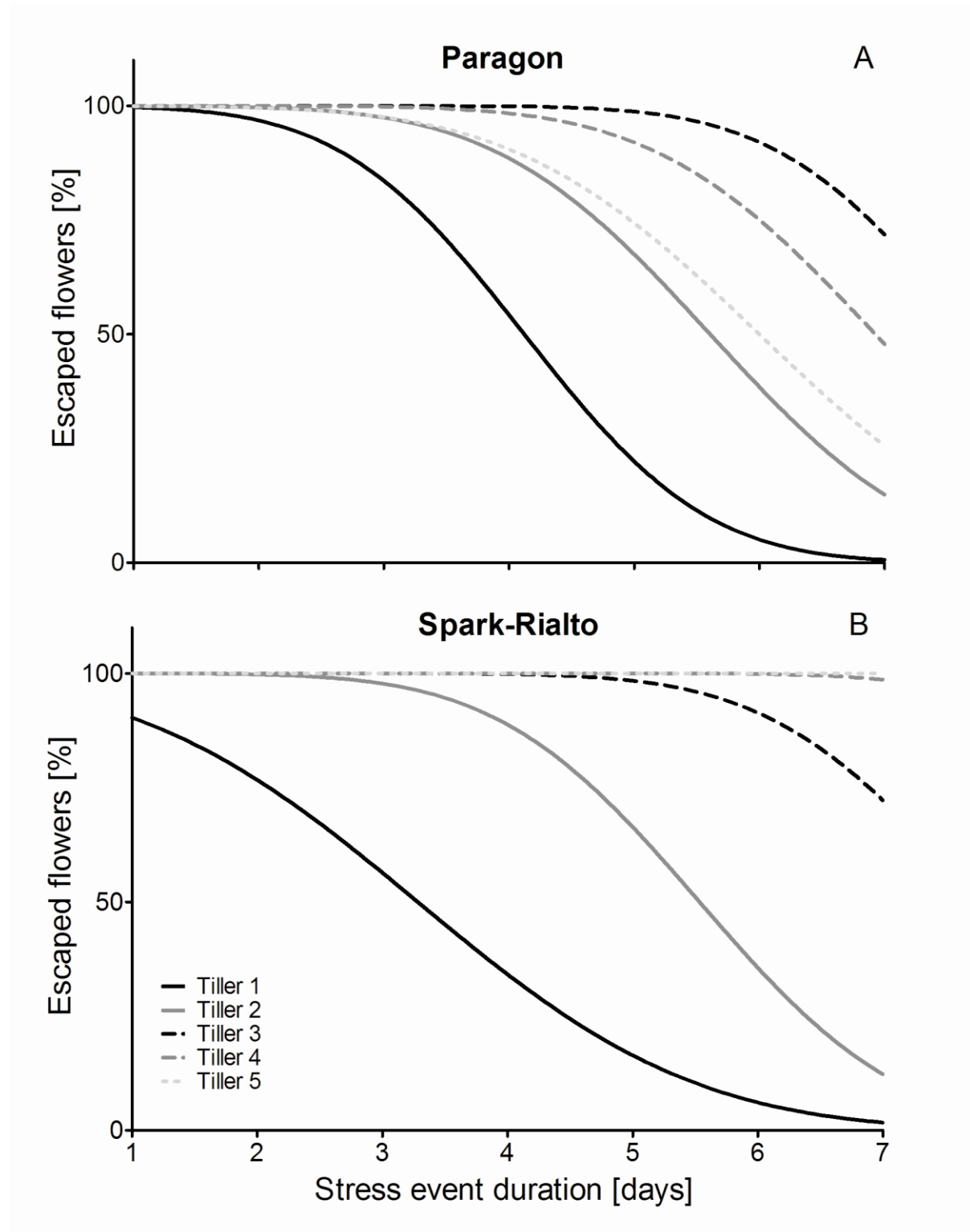
**Figure 3 a and b** – Ratio between the number of self-pollinated flowers and the total number of flowers where active stages of male and female flowers were observed. Self-pollinated flowers were defined as those where stigma collapsed within  $\pm 1$  day from pollen dehiscence. Mean values for Paragon lines (Figure 2A, N=11) and Spark-Rialto lines (Figure 2B, N=26) are shown.



