Mirid feeding preference as influenced by light and temperature mediated changes in plant nutrient concentration in cocoa

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[1]G.K. Awudzi*, [2]P. Hadley, [3]P.E. Hatcher, [2]A.J. Daymond.

[1]Cocoa Research Institute of Ghana (CRIG), Box 8, New Tafo-Akim, Ghana. [2]School of Agriculture Policy and Development, University of Reading, Whiteknights, Reading, RG6 6AR, UK. [3]School of Biological Sciences, University of Reading, Whiteknights, Reading, RG6 6AS.

Corresponding author*: godfred.awudzi@crig.org.gh ; anthocyanin22@yahoo.com

Abstract

Cocoa mirids are the most important insect pests of cocoa in West Africa. This study investigated the effect of environmental parameters that are modulated by overhead shade, i.e. light intensity and temperature, on nutrient and phenolic concentrations in cocoa and their subsequent effect on mirid feeding. Eight-month-old cocoa seedlings were maintained for 50 days in two growth chambers set to day temperatures of 25^{o}C or 30^{o}C. Each chamber had sections with different light intensities (541, 365 and 181 µmolm^{-2}s^{-1} PAR). For the field studies at Akim-Tafo in Ghana, eight-month-old plants of three cocoa clones were subjected to shaded (PAR= 180 µmol m^{-2} s^{-1}, between 11:00 and 12:00) and unshaded (PAR= 1767 µmol m^{-2} s^{-1} between 11:00 and 12:00) treatments for 50 days after which nutrient measurements and mirid choice tests were carried out. No significant effect of environment was observed on the phenolic concentration of stems under controlled environment chamber conditions. However, in the field, the phenolic concentration of stems was significantly greater for unshaded compared with shaded plants (P=0.04). Under controlled conditions, the leaf nitrogen concentration increased slightly with light intensity (P=0.003). The same trend was seen in stems but only at 30^{o}C. In the field, the impact of overhead shade on nitrogen varied between cocoa clones. The concentration of carbohydrates in both leaves and stems in the field was higher under unshaded conditions. When subjected to feeding tests, stems from unshaded cocoa had significantly more mirid feeding lesions (P=0.003) after 24 hours exposure to mirids compared to shaded cocoa. Mirid feeding therefore appears not to be deterred by the higher phenolic levels but rather there was a preference for cocoa tissue grown under unshaded conditions. These findings highlight the need to consider the growing environment of cocoa clones when screening for varieties with resistance to mirids.

Key words: cocoa, mirids, phenolics, plant nutrient, choice-test

1.0 Introduction

Plants have evolved mechanisms over time to reduce insect feeding. Many plant secondary metabolites are known to affect the feeding, growth and oviposition of insects (Halkier and Du, 1997, Ossipov et al., 2001, Lattanzio et al., 2009). Such plant defence compounds include proteinase inhibitors, which inhibit digestion of proteins in insects thereby causing retarded growth and may eventually result in insect mortalities due to starvation (Stotz et al., 1999). As a group, the mirid species, *Sahlbergella singularis* Haglund and *Distantiella theobroma* (Distant) (both Hemiptera: Miridae), are the most important insect pests on cocoa (*Theobroma cacao*) in West Africa. Since plant phenolics and nutrients influence insect herbivory in a number of plant species (Dudt and Shure, 1994, Duffey and Stout, 1996, Lattanzio et al., 2009), understanding the effects of environmental factors on plant nutrient concentration and plant defence compounds could aid mirid management on cocoa farms.

Campbell (1984) reported that knowledge of mirid nutrient requirements and defence compounds produced by cocoa against mirids is limited and this still remains the case today. Specifically, there is little information on the relationship between soluble carbohydrates in tissues and mirid feeding or the extent to which phenolics might deter feeding. On the other hand, nitrogen is suggested to be a limiting factor as feeding by mirids on cocoa tissue with a high nitrogen concentration has been associated with an increase in weight and overall growth of mirids as compared with mirids on nitrogen poor diets (Entwistle, 1972). Anikwe (2010) also showed that *Sahlbergella singularis* preferred cocoa pods that had high protein concentration. This might explain, in part, why fertilizer application generally has been associated with an increase in insect feeding (White, 1984, Thompson and Hagen, 1999, Lee et al., 2003) since nitrogen concentration would be expected to be higher in the leaves, chupons and young unhardened stems making them a preferred choice over food sources with a lower nitrogen concentration (Altieri and Nicholls, 2003).

Mirids are known to prefer unshaded areas of cocoa farms, where they create extensive damage referred to as pockets (Padi and Owusu, 1998, Bigger, 1981, Entwistle, 1985, Awudzi et al., 2009, Babin et al., 2010). High solar radiation in unshaded areas of cocoa farms or portions with a break in the shade canopy enhances photosynthetic rate and vegetative growth of the cocoa trees (Bos et al., 2007, Babin et al., 2010). These new shoots provide feeding and breeding sites which sustain mirid growth and development. The quality and quantity of light has also been reported to affect nutrient concentrations in plant tissues,

which has a consequent influence on insect feeding (Bryant et al., 1983, Dudt and Shure, 1994). However, the extent to which environmentally induced changes in cocoa tissue nutrient concentrations and defence compounds might impact on mirid feeding is not known.

Here, we hypothesise that different concentrations of defence compounds and/or nutrients in the leaves and stems of shaded compared with unshaded cocoa affect the feeding preference of mirids. The impact of environmental factors that are modulated by shade, i.e. temperature and light on defence compounds and nutrients were studied through a combination of controlled environment and field studies.

