hamster[2], mouse[3] and other species[4]. Since the ovulation of a large number of eggs is not expected to occur simultaneously and the time of ovulation for each individual egg would be more spread out during superovulation than after normal ovulation, the chances of delayed fertilisation and digynic triploidy would be higher after superovulation than normal ovulation. Most of the fertilised eggs from superovulated rabbits, however, can develop into normal young after transferring them to several recipient rabbits[5].

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Nitrate reductase as predictive test of crop yield

THE report of Johnson, Whittington and Blackwood[1] on using nitrate reductase as a predictive test of crop yield is probably valid but there are several problems with both the *in vitro* and *in vivo* assay they used which deserve attention.

The maximal *in vitro* activity shown in Fig. 1 of ref. 1 is about 0.1 μmol g^{-1} h^{-1}, a value considerably lower than the activities (~10–12 μmol g^{-1} h^{-1}) commonly observed in leaves of wheat[2–4]. The disparity is so large as to cause concern about the assay procedure. Although the latter activities were obtained without casein in the extraction medium it is conceivable that the inordinately low activities obtained by Johnson *et al.* were partially due to failure to protect nitrate reductase from a constitutive serine protease which rapidly degrades nitrate reductase *in vitro*[2,5,6]. In *Escherichia coli* a serine protease acts to remove nitrate reductase from membranes[7] and in higher plants this may be a reason why its half life is in the order of 2–4.2 h (refs 8, 9). Incorporation of casein or phenylmethylsulphonylfluoride (PMSF) into the extraction media[5,6] obviates this protease activity and yields activities approximately 100 times[10,11] those reported by Johnson *et al.* Therefore, if *in vitro* nitrate reductase activity is to be used as a predictive assay, it should be assayed as described above to avoid complications resulting from differential degradation during extraction.

The problem with the *in vivo* assay of nitrate reductase is also serious. This assay may or may not be proportional to the *in vitro* activity[12,13], but typically represents only about 20% of the *in vitro* activity[12]. Also, the electron donor for the *in vivo* assay has never been identified, nor is it clear that the electron donor *in situ* is identical to that which serves in the conditions employed in the *in vivo* assay. Therefore, it seems unwise to use this assay to estimate any parameter associated with nitrate assimilation unit it is established exactly what the *in vivo* assay is measuring.

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JOHNSON *et al.* REPLY—While a number of interesting points are raised by Butz, it is difficult to see why his criticisms are directed specifically at our work. In any study where enzyme activities are measured either *in vitro* or by the so-called *in vivo* assays it is difficult to state categorically whether or not the measured rates reflect accurately the true activities of the enzymes within the cells. The essential point surely is that our experiments[1] and in the many experiments from Hageman's group in Illinois[2,3], a correlation has been shown between nitrate reductase activity as measured, and the yield of a crop. For such a correlation to be valid or useful the precise biochemical basis is not necessarily important. In this case, however, the correlation does provide valuable evidence that the enzyme activity measured is related to the process of nitrogen metabolism.

Some of the points raised by Butz do require some further comment. First, the references to the work of Schrader *et al.*[4,5] are misleading. In their investigations the improvement in nitrate reductase activity obtained using casein was strongly dependent on the type and age of the tissue employed. With the youngest leaf tissue (similar to that used in our study) the improvement was only 10% compared with the 100-fold quoted. This latter value was obtained only with old tissue of one particular variety of maize and cannot be considered in any way typical. In our investigation some of the *in vitro* wheat nitrate reductase assays were duplicated with *in vivo* assays. These gave substantially similar results. Casein is nevertheless a useful protector of nitrate reductase *in vitro*, although it is by no means the most effective one in all tissues.

Second, Butz speculates that the protease which attacks nitrate reductase does so by removing the enzyme from membranes. This seems unlikely as neither in *E. coli* nor, judged by the majority of the available evidence[6], in higher plants is assimilatory nitrate reductase membrane bound.

Third, in view of the problems known to be associated with the *in vitro* assay of nitrate reductase, of which those referred to by Butz are by no means all, it seems to us unwise to criticise an *in vivo* merely because it sometimes produces results differing from those obtained with an *in vitro* procedure. The fact that the electron donor is not known does not seem important in this context. Recent reports of differences between results obtained using the two procedures may have obscured the fact that in the majority of cases very similar results are obtained with both methods. For example, Hageman's group[3] showed a correlation of 0.99 between *in vitro* and *in vivo* assays of nitrate reductase in several varieties of wheat. Furthermore, a good correlation was obtained between nitrate reductase activity and nitrogen assimilation, thus confirming our observations.

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