Fruit Number in Relation to Pollen Production and Viability in Groundnut Exposed to Short Episodes of Heat Stress

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Hot days and warm nights are important environmental factors limiting fruit yields of groundnuts in the semi-arid tropics. The objective of the present research was to quantify the effects of short episodes of heat stress on pollen production and viability, and fruit yield. Plants of cultivar ‘ICGV 86015’ were grown at a day/night temperature of 28/22 °C from sowing until 9 d after flowering. Cohorts of plants were then exposed to a factorial combination of four day (28, 34, 42 and 48 °C) and two night (22 and 28 °C) temperatures for 6 d. Thereafter, all plants were maintained at 28/22 °C until final harvest 9 d later. Number of flowers per plant (FN), the proportion of flowers setting pegs (fruit-set), the number of pegs and pods per plant (reproductive number, RN), pollen production per flower and pollen viability were determined during the 6 d stress period. There were strong negative linear relations between day temperature over the range of 28 to 48 °C and FN (slope, −1·1°C−1), fruit-set (−2·8 % °C−1), RN (−0·9°C−1), and pollen production (−390°C−1) and viability (−1·9 % °C−1). Warmer night temperature (28 to 22 °C) had no effect on FN, but reduced fruit-set (31 to 19%), RN (8 to 5), and pollen production (4380 to 2800) and viability (49 to 40%). There were no significant interactions between day and night temperature. Reduced fruit-set was a consequence of fewer pollen grains and reduced pollen viability. The threshold day temperature for pollen production and viability was 34 °C and there were strong negative linear relations between both pollen production and pollen viability and accumulated temperature > 34 °C.

Key words: Arachis hypogaea L., fruit-set, groundnut, heat-stress, peanut, pollen viability, pollen production, temperature.

INTRODUCTION

Groundnut (Arachis hypogaea L.) is an important oilseed and forage crop grown in the semi-arid tropics (SAT) of Africa and Asia. Heat- and/or drought-induced stresses are the major environmental factors limiting pod yields in the SAT (I.C.R.I.S.A.T, 1994). The optimum day/night temperature for vegetative and reproductive growth and development in groundnut ranges from 25/25 °C (Wood, 1968) to 30/26 °C (Cox, 1991) and from 25/20 °C (Wood, 1968) to 26/22 °C (Cox, 1991), respectively. Short episodes of hot days (> 35 °C) and warm nights (> 25 °C) are common in the SAT (e.g. in sub-Saharan Africa; Sivakumar, Masdoukia and Stern, 1993), and if these episodes coincide with critical stages of plant development they can be detrimental to seed production.

Studies in controlled environments have shown that both continuous hot days (35 °C), and short episodes of hot days (> 38 °C for 6 d), reduce the number of pegs and pods in groundnut (Ketring, 1984; Wheeler et al., 1991; Vara Prasad et al., 1998). Groundnut plants are particularly sensitive to hot days from 6 d before until 15 d after coming into flower, with maximum effects occurring 9 d after flowering (Vara Prasad et al., 1998). However, the mechanisms by which high temperatures reduce fruit number have not been identified. In grain legumes such as cowpea [Vigna unguiculata (L.) Walp.] and common bean (Phaseolus vulgaris L.), reduced fruit numbers at high temperatures are associated with a reduction in the number of flowers produced and in the proportion of flowers which set fruits (Konsens, Ofir and Kigel, 1991; Hall, 1992). In cowpea, reduced fruit-set was associated with poor pollen viability and reduced anther dehiscence (Mutters, Ferreira and Hall, 1989; Ahmed, Hall and DeMason, 1992), particularly when high temperatures were experienced at microsporogenesis (Warrag and Hall, 1984).

The objective of the research reported here was to determine and quantify the effects of short episodes of hot days and warm nights on pollen production and viability, and fruit number in groundnuts.

MATERIALS AND METHODS

The experiment was conducted between June and August 1997 in the controlled environment facilities of the Plant Environment Laboratory, Department of Agriculture, The University of Reading, UK (51°27’ N, 0°56’ W). It was undertaken in a polyethylene covered tunnel (poly-tunnel) maintained at near optimum day/night temperatures of 28/22 °C and in five modified Saxcii growth cabinets each maintained at a different temperature regime. The actual temperatures experienced in the different environments and
treatments throughout by transfers between cabinets.