2.0 Materials and methods

2.1 Controlled environment experiment

Eight-month-old seedlings (variety: Amelonado) from the International Cocoa Quarantine Centre, at the University of Reading were used. Seedlings selected were those whose new leaves were just about to emerge (flush). Plants were grown in pots (volume 800ml) and the potting medium used was an inert mixture of sand, gravel and vermiculite (1:2:2 v:v). They were fed daily with a modified Long Ashton nutrient solution developed for cocoa (End, 1990) with pH maintained between 5.5 and 5.7 and an electrical conductivity of 2mS. Two walk-in growth chambers were used (dimensions, 3.2m long 2.5m wide and 1.8m high; Fitotron WEISS Gallenkamp, Loughborough, UK). The chambers were set to provide two different day temperatures (30^{o}C or 25^{o}C day ± 0.5 ^{o}C) with a common night temperature (22^{o}C± 0.5 ^{o}C) and a 12-hour day length to mimic tropical daylength. Each chamber was sub-divided into sections to give three different light intensities: 541 µmol m^{-2} s^{-1}; 365 µmol m^{-2} $s^{-1;}$ and at 181 µmol m^{-2} s^{-1} photosynthetically active radiation (PAR). Light was provided by fluorescent lamps (MASTER/TL/D/Reflex-58W/840/1SL, Philips) and their intensities were adjusted with a dimmer switch. PAR in each treatment was measured with a LI-COR quantum sensor (LI-191SA; LI-COR, Lincoln, NE 68504, USA) attached to a quantum flux meter (Skye Instruments, Llandrindod Wells).

PAR was measured periodically, to note any changes incident on the plants as they grew taller; values recorded on day 30 were as follows: 550 µmol m^{-2} s^{-1}; 380µmol m^{-2} s^{-1} and 185 µmol m^{-2} s^{-1}. Even though plants grew taller as the experiment progressed, the resultant increases in PAR at the shoot apex were relatively small. The experiment was carried out for 50 days in a split plot design with temperature as the main plot and light intensity as subplots

with 5 plants in each treatment. The last six fully expanded leaves and stem cuttings were removed from plants from all treatments after day 50 and kept at -20 ^{o}C until required for analysis. These samples were later ground in liquid nitrogen and stored at -80 ^{o}C for subsequent analysis. Tissue concentrations of total phenolics, nitrogen and carbohydrates were determined on three replicate stem and leaf samples under each light and temperature treatment.

2.2 Field experiment

An experiment to study the effect of solar radiation and temperature on the nutrient and total phenolic concentration in cocoa stems and leaves was carried out in the field at the Cocoa Research Institute of Ghana (CRIG), Akim-Tafo (latitude 06° 13'N, longitude 0°22'W), in the Eastern Region of Ghana. The cocoa clones used were: CATIE 1000, IMC 67 and T 85/799, originally sourced from the International Cocoa Quarantine Centre, University of Reading, UK. Eight-month-old clonal plants in pots containing loamy soil were transplanted into field plots at a spacing of 2m x 2m in shaded and unshaded treatments with 5 replicate plants per clone per treatment. Shade was provided by shade cloths and plants were watered daily in the mornings at 8:00am. Plants were maintained for 6 months after which stem cuttings and the last six fully expanded leaves were sampled for nitrogen, soluble carbohydrates and total phenolic concentration. Three replicates of stem cuttings and leaf samples were taken from each treatment.

Measurements of light quantity and quality were taken between the hours of 11:00am and 12:00 noon under the shaded and unshaded conditions over 5 days and averaged. A light meter (Skye Instruments, Llandrindod Wells) fitted with a LI-COR light sensor was used to measure PAR, whilst UV radiation (UVA & UVB) was measured with a UV meter (Solartech Inc. Solar meter model 5.7, UK). Temperature and relative humidity measurements were recorded with miniature data loggers (Gemini Tiny Tags, UK) placed in Stevenson screens, set to log at 30 minutes intervals, for 5 days and averaged. Total phenolics, nitrogen and carbohydrates were determined in leaf and stem samples. The experiment was an un-replicated split plot design with shade regime as main plots and cocoa clones as sub-plots in replicates of five. The field experiment was carried out from February to July, 2012 and the whole experiment was repeated between August 2012 and January 2013.

Total phenolics extraction and analysis

Total phenolic concentration of samples taken from both the controlled environment and field studies were determined using a method described by Singleton and Rossi (1965), using Folin-Ciocalteu as the reactive reagent on samples ground while frozen under liquid nitrogen. Preparation of the calibration curve for total phenolic concentration determination was carried out using gallic acid at a concentration of 0.5g/500ml and diluted serially 8 times. The total phenolic concentration was expressed as Gallic acid equivalents (GAE).

Nitrogen analysis

Nitrogen concentration of dried ground samples from the controlled environment studies was determined by a micro-Kjeldhal method. This analysis was carried out by the Farm Advisory Services Team (FAST), Faversham, UK. Samples were subjected to sulphuric acid/selenium digest followed by dilution and analysis through a Foss Fiastar 5000 Flow Analysis Injection analyser. The digested solution was made highly alkaline by merging with a sodium hydroxide stream, which releases ammonia gas that permeates a gas permeable membrane and into an indicator stream. The intensity of the colour produced was read photometrically at 590nm and the concentration of ammonium nitrogen was read against a calibration curve.

Determination of nitrogen concentration for field samples in Ghana was carried out using a modified form of the Kjeldhal method as described by Bremner and Mulvaney (1982).

Carbohydrate analysis

The carbohydrate concentration of ground stem and leaf samples taken from the controlled environment experiment was determined using the method described by Yemm and Willis (1954) with anthrone as a reagent. The green colour produced when carbohydrates are heated with anthrone in acid solution is the basis for this test. The carbohydrate concentration in field samples was determined by the method described by Dubois et al. (1956). This method is based on the reaction between simple sugars and phenol and concentrated sulphuric acid, which generates a yellow-orange colour. Different methods for carbohydrate extraction had to be used for the controlled environment and field experiments as the same equipment was not available in both places. Thus, we do not compare absolute carbohydrate values between the two sets of data. However, de Toledo et al. (2012) demonstrated the different methods measure the same type of carbohydrates and give comparable results.

