Generous with his time, he has been the prime mover in innumerable projects to benefit the nursery and florist industries, numerous youth groups, farm organizations, local schools, and his church.

He is past president of the International Plant Propagators Society, past president of the Western Region, IPPS, a supporter of the Western Washington Horticultural Society, a past president of Rainier Chapter, Washington State Nurserymens' Association, and a member of the Board of Directors, WSNA. He has been chairman of the Legislative Committee, and chairman of the Highway Committee, WSNA; chairman of the Grades and Standards Committee of the American Association of Nurserymen, a member of the American Rhododendron Society, and he is on the Board of Directors of The Rhododendron Species Foundation. He was named citizen of the year of his home town. He is an avid football fan and at times a pretty fair salmon fisherman. His name is Bruce Briggs.

MODERATOR LARRY CARVILLE: We will now have five presentations dealing with the general topic of "Plant Growth Regulators". David Lane of the Summerland Research Station at Summerland, British Columbia, will give the first paper:

PLANT MANIPULATION *IN VITRO* WITH HORMONES

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Abstract. The experiments described in this paper illustrate some of the responses of shoot cultures to treatment with growth regulators and the manipulations made possible through their use. First examined are the growth regulator requirements of shoot cultures. Cytokinin, in particular, is required by most cultures but, in exceptional circumstances, may not have to be supplied in the medium. The optimum growth regulator concentration required for shoot or root growth varies considerably between species and cultivar; growth regulators supplied in the medium can interact with those produced by the cultures and result in dramatically different responses. Varient requirements probably caused by this effect influence rooting more than shoot growth, particularly in the cultivar M.9. Its roots initially develop into callus rather than roots when continually exposed to a normal concentration of auxin in the medium. Shoot cultivars can be manipulated by exploiting differences in their tolerances to growth regulator concentrations higher or lower than their optimum. This should make it possible to develop procedures for preventing back mutation of spur-type strains to standard growth habit and, used in reverse, may be useful for isolating and identifying new spur-type strains arising as induced mutations in shoot cultures.

INTRODUCTION

It is commonly known by tissue culture specialists that the plant growth regulators — i.e. plant hormones — are fundamentally important in determining the growth characteristics of shoot cultures *in vitro*. Usually, cultures die if one or more are not included in the medium (1). Plant growth regulators also influence the number and length of shoots (2) and root number, size and length (3). Also, they influence callogenesis and rate of callus growth (4) and are thought to have secondary effects on such things as rooting success and the ease with which plants can be transplanted because of carryover effects from the shoot proliferation medium.

This paper presents results from three sets of experiments. In the first, the influence of cytokinin, auxin and gibberellic acid alone, and in combination, on flax shoot cultures is examined and information is presented bearing on the growth regulator requirements of shoot cultures. In the second section, a comparative examination is made of the response by five apple cultivars to a range of cytokinin and auxin concentrations by measuring their shoot growth and rooting. The variation in response among the cultivars is discussed. Finally, an experiment is reported in which three strains of the apple cultivar McIntosh, with standard, spur-type and dwarf growth habit, were examined for tolerance to cytokinin concentrations below and above their normal optimum; and the implications for prevention of mutations in shoot cultivars are discussed.

MATERIALS AND METHODS

Standard techniques described previously (5) (6) were used to initiate and maintain mother cultivars. Briefly, explants were obtained from germinated seed (flax) or from field trees growing at the Summerland Research Station. They were surface sterilized and incubated in Murashige and Skoog nutrient medium (7) containing 5μM (apple) or 0.1μM (flax) benzyladenine. Incumbation was in a growth chamber adjusted for day/night: cycle 16:8 hours, with temperatures of 22° and 28°C.

