Responses and tolerance of sugar beet to stress

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Summary

A biochemical test has been devised which is at least as accurate as current methods for determining seed vigour, particularly under conditions of field stress. Tests are also being developed that can be used in breeding programmes to improve the stress tolerance of sugar-beet plants. Leaf discs are subjected to either heat shock, water deficiency stress or UV irradiation and stress tolerance is subsequently determined from their chlorophyll fluorescence signals. The parameter F_v/F_p (the ratio of variable to peak fluorescence) is proportional to the quantum efficiency of light utilisation by the thylakoids and can be used to quantify the extent of damage. Evaluation of 22 commercial sugar-beet varieties indicates that reductions in F_v/F_p due to stress can vary up to ten-fold, providing a useful indication of their potential stress tolerance. The use of excess nitrogen fertiliser in the field can mask yellowing symptoms caused by beet yellows virus (BYV) and beet mild yellowing virus (BMYV). However, the effects of the virus on yield are equally severe whether the symptoms are masked or not. The apparent health of the high nitrogen, virus infected leaves seems to be due to elevated chlorophyll synthesis which is not matched by an increased pool of PSII electron acceptors.

Introduction

Environmental factors such as light, temperature, atmospheric CO_2 concentration, water and the nutritional status of soils, affect the establishment and productivity of sugar-beet plants. Potential sugar production is affected by environmental inputs, acting as substrates for, and regulators of, biochemical processes. There is an optimum combination of conditions determined by complex interactions that exist between the environment, gene expression and productivity of a particular phenotype. Productivity decreases when environmental conditions depart from this optimum; these conditions are generally regarded as stresses.

In addition to the stresses imposed by the environment, damage by pests and diseases can also be regarded as a form of stress. Combinations of all these stresses will have a complex effect on a sugar-beet crop throughout its life cycle. The development of varieties tolerant and/or resistant to these stresses, and the ability to select seed of high vigour will have obvious benefits to the breeder, grower and processor. This paper describes recent research at Broom's Barn aimed at increasing the understanding of regulatory processes underlying seed vigour and plant responses to stress.

Seed vigour

The germination of seeds and the establishment of vigorous plants is the most critical phase in the production of a good crop. Whilst the viability of a seedlot can easily be determined using

the standard ISTA test under optimal conditions, vigour is far more difficult to assess. Definitions of vigour have been the subject of much controversy; they have included the ability of a seedlot to survive stress or non-optimal conditions, and the rate and uniformity of germination. However, a general definition of vigour is `the relative ability of a seedlot of known viability to produce the expected established crop under field conditions'. Seed vigour is determined by both genetic and environmental factors.

Laboratory germination tests are currently used to assess seed vigour. These tests can involve combinations of temperature, moisture status and a physically obstructive medium, but they all take considerable time. A rapid biochemical test for seed vigour has, therefore, been the goal of seed physiologists for many years. In the past, parameters such as solute leakage, energy charge, and lipid composition have been studied with limited success (Bewley and Black, 1982). Low vigour and viability seeds have been shown to have more chromosomal aberrations, greater nucleic acid fragmentation, and poor DNA synthesis and repair mechanisms (Rao *et al.*, 1988; Brocklehurst and Fraser, 1980; Dell'Aquila and Margiotta, 1986). Evidence from priming and ageing experiments has indicated that a test based upon the quantity of extractable high molecular weight nucleic acids correlates with the laboratory germination performance of leek seeds (Clarke and James, 1991). The potential of this biochemical test for estimating the vigour of sugar-beet seeds has been investigated. In a series of experiments the performance of seedlots in the test was compared with results from laboratory germination tests and field trials. The experiments examined the value of the test for determining vigour in situations relevant to the industry. Thus, the influences of variety, seedlots of the same variety, storage and processing (seed sizing, steeping and advancement) were investigated.

After preliminary laboratory germination tests, 14 seedlots from two sugar-beet varieties were selected (the seedlots were grown in different regions) to be assessed by field trials, laboratory germination tests and the biochemical test. The biochemical test correlated significantly ($p<0.05$) with both field performance (emergence rate) and laboratory germination tests. Correlations between the biochemical test and field performance were not significant when seedlots from only one variety were compared. This may be explained by small differences in seedlot performance being masked by background variation in the field. In the laboratory germination tests, background variation was minimal and intra-varietal differences between seedlots were more reliable. This was reflected in the biochemical test correlating significantly with our laboratory germination tests.