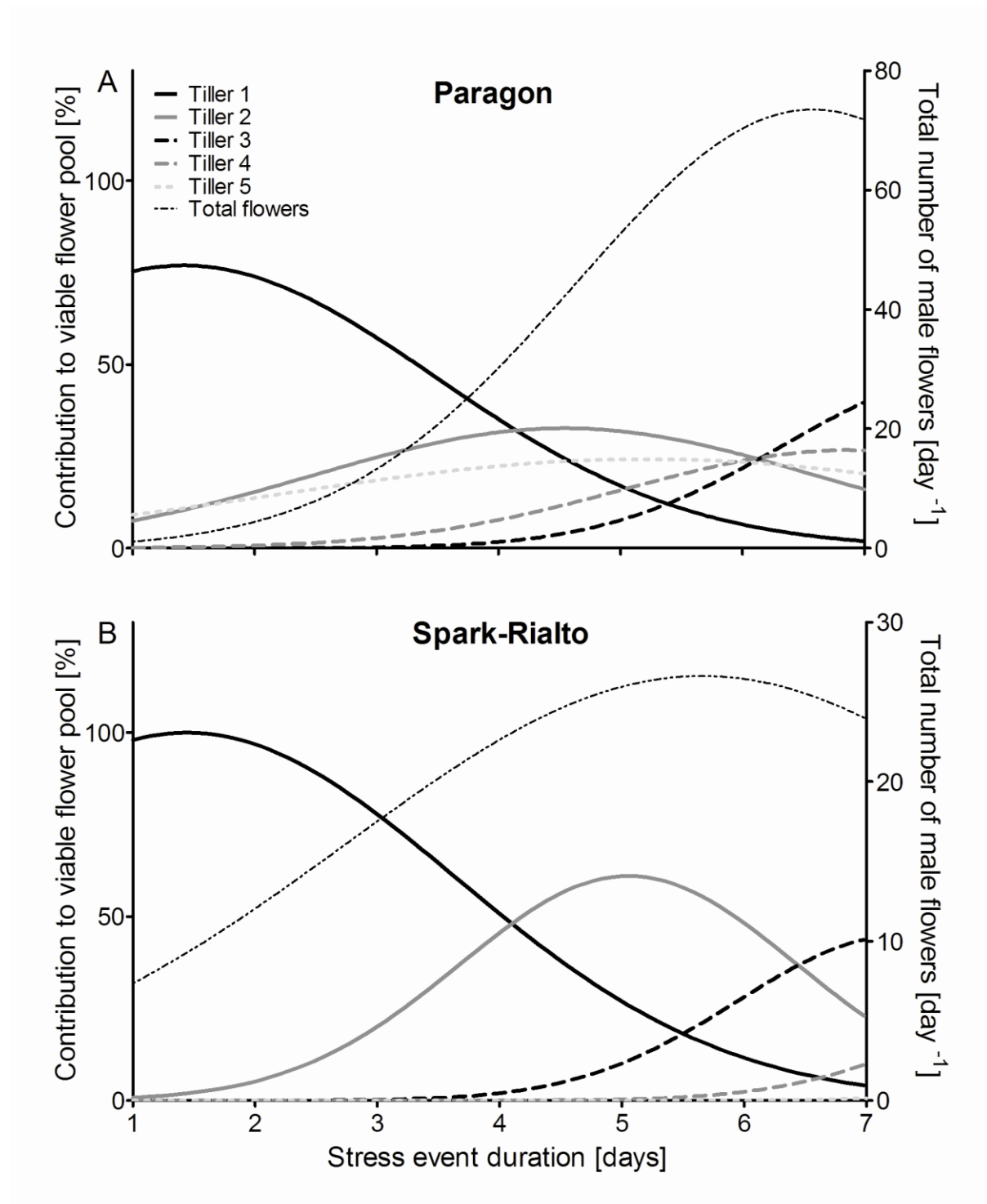
**Figure 4** – Individual flower synchrony affects the probability of successful seed set (P for trend equal to zero 0.015 in Paragon and 0.024 in Spark-Rialto). Positive values of flower synchrony indicate stigma collapse before pollen dehiscence, negative after dehiscence.