RESULTS AND DISCUSSION

Shoot Culture Requirements for Growth Regulators

Although plant species respond differently and differences among cultivars are common (8), particularly in the optimum concentrations they require, responses of a generalized nature form a fairly consistent pattern and are useful for defining most shoot culture requirements. These growth requirements

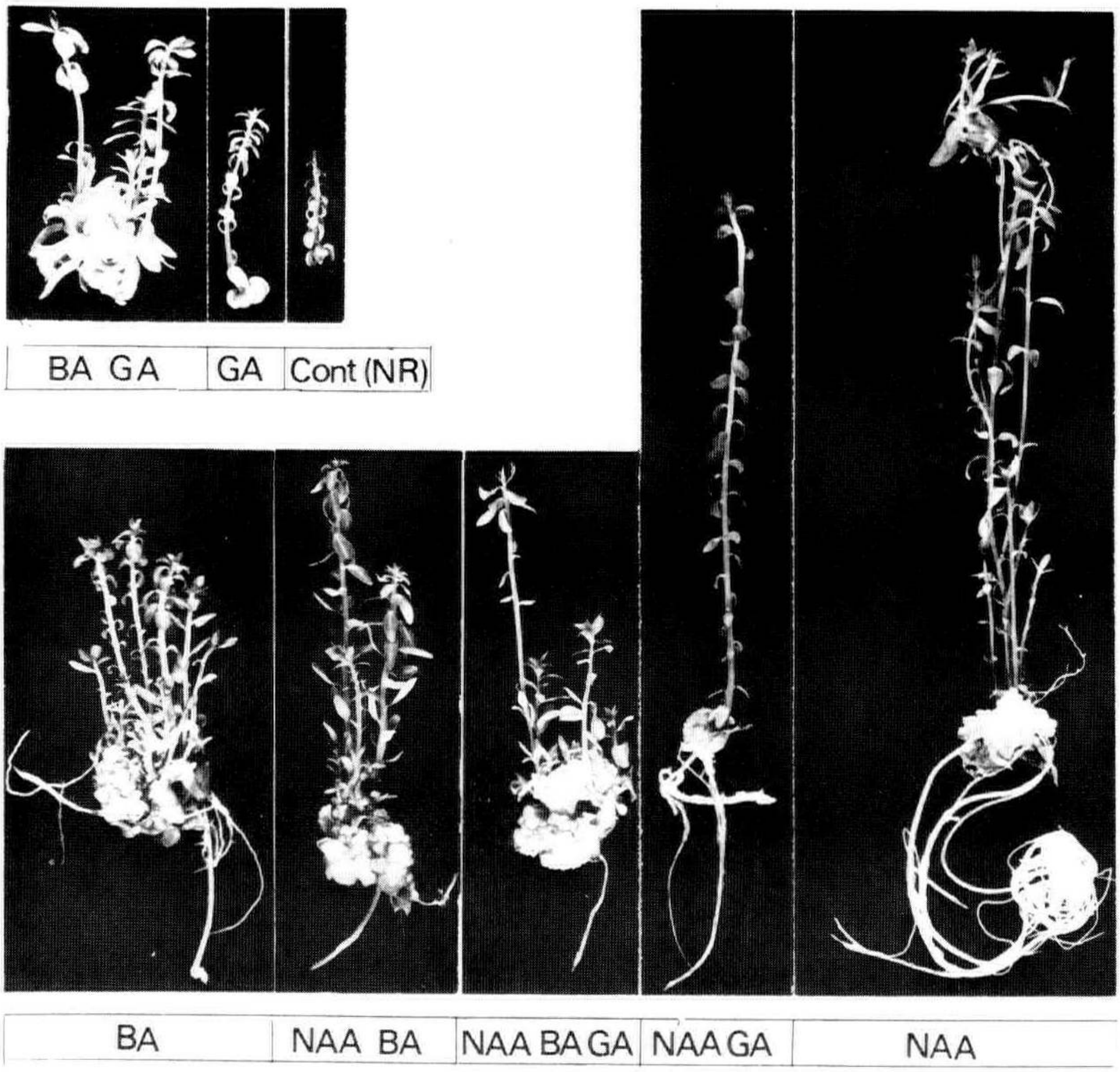

Figure 1. The influence of BA, NAA and GA alone and in combination and the no regulator control on the morphogenic development of flax meristem-tips. Concentrations: BA, 0.1 μm; NAA, 0.05 μM; GA, 5 μM.

are not consistent enough to be considered rules, though, as exceptions are known.

