Control of Digitaria insularis (L.) Fedde in eucalyptus forests: shading increases sensitivity to glyphosate applied alone and in a mixture with carfentrazone-ethyl

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Abstract

Shading, the predominant condition in most of the eucalyptus cultivation cycle, causes changes in the morphological and physiological weed's characteristics, which can alter their responses to glyphosate and carfentrazone-ethyl, important herbicides for the crop. The objective was to evaluate the influence of light on the efficiency of glyphosate and carfentrazone-ethyl used alone and in a mixture in *Digitaria insularis* control, a priority pest in the crop. The experiment was carried out in a 3x6 factorial scheme. The first factor corresponded to 3 cultivation environments (full sunlight, 45 and 63% shading) and the second factor to doses of glyphosate and carfentrazone-ethyl applied isolated (1920 and 40 g ai ha^{-1}) and mixed (1536 + 8; 1152 + 16; 768 + 24 and 384 + 32 g ai ha^{-1}), respectively. In shading, the glyphosate application alone at a dose of 1920 g ha^{-1} and in a mixture with carfentrazone-ethyl at doses of 1536 + 8 and 1152 + 16 g ha^{-1} were efficient in *D. insularis* management. In the environment of 63% shading, the dose of 768 + 24 g ha^{-1} was also efficient in this species management. None of the doses were effective in controlling *D. insularis* in full sunlight. Isolated carfentrazone-ethyl was inefficient in controlling *D. insularis*, regardless of the growth environment. Shading increases the quantum yield of photosystem II and reduces the electron transport rate, photosynthetic rate, stomatal conductance, and transpiration rate of *D. insularis*. In shady environments, it is possible to control *D. insularis* with lower glyphosate doses, used alone and mixed with carfentrazone-ethyl, reducing costs and environmental impacts.

1. Introduction

Eucalyptus is considered the most planted forest species worldwide [1, 2]. In this scenario, Brazil stands out as one of the largest producers [3], obtaining high wood productivity per hectare/year in a shorter rotation period and growing crop expansion in the country [4, 5].

In areas of crop expansion or even the oldest stands, weed management is one of the most important practices in eucalyptus cultivation. Without control, weed interference can lead to losses of up to 40% in forest productivity [6]. Of the five pests of economic importance and the greatest phytosanitary risk for eucalyptus, which are prioritized in the analysis of product registration processes and control technologies in Brazil, all are weeds [7]. Among them is *Digitaria insularis* [7], a perennial grass that is difficult to control, with biotypes resistant to glyphosate [8–10] and cross-resistance to ACCase-inhibiting herbicides [11]. *D. insularis* is also considered a weed in corn [12], soybean [13], cowpea [14], coffee [15], pastures [16] and in urban areas [9], which reinforces the species importance.

Glyphosate and carfentrazone-ethyl, herbicides that inhibit EPSPS and protox, respectively, are among the herbicides registered for eucalyptus in Brazil [17] and accepted by certifiers for use in the crop [18]. Both products have been used in eucalyptus plantations to control weeds between the rows, even mixed in the tank as an alternative to increasing the control spectrum. In shaded environments, a condition commonly found under the canopies of eucalyptus planted forests [19], some weeds are more sensitive to glyphosate action, such as *Euphorbia heterophylla* [20] and *Merremia cissoides* [21] and protox-inhibiting herbicides, such as *Commelina benghalensis* [22], and can be controlled with lower doses of these products. The herbicides application in lower doses with high control efficiency, as occurs in shading, helps to reduce the negative impacts of these pesticides on the environment, which is currently so questioned by society [23–29], in addition to reducing production costs.

Maintaining the viability of glyphosate and carfentrazone-ethyl is essential for successful weed management in eucalyptus crops. Mixing herbicides with different mechanisms of action increases the weed control spectrum, reduces selection pressure, and delays the resistance emergence [30–32], in addition to can also be used to control already resistant biotypes [33–36].

The mixture of glyphosate with protox-inhibiting herbicides has an additive and synergistic effect on the control of several species of broadleaf and grass weeds [37–39]. However, shading can interfere with the herbicide mixture's efficiency since the morphological and physiological plants characteristics [40–44] and herbicides efficiency when applied alone are altered under low light intensity [20–22].