Three seedlots from one variety were either thiram steeped or given a primed advancement treatment (PAT). PATing significantly improved ($p<0.001$) the emergence rate and establishment in the field. This enhancement of vigour was reflected in both higher results in the biochemical test and improved performance in laboratory germination tests. There was also a significant correlation ($p<0.01$) between the biochemical test and field performance (rate of emergence and total emergence - Fig 1).

Commercially available sugar-beet varieties in the UK are either diploid or triploid. To investigate the effect of ploidy on the biochemical test, bulks of seeds from different varieties were selected in pairs; each pair having similar laboratory germination test results but different ploidy. Within each pair there was no significant difference between their performance in the

biochemical test. Therefore, ploidy level does not affect the biochemical test within the range of varieties and vigour levels studied. Sugar-beet seed for the UK market also has to be within a specific size range. A positive correlation between fruit size, average true seed weight and vigour in the field has been demonstrated (Thomas and Yallop, 1994). Experiments with this seed have shown that increasing seed size also correlated with the biochemical test results.

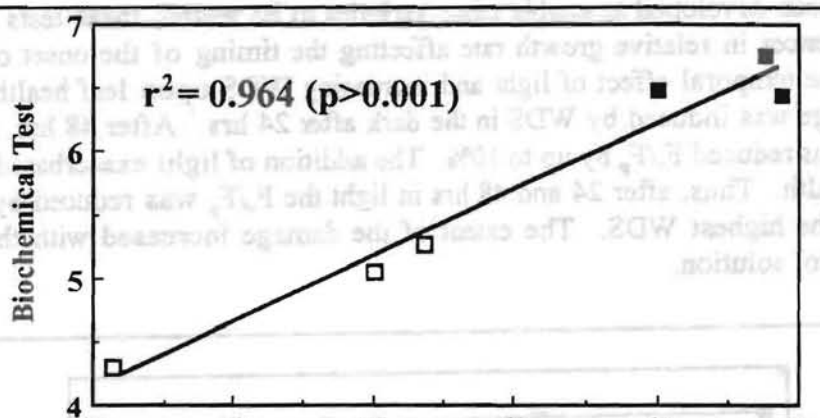

Figure 1 The correlation between the biochemical test and total emergence in the field. □ Thiram steeped seeds ■ PATed seeds.

Stress tolerance

- Drought stress

The principal cause of environmental stress to the UK sugar-beet crop is drought. Sugar-beet's survival strategy in such conditions is to reduce water loss through stomatal closure. Initially the oldest leaves wilt whilst the younger leaves maintain turgidity. Net photosynthetic rate and stomatal conductance both decrease as the leaves age and as stress is applied (Clarke *et al.*, 1993); the integrity of the photosystem II (PSII) of these leaves also decreased but showed a slight delay. Dehydration alone is a stress, but high temperatures and strong sunlight become additional stresses during drought. Stress tests have been developed that can be used in breeding programmes to improve the stress tolerance of leaves. In addition, the screening process has identified useful experimental material for the analysis of stress tolerance mechanisms.

Water deficiency stress (WDS) was imposed either to whole plants by withholding water or to leaf discs by floating them on solutions of mannitol. The interaction of light and WDS was also studied on the leaf discs. Stress tolerance of the leaf tissue was estimated from the chlorophyll fluorescence signal, which is a sensitive and early indicator of damage to PSII and the physiology of the plant in general (Bolhar-Nordenkampf *et al.*, 1989). Several components of the fluorescence induction kinetics were studied but F_v/F_p (the ratio of variable to peak fluorescence) was principally used to quantify the extent of damage. F_v/F_p is indicative of the integrity of PSII and has been shown to be proportional to the quantum yield of photochemistry.

Experiments with whole plants demonstrated the differential effect of WDS upon five varieties selected for their extreme responses to stress in the UK national trials (Clarke *et al.*, 1993). The variety identified as the most stress tolerant in the national trials was also the most tolerant as the relative water content of the leaves fell more slowly and the damage to PSII was reduced.