2.3 Mirid feeding preference test for cocoa clones (choice test)

Stem cuttings were taken from different clones to evaluate their attractiveness (defined as a combination of attraction and antixenosis) to mirids after exposure to either shaded and unshaded treatments in the field for six months in Ghana using the method described by N' Guessan et al. (2008). Healthy young twigs of each of the three cocoa clones from the shaded and unshaded treatments in the field experiment were cut into 5-cm sections and arranged randomly each time with each piece touching another in Petri dishes forming a hexagon of six sections. Cuttings were selected from plants of the same age and similar size at the mid-sections with similar circumference. Adult mirids were collected from CRIG plots at Tafo with hybrid cocoa and reared on chupons and pods in an insectary as described by Babin et al. (2008). One 4^{th} instar (nymph which has just developed wing buds) *S. singularis* mirid nymph of the next generation, starved for 24 hours to the time of screening, was placed in the middle of each Petri dish and the number of feeding lesions on stem cuttings counted and recorded after 24hrs. The test was conducted twice with 8 replicates on each occasion making a total of 16 cuttings per clone * shade treatment. Petri dishes were placed on insectary benches to obtain uniform distribution of light on test materials at an average room temperature of 25^{o}C.

3.0 Statistical analysis

The differences in the concentration of nitrogen, carbohydrate and phenolics in samples as a result of the different treatments under both controlled and field conditions were determined using an ANOVA. In the mirid feeding preference tests, the impact of shaded and unshaded treatments as well as the different cocoa clones on mirid feeding was also analysed by means of ANOVA. For the field experiment, the analysis was performed on the combined data of the two repeated experiments since initial analysis showed no significant differences in the repeated experiments for phenolics, nitrogen, carbohydrate concentrations and mirid feeding preference. Data was analysed with GenStat version 11.

4.0 Results

4.1 Controlled environment

4.1.1 Total phenolics

There was a non-significant trend of a reduction in phenolic concentration in stems with an increase in PAR (P=0.06) or temperature (P=0.79). There was also no significant effect of

light (P=0.9) or temperature (P=0.64) on the total phenolic concentration in leaf samples measured (data not shown).

4.1.2 Nitrogen

The nitrogen concentration of leaves was significantly greater in plants grown under higher light intensity (P=0.003) (Fig. 1 A). A significant interaction of light and temperature was observed on percentage nitrogen in stems (P=0.05) (Fig. 1 B). Stem nitrogen concentration of plants grown at 30^{o}C increased with increasing light intensity. However, this trend was not observed at 25^{o}C. As with leaves, stems under the highest light level also had the greatest percentage nitrogen (P=0.04) while temperature had no significant effect (P=0.28).

Figure 1 here

4.1.3 Soluble carbohydrates

A significant interaction of light and temperature on soluble carbohydrate concentration of cocoa leaves was observed (P=0.04) such that an effect of temperature (P=0.04) (Fig. 2A) was only observed at a PAR of 365 µmol m^{-2} s^{-1} (where carbohydrate concentration was higher at 25°C). There was also a significant interaction of light and temperature on the carbohydrate concentration of stems (P=0.03) whereby carbohydrate concentration was higher at 30°C at PAR levels of 181 and 365 µmol m^{-2} s^{-1} but no significant differences between temperatures were evident at the highest PAR (Fig. 2 B).

Figure 2 here

4.2 Field experiment

4.1. Microenvironment

UVA radiation was significantly higher in the unshaded treatment (mean=0.40 mw cm^{-2}) relative to shade treatment (mean=2.4 mw cm^{-2}) ($P<0.001$; Lsd= 0.18). UVB radiation was also significantly higher under the unshaded treatment (mean=289.67 µw cm^{-2}) than the shaded treatment (mean= 27.33 µw cm^{-2}) ($P<0.001$; Lsd=3.5). PAR (between 11:00 and 12:00) measured under unshaded conditions was significantly greater (mean= 1767 µmol m^{-2} s^{-1}) compared with the shade treatment (180 µmol m^{-2} s^{-1}) ($P<0.001$; Lsd=122.7). Day time mean temperature under unshaded conditions (mean=32 ^{o}C) was significantly greater than that measured under shade (mean= 25 ^{o}C) ($P=0.01$; Lsd=4.14), whilst there was no significant difference in relative humidity measured in unshaded (mean=57%) compared with the shaded treatment (62%) ($P=0.08$; Lsd=6).

4.2.1 Total phenolics

A significant interaction between clone and shade treatments was observed in the concentration of total phenolics in leaves ($P=0.03$). For all three cocoa clones, the concentration of phenolics was higher under non-shaded conditions but the magnitude of the difference was not consistent across clones (Fig**.** 3A). The difference between the phenolic concentration of unshaded and shaded IMC 67 was greater than 18mg g^{-1} while for CATIE 1000 and T85/799, the differences were approximately 12mg/g and 7mg/g, respectively. Phenolic concentration in stems was also influenced by the shade treatments ($P=0.04$) (Fig. 3 B). There was a significant effect of shade on the phenolic concentration of CATIE 1000 (higher under no shade conditions) but not on the other two clones. In all, the phenolic concentration of leaves (mean=89 mg/g) was significantly greater than that in stems (42 mg/g) ($P<0.001$; Lsd= 8.6).

Figure 3 here

4.2.2 Nitrogen

There was a significant interaction between shade treatments and clone on the nitrogen concentration of stems ($P=0.01$). The effect of shade was significant only for CATIE 1000 and IMC 67. However, the direction of response was inconsistent as under the shaded

condition the nitrogen concentration of CATIE 1000 in stems was significantly greater than in unshaded trees while the reverse was observed for IMC 67 (Fig. 4).

