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Carbon isotopic analysis of individual modern and fossil grass-pollen grains using a moving-wire combustion interface

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Pollen grains from grasses using the C_3 and C_4 photosynthetic pathways possess distinct δ^{13}C values that may be used to estimate the relative abundance of these grasses in paleorecords. Using typical methods, a single analysis using an elemental analyzer interfaced with an isotope-ratio mass spectrometer (EA-IRMS) requires 600 grains of pollen and $\sim$10-hours of preparation time. In addition, single data points representing mixed samples may prevent detection of C_4grasses at $< \sim$30% abundance because of assumptions associated with mixing models. We evaluated a moving-wire IRMS (mw-IRMS) system for δ^{13}C analysis of individual grass pollen grains. Pollen from four C_3 and four C_4 grass species was isolated through micromanipulation and analyzed as single grains suspended in water. The criterion to distinguish samples containing pollen ("pollen present") from those not containing pollen, was a carbon yield greater than the 2σ range of the carbon content of blanks containing only water. This criterion resulted in the exclusion of $\sim$45% of the 946 samples applied to the wire. The average δ^{13}C values ($\pm$ 1σ) of the remaining samples were -26.9‰ ($\pm$ 6.3‰) and -11.5‰ ($\pm$ 9.6‰) for C_3 grasses and C_4 grasses, respectively, after blank-correcting the δ^{13}C data. Such high variability in measured δ^{13}C values likely is caused by a combination of factors. These include natural isotopic variability among individual pollen grains; the relatively poor precision that can be obtained when determining δ^{13}C values of such small samples; and the large degree of uncertainty in the magnitude, isotopic composition, and stability of the analytical blank. Nonetheless, high percentages of individual pollen grains were correctly classified as being of either

C_3 or C_4 origin. On average, 90% (range=78-100%) of pollen grains from C_3 grasses had $\delta^{13}C$ values more negative than the empirically-determined cutoff threshold of -19.2‰; while 84% (range=77-90%) of pollen grains from C_4 grasses had $\delta^{13}C$ values more positive than 19.2‰. These results suggest that the mw-IRMS system can be used to distinguish C_3 from C_4 grass pollen. The number of pollen grains required for $\delta^{13}C$-based evaluation of C_3/C_4 grass composition is many times lower with mw-IRMS than with EA-IRMS, and $\delta^{13}C$ data from the mw-IRMS does not need to be incorporated into a mixing model to derive estimates of the abundance of C_3 and C_4 grass pollen. Carbon isotopic analysis of individual grass pollen grains may help improve our understanding of the evolutionary and ecological significance of grass taxa in the paleorecord. To further evaluate this proxy we will present $\delta^{13}C$ data obtained from individual grains of grass pollen from lake-sediment surface samples that span large gradients of C_3 and C_4 grass abundance in the North American Great Plains.