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Impact of increased ultraviolet-B radiation stress due to stratospheric ozone depletion on N_2 fixation in traditional African commercial legumes

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Reports of diminished nodule formation and nitrogenase activity in some Asian tropical legumes exposed to above-ambient levels of ultraviolet-B (UV-B: 280–315nm) radiation have raised concerns as to the impact of stratospheric ozone depletion on generally poorly developed traditional African farming systems confronted by the high cost and limited availability of chemical fertilisers. These rely on N_2-fixing legumes as the cheapest source of N for maintaining soil fertility and sustainable yields in the intrinsically infertile and heterogeneous African soils. In view of this, we examined the effects of supplemental UV-B radiation approximating 15% and 25% depletions in the total ozone column on N_2 fixation in eight traditional African commercial legume species representing crop, forest, medicinal, ornamental and pasture categories. In all categories examined, except medicinal, supplemental UV-B had no effect on root non-structural carbohydrates, anthocyanins and flavonoids, known to signal Rhizobiaceae micro-symbionts and promote nodule formation, or on nodule mass, activity and quantities of N fixed in different plant organs and whole plants. In contrast, in the medicinal category *Cyclopia maculata* (Honeybush) a slow growing commercially important herbal beverage with naturally high flavonoid concentrations, displayed decreased nodule activity and quantities of N fixed in different plant organs and whole plants with increased UV-B. This study's findings conclude negligible impacts of ozone depletion on nitrogen fixation and soil fertility in most traditional African farming systems, these limited to occasional inhibition of nodule induction in some crops.

Introduction

In Africa, the intrinsically low fertility and heterogeneous nature of soils is a major limitation of crop plant yields (Munns and Franco 1981). About 90% of the mineral N on the continent is found in living plants, with only a small mineral fraction left in the soils (Borlaug 1991). Increased use of chemical fertilisers has significantly increased food production world-wide, yet Africa's consumption of mineral fertilisers per ha is the lowest globally (Dakora and Keya 1997). This low use of chemical fertilisers, particularly N, is attributed in part to their high cost and limited availability in a generally poorly developed African infrastructure (Dakora and Keya 1997). Consequently, most traditional farming systems in Africa include N_2-fixing legume species, which contribute about 65% of N input into global agriculture (Vance and Graham 1995), as the cheapest source of N for maintaining soil fertility and sustainable yields in Africa (Dakora and Keya 1997).

Recent reports of substantial (up to 70%) reductions in nitrogenase activity and nodule production in some Asian tropical legumes, such as *Vigna radiata* and *Phaseolus mungo*, exposed to elevated levels of ultraviolet-B (UV-B: 280–315nm) radiation (Singh 1997) have raised concerns for biological N_2 fixation, since sustained depletion of the stratospheric ozone has led to increased levels of solar UV-B radiation in the troposphere of both the Southern and Northern Hemispheres (McKenzie *et al*. 1999). These concerns are especially pertinent to the Southern Hemisphere where stratospheric ozone destruction is more intense (Crutzen 1992) and solar UV-B fluxes are up to 50% higher than those at comparable Northern Hemisphere latitudes (Seckmeyer *et al*. 1995). Under current phase out schedules for chlorofluorocarbons (CFCs) and other ozone depleting halogens specified in the Montreal Protocol and its amendments, peak reductions of stratospheric ozone are expected early this century (Madronich *et al*. 1995) with a slow recovery over the next 50 years (WMO 1998). However, ozone recovery remains uncertain due to increased CFC production in some developing countries (Fraser and Prather 1999)

and recent discovery of other factors such as nitrogen oxides (Waibel *et al.* 1999, Tabazadeh *et al.* 2000) and global warming as mitigating factors in stratospheric ozone decline.

