THE INFLUENCE OF SUN AND SHADE ON HERBICIDE ACTIVITY AND TRANSLOCATION

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SUMMARY
The uptake, translocation, and biological activity of the herbicide imazapyr on *Paspalum conjugatum* was found to be strongly affected by the growing conditions experienced by the plants, with much better activity on sun grown plants, despite the higher leaf area ratio of plants growing in shade. Similar effects of shade on translocation patterns were found for the non-herbicidal compound, N-methyl-pyridinium chloride [MPC] in wheat, but in this case it was possible to show that low light conditions resulted in a significant increase in membrane permeability. The significance of light induced changes in cell membrane permeability, and its relationship to phloem translocation are discussed.

INTRODUCTION
Paspalum conjugatum Berg. is a creeping, stoloniferous perennial grass, considered to be one of the most important weeds particularly in estate crops in Malaysia. It can grow under conditions of deep shade or full sun (Beetle, 1974; Ward and Woolhouse, 1986). Shading conditions (0%, 50% and 75% shade) prior to treatment were shown to influence the morphology and physiology of *Paspalum conjugatum* (Ipor, 1989), and the activity of imazapyr on this species was significantly reduced by shading (Price and Ipor, 1990) . The adverse effects of shade on imazapyr activity have been attributed to reduced phloem translocation, probably related to reduced sugar transport (Ipor and Price, 1990), but data on the effects of light on the translocation of non-herbicidal model compounds suggests that changes in cell membrane permeability may also occur.

METHODS
Paspalum conjugatum was grown in pots in a greenhouse under 0%, 50%, and 75% shade using the procedures described by Price and Ipor (1990). Translocation was measured by applying ^{14}C labelled imazapyr to the third youngest leaf of *Paspalum conjugatum* plants at the five leaf stage grown under three shade levels (0%, 50%, and 75%). The labelled herbicides were applied at rates equivalent to 0.1 kg a.i/ha imazapyr in a spray volume of 211 L/ha. One μL of ^{14}C radiolabelled solution was applied to the leaf, avoiding the midrib. The treated plants were grown in the three shade levels until required for radioactivity determination. Surface deposits of imazapyr were removed by applying a 6% solution of cellulose acetate in acetone (Price, 1982). Radioactivity in the dry cellulose acetate film was measured by dissolving the film in 0.5 ml of acetic acid and adding 3 ml of scintillation fluid.

For translocation studies, the plants were sectioned into treated leaf, opposite treated leaf, leaf and stem above, leaves and stem below treated leaf, and roots.

The treated leaf was divided into three sections, the treated zone, above

the treated zone and below the treated zone. The radioactivity of separate sections of plants was determined by digesting with "NCS" tissue solubilizer (Amersham) and bleaching with benzoyl peroxide before adding scintillation fluid (Burell *et al.*, 1981).

The method of growing wheat, and treatment with N-methylpyridinium chloride [MPC] were as previously described (Price 1975). In this case the plants were grown in a phytotron under standard lighting conditions with a 16hr day length. Uptake and translocation of MPC [4 μL of a solution containing 2.0 μCi/ml, 0.217 mCi/mM], was measured over a 24 hour period either in standard conditions, constant light, or constant darkness.

Uptake and efflux studies were carried out on 400μ thick slices of wheat leaf from plants grown under standard conditions as described above. Such slices have approximately 80% of the mesophyll cells undamaged (Price 1979). The tissue slices were incubated in two mls of ^{14}C-methyl MPC solution containing 1 μCi with a specific activity of 1.8 mCi/mM at 20°C in a shaking water bath for 60 minutes before being washed with 1.75 mls of double distilled water. The water was changed at five minute intervals and the radioactivity measured by scintillation counting. After 25 washings the slices were left to equilibrate for 24 hours then the water was changed again and the tissue frozen and thawed several times to break all membranes. The tissue was extracted a further three times and the residue combusted and the residual radioactivity measured.

Figure 1.

