Table 1. Effect of cytokinin source and concentration on plating efficiency of 'Russet Burbank' leaf protoplasts after 17 days in culture.

Concn (mg·liter^{-1})	Plating efficiency (%)		
	BA	Kinetin	2iP
0	0 f	0 f	0 f
0.005	0.35 ef	0.35 ef	0 f
0.05	7.70 ab	1.44 de	0.11 f
0.5	8.82 a	4.97 c	5.63 bc
5.0	4.51 c	0.65 ef	2.09 d

Means separated by Bayes LSD ($K = 100$) or ($P = 0.05$).

What is noteworthy about this observation is the effectiveness of the other two sources. The 2iP source at the 0.5-mg·liter^{-1} concentration did not differ in plating efficiency from kinetin at the same concentration or the 0.05-mg·liter^{-1} BA treatment. Of the sources tested, BA was clearly the most effective. For example, BA at 0.05 mg·liter^{-1} did not differ significantly in plating efficiency from the 0.5-mg·liter^{-1} concentration, but was associated with greatly improved plating efficiency when compared with the other two sources at the same 0.05-mg·liter^{-1} concentration. Although the plating efficiency associated with the 5.0-mg·liter^{-1} BA treatment was significantly lower than that for the 0.5- and 0.05-mg·liter^{-1} BA treatments, it did not differ from the highest efficiencies for the other two sources.

These data show that BA is an effective cytokinin source for the culture of potato protoplasts in that it appears to be effective over a wide concentration range. The 2iP source, although providing a comparable yet reduced plating efficiency to BA for the 0.5-mg·liter^{-1} concentration, was associated with reduced efficiency values for concentrations both higher and lower than this value. Similarly, kinetin showed a very narrow effective concentration range, but with reduced plating efficiencies.

The foregoing results provide quantitative support for the legitimacy of Shepard and Totten's adoption for protoplasts of Lam's (7) value of 0.4 ppm BA, a concentration that had been effective for in vitro growth of potato tuber disks. Also using the potato disk system, Jarret et al. (6) observed the same order of effectiveness in stimulating shoot formation for the same three cytokinins used in the present study. That is, kinetin was the least effective, BA most effective, and 2iP intermediate to these in its shoot-promoting activity.

This experiment demonstrates that, of the three cytokinins evaluated, BA is the cytokinin source of choice for potato protoplast culture. This conclusion is further justified by the fact that Ramulu et al. (10) found that when BA was used as the cytokinin source in the culture of 'Bintje' protoplasts, fewer abnormal plants resulted than when another cytokinin, zeatin riboside, was used. These workers concluded that the choice of cytokinin can influence the stability of ploidy in potato culture. The present study confirms that the recent reports, in which workers used BA in the culture of potato protoplasts, were indeed correct in their choice and provides a method for quantitatively assessing protoplast development.

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Rubidium Uptake as an Indicator of Citrus Root Damage

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Abstract. **Sour orange (*Citrus aurantium* L.) seedlings grown in pots in a greenhouse had 1.8 times as much rubidium in the leaves when larvae of *Diaprepes abbreviatus* L., the West Indian sugarcane rootstalk borer, were feeding on their roots than weevil-free control trees. Manually inflicted damage to the roots simulating weevil damage had a similar effect. Rubidium uptake could be used to detect root damage as a nondestructive substitute for visual inspection of the roots.**

Radioisotopes have been used to measure root activity, i.e., the amounts of nutrients taken up from a common source of nutrient supply (4, 5). Work with radioactive materials has become increasingly difficult because of strict regulations. An alternative is the use of elements that normally occur in plants in minute quantities, are not toxic at low levels, and are readily absorbed. Rubidium is an element that has long been used as a potassium substitute in various types of experiments (2–4, 6, 8).

The larvae of the West Indian sugarcane rootstalk borer weevil attack citrus roots and

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Table 1. Rubidium concentrations in the leaves of sour orange seedlings 60 days after application of 50 mg Rb per pot, with and without *Diaprepes abbreviatus* larva infestation

Treatment	Mean no. larvae		Rb concn (ppm)
	No. trees	Recovered/plant	
Untreated controls	9	0	51 c[z]
Root system destroyed by larvae before Rb treatment	7	9	79 bc
Rb treated, no larvae	19	0	133 b
Rb treated, larvae added	12	3	239 a

[z]Mean separation by Duncan's multiple range test, $P = 0.05$.

