FACTORS AFFECTING THE USE OF MUNG BEAN CUTTINGS AS A RESEARCH TOOL IN VEGETATIVE PROPAGATION

NINA BASSUK

East Malling Research Station
Maidstone, Kent

A requirement in the study of vegetative propagation is a research technique which will facilitiate the detection of internal root promoting or inhibiting factors within the plants being propagated. Bioassay techniques are appropriate because their function is to detect growth factors by measuring responses induced in biological material. A bioassay for rooting substances is one in which an extract from the plant under test is applied to cuttings whose adventitious root development is measured as the response to the extract.

Went (5) published the first description of a bioassay for rooting factors using etiolated pea seedlings while Hemberg (1) and Luckwill (4) described bioassays using cuttings of dwarf bean seedlings. Hess (2) used the mung bean, *Phaseolus aureus,* as his test object and subsequently modified the technique (3). Seedlings are grown for 9 to 10 days in a controlled environment and then cuttings are prepared with 3 cm of hypocotyl, epicotyl, primary leaves and trifoliate bud. Ten cuttings are used per vial with 4 ml of test solution which is replaced by distilled water after 24 hours. Roots are recorded 5 to 6 days after treatment.

Characteristics of test material suitable for a bioassay. Test plants must be readily available, germinate uniformly, grow quickly and be easily handled, requirements which the mung bean fulfills adequately. Equally important is the need for uniformity of response in the test subject so that observed reactions can be attributed to the effect of the extract under test and not obscured by the natural variability within the bioassay. A criticism of the standard mung bean bioassay is the variable response among individual cuttings.

Sensitivity, defined as the ability of the bioassay material to respond quantitatively to a small amount of rooting factor, is another prerequisite. Ideally, mung bean cuttings should have inherently low rooting ability to avoid masking the response to the extract under test.

Sources of variability within the mung bean bioassay. Seeds from different sources were found to differ in size, and when cuttings from these were rooted under uniform conditions of daylength, temperature and IBA, cuttings from the larger seeds produced more roots than those raised from smaller seeds (Table 1).

Table 1. Seed size and cutting rootability related to seed source

Seed source	Weight of 500 seeds (grams)	Mean root number per intact cutting (mean of 12)
A	26.3	29.3
B	23.8	31.5
C	21.2	17.9
D	19.3	12.6
E	18.0	12.8
F	17.0	15.1

Cuttings from seedlings harvested between 2 and 8 days after sowing showed differing abilities to root in the same environment (Table 2).

Table 2. Age of cutting as related to mean root number

Age (as days from sowing) of cutting when treated	Mean root number (mean of 48)
2	29.0
3	23.7
4	42.4
5	38.2
8	33.4

Because cotyledons represent a high proportion of the mass of a seed and therefore might be expected to differ in size in different seed sources (Table 1), and because cotyledons degenerate within a few days of preparing the cuttings while primary leaves expand, effects of cotyledons and leaves were investigated to determine their influence on the rooting process. Cotyledons were found to be a source of root promoting factors and their removal caused a reduction in rooting (Fig. 1). This was especially evident in cuttings prepared from very young seedlings (2-4 days old). Removal of cotyledons from cuttings prepared from different seed sources reduced the range of roots per cutting from between 12.6 to 31.5 (Table 1) to between 5.7 and 10.8.