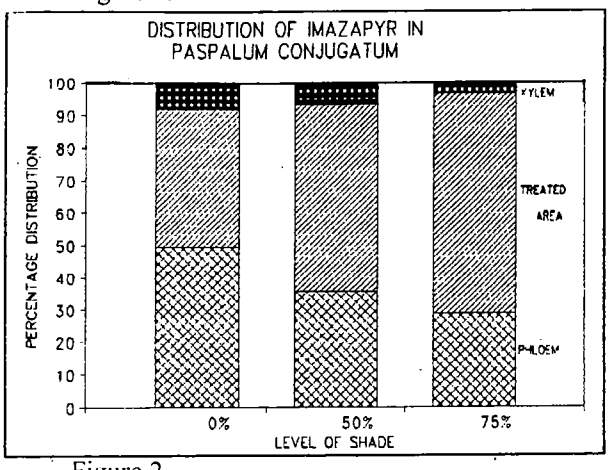


Figure 2.

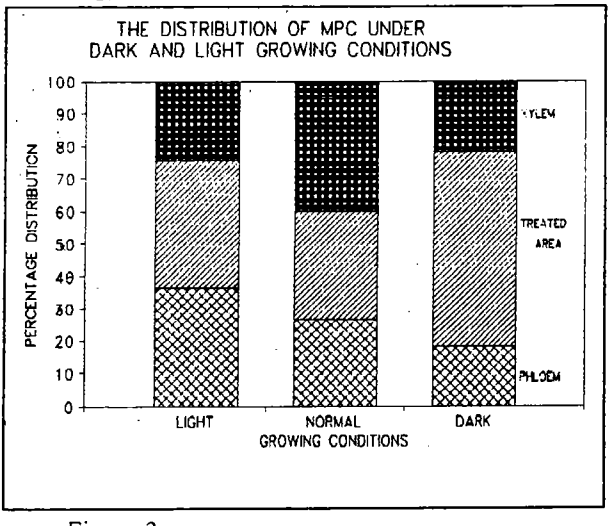
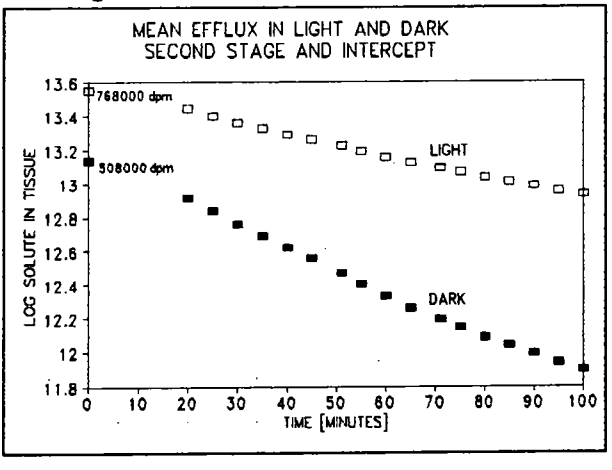


Figure 3.



The efflux data was analyzed by plotting the natural log of soluble radioactivity in the tissue against time. After 20 minutes the efflux approximated to a straight line from which it was possible to calculate a rate constant and intercept at zero time. The intercept gives the total content of the compartment and the rate constant gives the half time of efflux. An estimate of the statistical validity of the model is given by R squared, with 1.0 being the perfect agreement of data points to the regression equation.

RESULTS

Figure 1 shows the effect of increasing shade on the distribution of imazapyr in *Paspalum conjugatum*. As the light decreased there was a decrease in the relative amount of imazapyr translocating in the phloem and a corresponding increase in the amount remaining in the treated part of the leaf. Relatively little of the imazapyr moved in the xylem and this also decreased with decreasing light. Under all experimental conditions the imazapyr that translocated in the phloem entered the roots, stems and other leaves as well as the lower part of the treated leaf.

The distribution of MPC in the phloem of wheat plants was also reduced by reducing the amount of light, in this case by increasing periods of darkness, with correspondingly larger amounts remaining in the treated part of the leaf [Figure 2]. The amount of MPC translocating into the roots remained fairly constant at about 15% of the total MPC translocated in the phloem. MPC is not as phloem mobile as imazapyr and a significantly greater amount translocated in the xylem, especially under conditions of normal day length.