Figure 4 here

4.2.3 Soluble carbohydrates

Carbohydrate concentration of leaves was significantly influenced by clone ($P<0.001$) as well as by shade treatments ($P<0.001$) (Fig. 5A). Carbohydrate concentration was greater in unshaded conditions and highest for IMC 67 (25mg/g). There was no significant interaction between clone type and shade treatments. It can be seen from Figure 5B that there is a significant interaction between shade treatments and clones on carbohydrate concentration in stems ($P=0.01$). In all cases, carbohydrate concentration was greater under the unshaded treatment but the magnitude of the difference was greatest for CATIE 1000.

Figure 5 here

4.2.4 Mirid preference test for cocoa clones

Stem cuttings from unshaded cocoa clones had significantly ($P=0.003$) more mirid feeding lesions after 24hrs exposure to previously starved 4th instar mirids compared to stem cuttings from shaded cocoa clones (Fig. 6). The effect of shade on mirid feeding preference was greater for IMC 67 and T 85/799 than for CATIE 1000. There was however no significant effect of clone on mirid feeding preference.

Figure 6 here

5.0 Discussion

Most phytophagous insects have a narrow range of host plants on which they feed. This host range is often limited by the presence or absence of chemical (secondary metabolites) or physical feeding stimulants or deterrents. Such chemical stimulants or deterrents are usually complex in nature and may have more than one function depending on the plant species in question (Close and McArthur, 2002, Lattanzio et al., 2009). Plant phenolic compounds, an example of such secondary metabolites, are found mainly in the epidermis and its appendages and may act as the first line of defence absorbing the harmful UV region of the light spectrum (Caldwell et al., 1983, Grammatikopoulos et al., 1999, van Emden, 1966). However, phenolic compounds may have some other important functions. They are reported to function as antifungal agents and due to their bitter taste, are considered as potential feeding deterrents to

insect herbivores (Matern and Kneusel, 1988, Bernays et al., 1989, Berenbaum, 1995, Haukioja et al., 2002). On the other hand, nutrients such as nitrogen and carbohydrates have been reported to enhance insect growth and development (van Emden, 1966, Waring and Cobb, 1992, Entwistle, 1985). This study sought to clarify the effect of light and temperature on plant nutrients and phenolic compounds in cocoa, thereby potentially providing some understanding as to why mirids prefer unshaded to shaded cocoa. Mirid numbers increase under shaded cocoa when there is a break in the canopy permitting more light into the crop canopy (Padi and Owusu, 1998, Babin et al., 2010).

Differences in phenolic concentration of leaves and stems observed under different light and temperature treatments under controlled environment and shaded and unshaded cocoa in the field experiment suggest that light and temperature influences nutrients and phenolic concentrations in leaves and stems of cocoa. Under controlled conditions, there was a trend of increasing concentration of total phenolic compounds in young cocoa stems as PAR levels decreased. This result was different from that observed in the field where significantly more phenolic compounds were measured in unshaded compared to shaded cocoa. The difference in the quality and quantity of light that plants were exposed to could explain the difference in results obtained between controlled and field experiments. In the field, plants were subjected to a broader spectrum of light and high levels of UVA and UVB were measured, which are reported to influence the phenolic synthesis pathway in plants (Hatcher and Paul, 1994, Zavala et al., 2001). However, UV light was absent in fluorescent tubes used in providing light under the controlled environment experiment. As mirids preferred feeding on twigs kept under unshaded conditions with relatively high phenolic concentrations as observed in the mirid preference tests, high phenolic concentration of stems however, does not appear to be a major deterrent to mirid feeding. These results suggest that, whilst phenolic compounds in cocoa could provide protection against photo-damage from harmful rays from the sun they do not necessarily act as defence against insect herbivory. This is in agreement with the report of Close and McArthur (2002) as they concluded that plant phenolic compounds do not necessarily provide defence against insect herbivory, but rather provide protection from photo-damage. However, the results are not consistent with the report of Dudt and Shure (1994) that slow growing dogwood under shade produce more phenolics to act as feeding deterrents as they are unable to grow rapidly enough to recover from pest damage. Hatcher and Paul (1994) highlighted the risk in attributing changes in insect feeding preference only

to the effect of phenolic compounds and other plant secondary metabolites. It would appear from our results that, for cocoa, the presence of phenolics are not a major deterrent to insect feeding. Another hypothesis may be that mirids have evolved to be able to metabolize phenolic compounds.

The observation of higher levels of nitrogen in the controlled environment study with increased light intensity was not experienced in the field. Moreover, the direction of response to light conditions differed between cocoa genotypes. Entwistle (1972) and Anikwe *et al*. (2009) have reported enhanced mirid feeding and development under high levels of nitrogen. White et al. (1984), Ohmart *et al*. (1985) and Myers (1981) also reported enhanced insect (*Glycaspis* spp.) growth and activity under conditions that increased the amount of nitrogen available to insects in their food. Even though mirids preferred twigs obtained from unshaded conditions in the choice test, nitrogen level was only higher for one clone (IMC 67) under such conditions. Thus, the results did not produce conclusive evidence of an effect of nitrogen concentration of cocoa stem tissues on mirid feeding preference. A reduction in nitrogen in some plant species is related to an increase in carbon-based phenolic compounds (Kytö et al., 1996). Keski-Saari and Julkunen-Tiitto (2003) demonstrated that the concentration of phenolics was higher in different parts of juvenile mountain birch plants (*Betula pubescens* ssp. *czerepanovii* (N.I. Orlova) Hämet-Ahti) at lower levels of nitrogen than at moderate nitrogen level. However, in the present study the effect of the variation in cocoa nitrogen concentrations on the level of phenolics was not consistent across clones.