Numerous studies have shown that UV-B radiation above ambient, and even at ambient levels may depress plant photosynthesis, growth and yields (Teramura and Sullivan 1994, Rozema *et al.* 1997). Direct depression of photosynthesis by UV-B has been accredited to down-regulation of photosynthetic genes, photomodification of chloroplast thylakoid membranes (Strid *et al.* 1994), inhibition of photosynthetic enzymes, e.g. Rubisco, and disruption of electron transport in photosystem II (PSII) reaction centers via decreases in the Hill reaction (Murthy and Rajagopal 1995). Also, UV-B may affect photosynthesis indirectly by impairing stomatal function, altering photosynthetic pigments, leaf anatomy and canopy morphology (Teramura and Sullivan 1994). However, there exists considerable genotypic variation in sensitivity to UV-B radiation among agricultural and natural taxa (Sullivan *et al.* 1992) which suggests that ecotypic differentiation may have developed to increasing UV-B radiation over latitudinal and elevation gradients (Ziska *et al.* 1992). These differential sensitivities to UV-B radiation have been associated to a large extent with the different abilities of genotypes to produce secondary phenylpropanoid compounds, such as flavonoids, anthocyanins, hydroxycinnamic acid derivatives and related phenolics, which have substantial anti-oxidant (Dawar *et al*. 1998), energy dissipating (Smith and Markham 1998) and UV-B absorbing (Hoque and Remus 1999) properties that limit damage to the photosynthetic apparatus (Tevini *et al*. 1991) and to DNA (Stapleton and Walbot 1994). This is highlighted by the hypersensitivity to UV-B displayed by plant mutants deficient in the general phenylpronanoid or flavonoid pathway (Li *et al.* 1993). Also, increased production of various phenylpropanoids in response to UV-B irradiation have been widely reported (Rozema *et al*. 1997) and associated with inter- and intra-species differences in UV-B sensitivity (Day *et al*. 1994).

The link between UV-B, carbon assimilation and allocation and nodulation is uncertain. Current opinion is that biochemical and physiological changes induced in host plants by UV-B radiation may generate changes in their respective bacterial microsymbionts. For example, UV-B induced inhibition of photosynthesis may potentially alter carbohydrate resources available to microsymbionts, either as biologically functional root exudates or direct carbon supply to nodules (Van de Staaij *et al.* 1999), thereby leading to reduced nodule biomass and activity. Alternatively, increased concentration of phenylpropanoids in UV-B irradiated leaves (Tegelberg and Julkunen-Tiitto 2001, Kolb *et al.* 2001) and their subsequent accumulation in the roots may promote nodule formation (Muofhe and Dakora 1999), since some phenylpropanoids, particularly flavonoids, have been reported to act as plant signals to symbiotic bacteria in the Rhizobiaceae (Dakora and Phillips 1996, Phillips 2000, Cullimore and Dénarié 2003).

In view of these conflicting hypotheses and the important role of N_2 fixation in maintaining soil fertility and sustainable yields in African agriculture, we examined the effects of increased levels of UV-B radiation on N_2 fixation in some traditional African legumes representing five commercial categories.

Materials and Methods

Experimental plants and growing conditions

Eight species of traditional African legumes representing five commercial categories (crop, forest, medicinal, ornamental and pasture) were selected for study. These categories comprised the crop species *Vigna unguiculata* (L.) Walp. landlace Bengpilaa ex-Ghana, *Glycine max* (L.) Merr. cv. Prima and *Phaseolus vulgaris* (L.) cv. PAN 159, the forest species *Virgilia oroboides* Salter, the medicinal species *Cyclopia maculata* (L.) Vent., the ornamental species *Podalyria calyptrata* Willd., and the pasture species *Lupinus luteus* L. and *Vicia atropurpurea* Desv. Seeds were sown into 20cm high x 20cm diameter pots containing acid washed sand. *Vigna unguiculata* seeds were inoculated with *Bradyrhizobium* strain CB756, *G. max* seeds with *B. japonicum* strain CB 1809, *P. vulgaris* seeds with *Rhizobium leguminosarum* bv. Phaseoli strain UD2, *L. luteus* seeds with *Bradyrhizobium* lupin and *V. atropurpurea* seeds with *Rhizobium leguminosarum* bv. vicea at the sowing stage. *Cyclopia maculata, P. calyptrata* and *V. oroboides* seedlings were inoculated at time of seedling emergence with rhizobia isolated from nodules collected from species growing in the wild.

