and would reduce the amount of insecticide used when compared with other methods of application including folial spray, dusting and painting. The method is also one that can be applied in any season (rainy or dry) to obtain maximum efficiency as the insecticide goes directly into the tunnel. It is however limited to heights within reach but can be aided by the use of ladders. Since the devastating effect of the borer is more on younger plants than the very old ones, control at the stage of establishment is needed and can be carried out effectively using the methods described above.

Summary

Laboratory and field studies with oil can injectors as insecticide applicator have shown that kola stem borers *Phosphorus* sp. can be successfully controlled by the application of 0.25% a.i. Gammalin 20 E.C. (20% Gamma BHC) to the holes bored by the insects.

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Nitrate reduction in some tropical grasses in their natural environment.

Sumario. Diecisiete especies de pastos y otras nueve monocotiledóneas que crecían en su medio ambiente natural fueron sometidas a ensayo respecto a la actividad de nitrato-reductasa utilizando dos métodos en vivo. El método de infiltración al vacío dio valores más elevados en 19 especies que el método Jaworski. El significado y posibles aplicaciones de las técnicas de la prueba nitrato-reductasa para trabajos en pastizales son presentados en la presente comunicación.

Reduction of nitrate to nitrite by nitrate reductase is the rate limiting step in the overall reduction of nitrate to ammonia in plants (3). Some workers have made correlations between nitrate reductase activity and grain yield potential of a grass crop (1) and total dry weight accumulation in ryegrass. Hageman *et al* (6) suggested that estimation of nitrate reductase in seedlings was a useful approach in the screening of species in breeding programmes where protein yield was the objective. A similar approach (using a biochemical tool) in the assessment of nitrogen requirements is just as valid in the management of other ecological systems especially in grassland ecosystems. The object of this study was to develop a method for assessing the plant nitrate status by NRA assay. The NRA obtained in two *in vivo* assay methods were compared in seventeen grass species and nine other monocotyledons growing in their natural environments. The methods were chosen for their simplicity, relative inexpensiveness and suitability for assaying numerous samples.

Materials and Methods

Seventeen grass species and nine non-grass monocotyledons growing in their natural environment on the University of Ife Campus, Ile-Ife, Nigeria were used in this study. Leaves were harvested and chopped into large pieces about 1.5 cm squared and 0.5 g of tissue was used for each assay. NRA was determined by methods based on the *in vivo* assays of Jaworski (8) using propanol and Klepper *et al.* (10) using vacuum infiltration. Modifications were made to standardize some of the common basis of both methods e. g. weight of tissue 0.5 g; incubating medium 5.0 ml in 25 ml screw cap bottles; concentration of reagents (0.1 m phosphate buffer pH 7.5, 0.05 M KNO_3). For the vacuum infiltration technique, propanol (5 %) was excluded. Samples were vacuum infiltrated twice for 5-10 minutes each time by placing the bottles in a vacuum dessiccator and connecting it to a vacuum line. Samples were incubated

Abbreviation: NRA Nitrate reductase activity

at room temperature. In both methods, samples were assayed at 0, then 30, 40 and 50 minutes after incubation – the means of the 30, 40 and 50 minutes reading were used to estimate the nitrate reductase activity.

Results and Discussion

The results from this study (Table I) show that of the two *in vivo* methods, the infiltration technique on the average gave higher values than the Jarworski method. In close comparison these values were between 1½ – 4 times higher for *Pennisetum* sp., *Rotboellia, Setaria, Cynodon* sp., *Sporobolus, Digitaria, Leptochloa, Axompus, Cympopogon, Bambusa, Rheo, Roystenea, Hyphaena* and *Commelina*; 6, 10, 14 and 18 times higher for *Imperata, Pandanus,*

Table 1. Comparison of Nitrate Reductase Activity of two *in vivo* assays in different Grass and Monocotyledon Species: $\mu g\ NO_2^-$ formed (g fr. wt)$^{-1}$ hr^{-1}

Grasses	Jarworski	Infiltration
Panicum maximum	1.89	26.72
Pennisetum subagustrum	1.29	6.79
Rottboellia exaltata	8.91	32.31
Andropogon tectorium	2.61	0.86
Setarria longiseta	2.70	6.30
Pennisetum purpureum	10.89	45.00
Paspalum conjugatum	12.9	12.09
Cynodon plectostachyus	2.19	3.60
Cynodon dactylon	3.00	4.50
Sporobolus pyramidis	3.30	4.89
Paspalum orbiculare	2.31	4.50
Imperata cylindrica	0.30	1.80
Digitaria horizontalis	5.31	10.50
Leptochloa uniflora	1.50	3.69
Marantochloa purpurea	3.60	0.81
Axompus compressus (Carpet grass)	4.89	9.30
Cympopogon citratus (Lemon grass)	3.21	5.19
Bambusa vulgaris (bamboo)	5.91	8.10
Other Monocots		
Elaeis guineensis (oilpalm tree)	1.89	2.40
Musa sapientum (plantain)	5.28	2.10
Thaumatococcus danielli	0.51	9.39
Agave sisalana (ornamental sisal)	0.96	1.20
Rheo discolor	1.59	3.60
Roystenea borinquena (Royal palm)	0.90	2.79
Hyphaena thebaica	1.71	5.79
Commelina diffusa	3.39	6.60
Pandanus sanderi	0.09	0.90

Panicum and *Thaumatococcus* respectively; the values were approximately equal in *Elaeis, Paspalum* and *Agave;* while the Jarworski method gave higher values for *Andropogon* (3x) *Marantochoa* (4x) and *Musa* (2x). Results from laboratory grown plants are usually higher than the above which were growing in their natural environment. However, such laboratory results should be viewed as artificial as even field soils heavily fertilized with nitrogen never attain as high a flux as in the laboratory experiments.

It is now generally accepted that NR is the rate limiting step in nitrate reduction and therefore plays a major role in the regulation of protein production. Shaner and Boyer (11, 12) reported that in maize seedlings having adequate water supply, extractable NRA is regulated by the nitrate flux from the roots to the shoots rather than by the nitrate content of the leaves. Their work (12) implies that at low water potential the NRA response to the flux of nitrate is controlled by transpiration and root uptake effects. Similarly in tobacco callus cells NRA induction is more closely related to the nitrate entering the cells than to that stored within the cells (7). Finally, when all other environmental conditions are constant, NRA is inducible by flux (9). Brunetti and Hageman (5) report that the *in vivo* assay provided the closest approximation of the actual amount of N accumulated in wheat seedlings. As there is a close correlation between the NRA of a leaf and the nitrate status of the solution (soil) in which the plant grows (12), determination of NRA in leaves would be a rapid means of assessing the status of nitrate availability in soils. Protein production through dry matter accumulation is the goal in tropical savannah grassland and natural ecosystem management. In these, the use of NR-assay techniques which serve as a means of evaluating the cycling of nitrate nitrogen should be an integral part of the management of these systems.

Ongoing research in this laboratory will use these methods to establish a relationship between the NRA, nitrate status of the leaves of grasses and the soil nitrogen levels in the savannah region of Nigeria.

Summary

Seventeen grass species and nine other monocotyledonous plantas growing in their natural environment were assayed for nitrate reductase activity using two *in vivo* methods. The vacuum infiltration method gave higher values than the Jaworski method in 19 species. The implications and possible

Abbreviation: NR Nitrate reductase

applications of nitrate reductase assay techniques in grassland work is discussed.

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