**Figure 5 a and b** – Effect of a hypothetical heat stress event on the number of surviving male flowers on 5 tillers of Paragon (A) and Spark-Rialto (B) lines. Active male flower numbers are determined by observed flower distribution patterns, heat stress event starts 1 s.d. prior to mean peak flowering on tiller 1. See table T1 for model description and parameter estimates.



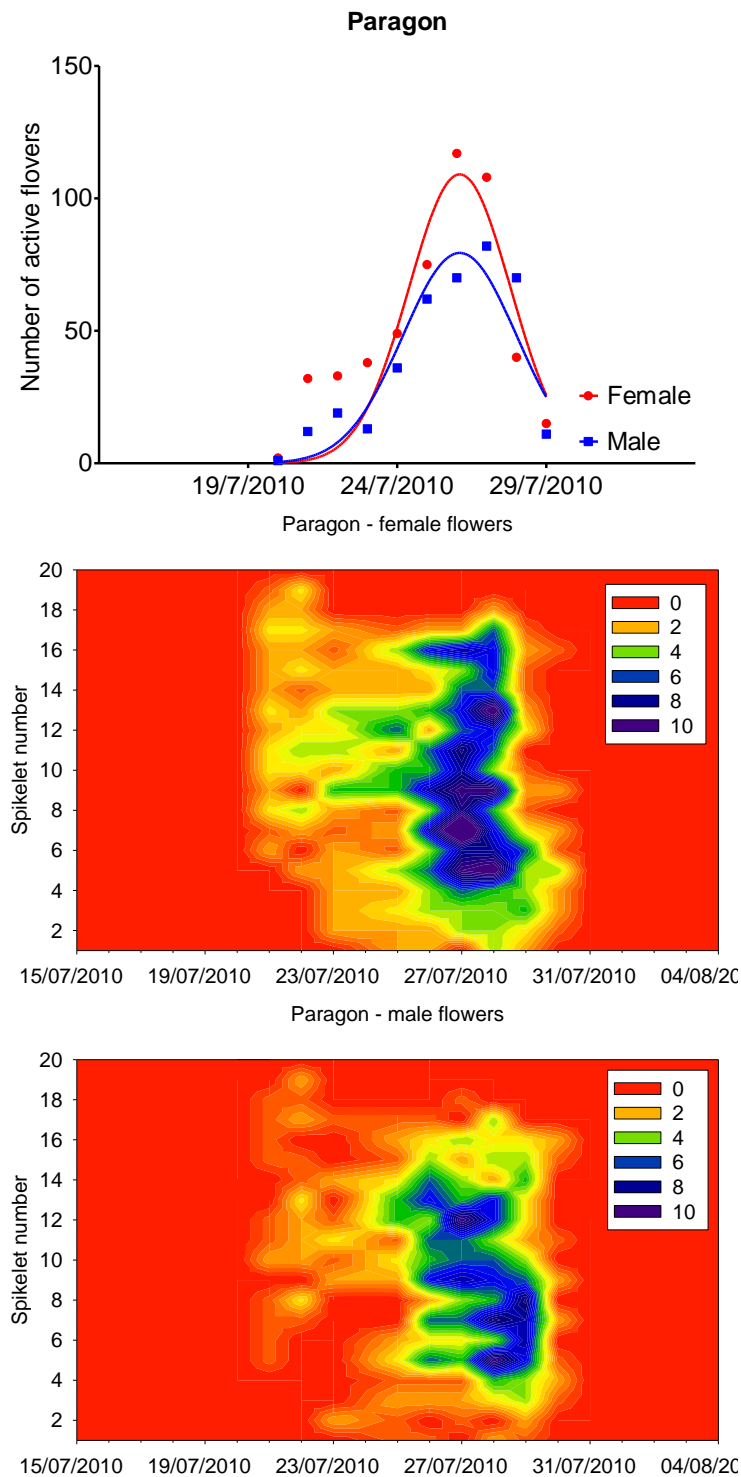
**Figure 6 a and b** – Relative contribution of surviving male flowers on each tiller to the total number of surviving flowers on a plant (left axis) and the total number of active male flowers per day (right axis), while x-axis represents the duration of a hypothetical heat stress event. See table T2 for model description and parameter estimates.