Leaf-disc tests have been developed to enable more varieties to be tested; these tests also avoid the problem of differences in relative growth rate affecting the timing of the onset of stress in whole-plant tests. The temporal effect of light and increasing WDS upon leaf health is shown in Fig 2. Little damage was induced by WDS in the dark after 24 hrs. After 48 hrs, increasing mannitol concentrations reduced F_v/F_p by up to 10%. The addition of light exacerbated the effect of WDS upon leaf health. Thus, after 24 and 48 hrs in light the F_v/F_p was reduced by 20% and 50% respectively at the highest WDS. The extent of the damage increased with the osmotic strength of the mannitol solution.

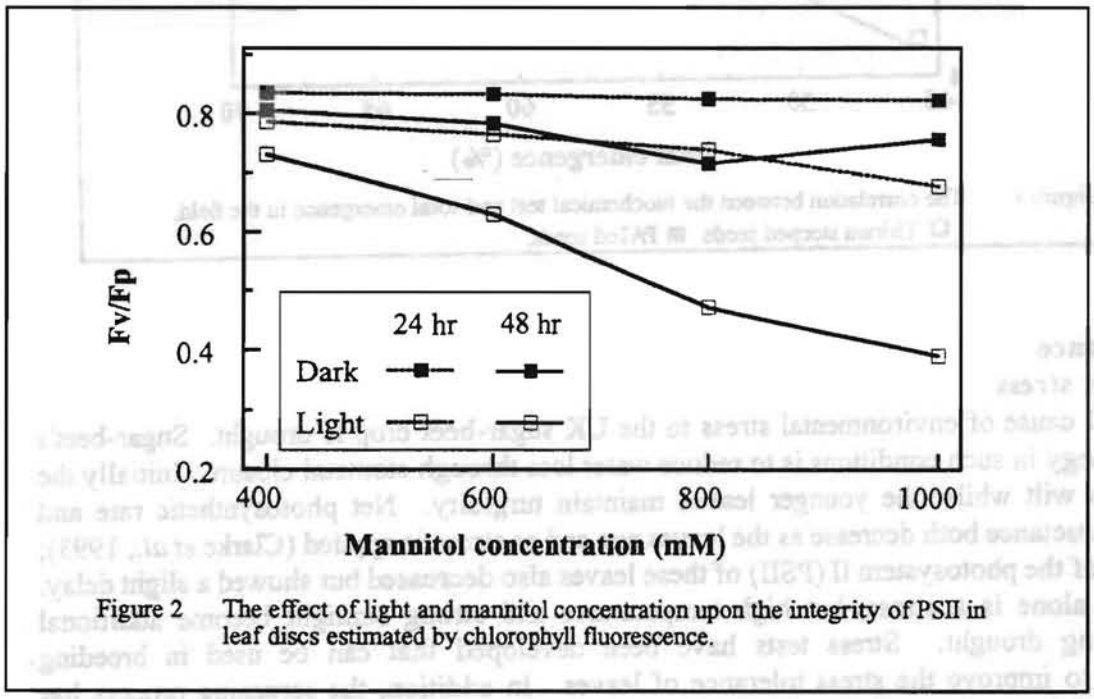

Figure 2 The effect of light and mannitol concentration upon the integrity of PSII in leaf discs estimated by chlorophyll fluorescence.

Over twenty varieties from around the world have been screened for stress tolerance using the leaf-disc test. Results indicate that reductions in F_v/F_p due to stress can vary up to threefold, providing a useful indication of their potential stress tolerance. The variety identified in the national trials as stress tolerant also proved to be the most tolerant in the leaf-disc experiments. Work in progress indicates that the varieties with extreme tolerances have significant differences in their physiological make-up.

- Heat stress

Similar experiments have been carried out to investigate the effects of heat shock on sugar-beet plants. Heat stress was imposed either on seedlings and whole plants in incubators, or on leaf discs in microcentrifuge tubes immersed in a water bath.

A four hour heat-shock given to four-day old seedlings indicated that the upper temperature limit (LT_{50}) for sugar beet is approximately 43°C. The LT_{50} was also estimated at 43°C when the chlorophyll *a* fluorescence signals of heat-shocked plants were used to quantify leaf damage. A heat-shock treatment (43°C, 4hr), followed by recovery at room temperature (3hr), resulted in complete inhibition of photosynthesis. Leaf disc experiments confirmed the LT_{50}. The effects of pre-treating the discs at elevated, but not heat-shock, temperatures were examined. A pre-incubation at 40°C followed by recovery at room temperature was shown to confer some tolerance to a subsequent heat-shock at 45°C (Fig 3). Twenty-five varieties have also been screened for heat stress tolerance using a modified leaf-disc test. Thirty percent less damage was present in the most tolerant variety compared with the least tolerant. Yet again the most tolerant variety was the same as that identified in the national trials, whole plant tests, and WDS tests.

