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PHOTOACOUSTIC ASSESSMENT OF PROSPECTIVE PLANT PRODUCTIVITY

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ABSTRACT

We present preliminary evidence that photoacoustic spectroscopy (PAS) can be a very useful complementary tool in assessing plant producitivity at some stage of its development. Experimental support comes from PAS of the red chlorophyl band in green leaves samples when correlated with the rate of eater loss due to spontaneous dehydration. Samples were selected from live plant leaves of both an inbred line (selfed five times) and an open pollinated UR-1 variety of corn grown under controlled conditions and known to have well differentiated crop yields. The analysis of the first experimental results seems to indicate some definite correlation between the data and the plant prospective productivity. This encouraged long term testing of our findings, which are now in progress to check for statistical significance through several plant generations.

INTRODUCTION

As is well know the photoacoustic (PA) effect probes essentially the nonradiative deexcitation process that occur in a system which is periodically excited by the absorption of modulated light. This selective sensitivity of the PAS technique to the nonradiative thermal deexcitation channel makes it a choice technique for investigating photosensitive materials. This is evident from the sucessful ise of PAS as a complementary tool in the study of a variety of relaxation processes in fluorescence[1-2] and photochemical[3] studies as well as in biological systems[4-8]. The basic ide a governing these studies is that the relaxation following the optical excitation of a given material involves both radiative (fluorescence and phosphorescence) and non-radiative (photochemical, thermal excitation, etc) processes. Now, since the PA effect couples only to the thermal deexcitation channel its efficiency is of course complementary to that of the other deexcitation processes. Hence, when an optically excited energy level decays through mechanisms suchh as fluorescence, phosphorescence or a photochemical process, then a weaker acoustic signal shows up in the PA-cell.

In this paper we further explore these ideas in the context of grain production in agriculture, by investigating the PAS signal of green leaves of different varieties of a given culture (in our case, corn). The idea is essentially centered on the characteristics of the PA signal generation outlined in the foregoing. The PAS of a green leaf turns out to be a function of both the absorbed optical energy and of the competition among the thermal, bioluminescence and photochemical channels. Here we note that for plant matter the luminescence efficiency is usually very low[9,10] and may therefore be disregarded as an effective deexcitation channel as

compared to the photochemical and thermal deexcitation. The complementary character of these two processes entails that if for two specimens of comparable thickness and moisture content the photochemical yield (e.g., photosynthetic activity) are different one should then also expect to observe different PAS signals on account that the acoustic signal should be smaller for the specimen with the largest photochemical yield. In other words, the PAS signal may, in principle, be used as a sensitive tool for discriminating in a collection of similar samples the specimen which shows the largest photochemical yield. This we assume must come from the live plant exhibiting the largest photosynthesis activity and therefore having the largest potential for grain production.

EXPERIMENTAL

Our PAS experimental set up consisted basically of a 300 W tungsten-filament lamp with a flat intensity from 600 to 727 nm, a variable-speed light chopper, an aluminum cylindrical cell fitted with an electrect microphone, a PAR 186A Synchro-Het lock-in amplifier and a Bausch and Lomb 6334 VK monochromator.

Our samples came from live specimens of both an open po-linated UR-1 variety and an inbred line (selfed 5 times) of corn (Zea mays). These specimens belong to an experimental agrostation where both kinds of corn plants are being studied with regards to crop production for five plant generations. The important point for our purpose is that for the specimens from where our samples were taken the inbred line has a certified crop yield that is almost three times smaller than that of the UR-1 variety.[11] The samples were cut in the form of equally thick green leaf disks of 10 mm diameter and were labelled according to the position of the leaf in the stem of the live plant

to which it belonged. The leaves were numbered sequentially from top to bottom. To distinguish among samples of the two kinds of plant we labelled A (B) the inbred line (open pollinated UR-1 variety). Thus an A_5 (B_5) sample means a sample cut from the fifth leaf from the top of a corn plant specimen of line A (B). While accompanying the time evolution of the PAS signal for a given sample we have also carefully monitored its mass with an analytical balance. To avoid mechanical deformation of the disk shaped sample as it undergoes spontaneous dehydration we kept the samples, between measurements, well stretched between glass plates. We have also assessed the pigment concentration on the various samples. This was done via transmission spsectroscopy of an acetone based solution of the samples pigment. The results showed that the pigment concentration were the same within a 15% margin.

