Street kept him constantly frustrated and unhappy while similar problems and uncertainties of the professional propagator have provided him the quietude and satisfaction he was seeking.

Our Awardee is not above insider trading, in fact, he has participated in it each year, willingly trading stock or information with his fellow insiders at these meetings. Through this insider trading he was able to accomplish a very successful merger. He has merged the boy, the farm, and business to produce an outstanding professional propagator.

Ladies and Gentlemen I present to you the 1986 Eastern Region IPPS Award of Merit recipient, James (Jim) E. Cross.

Friday Morning, December 12, 1986

The Friday morning session convened at 8:00 a.m. with Peter Vermeulen serving as moderator.

CYTOKININ CONSUMPTION BY MICROPROPAGATED SHOOTS

JOHN W. EINSET
Arnold Arboretum
Harvard University
Cambridge, Massachusetts 02138

Abstract. When shoots of *Actinidia kolomikta* were cultured on a basal medium supplemented with 30 μM N^6-(Δ^2-isopentenyl)adenine (i^6Ade), cytokinin was rapidly consumed from the medium at a rate corresponding to 100±23 nanomoles i^6Ade per g FW per day. At the same time, zeatin (io^6Ade) was excreted into the medium where it reached a level of approximately 8μM during 10 days of incubation. Rates of i^6Ade consumption, expressed as nanomoles consumed per g FW per day, for shoot cultures of other species were as follows: *Actinidia arguta*, 160±23; *Magnolia* × *soulangiana*, 18±3; *Metasequoia glyptostroboides*, 120±43; *Nicotiana tabacum*, 33±8; *Paulownia coreana*, 130±30; *Sassafras albidum*, 53±11; *Syringa* × *hyacinthiflora*, 62±10. Based on these consumption rates, one can expect that if one uses standard procedures for micropropagation (e.g. 30 μM i^6Ade, 20 ml per tube), the medium will become totally depleted of cytokinin within about 3 to 10 weeks depending on the species.

INTRODUCTION

Because cytokinin treatments are fundamental to micropropagation by shoot multiplication (1,4,7), improvements in technology will undoubtedly depend on advances in the basic understanding of these important phytohormones. During the last 3 yr, we have taken

advantage of the experimental characteristics of *Actinidia* species to develop well-defined hypotheses with regard to the biosynthesis of cytokinins, in particular, the metabolic pathway producing cytokinin (2) and the sites of biosynthesis within plants (3).

Theoretically, when one uses cytokinin as a growth regulator for micropropagation, one elevates the internal phytohormone level of shoots to cause controlled sympodial growth. At the end of each passage *in vitro,* clusters of shoots are subdivided and the individual branches are used either for further shoot multiplication or for the production of plantlets after inducing roots on cuttings with an auxin treatment. When utilized with responsive plants, these manipulations have the potential to result in a million-fold clonal multiplication of desirable individuals in less than a year.

In view of the obvious importance of micropropagation to horticulture, surprisingly little is known about the cytokinin status of propagules growing *in vitro*. Following an earlier determination of the "critical cytokinin concentration" in actively growing *Actinidia* shoots (1), this report examines *in vitro* cytokinin consumption by several woody species, including *Actinidia*, by monitoring the disappearance of phytohormone from the medium using HPLC. The results confirm the earlier finding of a substantial cytokinin requirement by micropropagated shoots in the range from 18 to 160 nanomoles per g fresh weight per day, depending on the species. Determining the exact cytokinin requirement of growing shoots is an important step in understanding the nutritional basis of micropropagation and in designing improved methods for unresponsive species (1).

MATERIALS AND METHODS

Shoot cultures of the following species have been maintained for 1 to 3 years with monthly passage onto a basal medium supplemented with cytokinin: *Actinidia arguta*, *A. kolomikta*, *Magnolia* × *soulangiana*, *Metasequoia glyptostroboides*, *Nicotiana tabacum* cv. Wisconsin #38, *Paulownia coreana*, *Sassafras albidium*, *Syringa* × *hyacinthiflora* cv. Excel (5). In all cases the basal medium contained inorganic (8) and organic (6) constituents as specified plus 5 mg/l nicotinic acid and 5 mg/l pyridoxine HCl. Media for stock cultures of *S. albidium* and *S.* × *hyacinthiflora* contained supplements of either 33 μM N^6-benzyladenine plus 0.6 μM indole-3-acetic acid or 2.2 μM thidiazuron, respectively. All other shoot cultures were maintained with basal medium plus 30 μm N^6-(Δ^2-isopentenyl)adenine (i^6Ade).