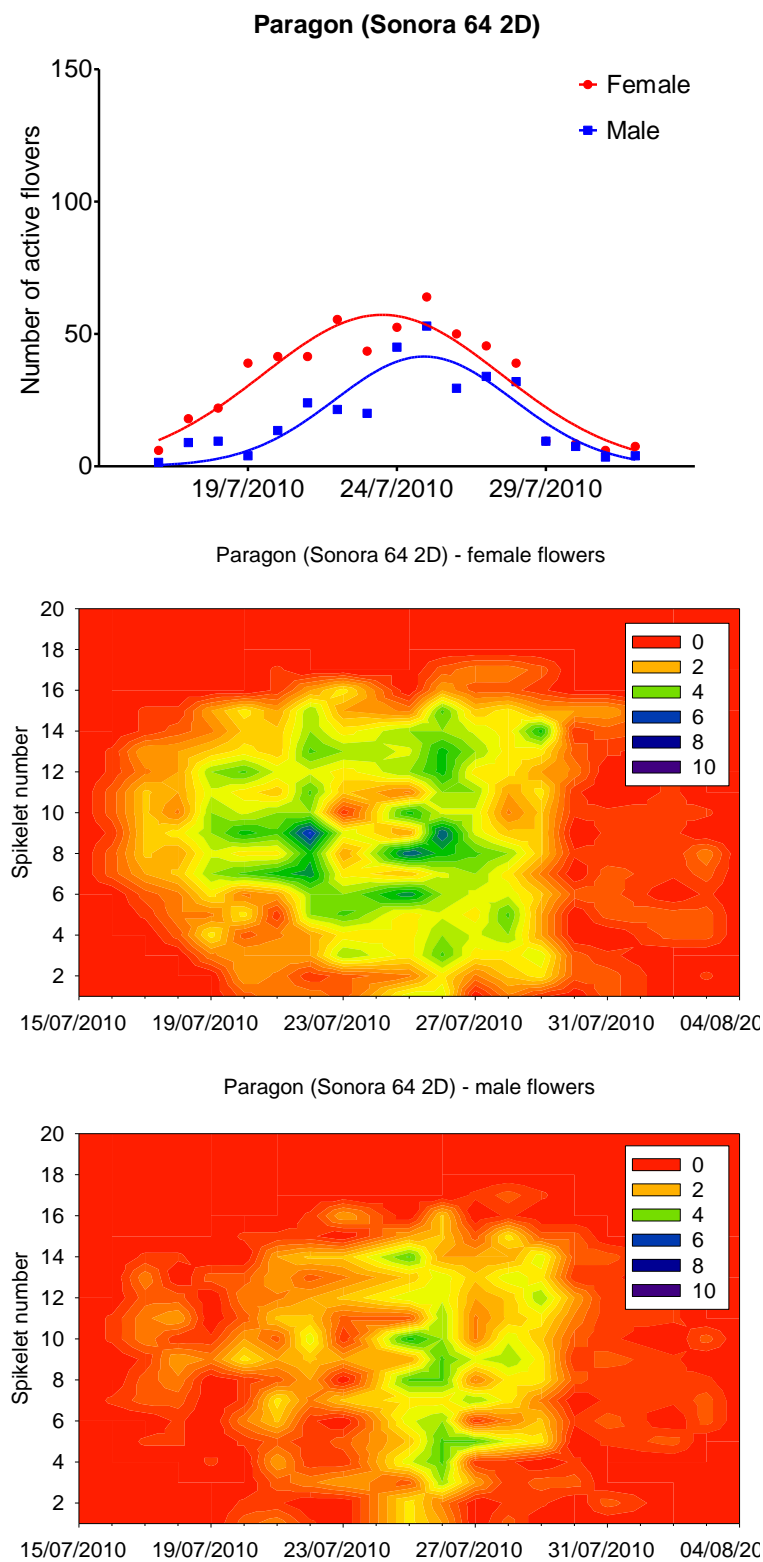


**Figures A1 to A3.** Flowering biology of three Paragon lines depicted as numbers of active male and female flowers. The values represent daily sums of pollen releasing anthers and receptive stigmas in each spikelet, aggregated for the first five tillers in a plant. Figures show contrasting flowering biology in the three selected lines; well defined and synchronised peak of male and female flowering activity (Figure A1), a wide temporal spread of both male and female flowering (Figure A2) and spread out but perfectly synchronised peaks of male and female active flowers (Figure A3).