Crop and pasture seedlings were reduced to one per pot, and forest, medicinal and ornamental species to two per pot. Pots were irrigated daily with equal volumes of water. Immediately after seedling emergence, 400ml of ½ strength N-free Hoagland's nutrient solution (Hewitt 1966) was supplied twice weekly to the crop and pasture plants whereas the forest, medicinal and ornamental plants received ¼ strength of the nutrient solution due to their low growth rates and natural occurrence on nutrient impoverished soils.

UV-B treatments

The experimental design comprised 12 separate banks of fluorescent sun lamps (Phillips TL/12 40W UV-B, The Netherlands) located in an open natural area in the Kirstenbosch National Botanical Gardens, Cape Town (36°56'S, 18°29'E). Four pots of each study species were randomised beneath each lamp bank. There were three UV-B treatments, which commenced immediately after seed sowing, each replicated four times. In the near-ambient controls ($UV\text{-}B_{CONTROL}$), lamps in four alternate banks were filtered (no transmission below 316nm) with 0.12mm thick Mylar-D film (DuPont De Nemours, Wilmington, Delaware, USA). In the two above-ambient ($UV\text{-}B_{134}$ and $UV\text{-}B_{166}$) treatments, lamps in intervening banks were filtered (transmission down to 290nm) with 0.075mm thick cellulose acetate film (Courtaulds Chemicals, Derby, UK). All filters were replaced weekly to ensure uniformity of UV transmission.

Artificial UV-B radiation was supplied for 8h per day, but graduated, with two-thirds of the total daily UV-B supplement spread over a 4h photoperiod centered on the solar noon (13h00 South African Standard Time). The remaining one-

third was applied equally over the two 2h early morning and late afternoon photoperiods. This was achieved by switching on fewer lamps in each bank during these photoperiods. This step-wise application of the supplemental UV-B was followed to account for diurnal alterations in ambient UV-B irradiance intensity due to changes in solar zenith angle. Spectral irradiances of filtered lamps were measured after sunset with a computer-interfaced spectroradiometer (IL-1 700 radiometer, IL760D detector, IL783 monochromator, International Light Inc., Newburyport, USA), calibrated for absolute response and checked for wavelength alignment. Measured irradiances were weighted with the generalised plant response action spectrum (Caldwell 1971), as mathematically formulated by Green *et al.* (1974), which was normalised at 300nm. Weighted irradiances were integrated over the wavelength range 280–315nm and expressed as a function of distance from the lamp source. Distances between cellulose acetate filtered lamps and median height of plants in each bank were adjusted to increase UV-B above modeled clear-sky background flux (range: 0.898–8.554kJ m^{-2} d^{-1}) by 34% (UV-B_{134}: 1.270–11.276kJ m^{-2} d^{-1}) and 66% (UV-B_{166}: 1.626–13.815kJ m^{-2} d^{-1}). These increases are expected from 15% and 25% depletions in total column ozone above Cape Town according to a computerised (Musil and Bhagwandin 1992) semi-empirical model (Green 1983). Lamps in the mylar-filtered controls were fixed at the same distances above plants as the UV-B_{134} treatment to provide similar UV-A exposures in both (Newsham *et al*. 1996). To avoid semi-empirical model overestimation of the level of supplementary UV-B irradiance (Musil *et al*. 2002) required for each UV-B treatment due to local variations in the amount and form of cloud and atmospheric aerosols (Theil *et al.* 1997), artificial UV-B supplements were supplied under predominantly clear-sky conditions. This was achieved by switching off lamps during cloudy periods caused by the passage of intermittent cold fronts. Also, heights of lamps were regularly adjusted to accommodate median increases in plant height in each bank and seasonal variations in UV-B exposure. Adjustments were checked with UV-B biometer sensor (Model 3D-600, Solar Light Company, Philadelphia, USA), calibrated against the spectroradiometer for the generalised plant action spectrum, which regularly checked percentage changes in UV-B flux at median plant heights beneath the lamps. Measured UV-B exposures above background fluxes during the course of the experiment averaged 95.0% (range: 86.7–104.8%) in the UV-$B_{CONTROL}$, 137.1% (range 127–149.2%) in the UV-B_{134} treatment and 169.3% (range: 154.0–186.0%) in the UV-B_{166} treatment (Figure 1).