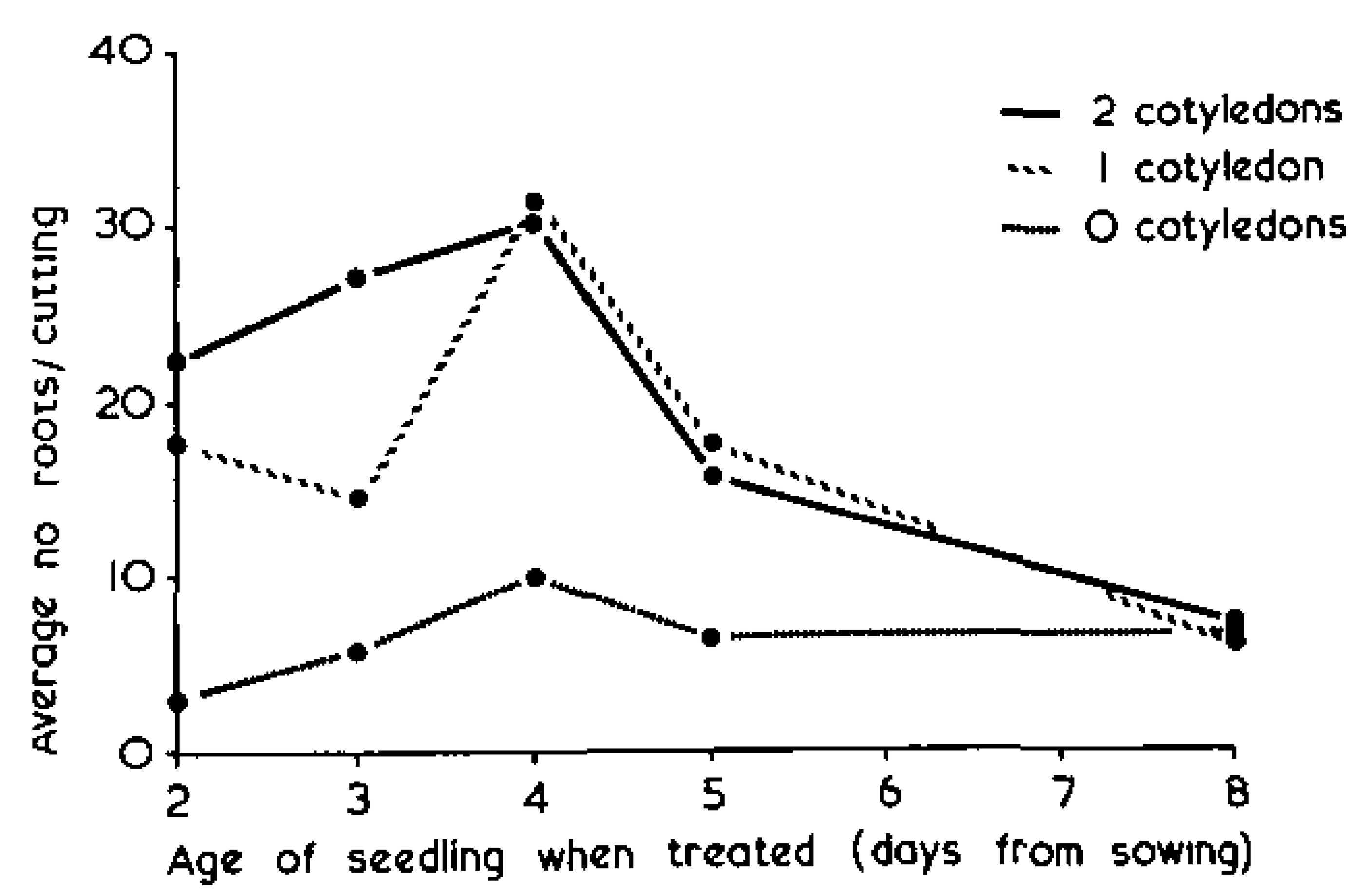

Figure 1. The effect on rooting of removing cotyledons from seedlings of increasing age.