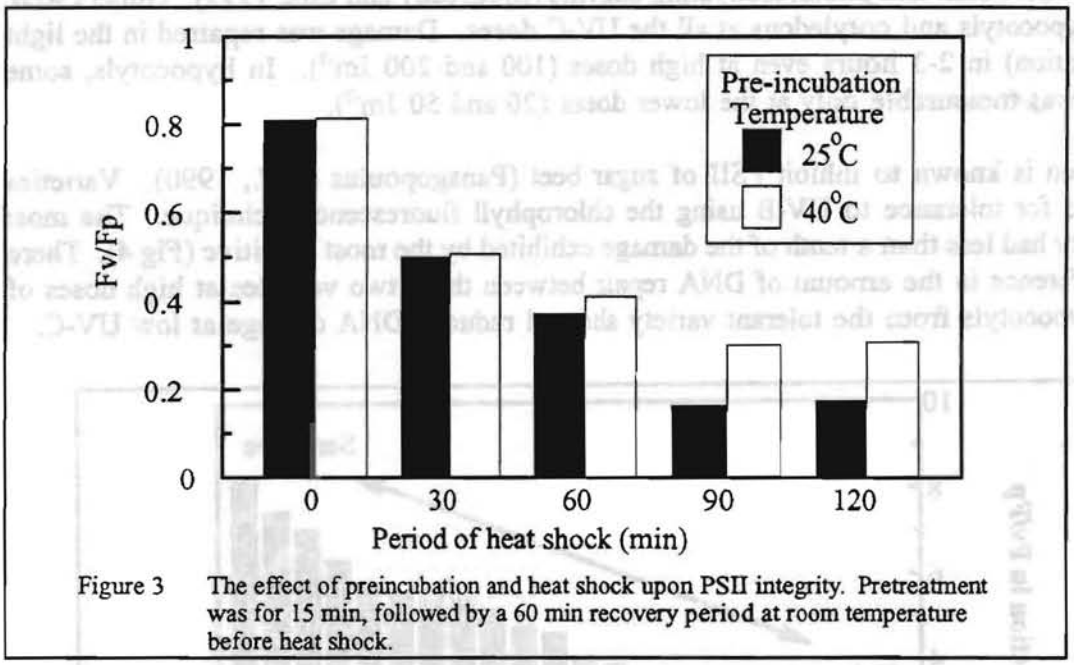

Figure 3 The effect of preincubation and heat shock upon PSII integrity. Pretreatment was for 15 min, followed by a 60 min recovery period at room temperature before heat shock.

- UV-stress

UV light can be divided into three classes UV-C, UV-B and UV-A. Ozone depletion in the stratosphere is leading to increased UV-B radiation on the Earth's surface. UV-B radiation is known to damage various plant processes (Stapleton, 1992). However, it is still debatable what effect an increase in total UV-radiation will have upon both wild and crop plants.

Mature plants are partially protected against UV-radiation by characteristics such as the thick cell walls of their surface tissues. Flavonoids and anthocyanins can also absorb UV-radiation from sunlight. These pigments are induced by UV-B exposure and generally accumulate in the epidermis. However, the cells particularly liable to UV-radiation damage must be those of the young meristem tissues of seedlings and those of mature pollen.

DNA is a very sensitive molecular target for UV-C and UV-B radiation because it absorbs quanta in those spectral regions. UV irradiation of DNA mainly leads to the formation of dimers

between adjacent pyrimidines. These are cyclobutane pyrimidine dimers (CPDs) and pyrimidine (6-4) pyrimidone dimers (6-4 photoproducts). Results from yeast and mammalian systems indicate that 6-4 photoproducts are repaired much more rapidly than cyclobutane dimers.