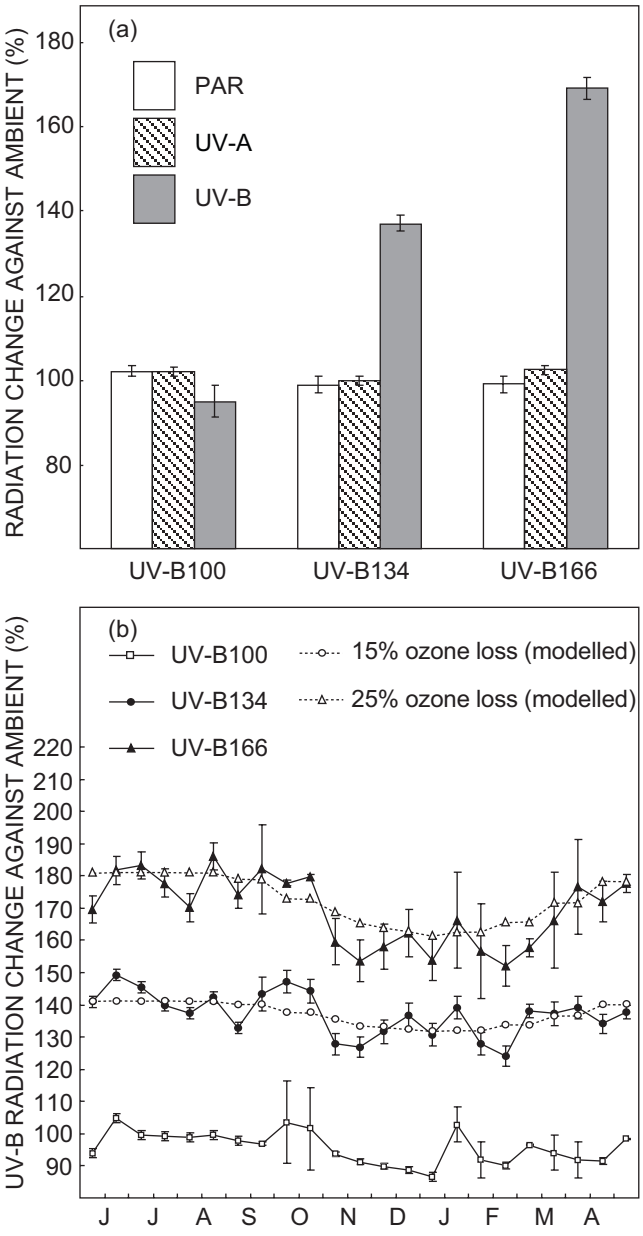

Figure 1: Measured changes in (a) UV-B, UV-A and PAR radiation beneath lamp systems and (b) measured and modeled UV-B radiation change at 15% and 25% ozone loss

Biomass accumulation

Crop species were harvested between 65 and 72 days after germination, pasture species between 108 and 128 days after germination and forest, medicinal and ornamental species between 184 and 194 days after germination. Only the crop and pasture species had reached reproductive maturity at harvest. Harvested plants were separated into nodule, root, stem and leaf fractions and dried at 60°C in a forced draft oven to a constant dry mass. The various plant fractions were weighed and ground in a mill.

Chemical assays

Percentage N content was determined in the oven dried, milled samples of different plant organs, and in the original seed samples from which species were grown, using a Carlo Erba NA 1 500 elemental analyser (Fisons Instruments SpA, Strada Rivoltana, Italy) coupled to a Finniggan MAT 252 mass spectrometer (Finnigan MAT GmbH, Bremen, Germany) via a Conflo II open-split device. The quantity of N fixed in each plant organ was estimated from the product of its measured %N content and dry mass. The total N fixed by each plant was calculated from the total N fixed in all

plant organs including that in the nodules minus the quantity present in the original seeds from which plants were grown. Division of this value by the nodule dry mass and the length of growing period from germination to harvest provided an estimate of the specific nodule N_2-fixing activity (mg N fixed g^{-1} nodule mass d^{-1}) in each plant.