RESULTS

Figs. 1 to 3 which correspond to three different stages of the plant development show some typical spectra. They have all been recorded at a modulation frequency of 45 Hz and were taken after the indicated times have elapsed since the respective sample had been cur from the live plant. The well defined absorption pattern peaked at 700 nm is characteristic of the chlorophyl band and will be of central importance in this work. When we took the spectra in Fig. 1 the samples A_5 and B_5 were collected from plants that had reached only about one third of their size. This corresponded to a stage of the plant growth when the plant was developing at a comparatively fast rate (growing stage). In contrast, in the case of Figs. 2 and 3 the samples were collected when the live plants were already fully grown. In the case of Fig. 2 the plants were in the flowering stage whereas in that of

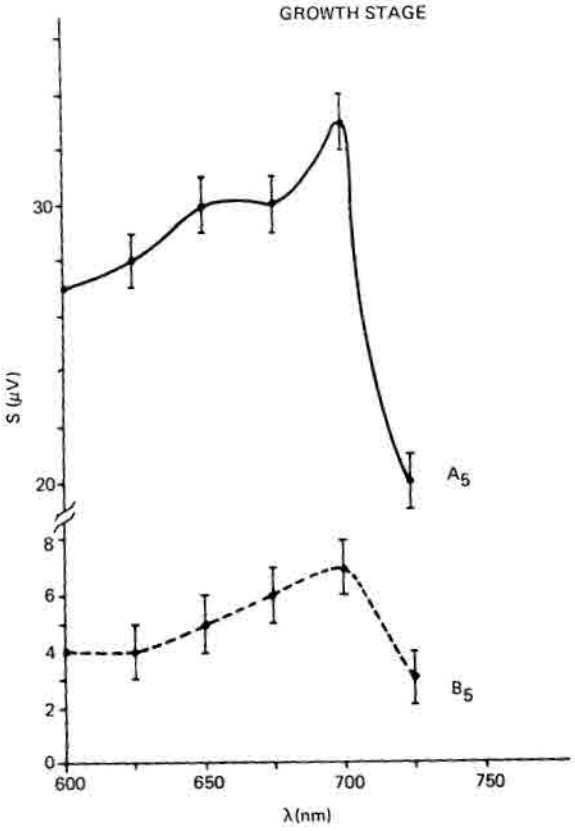

FIGURE 1 - Photoacoustic spectra of the A_5 and B_5 samples taken 28 hours after cur from the live plant. Chopping frequency was 45 Hz and we have spanned the chlorophyl band centered at 700 nm Plants were in growth stage.

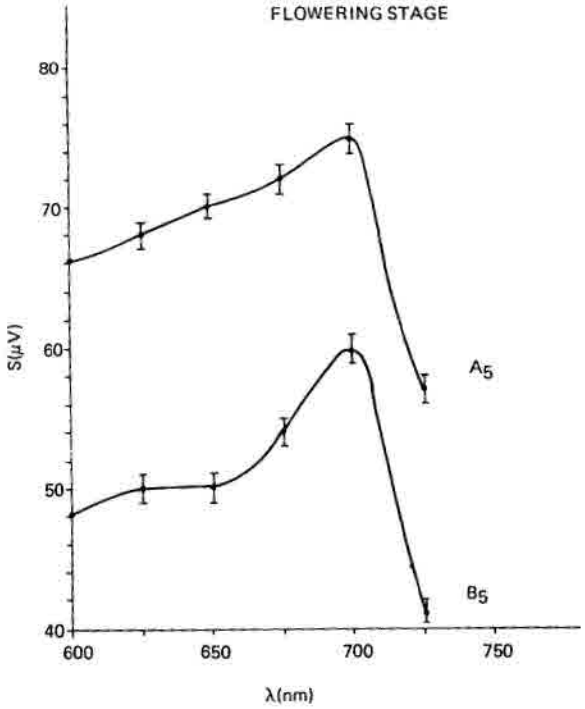

FIGURE 2 - Photoacoustic spectra of the A_5 and B_5 samples taken 5 hours after cur from the live plant. Chopping frequency was 45 Hz and we have spanned the chlorophyl band centered at 700 nm Plants were in flowering stage.

Fig. 3 the grain production stage was already in progress (seeding stage).

In Fig. 4 we show the time evolution of the ratio $R_i = S(A_i) / S(B_i)$ of the peak PAS signal from samples of line A to that from samples B recorded at 700 nm and a modulation frequency of 45 Hz, at each of the forementioned plant stages. Finally, in Fig. 5 we show a typical peak PAS signal of the A_5 sample at 700 nm, as a function of the modulation frequency.