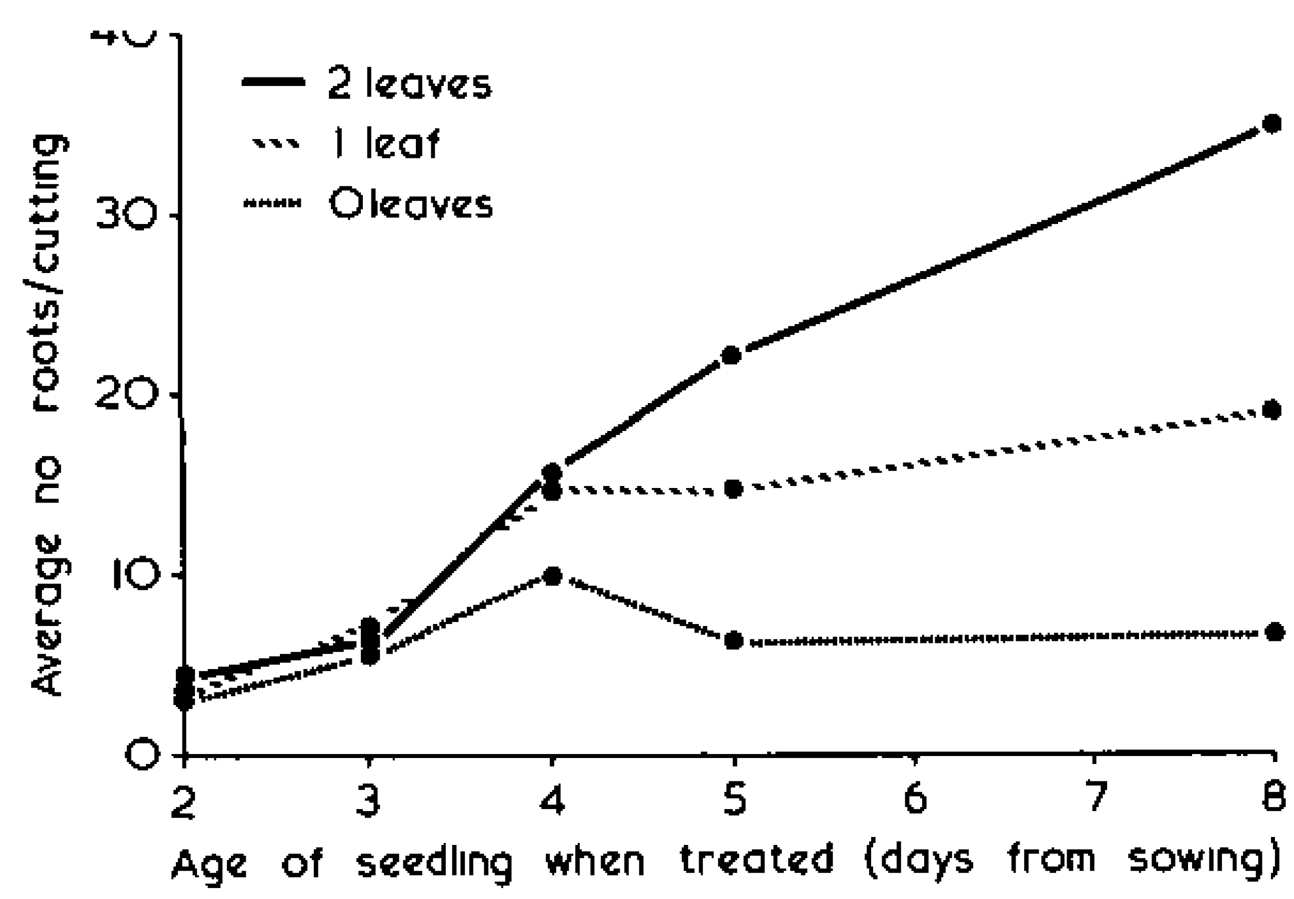

Figure 2. The effect on rooting of removing leaves from seedlings of increasing age.

Removal of leaves, especially from cuttings prepared from 4-day or older seedlings, depressed rooting (Fig. 2), suggesting that the root promotive effect of the cotyledons is taken over by the leaves as the material becomes older, although the stimulus may not be the same.

The effects upon rooting variability of removing cotyledons and leaves were measured by plotting the standard deviation (SD)[1] from the treatment means (Fig. 3). Standard deviations diminished as cotyledons aged and increased with increasing age of leaf.