The mixture between glyphosate and carfentrazone-ethyl is an interesting strategy to delay the emergence of resistant biotypes. However, the weed growth environment promoted by eucalyptus must be considered when evaluating these herbicides' efficiency, which has been neglected. The objective was to evaluate the effect of shading on the efficiency of glyphosate and carfentrazone-ethyl applied alone and in a mixture in the *D. insularis* control, a priority pest in the eucalyptus crop in Brazil.

2. Materials and methods

2.1 Site and plant material description

The experiment was carried out at the Instituto de Ciências Agrárias of the Universidade Federal de Minas Gerais, Brazil (16°40'58.1"S, 43°50'19.3"W). Köppen classifies the region's climate as Aw - tropical with a dry season in winter [45].

D. insularis seedlings were produced from tillers collected from plants in areas with frequent glyphosate applications. The seedlings were transplanted into 10 dm^3 pots containing a substrate of sandy soil and bovine manure in a 3:1 ratio (volume:volume). In each pot, two *D. insularis* plants were grown and taken to the cultivation environments after transplanting. The soil used had the following characteristics: pH (water) = 5.3; organic matter = 1.66%; sand = 72%; silt = 16%; and clay = 12%. The substrate was fertilized with NPK 4-30-10 fertilizer, as recommended for pots fertilization [46], and irrigated once a day to maintain humidity between 80 and 100% of field capacity.

2.2 Experimental design and treatments

The design adopted was randomized blocks with four replications. The treatments were established in a 3 x 6 factorial scheme, where the first factor consisted of plants in full sunlight and two shading levels (45 and 63%), and the second factor, by doses of glyphosate and carfentrazone-ethyl, applied isolated and in a mixture (Table 1).

Table 1
Glyphosate and carfentrazone-ethyl applied alone and in a mixture to control *D. insularis*

Herbicides common name	Herbicides commercial name	Doses (g ai ha^{-1})
Glyphosate	Roundup Original DI®	1920
Glyphosate + carfentrazone-ethyl	Roundup Original DI® + Aurora®	1536 + 8
Glyphosate + carfentrazone-ethyl	Roundup Original DI® + Aurora®	1152 + 16
Glyphosate + carfentrazone-ethyl	Roundup Original DI® + Aurora®	768 + 24
Glyphosate + carfentrazone-ethyl	Roundup Original DI® + Aurora®	384 + 32
Carfentrazone-ethyl	Aurora®	40
ai = active ingredient		

Shading was obtained with a black polypropylene shade screen, installed on structures built with wooden posts and wire 2 m high, closing the sides. The shading levels adopted in the study are those found in eucalyptus planted forests [47, 48]. The incidence of photosynthetically active radiation (PAR) in the growth environments was determined in 20 samples during the experiment execution, at 8:00, 12:00, and 16:00 hours, with the fluorometer device Y (II) meter (OPTI- SCIENCES, Hudson, USA) (Fig. 1).

The plants remained in the cultivation environments for 52 days. A standardization cut was performed during this period at 37 cultivation days, at 5 cm height. At 15 days after plant cutting, the herbicides were applied. The application was carried out with a backpack sprayer pressurized with CO_2 with a TTI 11002 nozzle model (Teejet, Wheaton, Illinois, USA) and a pressure regulating valve (Comam, Belo Horizonte, Brazil) constant at 300 kPa, calibrated to apply 116 L ha^{-1} of spray volume.

2.3 Assessments

At 3 and 6 days after application (DAA), photosynthetic rate (Pn, µmol CO_2 m^{-2} s^{-1}), stomatal conductance (gs, mol H_2O m^{-2} s^{-1}), and transpiration rate (E, µmol H_2O m^{-2} s^{-1}) of the plants were analyzed using an infrared gas analyzer (IRGA, model LI-6400xtr, LCI-PRO, Lincoln, Nebraska, USA) and the quantum yield of photosystem II (ΦPSII) and electron transport rate (ETR) with the fluorometer device Y (II) meter (OPTI-SCIENCES, Hudson, USA).