It is generally accepted that UV-C can be used as a model radiation for the induction and repair of these dimers in plants (Stapleton, 1992). We have investigated CPD and 6-4 photoproducts independently, as well as total dimers, in sugar-beet hypocotyls and cotyledons irradiated with UV-C at between 20-200 Jm^{-2}. The amount of damage to DNA and the speed of repair in both light and dark has been determined in time-course experiments over periods from 30 min to 3 hrs. The damage to DNA was quantified in collaboration with Dr Shirley McCready (Oxford University). The technique involves dot-blot immunoassay using a polyclonal antiserum raised against UV-C irradiated DNA; detection of the individual lesions was based on their differential sensitivities to hot alkali and photoreactivating enzyme (McCready and Cox, 1993). Dimers were detected in hypocotyls and cotyledons at all the UV-C doses. Damage was repaired in the light (photoreactivation) in 2-3 hours even at high doses (100 and 200 Jm^{-2}). In hypocotyls, some 'dark repair' was measurable only at the lower doses (20 and 50 Jm^{-2}).

UV-B radiation is known to inhibit PSII of sugar beet (Panagopoulus *et al.*, 1990). Varieties were screened for tolerance to UV-B using the chlorophyll fluorescence technique. The most tolerant variety had less than a tenth of the damage exhibited by the most sensitive (Fig 4). There was little difference in the amount of DNA repair between these two varieties at high doses of UV-C, but hypocotyls from the tolerant variety showed reduced DNA damage at low UV-C.

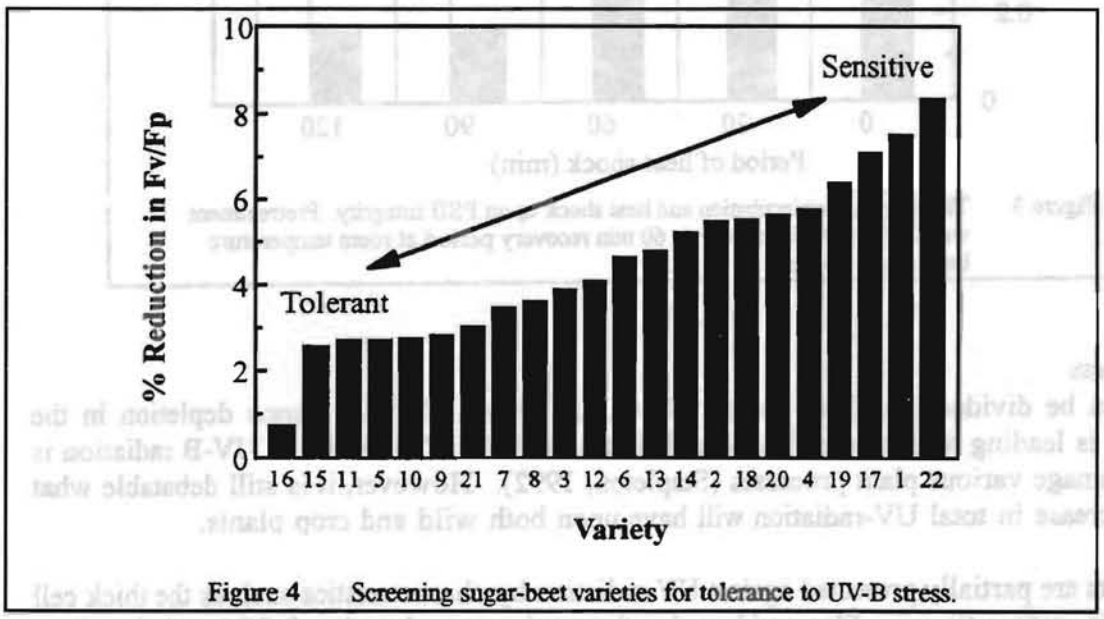

Figure 4 Screening sugar-beet varieties for tolerance to UV-B stress.

-Virus yellows induced stress

Relatively little is known about the effects of yellowing viruses on the physiology of sugar beet. Beet yellows virus (BYV) particles first accumulate in the phloem-parenchyma and companion cells, and then in the mesophyll (Esau *et al*, 1967). In the mesophyll cells, aggregates of virus

particles occur in the stroma of chloroplasts and are associated with the organelle's destruction, and the consequent development of yellowing symptoms. Beet mild yellowing virus (BMYV) particles can be detected first in sieve elements and phloem-parenchyma cells, and then in adjacent mesophyll; the particles are most numerous in the nuclei of the phloem cells (Esau and Hoefert, 1972). Thus, both viruses cause the destruction of chloroplasts and both accumulate in the phloem; this implies that infection interferes with sugar transport processes and for many years this was considered the main cause of damage.