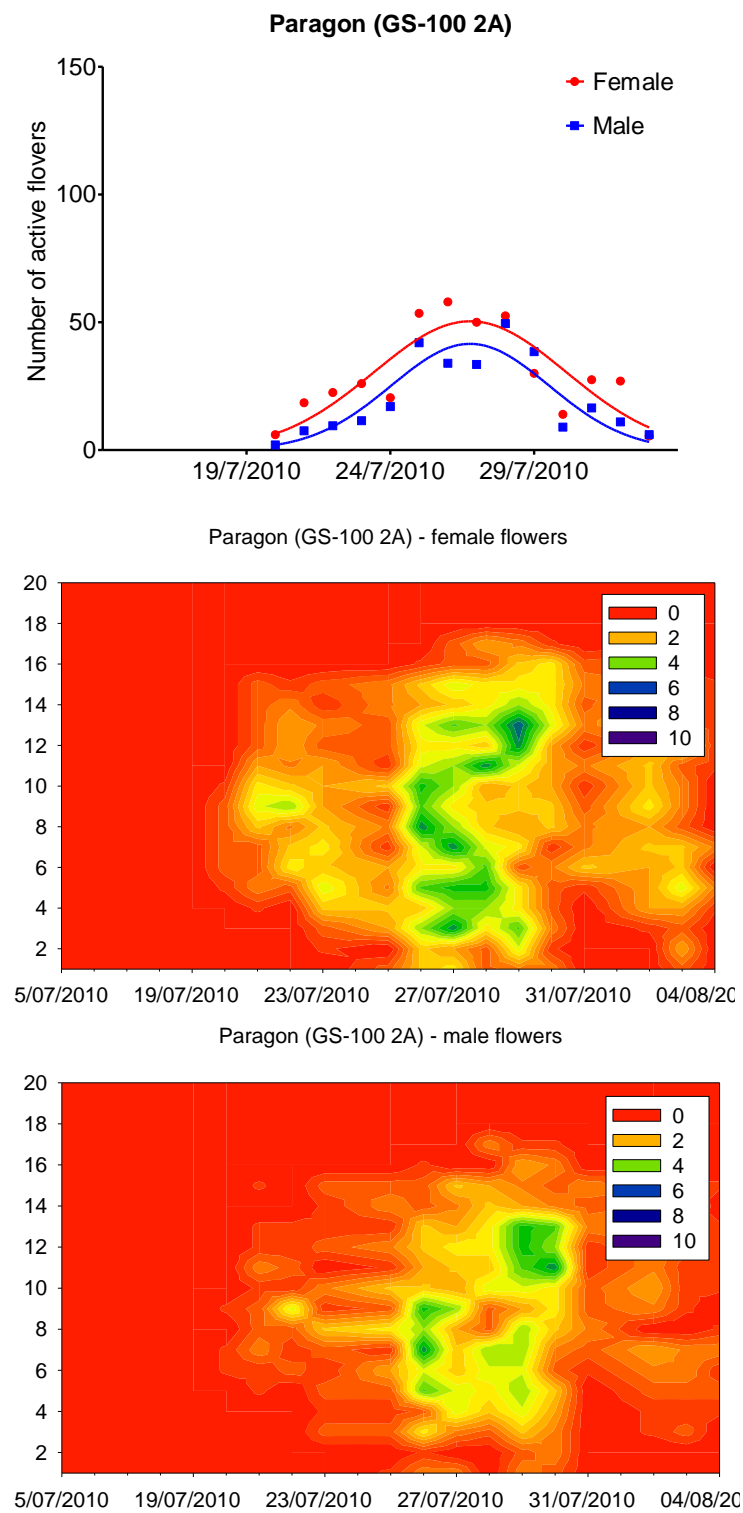
**Figure A1.**



**Figure A2.**

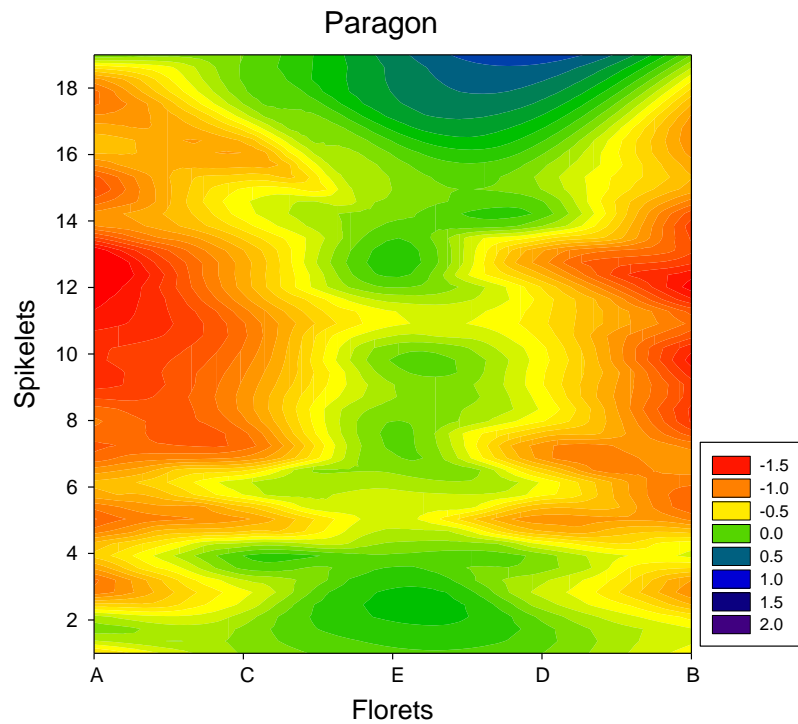