Soluble non-structural carbohydrates (glucose, fructose and sucrose) were extracted from oven dried, milled root stem and leaf samples suspended in two, 10ml volumes of 80% ethanol (80:20, v:v, ethanol:water) for at least 72h. After centrifuging, the supernatants were adjusted to 25ml in volumetric flasks for spectrophotometric determination of total soluble sugars (Buysse and Merckx 1993). For analysis of insoluble non-structural carbohydrates (starch), residues were hydrolysed for 3h in 5ml of 3.6% HCl at 100°C, centrifuged and supernatants adjusted to 25ml with 80% ethanol in the volumetric flasks before spectrophotometrically determining the resultant sugars in the extracts (Buysse and Merckx 1993). Total non-structural carbohydrate concentrations (NSC) were expressed as μg g^{-1} dry mass.

Phenylpropanoids (methanol extractable UV absorbing compounds) were extracted from oven-dried, milled root and leaf samples suspended in 10ml of acidified methanol (79:20:1, v:v, methanol:water:HCl). Absorbances (A_b) of appropriately diluted centrifuged extracts were measured with the spectrophotometer at 300nm, 530nm and 657nm. Flavonoid concentrations were computed as A_b at 300nm g^{-1} dry mass (Mirecki and Teramura 1984), and anthocyanins as A_b530nm — 1/3 A_b657nm g^{-1} dry mass (Lindoo and Caldwell 1978).

Statistical analyses

All measurements were $\log_e$ transformed to reduce inequality of variance in the raw data. A two factor ANOVA test measured differences in root chemical properties, nodule mass, specific activity, and quantities of N fixed in different plant organs and whole plants between UV treatments, commercial plant categories and their interactions. Significantly different ($P \leq 0.05$) means were separated using Duncan's multiple range test. Computed correlation coefficients and a student's t-test evaluated statistical correspondence between root flavonoid, anthocyanin and non-structural carbohydrate concentrations, nodule mass and specific activity.

Results

Nodule specific activity decreased ($P \leq 0.05$) linearly with increased UV-B in the medicinal category only (Table 1). This decrease was insignificantly ($P \geq 0.05$) correlated with corresponding changes in root non-structural carbohydrate ($t_{1,21} = -0.71$), flavonoid ($t_{1,21} = 0.36$) and anthocyanin ($t_{1,21} = 0.73$) concentrations, but did correspond with significantly ($P \leq 0.05$) reduced quantities of N fixed in different plant organs, except nodules, and whole plants (Table 2). Conversely, in the crop category, nodule specific activity

Table 1: Effects of supplementary UV-B approximating 15% (UV-B_{134}) and 25% (UV-B_{166}) depletions in total column ozone on root metabolites, nodule mass and specific activity in traditional African commercial legumes