The diurnal photo- and thermo-period in both the poly-tunnel and cabinets were coincident and equal at 12 h d⁻¹ (0800 to 2000 h). The photoperiod was controlled by a manually operated blackout facility in the poly-tunnel, and by automatic timer switches in the growth cabinets. Air temperatures were measured in the poly-tunnel and the cabinets using screened and aspirated copper constantan thermocouples positioned at the top of the plant canopy. Readings were taken at 10 s intervals and means were stored for successive 30 min periods using a data logger (Delta-T Devices Ltd, Cambridge, UK). Carbon dioxide concentration in the cabinets was maintained at 360 µmol mol⁻¹ of air. Relative humidity during the day was maintained close to 70 (± 5)% in the poly-tunnel using water sprinklers and ventilation. In the cabinets vapour pressure deficit during the day was maintained at 1-2 kPa by controlling relative humidity close to 70, 75, 85 and 90% in temperature regimes of 28, 34 and 48 °C, respectively. This was done either by removing the excess humidity by condensation, or adding moisture to air by passing through glycol maintained at a set temperature. The poly-tunnel transmitted 75% of incoming photosynthetically active radiation and photosynthetic photon flux density (PPFD) averaged 590 µmol m⁻² s⁻¹ during the experiment. The corresponding value of PPFD in each growth cabinet was 630 µmol m⁻² s⁻¹ obtained from a combination of cool white fluorescent and incandescent lamps (95 and 5% by wattage, respectively).

Temperature treatments

During the period from sowing until 9 d after first flower appearance (DAF), all plants were grown at a near optimum day/night temperature of 28/22 °C in the poly-tunnel. Thereafter, a factorial combination of four day (28, 34, 42 and 48 °C) and two night (22 and 28 °C) temperature treatments were imposed for 6 d by transferring plants to growth cabinets. The growth cabinets were maintained at day/night temperatures of 28/22, 28/28, 34/22, 42/22 and 48/22 °C (Table 1). The 34/28, 42/28 and 48/28 °C temperature treatments were imposed by transferring plants at 2000 h from 34/22, 42/22 and 48/22 °C to 28/28 °C, and back again at 0800 h each day. After the 6 d stress period in the cabinets, all plants were returned to the poly-tunnel maintained at 28/22 °C, where they remained until final harvest at 24 DAF. Plants maintained in the 28/22 °C cabinet for the 6 d treatment period served as controls.

Cultivar and plant husbandry

Uniform seeds of the Spanish botanical type (A. hypogaea subsp. fastigiata) 1CGV 86015 were selected and treated with Apron Combi 453 FS (Ciba, Agriculture, Cambridge, UK) as a precautionary measure against seed-borne diseases. Seeds were pre-germinated at 25 °C on moist filter paper in Petri dishes kept in the dark for 2 d until the radicles emerged. The germinated seeds were then sown on 3 Jun. 1997, one per 2.51 pot at a depth of 2.5 cm. The sides of pots were covered with aluminium foil to reduce radiative heating. The rooting medium comprised sand, gravel, vermiculite and leafless peat compost mixed in proportions of 4:2:2:1, by volume, respectively. A commercial controlled-release fertilizer (0.15 kg kg⁻¹ N, 0.10 kg kg⁻¹ P, 0.12 kg kg⁻¹ K, 0.02 kg kg⁻¹ MgO plus trace elements; Osmocote Plus, Scotts UK Ltd., UK) was incorporated into the mixture at the manufacturer’s recommended rate of 5 g l⁻¹. Seeds were not inoculated with rhizobia and plants were dependent on inorganic nitrogen. All pots were soaked with tap water and allowed to drain for 24 h before sowing; thereafter they were irrigated as necessary through an automatic drip irrigation system in the poly-tunnel or were hand-watered during the 6 d period in the cabinets. There were no disease problems and sporadic pest infestations were controlled by releases of the predators Phytoseius persimilis A.-Henriot against red spider mite (Tetranychus urticae Koch) and Amblyseius cucumeris Oudemans against thrips (Thrips tabaci Lindeman).

The experiment was sown with six replicates of each temperature treatment and with 12 replicates for the controls. Only uniform plants that flowered on same day (28 d after sowing) were selected and transferred to cabinets to remove any confounding effects of time of flowering, which gave four replicates of each temperature treatment and eight replicates of the controls. A subset of four plants from the control treatment were harvested at 18 DAF to estimate the numbers of pegs and pods produced from those flowers which had opened before the imposition of the target heat stress treatments.