Carbohydrate concentrations of leaves and stems in the field were higher under unshaded compared with shaded conditions. This could be attributed to enhanced photosynthetic activity and hence greater carbohydrate production under high light intensities. High concentrations of carbohydrates in young shoots/stems under no shade may be a reason why mirids prefer unshaded to shaded cocoa. The tender nature of such young stems with high moisture content may also be a reason why mirids prefer them to older shoots/stems. The fact that mirids preferentially fed on cocoa grown under unshaded conditions with higher carbohydrate concentrations suggests that nutrient concentration could be an important determinant of mirid feeding activity on cocoa. As there were no significant effect of clone on the number of mirid feeding lesions, the exposure of cocoa plants to different environmental conditions was the critical factor determining mirid feeding preference. The fact that nutrient status appears to impact on mirid feeding preference could explain

inconsistencies in reporting of which cocoa clones are resistant to mirid damage across West Africa. Mirid resistant clones in one country have been reported to be susceptible in another (N'Guessan et al., 2008, Anikwe et al., 2009). The effects of prevailing environmental conditions and hence stem carbohydrate levels are usually not considered when clones are tested. However, our results show that, in some cases, environmental conditions may override inherent genotypic factors that might incur pest resistance. Therefore, it is important that when screening for mirid resistance the cocoa clones should be grown and tested under a range of uniform conditions.

Conclusion

Light intensities and temperature both had an impact on nitrogen and carbohydrate concentrations in cocoa tissues, whilst UV radiation was associated with an increase in phenolics. Since mirids preferentially fed on cocoa stems that were higher in phenolics and nutrients, it is concluded that phenolics do not deter mirid feeding but that higher nutrient concentration, specifically carbohydrates, provides a plausible explanation for preferential feeding.

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Figure legends

Figure 1: Effect of light and temperature on percentage nitrogen concentration in the leaves (A) and stems (B) of young cocoa. Each bar represents a mean of three replicates.

Figure 2: Effect of PAR and temperature on carbohydrate concentration in cocoa leaves (A) and stems (B). Each bar represents a mean of three replicates. Note difference in scales between A & B.

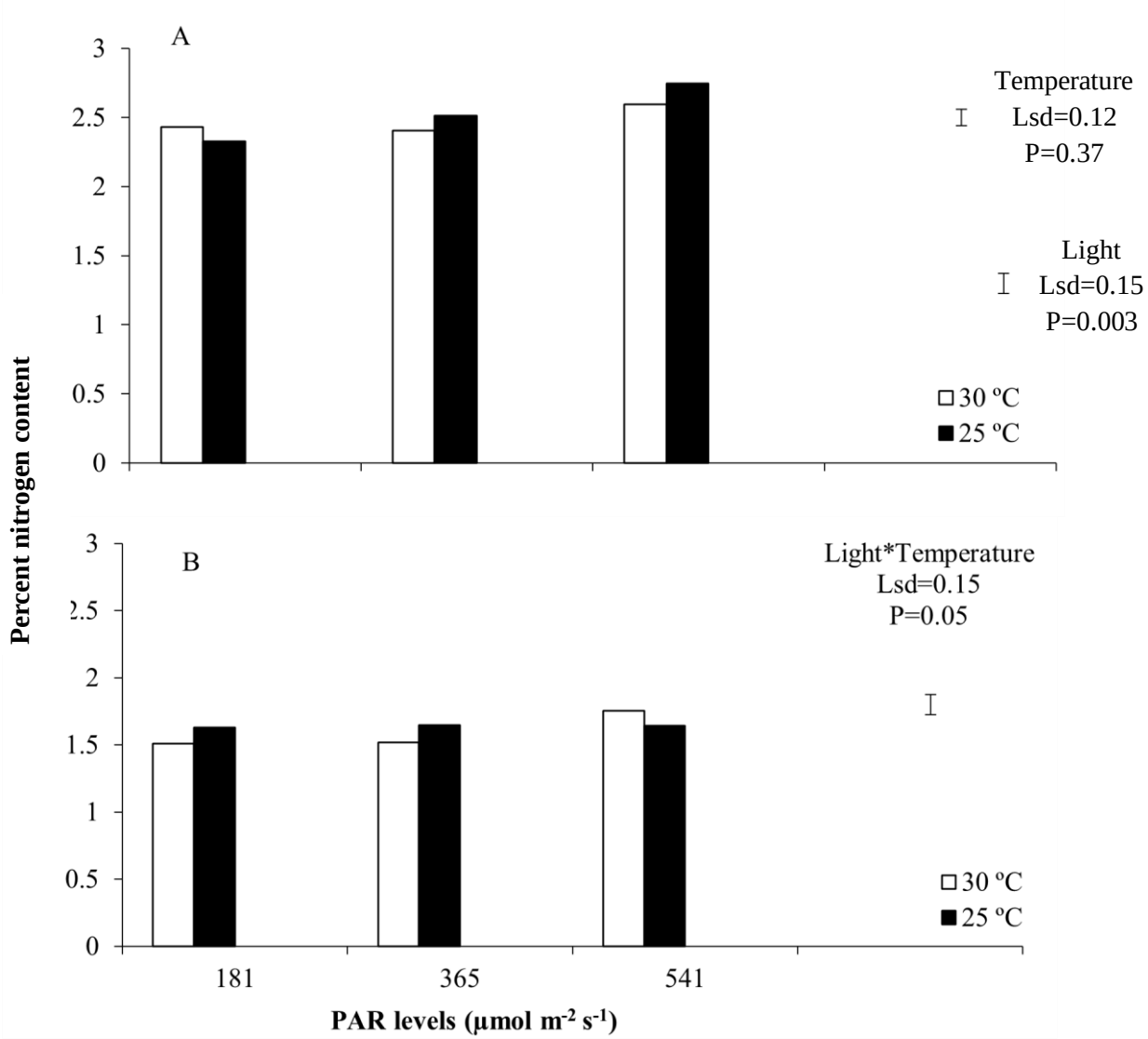
Figure 3: The interaction of shade treatments and clone on total phenolic concentration of young leaves (A) and stems (B). Note the difference in scales. Each bar represents a mean of six replicates.