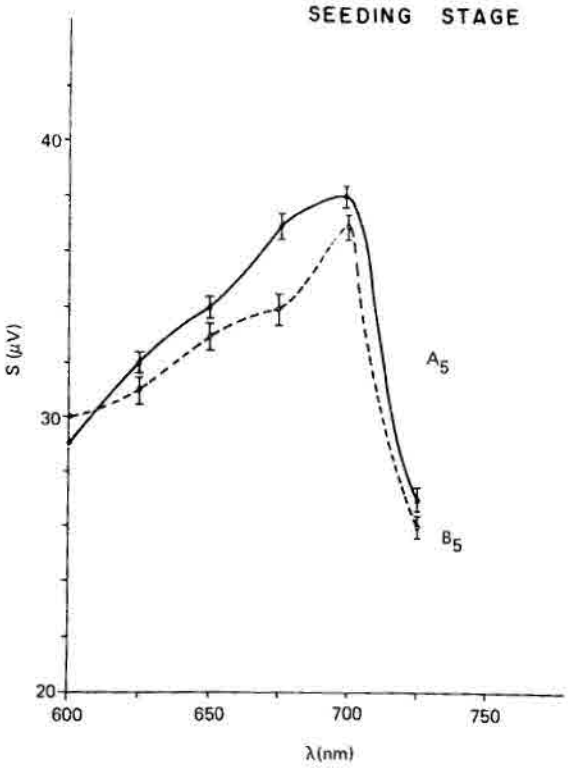

FIGURE 3 - Photoacoustic spectra of the A_5 and B_5 samples taken 27 hours after cut from the live plant. Chopping frequency was 45 Hz and we have spanned the chlorophyl band centered at 700 nm Plants were in seeding stage.

DISCUSSION

It is quite apparent from Figs 1-4 the sensitivity of the signal intensities in the PAS spactra to both the plant kind and the stage of its development. In the course of our work a systematic observation based on tens of spectra like those depected in Figs. 1 to 3

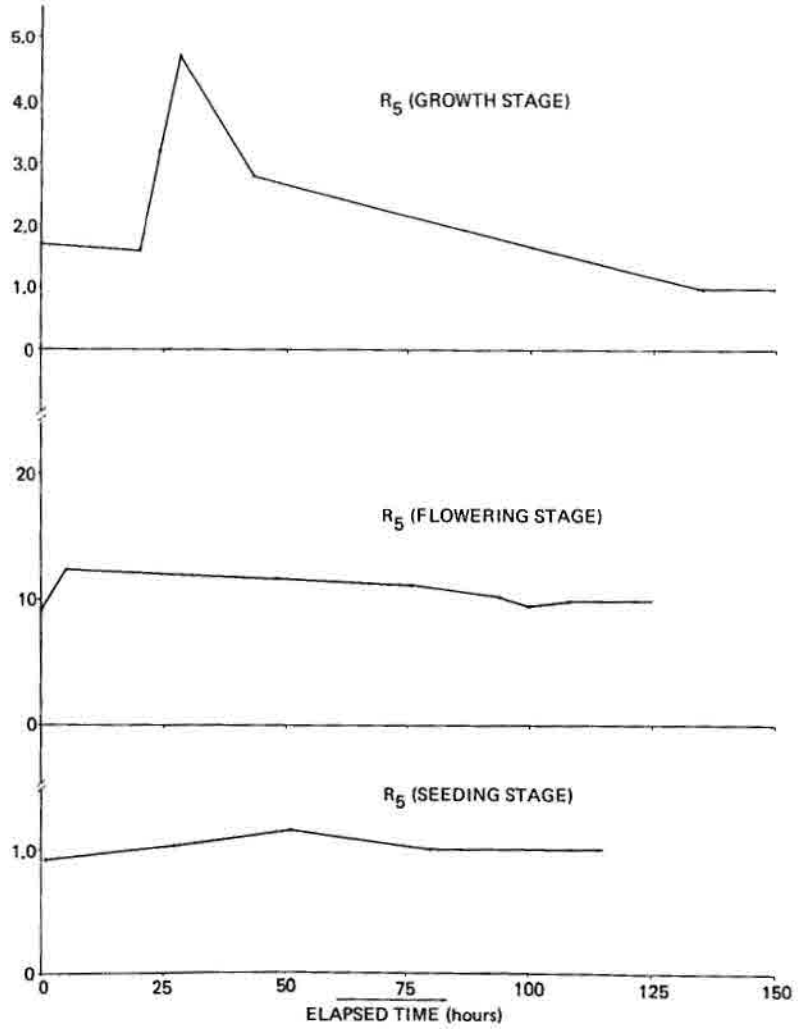

FIGURE 4 - Time evolution of the $R_5 = A_5 / B_5$ ratio between PAS 700 nm peak signals of the A_5 and B_5 samples, at the indicated plant development stages. Chopping frequency was 45 Hz.

