Media for germinating seeds — Germination tests using different media were being run as a laboratory experiment in a class in Horticulture. Following a suggestion by C. R. Burnham, sand was included with the perlite and peat moss for three lots of corn seeds: Hayes white sweet corn (1973 crop), seed from ears of A188 not fully matured at the time of a heavy frost in September, 1974, and a dent corn cross at least 10 years old. The test was not replicated, and only nine seeds were planted in each trial; the daytime temperature was 80^{o} F, nighttime 70^{o}. After five days, 8 of the 9 Hayes white seeds planted in sand were up, only 4 of the 9 in peat moss and 5 of the 9 in perlite; of the A188, 4 of the 9 emerged in sand, none in perlite and 2 in peat moss. With the old seed there were no plants up at the end of five days; but at the end of eight days, four had emerged in the peat moss.

Steve Ruce

Additional notes on seed germination — For some time I have had excellent success using fresh sand in a greenhouse bench to start plants from very old seed or seed from ears only partially developed at the time of harvest, in many cases advanced little beyond the milk stage. By planting in the sand bench a few days after the field plantings, the plants were ready to transplant to the field a few days after emerging in the sand.

There is evidence that silica stimulates the germination of wheat stem rust spores. I am almost convinced that sand may stimulate seed germination, but it may be that the conditions regarding moisture and texture are better in sand. I have had better success with tests in sand than in petri dishes.

Chas. R. Burnham

UNIVERSITY OF MISSOURI
Division of Biological Sciences, Columbia, Missouri

A positive selection technique for photosynthesis mutants of maize — We have previously reported a screening technique to isolate mutants of the photosynthetic light reaction (MGCNL 46:127-29, 1972). That procedure was an adaptation of one used for green algae that simply monitors the level of chlorophyll fluorescence (P. Bennoun and R. P. Levine, Plant Physiology 42:1284, 1967). The fluorescence technique works well with maize but there is one class of mutants for which it would not select, namely those lacking activity on the oxidizing side of photosystem II. On culture plates of algae this class of mutants has a lower than normal fluorescence yield, but selection of low levels of fluorescence is not practical with whole leaves since the normal green plant has a low level of fluorescence to begin with.

In order to isolate this class of mutant which the fluorescence technique misses, we have employed photodynamic herbicides or other known photodynamic inhibitors of photosynthesis. This class of compounds is only toxic to the plant tissues when reduced or otherwise changed by the photochemical reactions of photosynthesis. The herbicide which gave the best, most reproducible results was a commercial preparation of Diquat (Chevron Chemical Company, San Francisco, California).

The usual treatment procedure for two to three-week-old seedlings grown in vermiculite was to spray the leaves evenly with a common insect spray gun containing 10mM solution of the active ingredient, 6,7-dihydrodipyrido (1,2-a:2',1'-c) pyrazinediium dibromide. After a light spraying the plants were either put into the dark or exposed to normal growth chamber light (500 watt/m^2). Following 12 to 24 hr. in the light, treated plants showed multiple, often isolated lesions on the leaves. The plants which were kept in the dark for the same amount of time looked nearly normal. If the treated plants were allowed to remain in the dark 12 to 24 hours after spraying but before light exposure, there was considerable translocation of the herbicide and uniform killing of the leaf tissue followed light exposure. Therefore our technique usually involved spraying followed by 12 hours dark, 12 hours light treatment. During all this time herbicide treated dark control plants showed little damage.

Next, families segregating the high chlorophyll fluorescence mutants hcf and hcf3 (Miles and Daniel, Plant Physiology 53:589, 1974) were sprayed with Diquat. After the above treatment all normal plants were dead and all high fluorescent mutants were unharmed, showing only a few necrotic spots. With the high concentration of Diquat (10mM) mutants eventually also died in the light, but not before they could be clearly distinguished from wild type. 1 mM Diquat will allow the mutants to survive longer but this concentration is less effective in killing wild type.

We can conclude that with a photodynamic herbicide we should be able to select those families segregating photosynthesis mutants of all types. These mutants can include those blocked on the oxidizing side of photosystem II, whereas the high fluorescence technique will only select mutants in photosystem I and on the reducing side of photosystem II.

This type of treatment is reported to be an effective screen in green algae for photosynthesis mutants as well (R. K. Togasaki, Indiana University, personal communication).

Donald Miles & Kenneth Leto