Exceptional responses usually result from what is assumed to be the presence of growth regulators produced by the cultures themselves which interact with those supplied in the medium.

The effect of growth regulators on flax shoot cultures, summarized in Figure 1, shows the effects of individual and combined growth regulator treatments. Cytokinin (benzyladenine, BA) considered to be mainly synthesized by root tissue, was required by those flax shoot cultures which were devoid of roots. The control treatment with no BA died. Once the cytokinin requirement was met, study of the interaction of BA

with other growth regulators was possible. GA had no effect on shoot number, or it was inhibitory when combined with the treatments of BA alone, with the auxin, naphthaleneacetic acid (NAA) alone, or with BA plus NAA, depending on the concentration used. Addition of NAA often resulted in a reduced number of shoots, but those which did grow were longer. This effect could be used to advantage to give cultures with shoots which were less crowded and more easily manipulated *in vitro* than those with higher shoot number. In the flax shoot cultures, NAA alone resulted in root formation and gave rise to complete plants which grew similarly to germinated seedlings. Exogenously supplied cytokinin was no longer required in this situation, probably because cytokinin synthesis in the root supplies the requirement. Table 1 summarizes the results of these experiments and gives the calculated yield of shoots based on the frequency with which shoots became established and the average shoot production of those which did. The results indicate that in this system both NAA and GA have either neutral or, more often, inhibitory effects on shoot production.

Table 1. The influence of growth regulators on percent frequency of meristem-tip cultures forming multiple shoots; average number of shoots per proliferating culture; yield of shoots per 100 cultures; and average dry weight of cultures.

	Shoot Proliferation			
Growth Regulator	Frequency	Ave. No.	Yield	Dry wt.
BA *	93	5.7	530	57
BA NAA	79	4.6	363	53
BA NAA GA_3	80	3.3	264	48
BA GA_3	60	3.7	222	34
NAA	47	3.2	150	80
NO REGULATOR	33	2.8	92	29
NAA GA_3	13	3.9	51	29
GA_3	7	1	7	10

* Concentrations: BA, 0.1μM; NAA, 0.5μM; GA_3, 5μM. Previously published in reference 1.

Variation Among Clones in Response to Growth Regulators

Consistent requirements for growth regulators, such as cytokinin, applicable to most species commonly grown as shoot cultures *in vitro* are more universal than the specific growth regulator concentrations which give optimum growth (shoot number). In fact it is common for cultivars of a species to respond with different optimum concentrations of cytokinin for shoot proliferation and of auxin for root initiation, although, these differences are often small. The results of an experiment comparing the cytokinin requirements of four apple cultivars (three rootstock and one scion) for optimum shoot

production is presented in Table 2. Shoot production at the optimum BA concentration varied with the cultivar; the most prolific shoot producer, M.27, gave 249 percent more shoots than the least productive. The optimum BA concentration also differed, with M.26 and Macspur having an optimum of 5μM, and M.27 and M.111 producing best at 10μM. These figures would be modified downward somewhat if the most appropriate rate of shoot production for propagation, rather than for the maximum was recorded. The results demonstrate, however, that cultivar differences do occur even in closely related cultivars. There are several possible reasons why the cultivars responded differently. The effective levels of growth regulators produced by the cultures themselves could very well be different and, therefore, could influence the optimum concentration required in the medium. If the cultures are partially self sufficient, not as much added growth regulator would be required. Optimum concentrations could also be influenced by other characteristics of the cultures, such as the rate at which the growth regulator is taken up, the efficiency with which the culture transports it, or the speed with which it is broken down metabolically.