When cytokinin consumption was studied, shoots were transferred from stock cultures to basal medium (20 ml per tube) containing 30 μm i^6Ade. After 20 days incubation at 27°C with a 16 hr light (40 to 65 $\mu Em^{-2}s^{-1}$) and 8 hr dark photoperiod, the growing

shoots were moved to tubes containing 2 ml of liquid medium consisting of the basal constituents plus 30 μM i^6

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RESULTS AND DISCUSSIONS

The *Actinidia* system. Among the many advantages of *Actinidia* as an experimental subject for cytokinin studies is the fact that the phytohormone levels of tissues can be determined rapidly and definitely using straightforward HPLC methodology. By exploiting this characteristic of *Actinidia*, we have developed well-defined concepts about the mechanism (2) and sites of cytokinin production (3) in this plant. As indicated in the proposed scheme (Fig. 1), the cytokinin zeatin (io^6Ade) is formed by the 0_2-dependent hydroxylation of i^6Ade which, in turn, is produced in 2 steps from AMP with i^6AMP as an intermediate: AMP → i^6AMP → i^6Ade → io^6Ade. This pathway appears to be localized in roots and in the region of cellular differentiation behind the growing tip in the stem on the basis of an extensive analysis of the distribution of i^6Ade hydroxylase within plants (3).