Category	UV-B treatment	Roots			Nodules	
		Flavonoids Abs 300nm g^{-1} dry mass	Anthocyanins Abs 540nm g^{-1} dry mass	Non-structural carbohydrates μg mg^{-1} dry mass	Mass g	Specific activity mg N fixed g^{-1} nodule mass d^{-1} x 10^3
Crop						
	UV-B_{100}	48.7 ± 2.8[a]	0.580 ± 0.033[a]	118.4 ± 8.3[a]	0.681 ± 0.075[a]	7 445.4 ± 510.0[ab]
	UV-B_{134}	51.4 ± 2.9[a]	0.690 ± 0.059[a]	116.3 ± 8.2[a]	0.611 ± 0.085[a]	7 020.9 ± 490.4[a]
	UV-B_{166}	52.8 ± 4.9[a]	0.686 ± 0.079[a]	120.1 ± 9.6[a]	0.542 ± 0.072[a]	9 186.3 ± 840.3[b]
F-ratios		$F_{2,9} = 0.4$	$F_{2,9} = 0.8$	$F_{2,9} = 0.1$	$F_{2,9} = 0.3$	$F_{2,9} = 3.1$*
Forest						
	UV-B_{100}	55.9 ± 6.8[a]	0.921 ± 0.055[a]	116.3 ± 3.1[a]	1.671 ± 0.113[a]	1.7 ± 0.2[a]
	UV-B_{134}	57.2 ± 8.9[a]	0.984 ± 0.026[a]	122.1 ± 5.8[a]	1.680 ± 0.186[a]	1.9 ± 0.2[a]
	UV-B_{166}	62.5 ± 7.6[a]	0.886 ± 0.057[a]	118.2 ± 5.9[a]	1.503 ± 0.147[a]	1.6 ± 0.2[a]
F-ratios		$F_{2,9} = 1.0$	$F_{2,9} = 1.9$	$F_{2,9} = 0.2$	$F_{2,9} = 0.6$	$F_{2,9} = 0.9$
Medicinal						
	UV-B_{100}	158.5 ± 19.4[a]	1.520 ± 0.121[a]	118.1 ± 5.1[a]	1.207 ± 0.077[a]	2.6 ± 0.3[a]
	UV-B_{134}	178.8 ± 15.3[a]	1.658 ± 0.121[a]	128.2 ± 5.7[a]	1.580 ± 0.197[a]	2.1 ± 0.3[ab]
	UV-B_{166}	142.4 ± 14.4[a]	1.387 ± 0.098[a]	111.2 ± 6.1[a]	1.332 ± 0.259[a]	1.5 ± 0.2[b]
F-ratios		$F_{2,9} = 4.7$*	$F_{2,9} = 1.4$	$F_{2,9} = 2.6$	$F_{2,9} = 1.5$	$F_{2,9} = 4.8$*
Ornamental						
	UV-B_{100}	85.4 ± 8.2[a]	0.626 ± 0.059[a]	145.1 ± 3.8[a]	1.636 ± 0.173[a]	1.5 ± 0.1[a]
	UV-B_{134}	74.5 ± 7.7[a]	0.571 ± 0.050[a]	132.7 ± 8.7[a]	1.264 ± 0.194[a]	1.6 ± 0.1[a]
	UV-B_{166}	87.7 ± 5.0[a]	0.744 ± 0.064[a]	149.9 ± 2.1[a]	1.454 ± 0.264[a]	1.7 ± 0.3[a]
F-ratios		$F_{2,9} = 1.3$	$F_{2,9} = 2.9$	$F_{2,9} = 1.8$	$F_{2,9} = 1.0$	$F_{2,9} = 0.6$
Pasture						
	UV-B_{100}	82.9 ± 7.9[a]	1.039 ± 0.125[a]	71.3 ± 4.0[a]	0.337 ± 0.056[a]	43.4 ± 26.3[a]
	UV-B_{134}	85.0 ± 7.2[a]	0.931 ± 0.105[a]	69.3 ± 4.1[a]	0.268 ± 0.049[a]	25.1 ± 6.3[a]
	UV-B_{166}	111.0 ± 13.6[a]	1.317 ± 0.089[a]	78.4 ± 3.5[a]	0.332 ± 0.042[a]	14.2 ± 2.2[a]
F-ratios		$F_{2,9} = 1.6$	$F_{2,9} = 2.6$	$F_{2,9} = 1.9$	$F_{2,9} = 0.6$	$F_{2,9} = 0.4$

Table 2: Effects of supplementary UV-B approximating 15% ($UV\text{-}B_{134}$) and 25% ($UV\text{-}B_{166}$) depletions in total column ozone on the quantities of nitrogen fixed in different organs of traditional African commercial legumes