Table 2. Average number of shoots per culture of apple shoot cultures grown at 1.0, 5.0 and 10 μM BA.

	BA μM		
	1.0	5.0	10.0
M.27	1.22	6.16	7.54
M.9	0.50	3.03	3.91
M.26	0.50	3.41	2.72
Macspur	1.47	4.56	3.10

Standard error = 0.37

Rooting responses of apple were much more variable than shoot growth. Table 3 shows that the reaction of M.9 is quite different from the other rootstock cultivars tested here and that acute exposure to NAA, rather than chronic, is required (Table 4). It was observed that M.9 formed callus much more readily than the others when exposed to auxin, perhaps because its shoot cultures either respond to lower concentrations or had higher internal levels. As the root initials formed they developed into callus rather than roots. Acute exposure to NAA gave good root initiation but reduced callus growth because callus requires continuous supplies of auxin for its growth. Root initiation also requires auxin but initiation occurs within a few days. Once initials are formed root extension, which does not require auxin, can proceed unhindered.

Table 3. Percent rooting of four apple cultivars at a range of NAA concentrations.

	NAA μM					
Cultivar	0.1	0 33	1.0	3.3	10	33
M.27	10%	35%	85%	26%	15%	0%
M.26	5	16	85	30	15	0
M.111	20	20	58	84	7	5
MACSPUR	0	34	58	31	43	0
M.9	0	0	0	0	0	0

Table 4. Percent rooting of M 9, acute versus chronic exposure to NAA.

NAA Treatment							
Acute, mM NAA		Chronic, μM NAA					
0 54	1.61	0.1	0.33	1.0	3.3	10	33
45 (7.8)*	52 5 (7 9)	0	0	0	0	0	0

Standard error of percent rooting

Tolerance to BA Concentrations Higher and Lower than Optimum

In addition to having requirements for growth regulators as well as optimum concentrations, shoot cultures respond, and can be manipulated, by their response to BA supplied at below or above the optimum concentration. We compared the response of three strains of 'McIntosh' apple, with either standard, spur-type or dwarf growth habit, to a range of BA concentrations. Although the optimum level for all three strains was the same, there were striking differences in tolerance to high concentrations (Figures 2 and 3). The strains with standard growth habit ('Summerland Red McIntosh') could tolerate only a slightly higher level than the optimum, the spur-type ('Macspur') considerably higher, and the dwarf 'McIntosh Wijcik' a very high level without showing phytotoxicity or dying. It is apparent that the more extreme the growth habit the greater is the tolerance to supra-optimal BA concentration. At less than optimum concentrations, the response was reversed; the standard grew more vigorously than the spur type or dwarf. These experiments show, therefore, that by manipulating the growth regulator concentration, growth of one form can be encouraged while other forms are inhibited. A selective medium such as this could have valuable practical uses. In many tissue culture propagation laboratories a concern is mutation of spur-type forms, which arose as natural mutations in the field, to the standard growth habit types from which they originated. This is reenforced by the notion that spur-type growth habit is a chimeral characteristic. The selective medium could be used to advantage to encouraging faster growth of the desirable form by choosing an appropriate concentration of

BA. Any mutated sectors which should arise would soon become diluted because of their slower growth — and the faster growing, desirable form, would predominate. Using this mechanism, accumulation of mutated tissues could be prevented.

A second use is for selection and breeding. Using the differences in tolerances identified in these experiments it may be possible to encourage growth of mutated cells and develop this into a new technique for obtaining new mutants with spur-type or dwarf growth habit from standard growth habit form.