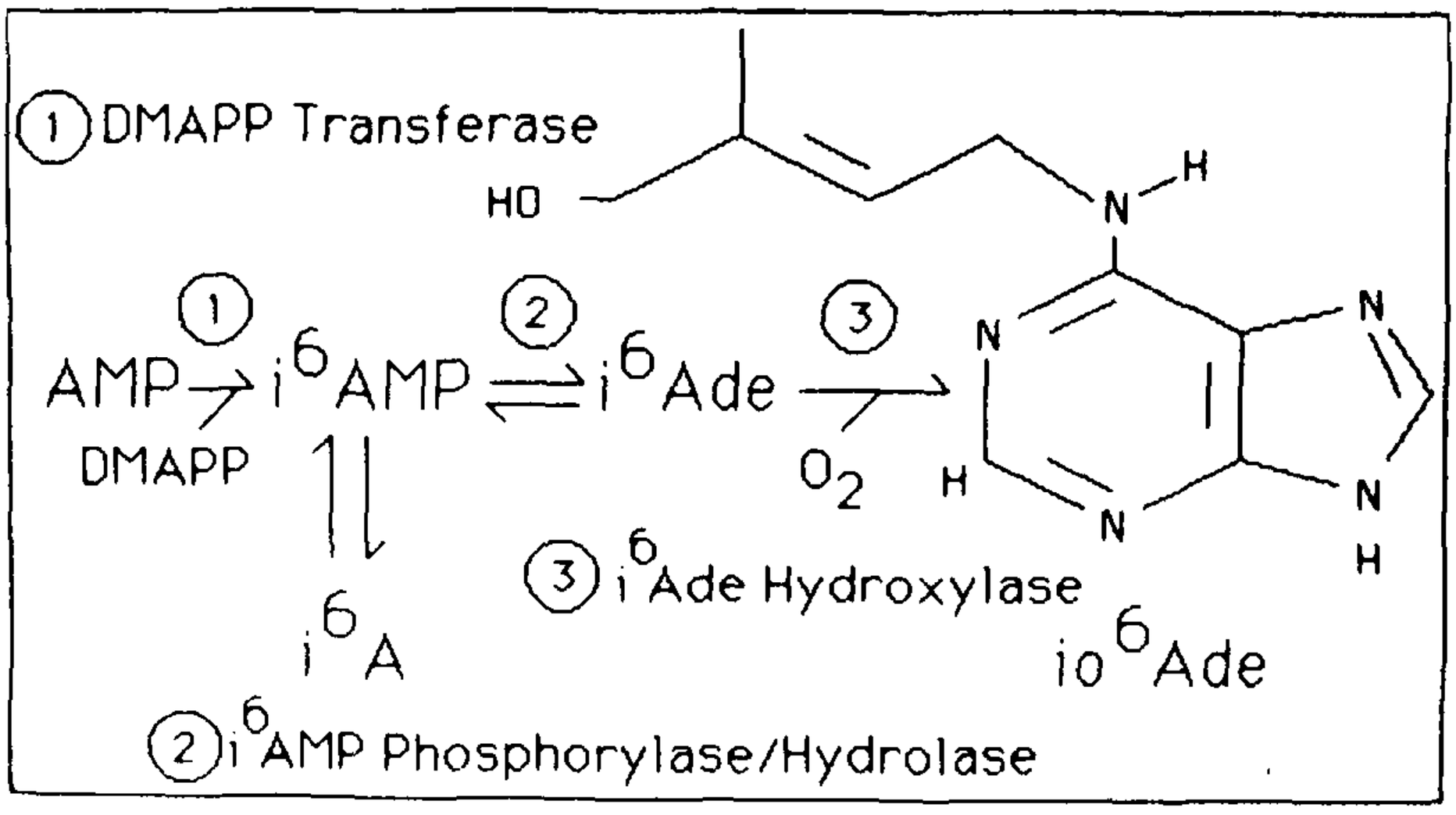

Figure 1. Proposed mechanism for cytokinin biosynthesis in *Actinidia*

Using i^{6}Ade as the cytokinin supporting *in vitro* shoot growth and multiplication, we have determined phytohormone consumption rates by various plant tissue cultures. As Fig. 2 shows, shoots of *A. kolomikta* rapidly depleted the i^{6}Ade content of the medium when they were incubated with 30 μM i^{6}Ade. Within 2 days the i^{6}Ade concentration of the medium had declined to about 20 μM and i^{6}Ade was almost totally consumed by 6 to 8 days. From the data in Fig. 2 and similar experiments, the rate of i^{6}Ade consumption by *A. kolomikta* is calculated to be 100±23 nanomoles per g FW per day. Not surprisingly, based on the biosynthetic pathway for cytokinin in this plant, *A. kolomikta* shoots excreted io^{6}Ade into the medium as they grew in the presence of i^{6}Ade.