Visual control assessments were carried out at 28 and 60 DAA, adopting a scale from 0 to 100%, where 0 is the absence of herbicide injuries, and 100 is the plant death. Three evaluators assigned control scores. The values per plot were determined by the arithmetic mean of the three scores. At 60 DAA, the plant biomass remaining in the pots was collected and weighed to determine the fresh biomass. For *D. insularis*, fresh biomass has the same behavior as dry biomass [49] and therefore was used as a parameter.

2.4 Statistical analysis

Data were submitted to variance analysis (ANOVA), and when significant, means were grouped using the Scott-Knott test ($p \leq 0.05$). ANOVA and the Scott-Knott mean clustering test were performed using the R Studio statistical program [50] and the ExpDes.pt package [51].

3. Results

3.1 Control and fresh biomass of *D. insularis*

Shading increased the *D. insularis* sensitivity to glyphosate applied alone and in a mixture with carfentrazone-ethyl (Tables 2 and 3). At 28 and 60 DAA, in environments with 45 and 63% shading, the isolated glyphosate application at a dose of 1920 g ha^{-1} and in a mixture with carfentrazone-ethyl at doses 1536 + 8 and 1152 + 16 g ha^{-1} were efficient in *D. insularis* management, with control levels above 80%. In the environment of 63% shading, the application of 768 + 24 g ha^{-1} was also efficient in managing this species (Table 2). These doses totally reduced the plant's fresh biomass at 60 DAA (Table 3). Although at 28 DAA, the doses 768 + 24 and 384 + 32 g ha^{-1} were efficient in environments with 45 and 63% shading, respectively, at 60 DAA, the plants recovered, and the control was less than 80%, considered unsatisfactory (Table 2). In full sunlight, none of the applied doses effectively controlled *D. insularis* (Tables 2 and 3). In this environment, the plants recovered from the herbicide injuries, with drastic control reductions at 60 DAA compared to 28 DAA (Table 2). At 60 DAA in full sunlight, *D. insularis* control levels were less than 18% at all applied doses. Carfentrazone-ethyl applied alone is not efficient in controlling *D. insularis*, regardless of the growth environment (Tables 2 and 3).

Table 2

D. insularis control (%) at 28 and 60 days after application (DAA) of glyphosate (Gly) and carfentrazone-ethyl (Car) alone or in a mixture in environments with different light intensities

28 days after application				
Herbicides	Doses	Growth environments		
	(g ai ha^{-1})	Full sunlight	45% shading	63% shading
Gly	1920	75.41 Ab	100.00 Aa	100.00 Aa
Gly + Car	1536 + 8	70.83 Ab	100.00 Aa	100.00 Aa
Gly + Car	1152 + 16	54.58 Bb	87.91 Aa	100.00 Aa
Gly + Car	768 + 24	45.83 Bb	91.25 Aa	96.25 Aa
Gly + Car	384 + 32	15.83 Cc	38.54 Bb	87.91 Aa
Car	40	7.50 Ca	5.00 Ca	3.33 Ba
CV (%) = 14.2				
60 days after application				
Herbicides	Doses	Growth environments		
	(g ai ha^{-1})	Full sunlight	45% shading	63% shading
Gly	1920	17.91 Ab	100.00 Aa	100.00 Aa
Gly + Car	1536 + 8	13.75 Ab	100.00 Aa	100.00 Aa
Gly + Car	1152 + 16	17.08 Ac	85.00 Bb	100.00 Aa
Gly + Car	768 + 24	13.33 Ac	34.33 Cb	100.00 Aa
Gly + Car	384 + 32	10.41 Ab	14.58 Db	57.91 Ba
Car	40	5.00 Aa	5.00 Da	5.00 Ca
CV (%) = 17.43				

Means followed by the same letter, uppercase in the column and lowercase in the row, do not differ by the Scott-Knott mean clustering test ($p \leq 0.05$). ai = active ingredient; CV = coefficient of variation.