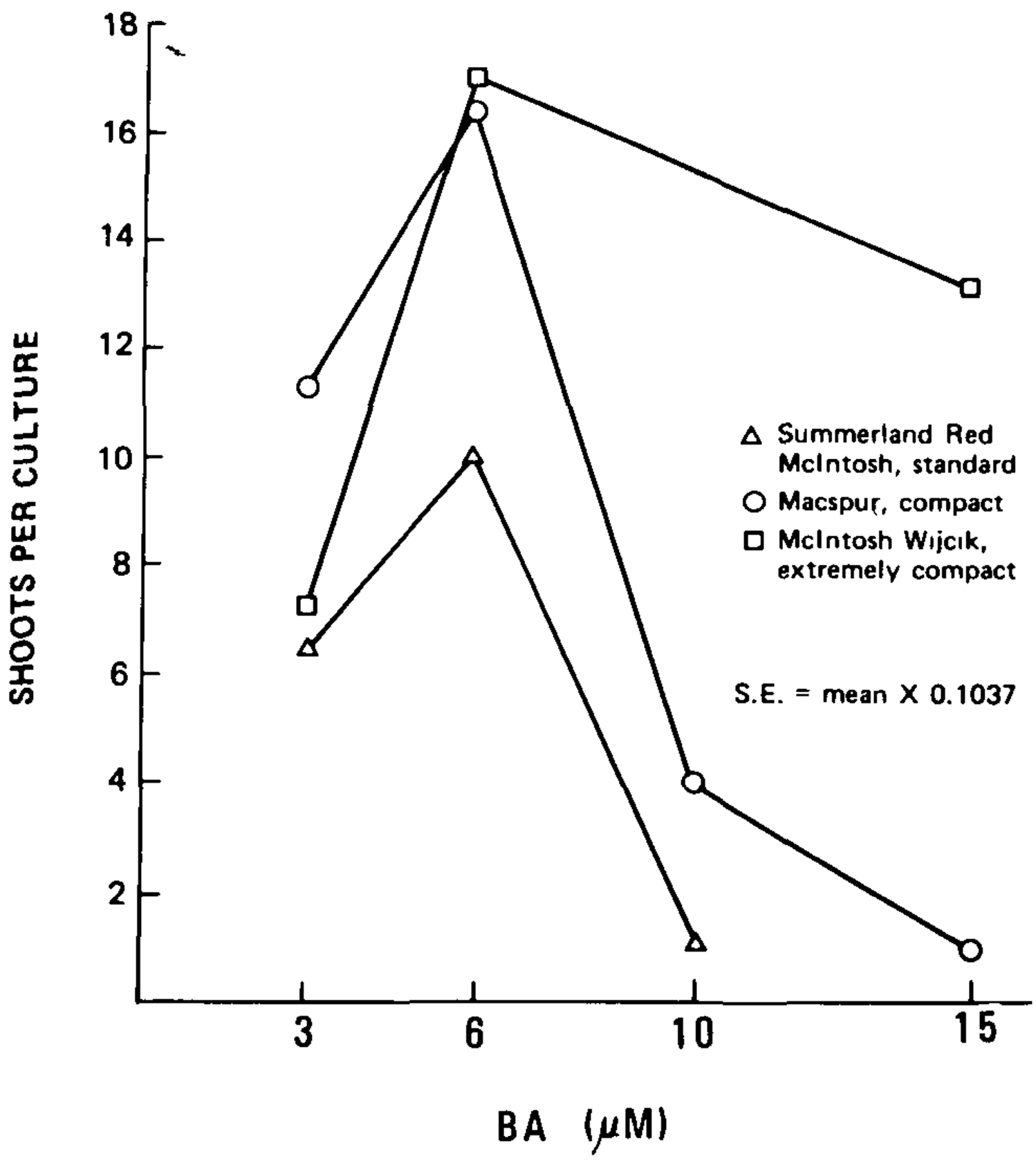

Figure 2. Number of shoots produced by meristem-tip cultures after 42 days growth by the three strains of apple with standard, compact or extremely compact growth habit as influenced by BA concentration.