Observations within the industry have suggested that the yellowing symptoms of virus infection are masked in fields where poultry manure is applied. This observation has been examined in a series of field experiments at Broom's Barn (1989-92). The results indicate that, whilst poultry manure visibly reduced yellowing, the effects of virus on yield were equally severe. Reduced phloem transport in virus infected crops can be used to explain this phenomenon. However, analysis of chlorophyll fluorescence studies on this field experiment provided a further explanation related to chloroplast damage. The ratio of variable to peak fluorescence (F_v/F_p) was again used as an estimate of PSII efficiency. Plants were inoculated with virus in early May. By early July the PSII of the leaves was severely damaged by virus infection (Fig 5a). The damage to PSII followed the classical pattern of virus infection developing in the leaf tip and spreading down to the base. Poultry manure had no significant effect upon PSII efficiency when compared with either no nitrogen application or the recommended rate of inorganic fertiliser.

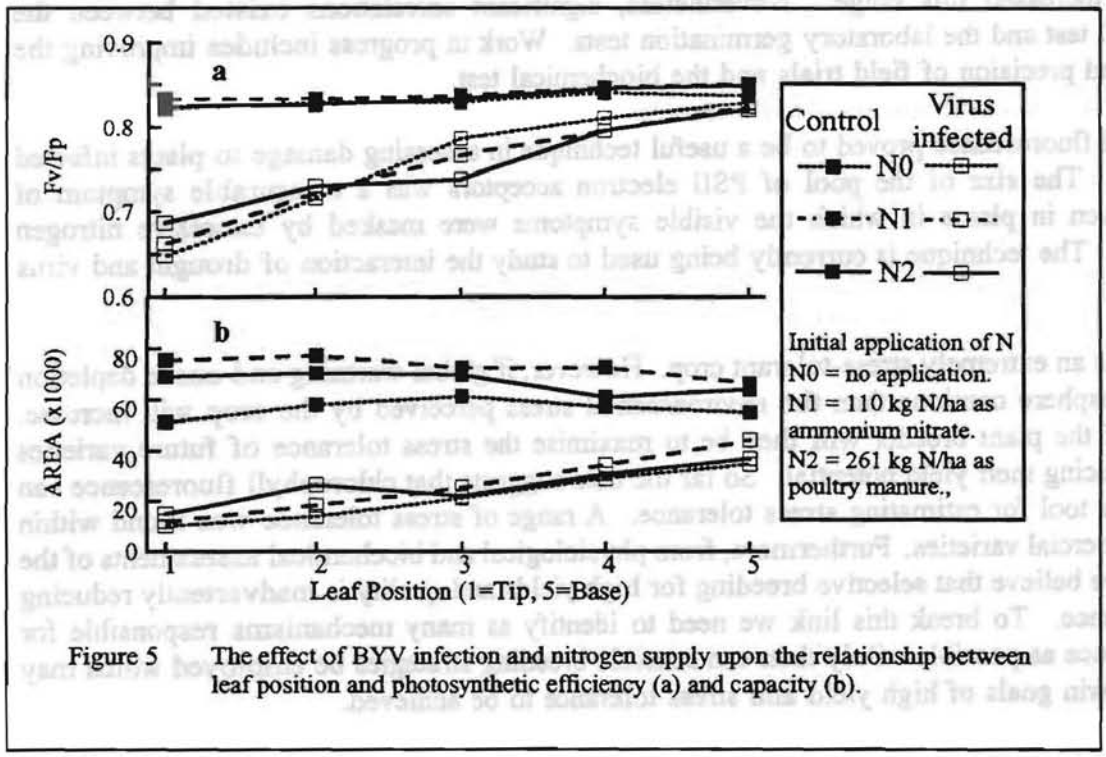

Figure 5 The effect of BYV infection and nitrogen supply upon the relationship between leaf position and photosynthetic efficiency (a) and capacity (b).

The pool size of electron acceptors on the reducing side of PSII (PSII capacity) can be estimated from the area over the chlorophyll fluorescence curve between F_o and F_p (Bolhar-Nordenkampf

et al., 1989). The effect of virus on PSII capacity showed a similar trend to PSII efficiency (cf. Fig 5a&b). In the virus-infected leaves there was again no significant difference between any of the nitrogen treatments. This suggests that the apparent health of infected plants grown with poultry manure was simply due to elevated chlorophyll synthesis that was not matched by an increased pool of PSII electron acceptors. This observation supports work on peanut plants, which provided evidence that the electron acceptor, plastoquinone, was the probable site of photosynthetic inhibition by peanut green mosaic virus (Naidu *et al.*, 1984).