**Figure A3.**

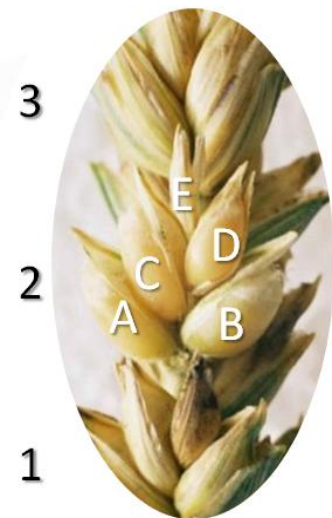
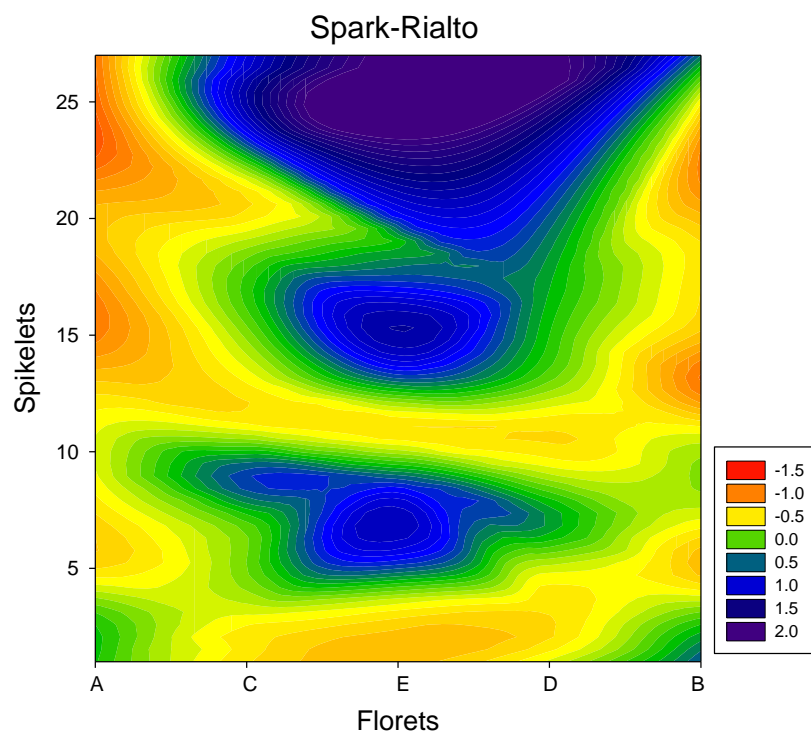


