Proxy methods to estimate UV

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Abstract. Depletion of stratospheric ozone since the mid 1970s has led to significant increases in Ultraviolet B (UV-B) irradiation over Antarctica. Plants produce photo-protective flavonoids that act as sunscreens to reduce cellular damage. These compounds are stable once dried and may be used as a proxy method to reveal historical levels of UVB radiation. The flavonoid contents of herbarium samples of the moss *Bryum argenteum* collected in Antarctica before and after the formation of the ozone hole were compared. Regression analysis showed the ratio of di-hydroxylated to mono-hydroxylated flavones increased significantly with increasing modelled midday UV-B/PAR ratio ($p<0.001$), and decreasing ozone concentration ($p<0.001$). We emphasise the utility of this ratio in interpreting historical ozone trends rather than relying on changes in total flavone concentrations alone. Factors such as cloud cover can have a significant influence on UV-B dose at ground level, modifying the flavonoid content of the specimen and adding considerable variability to the results.

Introduction

UV-B radiation damages a variety of biologically significant molecules including nucleic acids, proteins and lipids. Secondary plant metabolites are often implicated in the protection of plants from damaging UV-B radiation (Caldwell *et al.,* 2007), and their up-regulation is a most common response to UV–B stress in land plants (Ryan *et al.,* 1998). Flavonoids absorb strongly in the UV-B spectrum and harmlessly dissipate the absorbed energy, preventing photochemical damage. They are also effective free radicals scavengers.

Di-hydroxylated flavonoids, which have an extra hydroxyl group attached to the core of the flavonoid molecule, are preferentially up-regulated by UV-B radiation compared to their mono-hydroxylated counterparts. Their absorption spectra are similar, and the extra OH may confer improved antioxidant scavenging (Smith & Markham, 1996).

We used High Performance Liquid Chromatography (HPLC) to compare flavonoid concentrations in herbarium specimens of the moss *Bryum argenteum* collected from the Ross Sea region before and after the formation of the ozone hole in the mid 1970's. In particular, we examine whether ozone-induced increases in ambient UV-B radiation have caused an increase in the ratio of di-hydroxylated to mono-hydroxylated flavones in preserved specimens of Antarctic mosses.

Results and discussion

Flavonoids may be particularly useful indicators of environmental change as they are rapidly produced in higher plants in response to stressors such as UV-B, and are unusually stable over very long periods of time (Markham, 1982).

Our initial findings, using moss specimens from the Ross Sea region show that total flavonoid concentrations increased significantly after the development of the annual ozone hole ($P<0.001$, data not shown, see Ryan *et al.*, 2009). Data from the two years with the most data are shown in Fig. 1. Di and mono-hydroxylated flavones were present in approximately equal concentrations in the pre-ozone hole samples of 1965, but in 2004 the ratio increased to approximately 2:1.

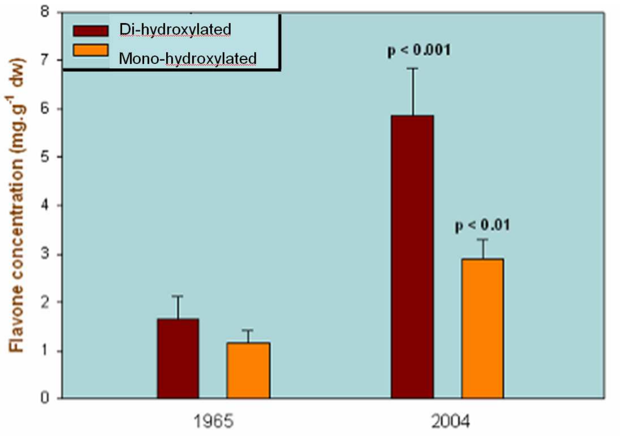

Figure 1. Mean Di- and Mono-hydroxylated flavone concentrations from year 1965 and 2004. Error bars are standard error of the mean.

Lower pigment compositions in our extracts of older herbarium specimens may have been due simply to degradation with time. However, flavonoids are perhaps the most stable plant metabolites, and have been useful in a number of long-term studies (Björn & McKenzie, 2007). Nevertheless, if samples are not stored correctly, there is still potential for degradation.

Elevated levels of UV-B result in up-regulation of di-hydroxylated flavonols (Ryan *et al.*, 1998). We propose that since there is no reason that di- and mono-hydroxylated flavonoids should degrade at different rates, a comparison of the ratios of the concentrations of the two should eliminate any effect of pigment loss. Even if some degradation has occurred during herbarium storage, we argue that the ratio will still reflect the native state at collection and thus provides an important new method for the analysis of historical ozone trends.

Ozone data were obtained from Halley Station (75^{O}S) (J. Shanklin, pers. comm.) and the mean daily ozone concentration for the two weeks prior to collection was calculated for each sample. A regression analysis shows a highly significant negative relationship between di:mono ratio and mean daily ozone (Fig. 2, $P<0.001$). Considerable variability in the data is clearly evident.