In healthy plants, the absence of nitrogen fertiliser caused a significant reduction in PSII capacity compared with the other two fertiliser treatments (Fig 5b). The visible effect of increasing greening of the canopy with nitrogen fertilisation is therefore accompanied by an increase in PSII capacity. However, the extra nitrogen applied in the poultry manure application was not matched with an increase in PSII capacity. Over a wide range of nitrogen fertilisation (0-125 kg ha^{-1}) sugar beet grown at Broom's Barn produce dry matter from intercepted solar radiation at a consistent rate of 1.7-1.8 g MJ^{-1}. This indicates that under UK conditions excess chlorophyll and PSII capacity (per unit area of leaf) are present at high nitrogen rates.

Conclusions

The biochemical test for seed vigour has proved to be a useful tool in predicting the vigour of a seedlot. The range of vigour between seedlots within each variety was limited, although seed treatments increased this range. Nevertheless, significant correlations existed between the biochemical test and the laboratory germination tests. Work in progress includes improving the accuracy and precision of field trials and the biochemical test.

Chlorophyll fluorescence proved to be a useful technique in assessing damage to plants infected with BYV. The size of the pool of PSII electron acceptors was a measurable symptom of infection even in plants in which the visible symptoms were masked by excessive nitrogen application. The technique is currently being used to study the interaction of drought and virus yellows.

Sugar beet is an extremely stress-tolerant crop. However, if global warming and ozone depletion in the stratosphere continue then the environmental stress perceived by the crop will increase. The goal of the plant breeder will then be to maximise the stress tolerance of future varieties without reducing their yield potential. So far the data suggests that chlorophyll fluorescence can be used as a tool for estimating stress tolerance. A range of stress tolerance was found within recent commercial varieties. Furthermore, from physiological and biochemical assessments of the varieties, we believe that selective breeding for high yields and quality is inadvertently reducing stress tolerance. To break this link we need to identify as many mechanisms responsible for stress tolerance as possible. Only then can sensible breeding strategies be employed which may enable the twin goals of high yield and stress tolerance to be achieved.

References

Bewley J.D. and Black M. (1982) *Physiology and biochemistry of seeds in relation to germination*. Vol 2, Springer-Verlag, Berlin.

Bolhar-Nordenkampf H.A., Long S.P., Baker N.R., Oquist G., Schreibers U., and Lechner E.G. (1989) *Functional Ecology*, 3,497-514.

Brocklehurst P.A. and Fraser R.S.S. (1980) *Planta,* **148,** 417-421.
Clarke N.A. and James P.E. (1991) *Journal of Experimental Botany*, **42,** 261-268.
Clarke, N.A., Hetschkun, H., Jones, C., Boswell, E. and Marfaing, H. (1993) Identification of stress tolerance traits in sugar beet. In *'Interacting stresses on plants in a changing climate'*. Eds M.B.Jackson and C.R.Black, Springer-Verlag, Berlin. Pp 511-524.
Dell'Aquila A. and Margiotta B. (1986) *Environmental and Experimental Botany*, **26,** 175-184.
Esau K., Cronshaw J. and Hoefert L.L. (1967) *Journal of Cell Biology*, **32,** 71-87.
Esau K. and Hoefert L.L. (1972) *Virology*, **48,** 724-738.
McCready S. and Cox B. (1993) *Mutation Research, DNA Repair*, **293**, 233-240.
Naidu R.A., Krishnan, M., Ramanujam, P., Gnanam A., and Nayudu M.V. (1984) Quoted by R.S.S. Fraser in *Biochemistry of virus-infected plants* (1987) John Willey and Sons, p91.
Panagopoulos I., Bornman J.F. and Björn L.O. (1990) *Journal of Photochemistry and Photobiology. B: Biology*, **8,** 73-87
Rao N.K., Roberts E.H. and Ellis R.H. (1988) *Annals of Botany*, **62,** 245-248.
Stapleton A.E. (1992) *The Plant Cell*, **4**, 1353-1358.
Thomas T.H. and Yallop S.J. (1994) Proceedings of the 15th Annual Seed Biology Meeting (Abstract).