Table 1. Mean day and night air temperatures (°C) in the poly-tunnel from planting to 36 d after sowing (DAS) and from 43 to 53 DAS, and in the eight 6 d temperature treatments imposed in the growth cabinets between 37 to 42 DAS

<table>
<thead>
<tr>
<th>Time (DAS)</th>
<th>Location</th>
<th>Target temperature (day/night °C)</th>
<th>Actual mean air temperature (day/night °C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 to 36</td>
<td>Poly-tunnel</td>
<td>28/22</td>
<td>28/22</td>
</tr>
<tr>
<td>37 to 42</td>
<td>Cabinets</td>
<td>28/22 (control)</td>
<td>28/22</td>
</tr>
<tr>
<td>42/22</td>
<td></td>
<td>34/28*</td>
<td>34/28</td>
</tr>
<tr>
<td>48/22</td>
<td></td>
<td>42/28*</td>
<td>42/28</td>
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<tr>
<td>28/28</td>
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<td>48/28</td>
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<td>34/28*</td>
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<td>48/28*</td>
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<td>28/28</td>
</tr>
<tr>
<td>43 to 53</td>
<td>Poly-tunnel</td>
<td></td>
<td>28/22</td>
</tr>
</tbody>
</table>

* Obtained by transfers between cabinets.
(24 DAF). At both harvests the numbers of pegs and pods per plant were counted and the dry weights of roots, stems (including petioles), leaves, pegs, and pods were determined after oven-drying at 80 °C for 3 d.

Individual flowers were collected between 0800 and 0815 h each day during the 6 d stress period in all the temperature regimes to count the number of pollen grains and to test pollen viability. The number of pollen grains per flower was counted using a haemocytometer (Fisher Scientific, Pittsburgh, USA) as described by Kearns and Inouye (1993) and Freshney (1994). The viability of the pollen was determined by the reaction of grains with triphenyl tetrazolium chloride as described by Kearns and Inouye (1993). Anthers were collected before dehiscence and were split open on a glass slide and stained. Pollen grains that stained red were classified as viable, whereas those that remained transparent were classified as dead. The numbers of viable and non-viable grains were counted.

**Data analysis**

The fate of individual flowers (i.e. whether they produced a peg or a pod) was not monitored and so the total number of pegs and pods at final harvest, hereafter referred to as the final reproductive number (RNf), had been produced from flowers opened at 28 °C (i.e. in the poly-tunnel) and in the 6 d temperature treatments in the cabinets. In order to determine the fate of those flowers which opened during the 6 d temperature treatment, it was assumed that for the cultivar 'ICGV 86015' the time from flower opening to peg appearance was 9 d (unpubl. res.). Therefore, all flowers that opened and were fertilized between the onset of flowering and 9 DAF should have produced a peg or a pod by the time the first harvest was taken at 15 DAF. The number of pegs and pods at this time is referred to as the initial reproductive number (RNi). Similarly, any flower that opened between the end of the temperature treatments (15 DAF) and the final harvest 9 d later (24 DAF), should not have formed a peg. Therefore, the numbers of pegs and pods arising from those flowers that opened during the 6 d temperature treatment (the treatment reproductive number, RNt) were estimated as the difference between RNi and RNf. Fruit-set during the temperature treatments was calculated as the ratio of RNf to the cumulative number of flowers produced during the 6 d temperature treatment (FN). Values of pollen viability and fruit-set were subject to angular transformation before analysis to ensure homogeneity of variances.

The effects of day and night temperature on FN, fruit-set, RNf, and pollen production and viability were examined by comparing linear regressions (Mead, Curnow and Hasted, 1993) using routines in Genstat 5 (Genstat 5 Committee, 1997).

**RESULTS**

There was no significant difference among plants in the number of flowers produced (19 ± 2.5) before the start of the heat stress treatments at 9 DAF and the number of pegs and pods produced from those flowers was 11 (s.d. ± 1.8).

**Table 2. Effect of night temperature on the cumulative number of flowers opened during the 6 d stress period (FN), the proportion of those flowers which set pegs or pods (fruit-set, angular transformed), and the number of pegs and pods (RN), and pollen production per flower and proportion of viable pollen (angular transformed) on day 6 of the stress period in groundnut**

<table>
<thead>
<tr>
<th>Trait</th>
<th>22</th>
<th>28</th>
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<tbody>
<tr>
<td>FN (per plant)</td>
<td>17.8 (± 2.21)</td>
<td>17.7 (± 2.55)</td>
</tr>
<tr>
<td>Fruit-set (%)</td>
<td>31.0 (± 3.39)</td>
<td>19.5 (± 3.00)</td>
</tr>
<tr>
<td>RNf (per plant)</td>
<td>7.7 (± 0.80)</td>
<td>5.0 (± 1.02)</td>
</tr>
<tr>
<td>Pollen production per flower</td>
<td>4389 (± 65.0)</td>
<td>2800 (± 267)</td>
</tr>
<tr>
<td>Pollen viability (%)</td>
<td>48.6 (± 3.03)</td>
<td>40.1 (± 2.02)</td>
</tr>
</tbody>
</table>

Data are the means (± s.e.) of four day temperature treatments and four replicates.