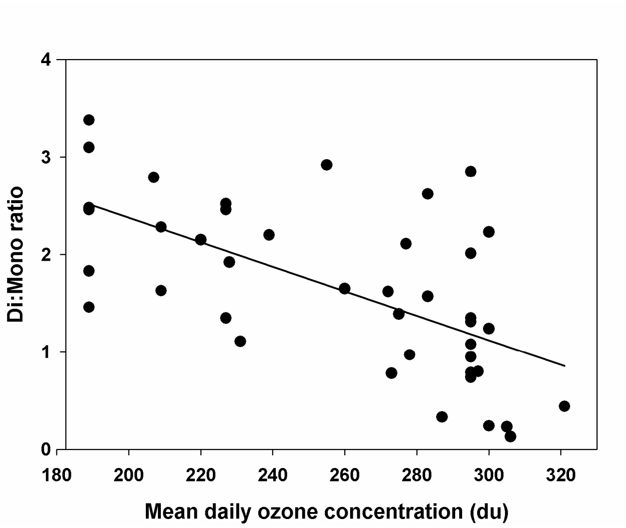

Figure 2. Linear regression between Di:Mono ratio and mean ozone level for 2 weeks prior to sample collection. $R^2 = 0.39$.

An attempt was made to eliminate this variability further by comparing the di:mono ratio with UV-B radiation estimated for each site. While some measurements of UV-B radiation are available for the Ross Sea region from Arrival Heights (Lat. 78°S), this dataset begins in 1990. Modelled clear sky UV-B and photosynthetically active radiation (PAR) data for the period covered by these samples were obtained using the interactive online radiative transfer model, TUV (Madronich & Flocke 1997). The model includes a latitude parameter and we argue that this should reduce variability further. While the relationship between L:A ratio and UV-B/PAR was also highly significant (Fig 3, $P<0.001$) the R2 value (0.28) was lower than that for the ozone comparison indicating more variability. The reasons for this are not understood.

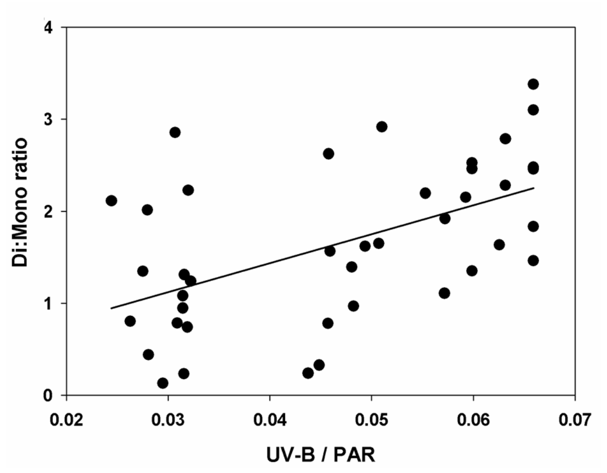

Figure 3. Linear regression between Di:Mono ratio and UV-B/PAR. $R^2 = 0.28$.

Although the relationship between ozone and L:A ratio in our data was highly statistically significant, more than 60% of the variation is still unexplained. In many of our samples, low ratios may have been due to local environmental factors, rather than changes in ozone. Unfortunately, apart from the 2004 samples we collected, we do not know the local weather conditions in the two weeks prior to the collection of the herbarium samples.

Even though tropospheric UV-B levels may be high due to depleted stratospheric ozone, clouds or snow cover can attenuate the UV-B radiation received at ground level. Furthermore, while depletion of Antarctic stratospheric ozone occurs in spring, near normal levels are recorded later in summer. Thus, flavonoid levels from more recent herbarium samples collected in late summer may reflect non-ozone hole conditions. Thus, while herbarium samples may offer tantalising possibilities in revealing historical ozone levels, it must be accepted that there will be unavoidable variability among the samples that reflect the combined influences of cloud cover, snow cover, season, temperature and shade at the time of collection. While up-regulation of phenylpropanoid metabolism may be primarily induced by exposure to UV-B, these other factors will modify rates of flavonoid accumulation and add scatter to the data. In spite of these confounding factors, the influence of changes in ambient UV-B radiation due to differences in overhead ozone concentration is still apparent in our data. The variability may be reduced by employing ratios of di : mono-hydroxylated flavonoids rather than using changes in total concentration, and this ratio provides a good proxy for recording past changes in stratospheric ozone concentration.

References

Björn LO, McKenzie RL (2007) Attempts to probe the ozone layer and the ultraviolet-B levels of the past. *Ambio*, 36, 366-371.

Caldwell MM, Bornman JF, Ballare CL *et al.,* (2007) Terrestrial ecosystems, increased solar ultraviolet radiation, and interactions with other climate change factors. *Photochem. Photobiol. Sci.*, **6**, 252-266.

Madronich S, Flocke S (1997) Theoretical estimation of biologically effective UV radiation at the Earth's surface. In: *Solar Ultraviolet Radiation-Modelling, Measurements & Effects* (eds Zerefos, CS & Bais, AF), NATO ASI Series I: Global Environmental Change, Vol. 52, Springer-Verlag, Berlin

Markham KR (1982) *Techniques of Flavonoid Identification.* Academic Press. London, 113 pp.

Ryan KG, Markham KR, Bloor SJ *et al.,* (1998) UVB radiation induced increase in quercetin: kaempferol ratio in wild-type and transgenic lines of *Petunia*. *Photochemistry & Photobiology*, **68**, 323-330

Ryan KG, Burne, A and Sepelt RD (2009). Historical ozone concentrations and flavonoid levels in herbarium specimens of the Antarctic moss *Bryum argenteum. Climate Change Biology* **15**, 1694-1702

Smith GJ, Markham KR (1996) The dissipation of excitation energy in methoxyflavones by internal conversion. *Journal of Photochem. Photobiol. A* **99,** 97-101