Table 2. Rubidium concentrations in the leaves of sour orange seedlings 30 days after simulating larva damage by removing two-thirds of the lateral and feeder roots or lacerating the taproot.

Treatment	Rb concn in leaves[z] (ppm)
Removal of part of lateral and feeder roots	83 a[y]
Lacerating taproot	67 a
Undamaged controls	44 b

[z]Means of 12 trees.
[y]Mean separation by Duncan's multiple range test, $P = 0.05$.

the roots of many other crops (7, 9–11). The weevil has long been a major agricultural pest in the Caribbean (1). *D. abbreviatus* was found in the United States in 1964, and it has caused extensive damage to citrus in central Florida (12). Evaluation of root damage usually means extraction of the root system for visual inspection, which is laborious and often fatal to the tree. A nondestructive method to indicate root weevil feeding would be valuable for determining infestation and damage.

In Jan. 1985, 1-year-old sour orange seedlings were transplanted into commercial potting mix (sphagnum moss, vermiculite, bark, composted pine bark, granite sand) in 5.6-liter clay pots in a greenhouse maintained at 26° ± 5°C. After 30 days, 50 neonate West Indian sugarcane rootstalk borer weevil larvae were introduced into each of 19 pots—28 pots were left untreated. Thirty days after placing the larvae, 7 of the 19 plants with weevil larvae wilted irreversibly because of extensive feeding damage to their root systems. Fifty milligrams of Rb as rubidium nitrate in 1 liter of water was added to the 19 larvae-infested and 19 larvae-free plants. Nine plants were left untreated as controls. After 60 days, 20 leaves were collected from each plant, washed, dried at 65°, ground to pass a 20-mesh screen, ashed at 450°, and analyzed for Rb by atomic absorption. The plants were removed from the pots, their root systems were examined, and the number of larvae per seedling was recorded. The data were analyzed statistically by analysis of variance and the means separated by Duncan's multiple range test.

Table 1 shows that the sour orange plants with actively feeding larvae had significantly ($P = 0.05$) higher Rb concentrations in the leaves than the plants without weevil larvae. The seven plants whose root systems were severely damaged by a greater number of larvae (Table 1) before Rb treatment had intermediate Rb levels, probably because the callused-over remnants of their root systems were largely nonfunctional. The Rb concentration in the leaves of undamaged Rb-treated plants was 2.6 times higher than in plants not treated with Rb; it was 4.7 times higher in Rb-treated plants on which larvae were feeding.

In a 2nd test in Apr. 1985, 36 sour orange seedlings grown similarly were removed from the pots. To simulate *Diaprepes* feeding injury, the outer layer of bark of 12 trees was cut off just below the soil line on one side of the taproot. On a 2nd group of 12 plants, two-thirds of the lateral and feeder roots were removed. The 3rd group of 12 plants was repotted uninjured. Again, 1 liter of water containing 50 mg of Rb was added to each pot, and 20-leaf samples were taken 30 days after treatment for Rb analysis.

The manually inflicted injury intended to simulate larval feeding also increased the Rb concentration in the leaves, but not as strongly as the actual feeding (Table 2). It was a less than perfect substitute, because the larvae destroy the roots system in small increments over a period of time, with formation of loci of cell division when callus is formed. The effect on Rb uptake of the massive injury by cutting off part of the root system, especially after it had healed, was more comparable to that of trees whose root system was severely reduced by greater numbers of feeding larvae before Rb treatment (Table 1).

The lower Rb levels in these plants are probably due to reduced metabolic activity, reduced absorption surface, and healing of the injuries. The particularly strong enhancement of Rb uptake of plants with actively feeding larvae is apparently the result of the impaired capacity of damaged root areas to absorb ions selectively, allowing mass flow of Rb into the plants. The continuous stimulation of callus growth may also increase Rb absorption. The fact that actively feeding larvae strongly increased Rb uptake indicates that the procedure described may be helpful in detecting insect feeding on the underground parts of trees. *Diaprepes* larvae tend to attack trees sporadically; only rarely are all trees in a given planting affected, so healthy controls are usually available.

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