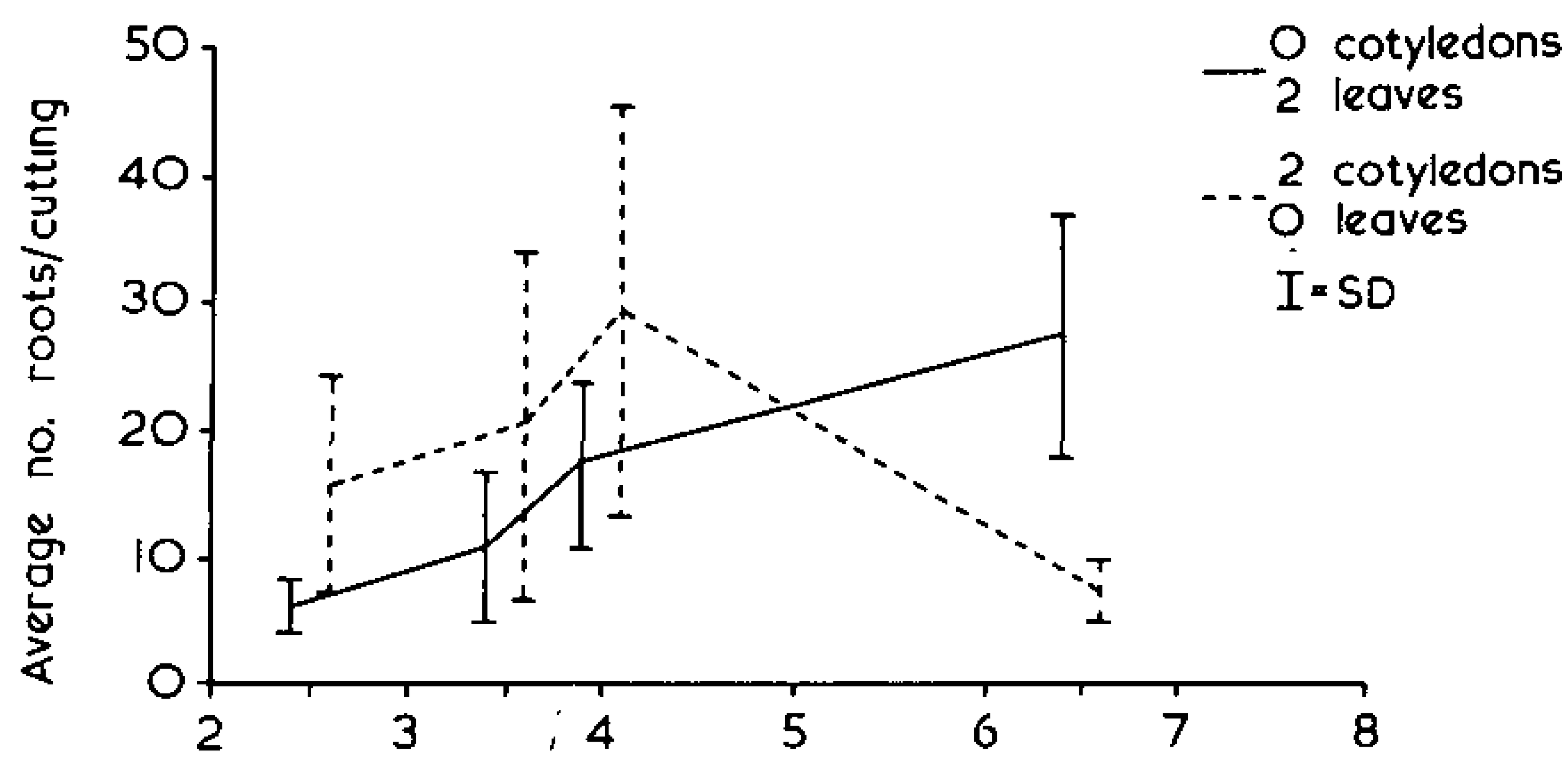

Figure 3. The effect on treatment variability due to cotyledons and leaves with increasing age.

Conclusions. It appears possible to improve the mung bean bioassay test object by removing the masking effects of rooting factors already present in the cutting and, in doing so, reduce the associated sources of variation, by either removing the cotyledons at an early stage, or the leaves at a later stage of cutting preparation. Removing cotyledons at an early stage is preferable both because the test can be completed sooner with a saving of about 5 days, and because the development of leaves assists uptake of test solutions and provides a low background level of rooting.

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[1] Standard deviation is a statistical measure of the range of values, shown as root numbers, obtained from individual cuttings in a similarly treated sample. Small standard deviations indicate uniform material and increase the precision of the test.

Preliminary results from using cuttings from seedlings about 3 days old, with cotyledons removed, suggest that they provide a sensitive system. Extracts of cotyledons applied in the modified bioassay have induced rooting responses whose magnitude was closely related to the amount of cotyledon extract present.

LITERATURE CITED

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