Table 3
D. insularis fresh biomass (g/pot) at 60 days after application (DAA) of glyphosate (Gly) and carfentrazone-ethyl (Car) alone or in a mixture in environments with different light intensities

Herbicides	**Doses**	**Growth environments**		
	(g ai ha^{-1})	**Full sunlight**	**45% shading**	**63% shading**
Gly	1920	150.20 Ba	0.00 Bb	0.00 Cb
Car	40	168.42 Bb	243.18 Aa	269.20 Aa
Gly + Car	1536 + 8	146.32 Ba	0.00 Bb	0.00 Cb
Gly + Car	1152 + 16	142.25 Ba	0.00 Bb	0.00 Cb
Gly + Car	768 + 24	222.46 Aa	211.89 Aa	0.00 Cb
Gly + Car	384 + 32	202.53 Aa	221.54 Aa	101.89 Bb
CV (%) = 35.51				

Means followed by the same letter, uppercase in the column and lowercase in the row, do not differ by the Scott-Knott mean clustering test ($p \leq 0.05$). ai = active ingredient; CV = coefficient of variation.

3.2 Quantum yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR)

Shading increased Φ_{PSII} and reduced the ETR of *D. insularis* at 3 and 6 DAA (Table 4). The glyphosate application alone at a dose of 1920 g ha^{-1} and mixed with carfentrazone-ethyl at doses 1536 + 8 and 1152 + 16 g ha^{-1} caused the most significant reductions in these variables at 3 and 5 DAA (Table 5). The carfentrazone-ethyl applied alone caused minor impacts on Φ_{PSII} and ETR of *D. insularis*.

Table 4
Quantum yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR) of *D. insularis* in different environments, at 3 and 6 days after application (DAA) of glyphosate and carfentrazone-ethyl alone or in a mixture

Growth environments	Variables			
	3 DAA		6 DAA	
	Φ_{PSII}	ETR	Φ_{PSII}	ETR
Full sunlight	0.2093 c	134.46 a	0.1255 c	91.98 a
45% shading	0.2780 b	92.95 b	0.2031 b	58.94 b
63% shading	0.3566 a	78.82 b	0.3473 a	50.50 b
CV (%)	33.66	35.13	48.70	63.98

Means followed by the same letter in the column do not differ by the Scott-Knott mean clustering test ($p \leq 0.05$). CV = coefficient of variation.

Table 5
Quantum yield of photosystem II (Φ_{PSII}) and electron transport rate (ETR) of *D. insularis* at 3 and 6 days after application (DAA) of glyphosate (Gly) and carfentrazone-ethyl (Car) alone or in a mixture

Herbicides	Doses	Variables			
	(g ai ha^{-1})	3 DAA		6 DAA	
		Φ_{PSII}	ETR	Φ_{PSII}	ETR
Gly	1920	0.1716 b	62.87 c	0.0672 c	25.43 c
Car	40	0.3975 a	152.10 a	0.4064 a	117.15 a
Gly + Car	1536 + 8	0.2201 b	69.55 c	0.1712 b	49.74 c
Gly + Car	1152 + 16	0.2504 b	95.58 c	0.2115 b	57.36 c
Gly + Car	768 + 24	0.3006 a	111.56 b	0.2360 b	78.81 b
Gly + Car	384 + 32	0.3475 a	120.80 b	0.2596 b	74.35 b
CV (%)		33.66	35.13	48.70	63.98

Means followed by the same letter in the column do not differ by the Scott-Knott mean clustering test ($p \leq 0.05$). ai = active ingredient; CV = coefficient of variation.

3.3 Photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E)

At 3 and 6 DAA, shading reduced the gs and E of *D. insularis* (Table 6). At 3 DAA, there was no difference between the growth environments in the plants P_n. However, at 6 DAA, plants in shading showed lower P_n. At 3 DAA, the doses that most reduced P_n, g_s, and E were glyphosate applied alone at a dose of 1920 g ha^{-1} and mixed with carfentrazone-ethyl at doses 1536 + 8, 1152 + 16 and 768 + 24 g ha^{-1} (Table 7). At 6 DAA, the doses that most reduced these variables were glyphosate applied alone at a dose of 1920 g ha^{-1} and mixed with carfentrazone-ethyl at doses 1536 + 8 and 1152 + 16 g ha^{-1} (Table 7). Carfentrazone-ethyl applied alone was the treatment with the least impact on P_n, g_s, and E of *D. insularis* (Table 7).