revealed the existence of interesting patterns in their time evolution. Our observations centered on both the temporal changes in the spectra of a single sample as a function of the time elapsed since it was cut and the behavwor of the PAS spectra of both kind of plant as a function of the stage of development. These patterns, to be discussed below, can be simultaneously appreciated in Fig. 4 which shows typical signal ratios vs. elapsed time at various plant stages (from now on by "elapsed time" we will always mean the time interval since the cut of the sample from the live plant). This is one very many similar plots from where a systematic behavior of the green plant PAS spectra and its relation to plant productivity was inferred, as will become apparent in what follows.

We begin by mentioning that during the growth stage the UR-1 variety already demonstrated a much more conspicuous development than the inbred line. The photoacoustic signal of UR-1 leaf samples exhibited a consistently smaller value than the signal from the inbred line sample. This was in agreement with our prediction that a larger photosynthetic activity implies in a smaller acoustic signal. The part in Fig. 4 which refers to the growth stage depicts this fact rather dramatically. However, while at the flowering stage differences in the PA peak signal intensities for the species can still be seen, they have clearly desappeared when the plants reached the seeding stage (grain formation). This fact is indicative that if differences in PAS signal are to be construed in some way to tell apart plants with potentially different crop fields this should be done at an early stage of plant development. Under some circunstances this ability for early discrimination can prove to be quite an advantage as regard to specimen selection for agro-experimentations in plant breeding for increasing productivity.

As we mentioned before the crop production of the two kinds of plants selected for our testings were known to be markedly different as established from several precious observations at the agrostation. The average grain yield of the inbred line has been 938 kg/ha, whereas that of the UR-1 variety is roughly 3946 kg/ha. From these results it was predicted that line B should have a much larger photosynthetic activity than line A since photosynthesis has quite a direct bearing on plant matter production and therefore on grain productivity. There remains, however, the question of why well differentiated photoacoustic responses are only seen in the growth stage. True enough the growth stage, being one of an intrinsically higher photosynthetic activity as compared to the other phases, could well be the main phase in determining the crop yield. But this should affect both specimens of plants. There must be some other (s) factor (s) that has a direct bearing on the photosynthetic activity and which is quite different among the two specimens. Of course this would reflect on different PAS responses for them in the growth stage. It turned out that while studying differences in PAS spectra as a function of the elapsed time we noticed sensible differences in the samples state due to spontaneous dehydration. We proceeded then to carefully monitor the samples masses to evaluate the water loss. Two facts soon became evident to us from our measurements: first, the two kinds of plant loose water at markedly different time rates; second, the product of the peak signal intensity and the sample mass was constant within reasonable error margins. This product had quite different values for each of the two kinds of plant. This pattern, however, was absent in the measutements made during the plants seeding (production) stage.

The second of the abovementioned facts is indeed quite important in our context. In fact it seems to be

clearly indicative that the PA signal intensity for our examined samples is inversely proportional to the sample heat capacity. The latter is varying in time due to the loss of water.

To further understand those differences in the PAS signals in the growing stage we have measuted the 700 nm peak signal dependence on the modulation frequency up to 150 Hz. From the results we concluded that within our working range of chopping frequencies (i.e, 45 Hz) our samples were thermally thin since the acoustic signal exhibited the expected f^{-1} law as shown in Fig. 5. Hence, according to the existing theories[12], the PAS signal can be written as

$$S = \frac{A \eta \beta}{f}$$

where β is the optical absorption coefficient, f is the modulation frequency, η is the efficiency at which the absorbed light is converted into heat ($\eta = 1 - \varepsilon$, ε being the photochemical efficienty) and A is a constant factor dependent upon the light intensity and the thermal properties of both the gas and the backing materials. The differences in the relative peak PAS signal in the growing stage may then be attributed to differences in the thermal conversion efficiences of the two kinds of plant. This difference in η (or ε) and consequently, on the heat transfer to the bulk of the sample and the ensuing temperature fluctuation in the PA cell gas, seems to suggest that the chlorophyl pigment have different macroenvironment in the UR-1 variety as compared to the inbred line. We suppose that this macroenvironment difference is most likely to be responsible for the differentiated abilility of the two species to retain water.