**Figures B1 and B2.** Mean synchrony between male and female flowers of Paragon (B1) and Spark-Rialto (B2) varieties. Flower synchrony was calculated as the difference between the day of pollen dehiscence and the day of stigma collapse. Negative values denote stigma collapse after pollen release from the same flower.

**Figure B1.**



**Figure B2.**



**Table T1.** Estimates of model parameters ( $\pm$  s.d.) describing the proportion of escaped flowers in Paragon and Spark-Rialto varieties. Reverse cumulative Gaussian distribution models were fitted into calculated percentages of flowers which escaped heat stress of specific length. The following formula was applied:

$$Y = \frac{1}{1 + \exp(-zdist \cdot (X - Mean) / SD)}$$

[Equation 1]

where  $Y$  is the % of flowers which escaped heat,  $zdist$  is one-tailed P value,  $X$  is the length of heat stress in days and  $Mean$  and  $SD$  are the parameters to be fitted.

		Tiller 1	Tiller 2	Tiller 3	Tiller 4	Tiller 5
Paragon	<i>Mean</i>	4.121	5.607	7.694	6.926	5.999
		(0.0002662)	(2.000e-005)	(7.616e-005)	(2.762e-005)	(6.974e-005)
	<i>SD</i>	1.146	1.338	1.204	1.372	1.527
		(0.0003765)	(2.986e-005)	(9.566e-005)	(4.694e-005)	(0.0001129)
	<i>R</i> <sup>2</sup>	1.000	1.000	1.000	1.000	1.000
Spark-Rialto	<i>Mean</i>	3.281	5.534	7.762	9.814	11.27
		(0.04933)	(3.161e-005)	(5.763e-005)	(6.667e-005)	(0.000)
	<i>SD</i>	1.759	1.263	1.292	1.286	1.232
		(0.07245)	(4.634e-005)	(7.042e-005)	(3.023e-005)	(0.000)
	<i>R</i> <sup>2</sup>	0.9973	1.000	1.000	1.000	1.000

**Table T2.** Estimates of model parameters ( $\pm$  s.d.) describing the contribution of individual tillers to the total active male flower pool in Paragon and Spark-Rialto varieties. Gaussian distribution models were fitted, where *Peak* denotes the flowering intensity, *Mean* is the location of the peak and *Variance* is the width of the distribution.

		Tiller 1	Tiller 2	Tiller 3	Tiller 4	Tiller 5
Paragon	<i>Peak</i>	76.98	32.69	46.64	26.71	24.28
		(1.897)	(1.719)	(0.2292)	(0.2060)	(1.425)
	<i>Mean</i>	1.415	4.539	7.848	6.867	5.209
		(0.1658)	(0.1312)	(0.01641)	(0.05050)	(0.3448)
	<i>Variance</i>	2.065	2.067	1.500	1.824	3.004
		(0.1349)	(0.1565)	(0.007674)	(0.03792)	(0.4386)
	<i>R</i> <sup>2</sup>	0.9954	0.9458	1.000	0.9997	0.8800
Spark-Rialto	<i>Peak</i>	99.98	61.06	44.83	39.24	39.32
		(1.214)	(0.5908)	(0.1644)	(0.5348)	(0.1348)
	<i>Mean</i>	1.444	5.054	7.274	9.337	11.14
		(0.08760)	(0.01552)	(0.01729)	(0.02222)	(0.008415)
	<i>Variance</i>	2.198	1.380	1.319	1.414	1.448
		(0.07085)	(0.01654)	(0.01080)	(0.02259)	(0.009111)
	<i>R</i> <sup>2</sup>	0.9988	0.9993	1.000	0.9981	0.9999