Table 6
Photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) of *D. insularis* in different environments at 3 and 6 days after application (DAA) of glyphosate and carfentrazone-ethyl alone or in a mixture

Growth environments	Variables					
	3 DAA			6 DAA		
	P_n	g_s	E	P_n	g_s	E
Full sunlight	9.18 a	0.0945 a	2.85 a	9.01 a	0.1333 a	2.89 a
45% shading	7.99 a	0.0629 b	2.28 b	6.74 b	0.0616 b	1.88 b
63% shading	7.18 a	0.0516 b	1.87 b	4.91 b	0.0433 b	1.24 c
CV (%)	42.63	40.23	30.77	46.53	51.16	35.44

Means followed by the same letter in the column do not differ by the Scott-Knott mean clustering test ($p \leq 0.05$). CV = coefficient of variation.

Table 7
Photosynthetic rate (P_n), stomatal conductance (g_s), and transpiration rate (E) of *D. insularis* at 3 and 6 days after application (DAA) of glyphosate (Gly) and carfentrazone-ethyl (Car) alone or in a mixture

Herbicides	Doses ($g\ ai\ ha^{-1}$)	Variables					
		3 DAA			6 DAA		
		P_n	g_s	E	P_n	g_s	E
Gly	1920	4.62 c	0.050 c	1.77 c	2.84 c	0.039 b	1.19 c
Car	40	17.33 a	0.139 a	4.04 a	17.21 a	0.160 a	3.52 a
Gly + Car	1536 + 8	4.67 c	0.045 c	1.66 c	3.43 c	0.054 b	1.55 c
Gly + Car	1152 + 16	4.46 c	0.038 c	1.52 c	4.26 c	0.064 b	1.77 b
Gly + Car	768 + 24	6.03 c	0.055 c	1.97 c	6.37 b	0.084 b	2.12 b
Gly + Car	384 + 32	11.59 b	0.089 b	3.04 b	7.24 b	0.074 b	1.87 b
CV (%)		42.63	40.23	30.77	46.53	51.16	35.44

Means followed by the same letter in the column do not differ by the Scott-Knott mean clustering test ($p \leq 0.05$). ai = active ingredient; CV = coefficient of variation.

4. Discussion

D. insularis showed considerable fresh biomass accumulation in environments with 45 and 63% shading (Table 3), which correspond to the average levels of incident radiation of 607 and 394 μmols m^{-2} s^{-1} (Fig. 1), respectively, indicating the ability to grow even under low light intensity. Caron [48] did not identify the need for weed control in eucalyptus planted forests when the shading level was greater than 60% due to low plant growth. However, the study does not report the radiation level corresponding to the shading levels studied, a factor that depends on the time and region where the study was conducted and that directly impacts weed growth and the decision to manage them. In addition, the weed community in the study was mainly composed of the species *Sida rhombifolia*, *Stellaria media*, *Sonchus oleraceus*, and *Echium plantagineum*, different species from the present study, and which have an unknown ability to adapt to shading. Therefore, the decision to manage or not to manage weeds depends more on the incident radiation level and the species' ability to grow in the shade than just the shade level imposed by the forest canopy. From the observed *D. insularis* growth in shaded environments and full sunlight, it was observed that the species has phenotypic plasticity and good adaptation to environments with light restriction, which reinforces the need for attention with the species in forest areas.

The increased *D. insularis* sensitivity to glyphosate applied alone and in a mixture with carfentrazone-ethyl in shading may be linked to lower ETR and P_n of plants in these environments (Tables 4 and 6).

These variables are related to carbon fixation and plant energy availability [52]. Plants with lower energy reserves are less likely to recover from the herbicides' injuries, becoming more sensitive to these products' actions [53]. The doses considered efficient in controlling *D. insularis* (1920 g ha^{-1} of glyphosate alone and mixed with carfentrazone-ethyl at doses 1536 + 8 and 1152 + 16 g ha^{-1}) were the ones that most negatively affected the physiological variables analyzed (Tables 5 and 7).