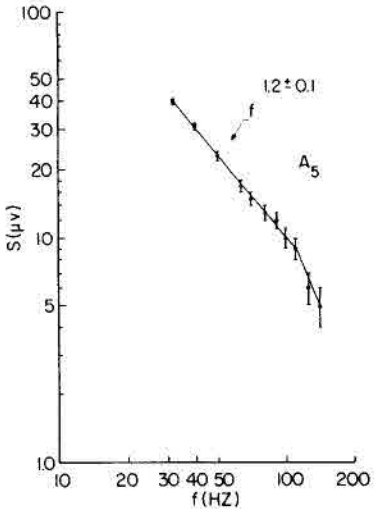

FIGURE 5 - PAS 700 nm peak signal of the A_5 sample, taken 6 hours after cut from the live plant, as a function of the modulation frequency.

When we correlate these results with the observed signal times mass time independence in both the growth and flowering stages plus the forementioned thermally thin character of our samples we are led to the conclusion the thermal efficiency itself can be written as $\eta = \frac{\chi}{C}$. Here C is the sample's heat capacity and χ is a factor related to the plant kind ability to retain water for possible photosynthetic use. This hypothesis seems to have some support from its consequences being in direct agreement with our experimental observations regarding the time behavior of the PAS spectra. For instance, the absence of marked differences between the peak signal intensity of the two kinds of plant during the seeding stage could be accounted for as follows. Assume that at this stage the water storage on the plant leaf is brought

to a minimum due to high photosynthetic demand. This would render the thermal conversion efficiency at this stage rather insensitive to the water content. In other words, the lack or the very low level of residual water in the sample make it irrelevant, for the PA signal build up, the eventual differences in macroenvironment of the chlorophyl pigments which otherwise would sensibly affect the acoustic signal on account of the differences on the plant ability to retain water. Again, on account of the $\eta = \chi/C$ relation one understands all of the forementioned time patterns exhibited by the spectra both in regard of the plant stage and of the elapsed time.

By this explanation the observed S x m constant value in both the growth and early flowering stages are unequivocally correlated with the observed time patterns of the PAS spectra. It would then seem possible, at least for preliminary evaluations, to tell apart plants of prospectively differentiable crop yields on account of the observed value for that product. In our observations with the two kinds of corn plants studied we recorded average values of the S x m products, along those stages, that were consistently more than twice as large for the UR-1 variety as compared to the inbred line.

Even though to make a well founded claim on our having a PAS based method to assess crop productivity will call for continued observations for statistical evaluation of our findings it is definitely worth noticing that such a ratio is in good agreement with the known ratio of crop productivity for these two corn species and in complicance with our previewed behavior of the PAS peak signal in the chlorophyl band spectra of plant green leaves.

CONCLUSION

We have produced some evidence that PAS can be of use in agronomics. Yet a definite statement as to the

aplicability of our procedure, described in the foregoing, calls for an statistical evaluation of our findings to rule out the chances of the observed time evolution of the PAS spectra being random. We have already set out, in association with our experimental agrostation, to make extensive studies of our findings along several plant generations, in order to establish their statistical significance. We should finally remark that though we have made our experiments using only corn plant our method should apply equally well to any other kind of green plant. Work is also in progress in this connection.

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DEPTH-PROFILING OF DOPANT REGIONS IN SILICON WITH THERMAL-WAVE MICROSCOPY

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ABSTRACT

Thermal-wave microscopy can be used to image, and to obtain depth-profiles of, dopant regions in semiconductors.

There has been considerable activity recently in thermal-wave microscopy, wherein images of microscopic surface and subsurface features are recorded when an intensity-modulated optical (laser) or electron beam is focused and scanned across the surface of an opaque sample.[1,2] Thermal waves are generated in the sample through the localized periodic heating that results from absorption of the sample of the intensity-modulated beam. These thermal waves have a wavelength $\lambda = 2\pi\mu$ where μ is the thermal diffusion length. The wave-like aspects of these thermal disturbances (see Fig. 1) are a result of the fact that the thermal flow is occurring at a single harmonic frequency. Under these conditions, the thermal disturbances can be treated, mathematically,as highly-damped hemispherical wavelets that emanate from the beam spot, and that undergo "reflection" and "scattering" processes when they encounter regions of different thermal characteristics.[2,3] In analogy with optics and acoustics, the resolution attainable in

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