The efflux of MPC was significantly faster from leaf slices cut from plants growing in the dark (half time of elution of 54 mins), than from slices cut from plants growing in the light (half time of elution of 109 mins) [Figure 3]. The amount of chemical accumulating in the main elution phase of sun grown plants, measured as the intercept of the efflux line (768,000 dpm), was 50% greater than that of shade grown plants (508,000 dpm). In both cases the R^2 exceeded 0.99. The 24 hr equilibrations gave tissue: medium concentration ratios of 3554:1 for light, and 910:1 for dark conditions, equivalent to cell potentials of -206mv and -172mv respectively.

DISCUSSION

Plants growing under shade conditions tend to have a much higher leaf area index, compared with plants growing in full sunlight [Price and Ipor 1990]. This should make them much more susceptible to herbicide applications because at any concentration of herbicide in the spray solution, the amount of herbicide per gram of weed tissue is directly proportional to the leaf area index. This conclusion is confirmed for a contact herbicide such as paraquat [Ipor and Price, 1990] but the opposite is found for herbicides such as imazapyr, which have to translocate to the growing points to kill the plant.

The beneficial effects of light on the activity of a phloem systemic herbicide such as imazapyr may be a direct result of its improved translocation under high light conditions. Under such lighting conditions the target species is synthesising, and translocating, sugars at a high rate and it is reasonable to assume that it is the sugar flux that provides the mechanism for the phloem translocation of the herbicide.

The use of biologically active compounds to study translocation is

complicated by the possible interaction of the compound with cell membrane permeability and phloem translocating mechanisms. This problem is reduced by using model compounds that have little or no observable effect on the plant. In this case, the model compound, MPC, showed very similar responses to light as the herbicide, imazapyr, with increasing periods of darkness reducing translocation in the phloem. The symplast concept implies that the cytoplasm of different cells, including the sieve tubes, forms a continuum within which a solute can effectively translocate from the outermost leaf cell to the root, but from the data on whole plant translocation it may be argued that under shade conditions imazapyr and MPC did not translocate in the phloem because they were retained behind the membranes of the nonphloem cells. The data on accumulation and efflux of MPC using leaf slices showed quite conclusively that the solute was more extensively accumulated and crossed the membrane with more difficulty when the plants were growing in the sun than in the shade. The reduced translocation observed under conditions of darkness or low light conditions is more likely to be the result of low sugar fluxes in the phloem combined with less effective retention behind membranes. The fact that high phloem translocation occurs under conditions favouring high retention behind mesophyll cell membranes suggests that the two may be related.

ACKNOWLEDGEMENT

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REFERENCES

BEETLE, A.A. (1974) . Sour *Paspalum* - tropical weed or forage. *Journal of Range Management.*, 27(2): 347-349.

BURREL, M.M. & BRUNT, P, (1981). Preparation of green plant material for liquid scintillation counting. *Ann. Bot, 48: 395-397.*

IPOR, I.B., (1989). Action of herbicides on *Mikania micrantha* H.B.K. and *Paspalum conjugatum* Berg. *PhD thesis. University of London.*

IPOR, I.B., PRICE, C.E. (1990). Effect of shade on the uptake and translocation of ^{14}C-imazapyr in *Paspalum conjugatum* Berg. *Proc. Tropical Weeds* Conference, *Kuala Lumpur, 1990. (in* press).

PRICE, C.E. (1975). The uptake and translocation of 1-methylpyridinium chloride and related model compounds in wheat. *J.exp.Bot.26, p.521-532.*

PRICE, C.E. (1979) Movement of Xenobiotics in Plants - Perspectives. *Advances in Pesticide Science. pp 401-409. Edd. H.Geissbuhler,*

PRICE, C.E., (1982). A review of the factors influencing the penetration of pesticides through plant leaves. In *Plant Cuticle, pp 237-252 eds. D.F Cutler, K.L. Alvin, & C.E. Price. Linnaean Society Symposium Series No. 10 Academic Press.*

PRICE, C.E., IPOR, I.B. (1990). The effect of shade on the activity of paraquat and imazapyr on *Paspalum conjugatum. Proc. Tropical* Weeds *Conference, Kuala Lumpur, 1990. (in press).*

WARD, D.A. & WOOLHOUSE, H.W., (1986). Comparative effects of light during growth on the photosynthetic properties of NADP-ME type C4 grasses from open and shaded habitats. Gas exchange, leaf, anatomy and ultrastructure. *Plant Cell and Environmental, 9: 261-270.*