In addition to the energy deficit, changes in *D. insularis* growth caused by shading may also be associated with greater herbicide sensitivity. *D. insularis* has rhizomes, reserve organs that, when present, make it difficult to control [54]. *D. insularis* begin to produce rhizomes 45 days after emergence [55]. In the present study, the herbicides were applied at 52 cultivation days, after the formation beginning of these structures in full sunlight. However, shading alters the dry matter partition of grasses, investing more resources in shoot development as a function of root growth [56, 57], which, combined with lower ETR and P_n (Tables 4 and 6), may have delayed or compromised rhizome development and increased *D. insularis* sensitivity.

D. insularis is a C4 metabolism grass. C4 metabolism plants have a high light and temperature saturation point [58]. The reductions in ETR, P_n, g_s, and E in *D. insularis* grown in shading are due to the lower light incidence in these environments (Fig. 1). These results align with those found in other grasses grown in the shade [59–62].

Shading also alters wax deposition on the leaf surface. Wax has a variety of functions, such as reducing water loss and pesticide uptake [63, 64]. Due to reduced transpiration and water loss in shading (Table 6), wax deposition is also lower in these environments [65]. The smaller wax amount in shading can increase herbicide penetration and, consequently, its efficiency.

Increased sensitivity in shading to glyphosate applied alone was also observed in *Euphorbia heterophylla* [20] and *Merremia cissoides* [21], and the mixture of glyphosate and carfentrazone-ethyl in *Macroptilium atropurpureum* [66]. Unlike what was observed in the present study, where no increase in the efficiency of carfentrazone-ethyl isolated under shading was found, Santos Júnior [22] found an increase in saflufenacil efficiency, another protox-inhibiting herbicide, in controlling *Commelina benghalensis*.

The low control obtained by isolated carfentrazone-ethyl, regardless of the growth environment, is due to the advanced plant stage at the application time. Carfentrazone-ethyl is a contact herbicide efficient in controlling plants in the early development stages [67]. The low control obtained by the mixture of glyphosate and carfentrazone-ethyl at the dose of 384 + 32 g ha^{-1}, regardless of the growth environment, is associated with the low glyphosate dose used in the mixture. Carfentrazone-ethyl applied alone at a dose of 40 g ha^{-1} and mixed with glyphosate at a dose of 384 + 32 g ha^{-1} were the doses that had the least impact on *D. insularis* physiology (Tables 5 and 7).

Although isolated carfentrazone-ethyl has low control efficiency against *D. insularis*, it has good efficiency against other glyphosate-resistant weeds, such as *Commelina* ssp. [37]. Moreover, its use in a mixture with glyphosate can benefit *D. insularis* control in shading, as in the present study, and against other important weeds, like the glyphosate-tolerant species [37, 68]. Additionally, the constant glyphosate application, the most widely used herbicide in the world, led to the selection of resistant biotypes over time [69–71]. The use of two or more herbicides with different mechanisms of action has been an interesting agronomic practice because, in addition to improving the management efficiency of tolerant weeds [72–74], it decreases selection pressure for resistant weeds [30].

The application of glyphosate alone and in a mixture with carfentrazone-ethyl showed different behavior depending on the light intensity in the growth environments, indicating the need to consider this factor when defining weed control doses in eucalyptus areas. Studies on the absorption and translocation of glyphosate and carfentrazone-ethyl in *D. insularis* cultivated in environments with different light intensities are necessary to elucidate the mechanisms involved in species tolerance when cultivated in full sunlight.

Conclusions

D. insulares showed high biomass accumulation under low light intensity in environments with 45 and 63% shading, corresponding to the average incident radiation levels of 607 and 394 µmols m^{-2} s^{-1}, respectively, demonstrating the species' importance in different phases of the eucalyptus cycle. The shading of 45 and 63% of the photosynthetically active radiation increases *D. insularis* sensitivity to glyphosate applied alone and mixed with carfentrazone-ethyl, requiring lower doses for its control in the understory of eucalyptus plantations. Considering the light availability in growing environments in the definition of herbicide doses for weed control creates a new possibility for efficient dose reduction and, consequently, gains in economy and sustainability. Weeds in shading conditions are widely found in crops of forest species, orchards, and integration systems, adding up to millions of hectares where this new management approach could be applied. The light intensity in the growing environments must be considered when defining glyphosate doses, applied alone or mixed with carfentrazone-ethyl.