Figure 4: The interaction of shade and unshaded treatments and clone types on nitrogen concentration of stems. Each bar represents a mean of six replicates.

Figure 5: Interaction between shade treatments and clone type on soluble carbohydrate concentration in the leaves (A) and stems (B) of young cocoa. Each bar represents a mean of six replicates.

Figure 6: Mirid feeding preference on stem cuttings from shaded and unshaded cocoa. Each bar represents a mean of sixteen replicates.

Figures



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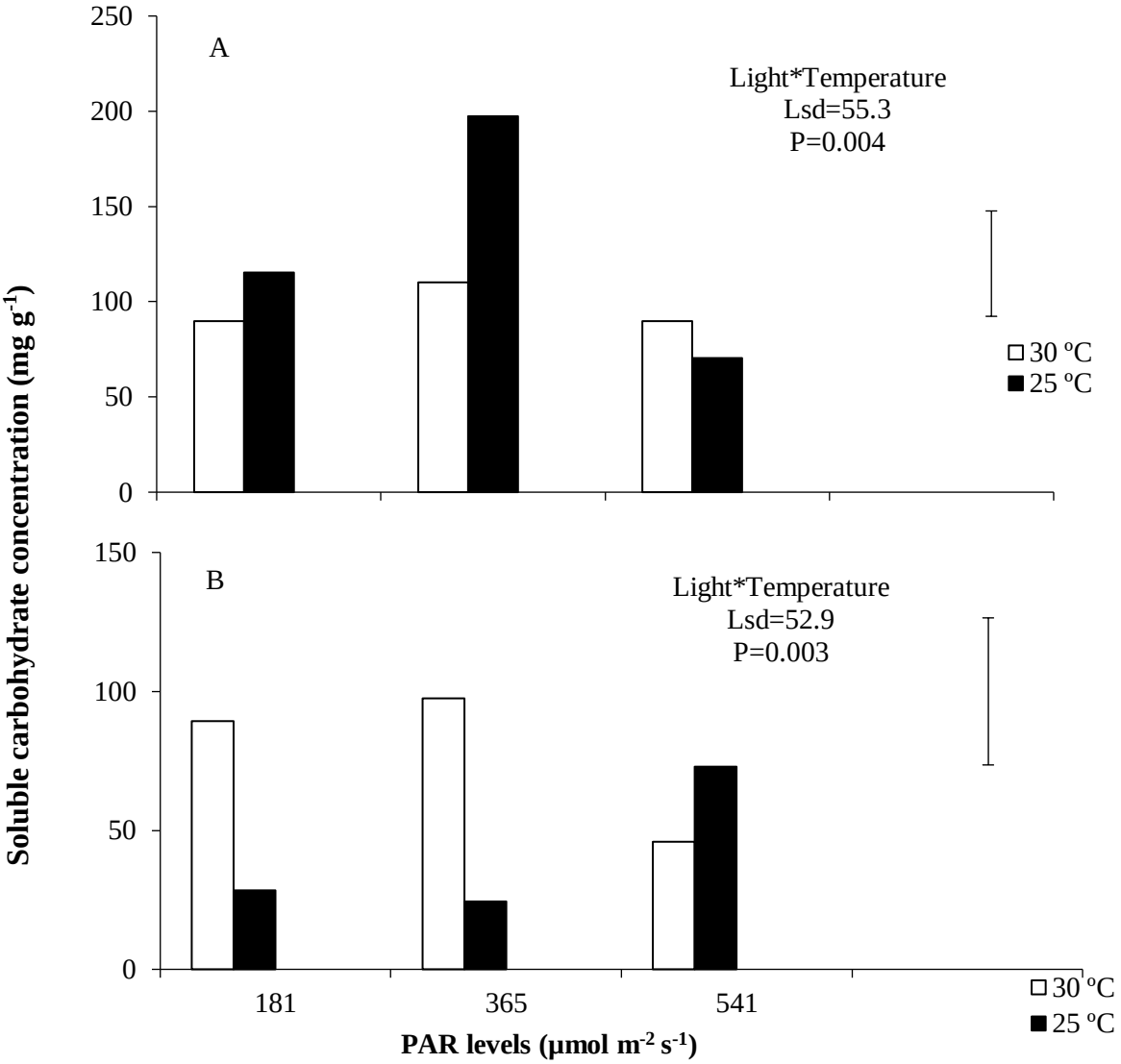


Figure 2: Effect of PAR and temperature on soluble carbohydrate concentration in cocoa leaves (A) and stems (B). Each bar represents a mean of three replicates. Note difference in scales between A & B.