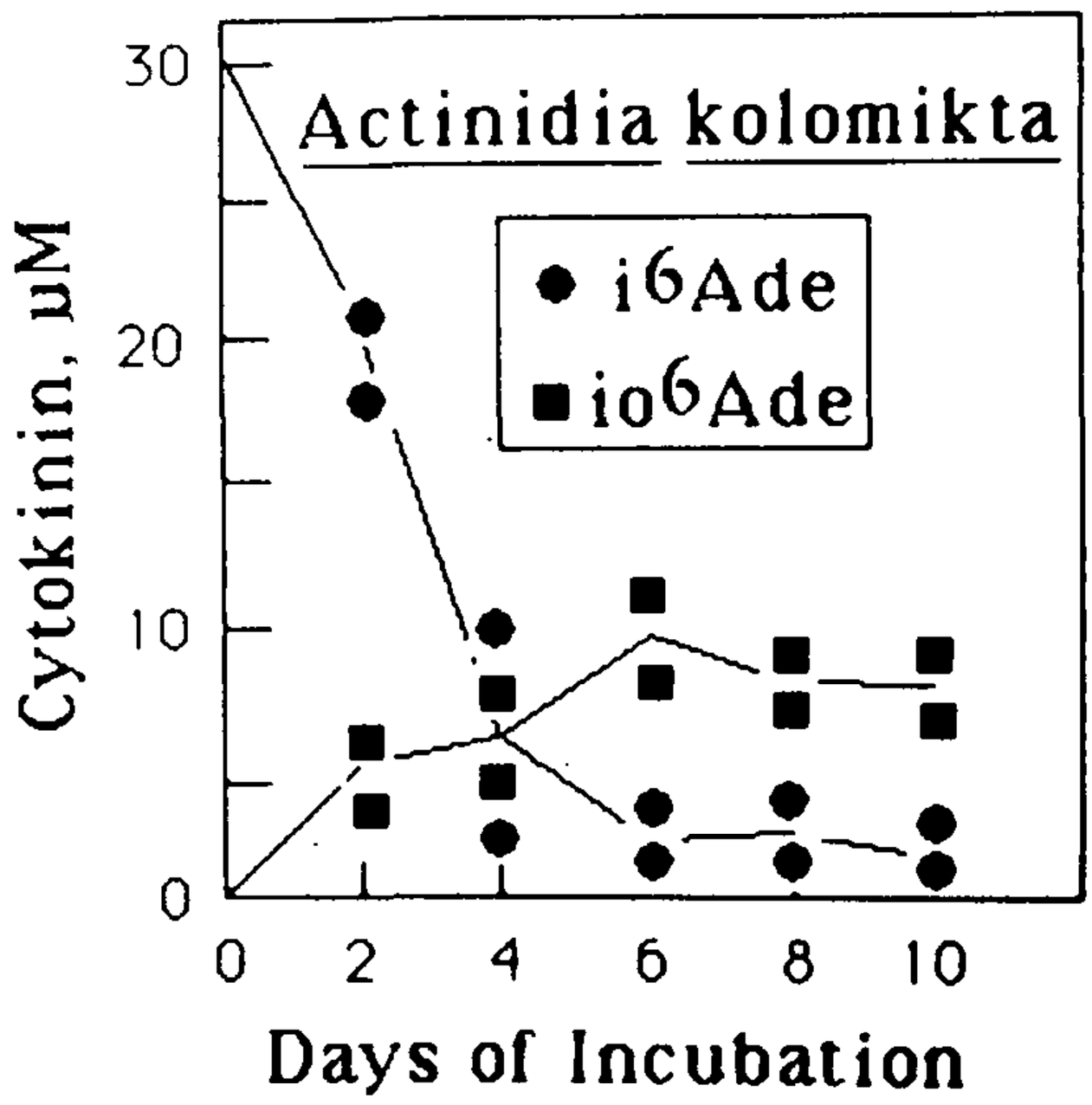

Figure 2. Cytokinin concentrations in nutrient medium supporting the growth of *Actinidia kolomikta* shoots.

Comparative cytokinin consumption. Table 1 lists i^{6}Ade uptake rates by shoot cultures of several different species. Rates of consumption ranged from a maximum corresponding to 160 nanomoles per gFW per day for *A. arguta* to a low of 18 nanomoles per gFW per day for *M. soulangiana*. Interestingly *M.* ×*soulangiana* had the slowest growth rate of any of the plants studied. On the other hand, *N. tabacum*, which was the most rapidly-growing species, also had a low rate of i^{6}Ade consumption. There did not appear, therefore, to be a clear-cut relationship between growth rate and cytokinin consumption by shoots. As expected (2), both species of *Actinidia*, as well as *S.* ×*hyacinthiflora*, excreted io^{6}Ade into the medium during growth on i^{6}Ade.

Table 1. *Cytokinin consumption rates by micropropagated shoots growing in a nutrient medium supplemented with 30 μM i⁶Ade. Stock cultures were subdivided and individual shoots (0.1 g per shoot) were transferred to fresh medium as described; in each case values are means of at least 4 independent determinations ± SE.*

Family	Species	Rate of i⁶Ade Consumption (nmoles/gFW/day
Taxodiaceae	*Metasequoia glyptostroboides*	120±43
Magnoliaceae	*Magnolia × soulangiana*	18±3
Lauracae	*Sassafras albidum*	53±11
Actinidiaceae	*Actinidia arguta*	160±50
	Actinidia kolomikta	100±23
Oleaceae	*Syringea × hyacinthiflora*	62±10
Solonaceae	*Nicotiana tabacum*	33±8
Scrophulariaceae	*Paulownia coreana*	130±30

SUMMARY

Cytokinin consumption rates can be used to determine the phytohormone concentrations needed by shoot cultures. For example, given an uptake rate of 160 nanomoles per gFW per day (*A. arguta*) and an increase in fresh weight corresponding to a doubling per week, a typical micropropagated shoot (0.1 g) would deplete the cytokinin in 20 ml of 30 μM i⁶Ade within 18 days. Under the same conditions, shoots with consumption rates corresponding to 150, 100,50 and 25 nanomoles per g FW per day would deplete the i⁶Ade in a standard medium in 20, 28, 56 and 112 days, respectively. Thus, it is probable that, in several instances, micropropagators transfer their shoot cultures to fresh medium long after the cytokinin in the medium has been exhausted. The so-called "maturation" phase of tissue culture growth, for example, conceivably could involve a period of cytokininless development.

Unfortunately, cytokinin consumption rates reveal little about the biochemistry involved in cytokinin action. It is not possible, for example, to know the exact amount of phytohormone that the plant actually uses in meeting its nutritional requirement for cytokinin since only a fraction of the amount consumed may be utilized internally. Nevertheless, a daily rate of cytokinin consumption of 100 to 160 nanomoles per g FW determined for *Actinidia* is in the range expected for a "critical concentration" corresponding to 150 nanomoles io⁶Ade per g FW (1). This rate is also consistent with estimates of the rate of cytokinin disappearance from within shoots that were transferred from stock cultures to cytokininless mediums.

Having developed the technology to study cytokinin consumption by micropropagated shoots and having determined the rates of i⁶Ade uptake by several species that can be grown successfully *in vitro*, we are now at the stage to test some possible explanations

why some woody species are not responsive (1): that is,

1) The cytokinin requirement of non-responsive explants is greater than the amount supplied in the medium.
2) Non-responsive explants fail to assimilate cytokinin from the medium.

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