Category	UV-B treatment	Nitrogen fixed mg				
		Nodules	Roots	Stems	Leaves	Whole plant
Crop						
	$UV\text{-}B_{100}$	38.4 ± 4.4[a]	49.7 ± 5.0[a]	58.8 ± 5.8[a]	108.8 ± 10.4[a]	274.7 ± 22.1[a]
	$UV\text{-}B_{134}$	34.3 ± 4.8[a]	41.8 ± 5.4[a]	45.9 ± 6.5[a]	85.6 ± 12.3[a]	234.6 ± 25.7[a]
	$UV\text{-}B_{166}$	30.1 ± 4.3[a]	40.0 ± 4.5[a]	55.1 ± 6.2[a]	111.6 ± 11.9[a]	264.4 ± 21.9[a]
F-ratios		$F_{2,9}$ = 0.4	$F_{2,9}$ = 0.8	$F_{2,9}$ = 1.6	$F_{2,9}$ = 2.1	$F_{2,9}$ = 1.6
Forest						
	$UV\text{-}B_{100}$	77.9 ± 8.1[a]	145.6 ± 26.2[a]	63.4 ± 13.2[a]	197.1 ± 40.3[a]	484.1 ± 81.9[a]
	$UV\text{-}B_{134}$	86.0 ± 9.0[a]	153.4 ± 22.6[a]	69.1 ± 9.7[a]	233.5 ± 37.5[a]	542.0 ± 71.2[a]
	$UV\text{-}B_{166}$	66.9 ± 7.0[a]	128.4 ± 20.4[a]	55.4 ± 14.9[a]	152.8 ± 30.6[a]	403.4 ± 69.5[a]
F-ratios		$F_{2,9}$ = 2.1	$F_{2,9}$ = 0.5	$F_{2,9}$ = 0.9	$F_{2,9}$ = 2.3	$F_{2,9}$ = 1.7
Medicinal						
	$UV\text{-}B_{100}$	45.4 ± 2.6[a]	170.9 ± 14.5[a]	94.6 ± 10.6[a]	269.3 ± 25.1[a]	580.2 ± 45.3[a]
	$UV\text{-}B_{134}$	58.9 ± 5.0[a]	185.2 ± 12.2[a]	74.4 ± 5.9[a]	258.7 ± 40.1[a]	577.2 ± 55.0[a]
	$UV\text{-}B_{166}$	45.2 ± 9.6[a]	127.9 ± 29.7[b]	49.8 ± 15.1[b]	169.7 ± 43.8[b]	392.6 ± 95.2[b]
F-ratios		$F_{2,9}$ = 3.8	$F_{2,9}$ = 5.7*	$F_{2,9}$ = 12.3**	$F_{2,9}$ = 6.5*	$F_{2,9}$ = 8.5**
Ornamental						
	$UV\text{-}B_{100}$	73.8 ± 9.8[a]	155.0 ± 23.0[a]	53.1 ± 11.1[a]	179.3 ± 34.1[a]	461.1 ± 74.7[a]
	$UV\text{-}B_{134}$	60.9 ± 11.1[a]	138.1 ± 26.3[a]	49.6 ± 15.0[a]	152.7 ± 37.4[a]	401.3 ± 87.6[a]
	$UV\text{-}B_{166}$	71.6 ± 12.9[a]	141.5 ± 26.7[a]	59.4 ± 15.8[a]	204.3 ± 50.6[a]	476.8 ± 101.3[a]
F-ratios		$F_{2,9}$ = 0.4	$F_{2,9}$ = 0.4	$F_{2,9}$ = 0.3	$F_{2,9}$ = 0.3	$F_{2,9}$ = 0.4
Pasture						
	$UV\text{-}B_{100}$	22.2 ± 4.4[a]	114.4 ± 20.8[a]	103.9 ± 9.8[a]	177.6 ± 21.0[a]	429.0 ± 41.9[a]
	$UV\text{-}B_{134}$	18.1 ± 3.9[a]	112.0 ± 21.1[a]	104.6 ± 10.2[a]	162.3 ± 17.6[a]	412.6 ± 43.4[a]
	$UV\text{-}B_{166}$	21.9 ± 3.7[a]	114.1 ± 19.3[a]	103.9 ± 8.7[a]	174.6 ± 21.9[a]	425.0 ± 43.1[a]
F-ratios		$F_{2,9}$ = 0.5	$F_{2,9}$ = 0.1	$F_{2,9}$ = 0.1	$F_{2,9}$ = 0.1	$F_{2,9}$ = 0.1