Declarations

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Figures

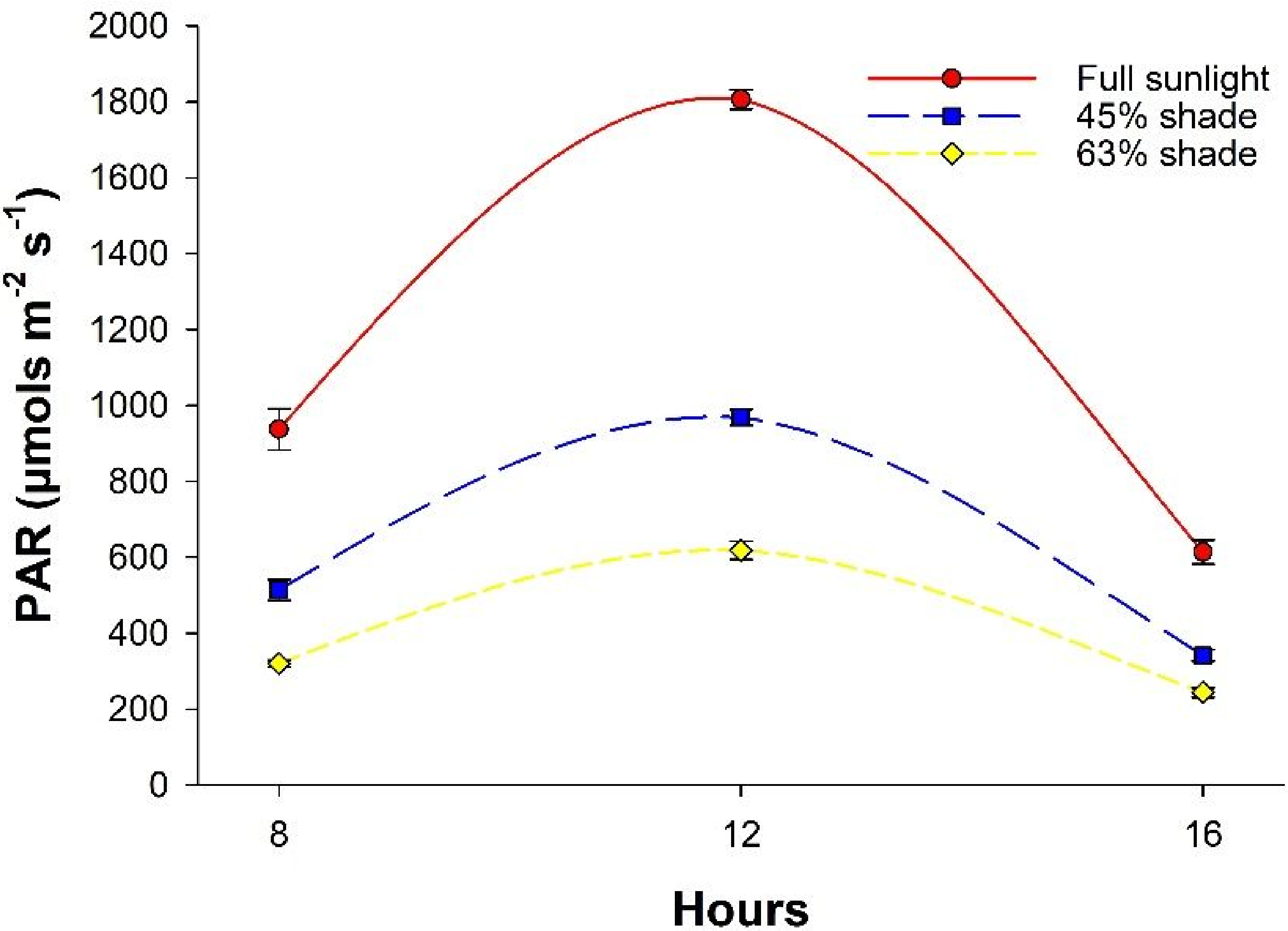

Figure 1

Availability of photosynthetically active radiation (PAR) in growing environments at different times. Bars = mean standard error