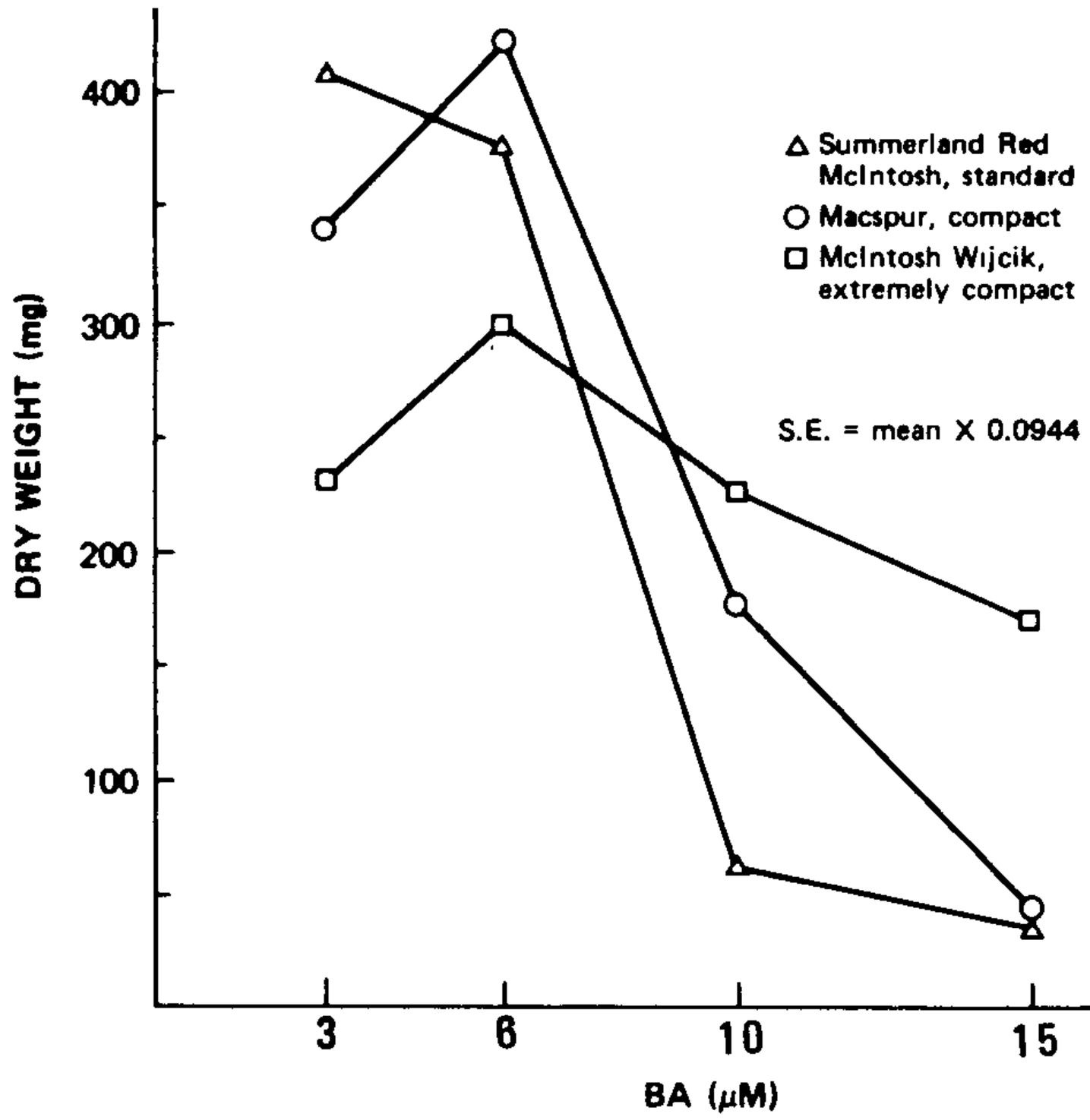

Figure 3. Dry weight of meristem-tip cultures after 42 days growth of the three strains of apple with standard, compact or extremely compact growth habit as influenced by BA concentration.

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