increased ($P \leq 0.05$) with increased UV-B (Table 1). However, the increase was only statistically significant ($P \leq 0.05$) between the moderately ($UV\text{-}B_{134}$) and highly ($UV\text{-}B_{166}$) elevated UV-B treatments (Table 1), and significantly ($P \leq 0.05$) negatively correlated ($t_{1,102} = -1.69$) with root flavonoid concentrations only. Also, the increase in nodule activity did not correspond with any significant ($P \geq 0.05$) changes in the quantities of N fixed in different plant organs and whole plants. The remaining three commercial categories displayed insignificant changes in nodule mass and activity, quantities of N fixed in different plant organs and whole plants, as well as in their root flavonoid, anthocyanin and non-structural carbohydrate concentrations with increased UV-B (Tables 1 and 2).

Discussion

Phenylpropanoid compounds such as flavonoids and anthocyanins have been functionally linked to nodulation in some legume species (Hungria and Phillips 1993, Zhang and Smith 1997, Dakora 2000, Cullimore and Dénarié 2003). Under ambient levels of UV-B radiation, elevated flavonoid concentration in *Pisum sativum* and *P. vulgaris* roots have been associated with increased nodule production and N_2 fixation (Shiokazi *et al.* 1999, Pinto *et al.* 2002). Consequently, the insignificantly unaltered root flavonoid concentrations observed in all traditional commercial categories, except the medicinal category, in this study could partly explain the unchanged nodulation and N_2 fixation in response to UV-B supplementation. These findings concur with reports of an absence of a UV-B effect on symbiotic function in *P. sativum* (Allen *et al.* 1998, Stephen *et al.* 1999), in *Vicia faba* (Al-Oudat *et al.* 1998) and *Acacia karroo* (Ernst *et al.* 1997), which together represent crop, pasture and forestry categories. However, they contrast with the decline in N_2 fixation with increased UV-B measured in *C. maculata* (Honeybush), a slow growing commercially important herbal beverage representing the medicinal category, and with previous reports of UV-B induced reductions in nitrogenase activity in the angiosperms *Vigna radiata* cv. Pan U-30, *Phaseolus munga* cv. Mash 48 (Singh 1997) as well as in the N_2-fixing cyanobacteria *Nostoc calciola* (Kumar *et al.* 1996), *Anabina flosaquae* and *Nostoc spongiaeforme* (Newton *et al.* 1979, Tyagi *et al.* 1992). These contrasting symbiotic responses to UV-B enhancement have been attributed to genotypic variation in UV-B sensitivity (Jansen *et al.* 1998), and/or differences in environmental conditions, including the intensity of UV-B under which plants are grown (Fiscus and Booker 1995). For example, the total daily supplemental UV-B exposure dose applied by Singh (1996, 1997) was similar to that in our moderately elevated UV-B treatment, but applied more intensely over much shorter (2h) daily photoperiods.

The unusual sensitivity of N_2 fixation in *C. maculata* to increased UV-B was unclear, since this medicinal species contained much higher flavonoid concentrations, implicated both in UV-B protection (Rozema *et al.* 1997) and nodule induction (Phillips 2000), than in the other commercial categories examined. Indeed, total flavonoid content in this species displayed no statistically significant separation

between the three UV-B treatments applied. However, compositional changes in flavonoid accumulation have also been reported in response to UV-B radiation in both pasture and forestry species (Hoffman *et al.* 2000, Tegelberg *et al.* 2001). Such changes in the proportions of different flavonol derivatives could potentially result in an inhibition rather than promotion of nod gene induction (Djordjevic *et al.* 1987), which might explain the decline in N_2 fixation observed in *C. maculata*, and also alter the taste and therapeutic properties of Honeybush that might threaten this newborn South African tea industry.

In conclusion, this study's findings indicate negligible impacts of ozone depletion on nitrogen fixation and soil fertility in most traditional African farming systems, these being limited to occasional inhibition of nodule induction in some commercial crops.

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