**Figure 1. Relationship between mean day temperature (°C) and number of flowers opened during the 6 d stress period (FN) (A), proportion of those flowers which set pegs or pods (fruit-set, angular transformed) (B), and number of pegs and pods (RNf) (C) of groundnut ‘ICGV 86015’ grown at night temperatures of 22 °C (●) or 28 °C (○). Fitted lines: A, $y = 58.20(± 5.16) - 107.7(± 0.133)x$, $r^2 = 0.93$, $P < 0.01$; B, at 22 °C: $y = 137.8(± 14.2) - 281.7(± 0.36)x$, $r^2 = 0.93$, $P < 0.01$ and at 28 °C: $y = 123.5(± 14.3) - 281.7(± 0.36)x$, $r^2 = 0.92$, $P < 0.01$; and C, at 22 °C: $y = 40.21(± 1.81) - 0.856(± 0.046)x$, $r^2 = 0.98$, $P < 0.01$ and at 28 °C: $y = 36.42(± 0.66) - 0.856(± 0.046)x$, $r^2 = 0.98$, $P < 0.01$. Bars denote s.e. and are shown where they exceed the size of the symbol.
Number of pegs and pods per plant (RN), as for fruit-set, was also affected by day ($P < 0.001$) and night ($P < 0.01$) temperature, but not by their interaction ($P > 0.10$), and so the response of RN to temperature was also described by two parallel lines (Fig. 1C). Thus, as day temperature increased from 28 to 48 °C, RN was reduced by 0.9 per plant °C⁻¹ at both 22 and 28 °C night temperature. Overall, warm nights reduced RN, from 7.7 to 5.0 per plant (Table 2). The ceiling temperature was 47 and 42.5 °C at 22 and 28 °C night temperature, respectively.

Pollen production and viability

There were strong positive linear relationships between fruit-set and both pollen production ($r^2 = 0.95$; Fig. 2A) and pollen viability ($r^2 = 0.94$; Fig. 2B) indicating that day and night temperature had similar effects on pollen production and pollen viability (both measured on day 6 of the stress period) as on fruit-set and RN (Fig. 2, Table 2). Pollen production and viability were reduced by 390 per flower °C⁻¹ and 1.9 °C⁻¹ at both 22 and 28 °C night temperature, respectively, as day temperature increased from 28 to 48 °C. Warmer nights reduced mean pollen numbers from 4389 to 2800 per flower and mean pollen viability from 49 to 40% (Table 2). The ceiling day temperature for pollen production and viability was 49 and 67 °C, respectively.

The viability of pollen grains is determined during the early stages of floral bud development (De Beer, 1963) and the effects of temperature on pollen viability vary with stage of pollen development (Sugiyama, Iwahori and Takahashi, 1996). In the present work, the effects of day and night temperature on pollen production and viability varied over time, but without interaction, and therefore the mean effects of night temperature are presented in Fig. 3 to illustrate these trends. The respective values for pollen production
and viability at 28 and 34 °C day temperatures were similar and constant during the 6 d stress period. At 42 °C, pollen production and viability were significantly reduced after 3 and 4 d of exposure, respectively, and after 6 d only 1524 pollen grains per flower were produced compared with 7346 at 28 °C (Fig. 3A). However, the viability of the pollen grains produced at 42 °C remained moderate at 43% (Fig. 3B). At 48 °C, pollen production and viability were lower after 3 d of exposure, and no flowers were produced after 4 d. The effects of 22 and 28 °C night temperatures on pollen production and viability followed a similar trend, with values at 28 °C being consistently smaller than those at 22 °C (Table 2). Clearly, pollen production is relatively more sensitive to hot days and warm nights than pollen viability.

**DISCUSSION**

Warmer days and nights, but not their interaction, significantly reduced RN, fruit-set, RN, and pollen production and viability. The fact that these responses were all well described by simple linear regressions over the range from 28 to 48 °C should be useful in the modelling of temperature responses in groundnut. The effects of temperature on RN, reported here agree with findings of other studies in controlled environments (Wood, 1968; Cox, 1979; Ketring, 1984). For example, Ketring (1984) reported that a day/night temperature regime of 35/22 °C relative to 30/22 °C reduced fruit numbers by 33%, similar to the reduction of 35% at 34/22 °C relative to 28/22 °C in the present study. The reduction in RN, at hot days and warm nights in the present study was mainly due to the production of fewer flowers and a reduction in the proportion of flowers which set fruits. This finding confirms that a day temperature of 35 °C is supraoptimal for fruit production in groundnuts, even under well watered conditions.