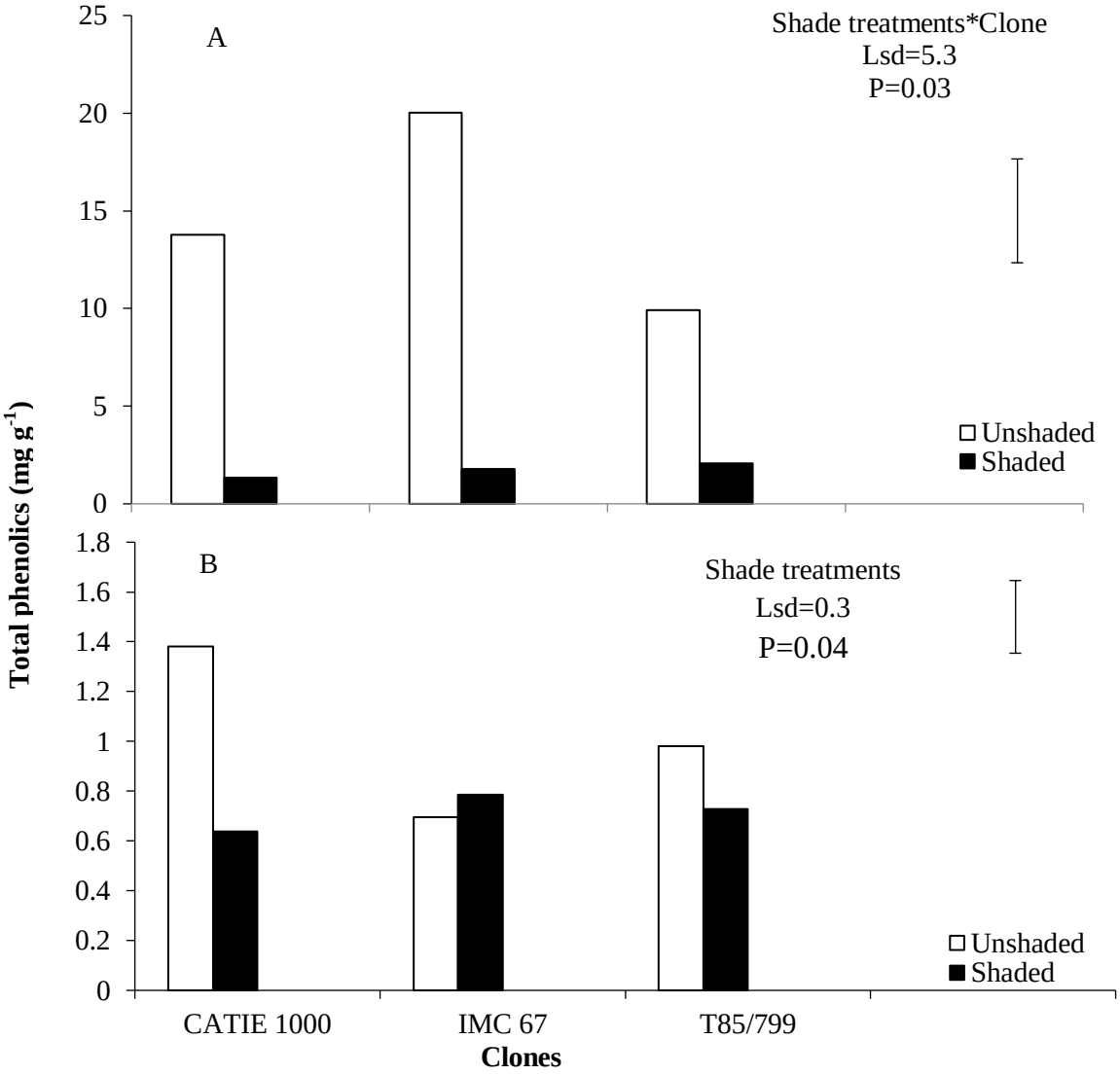


Figure 3: The interaction of shade treatments and clone on total phenolic concentration of young leaves (A) and stems (B). Note the difference in scales. Each bar represents a mean of six replicates.

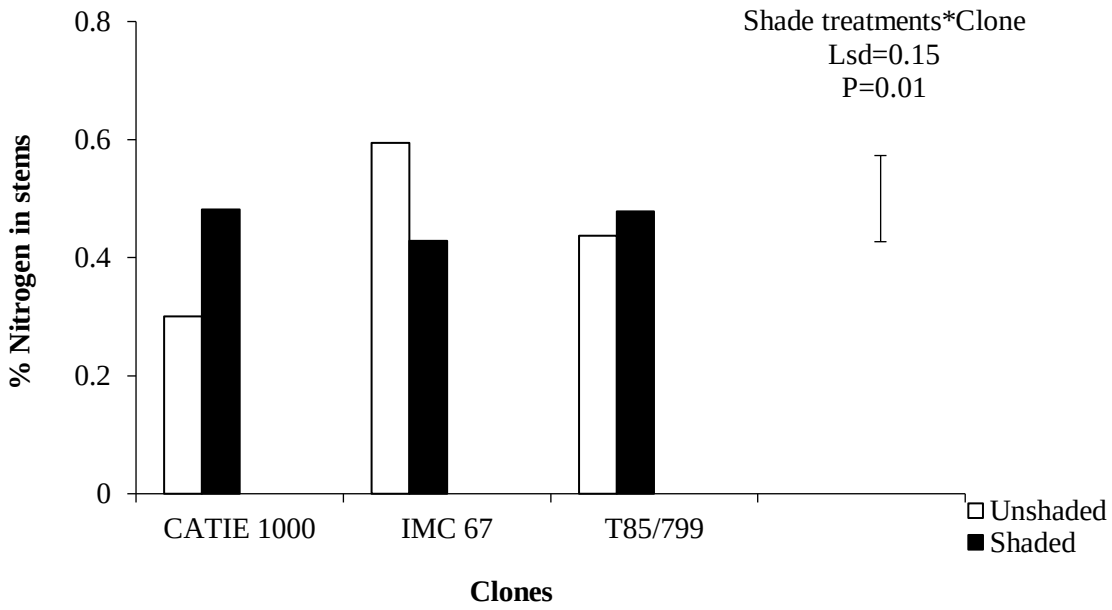


Figure 4: The interaction of shade and unshaded treatments and clone types on nitrogen concentration of stems. Each bar represents a mean of six replicates