De Beer (1963) reported that the number of pollen grains of groundnut 'Schwarz 21' was reduced by 71%, from 3388 to 987 grains per flower when the constant day and night temperature was increased from 24 to 33 °C, and no viable pollen was produced at 33 °C. This is consistent with a 69% reduction, from 8156 to 2500 grains per flower, when mean temperature was increased from 25 °C (28/22 °C) to 32 °C (42/22 °C), and a 93% reduction when mean temperature was increased to 35 °C (42/28 °C). The variation in pollen production per flower in optimal environments may have been due to cultivar differences (Palmer, Albertsen and Heer, 19/8), diurnal variation in the temperatures or the length of the stamens (Trivedi and Verma, 1975). Other studies on cowpea (Ahmed et al., 1992) and common bean (Gross and Kigel, 1994) have also shown that heat stress reduces fruit-set. In cowpea, poor fruit-set in hot nights (30 °C) was associated with impaired anther dehiscence and reduced pollen viability (Warrag and Hall, 1984). This is also the case in groundnuts, as shown by the strong and significant positive relations between fruit-set and both pollen production and pollen viability (Fig. 2). Similarly, studies in tomato have shown that both fruit weight and number, and fruit set, were decreased as daily mean temperature increased from 25 to 29 °C (Peet, Willlits and Gardner, 1997). This was associated with the effects of heat stress on pollen development and pollen release (Peet, Sato and Gardner, 1998).

Hot days (> 34 °C) reduced pollen production and viability only after 3 d of exposure (Fig. 3). This suggests that groundnut flowers are either sensitive to hot days and warm nights at a specific stage 3 to 4 d before flower opening, or that floral buds need to be exposed to day temperatures > 34 °C for more than 2 d to elicit effects on pollen production and pollen viability. To test these hypotheses, the pollen number data presented in Fig. 3A were re-plotted against accumulated day temperature > 34 °C (Fig. 4). There was a strong and significant \( r^2 = 0.88, n = 18; P < 0.001 \) negative relationship between pollen production and day temperature, such that pollen number was reduced by 164 grains per flower °C⁻¹ of accumulated temperature > 34 °C. A comparison of regressions at 22 or 28 °C night temperatures revealed no significant difference in the values of slope \( (P > 0.10) \) or intercept \( (P > 0.10) \) and so a common line described the data. No pollen grains were produced when flower buds accumulated > 55 °Cd above 34 °C. Pollen viability was also linearly related to cumulative temperature > 34 °C \( \left[ y = 72.4(± 3.0) – 0.54(± 0.1)x; r^2 = 0.65; P < 0.01; n = 16 \right] \), although, as noted previously, the effects of high temperature on pollen viability were not as marked as those on pollen number. We conclude that pollen production and viability are determined by the cumulative effects of temperature above a critical value, not by temperature effects at a specific stage of development before flower opening.

The precise mechanisms which reduce pollen production and viability in groundnut are not known. In cowpea, reductions in pollen viability and poor anther dehiscence reflect the premature degeneration of the tapetal layer (Ahmed et al., 1992), which plays an important role in microsporogenesis (Echtin, 19/1). Recent studies on groundnut have shown that flower buds are particularly sensitive to heat stress at a stage 3 to 5 d before anthesis (Talwar, 1997), which is known to coincide with microsporogenesis (Xi, 1991). Studies on common bean (Gross and Kigel, 1994) have also shown that microsporogenesis is sensitive to heat stress.

**Fig. 4.** Relationship between pollen production per flower and cumulative day temperature (> 34 °C) after the start of the 6 d temperature treatment at a night temperature of 22 (○) and 28 °C (●). Fitted line: \( y = 9055(± 490) – 164(± 14.2)x; r^2 = 0.88, n = 18, P < 0.001 \).
In the present study, research plants were not given the opportunity to acclimate to high temperatures and the transition from optimum to high temperature was rapid. Seasonal effects may be gradual and some plants may have the ability to acclimate to high temperature. Further research in groundnut is clearly needed to understand these effects of acclimation and mechanisms responsible for reduced pollen production and viability.

In summary, this research has shown that short episodes (6 d) of hot days (> 34 °C) and/or warm nights (28 °C) reduced flower production and fruit-set, and hence the number of fruits in groundnut. Reduced fruit-set was a consequence of fewer pollen grains and poor pollen viability. The threshold or critical day temperature for pollen production and viability was 34 °C and there was a strong linear negative relationship between both pollen production and pollen viability and accumulated temperature > 34 °C.

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LITERATURE CITED