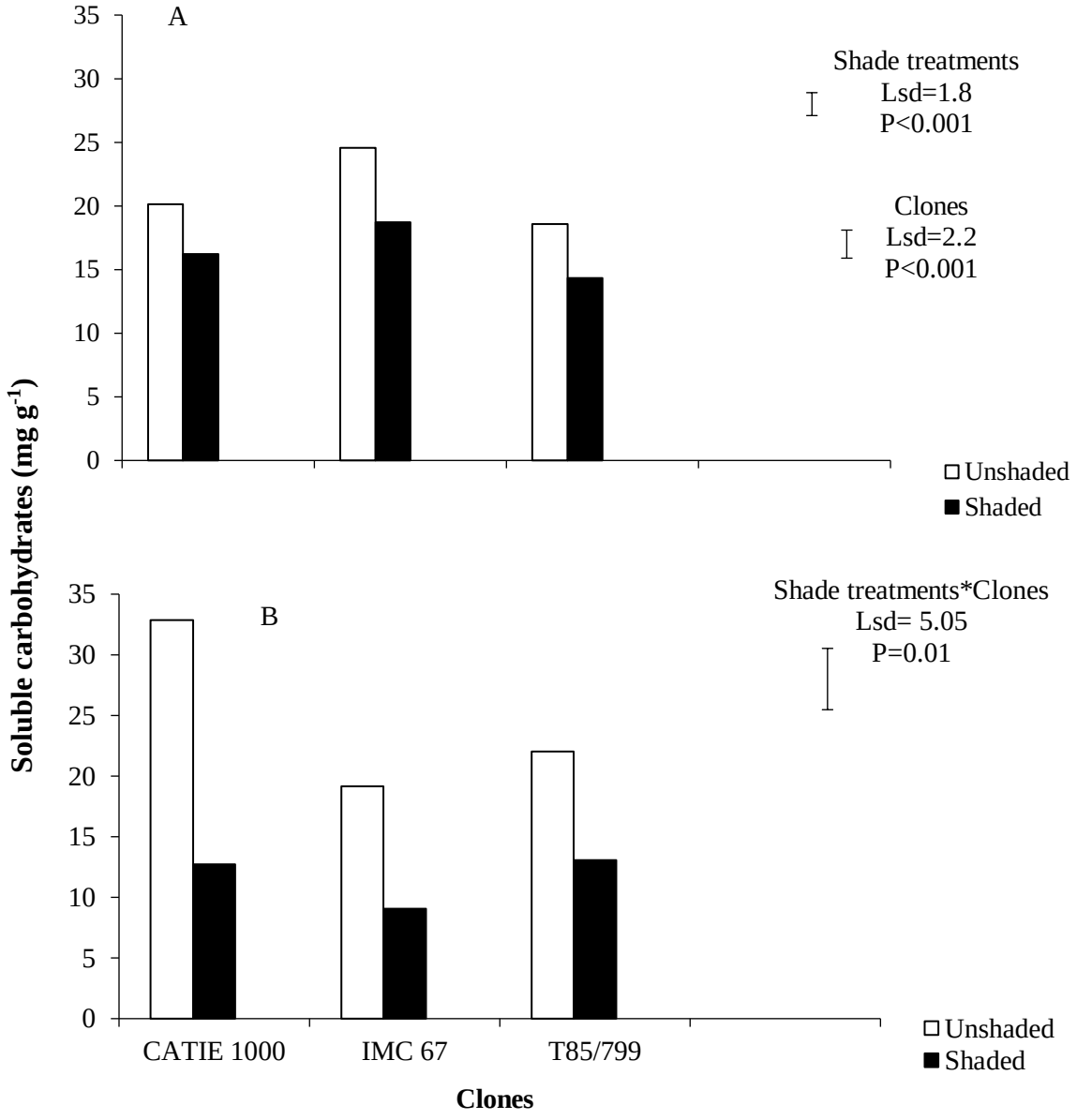
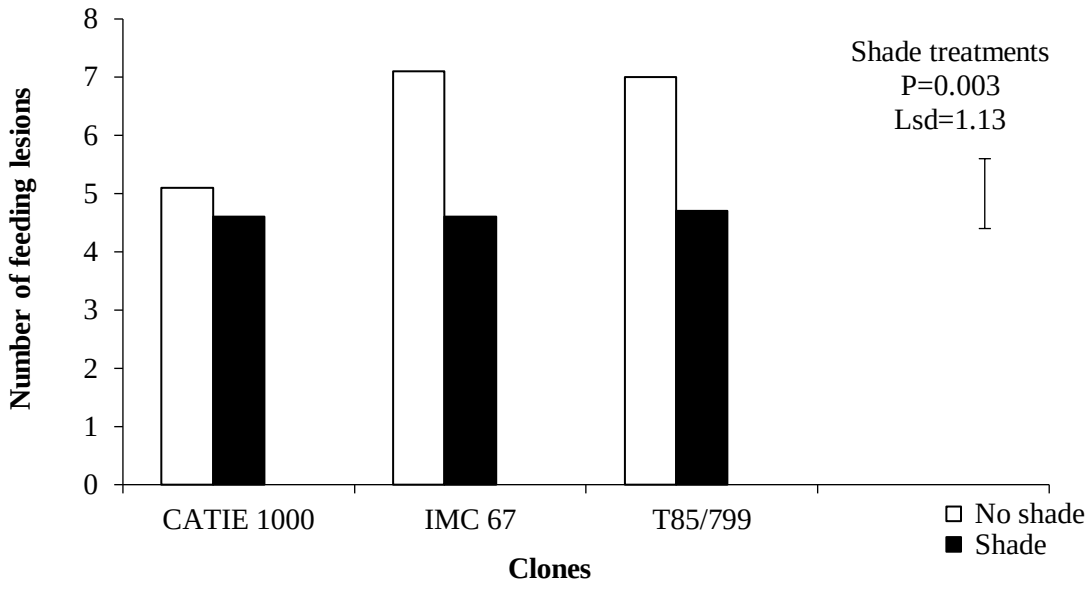


Figure 5: Interaction between shade treatments and clone type on soluble carbohydrate concentration in the leaves (A) and stems (B) of young cocoa. Each bar represents a mean of six replicates.



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