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Mineral Nutrient Status of Crabapple and Pear Shoots Cultured in Vitro on Varying Concentrations of Three Commercial Agars

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Abstract. **Shoot tips of 'Almey' crabapple [*Malus baccata* (L.) Borkh. x *M. pumila* var. Niedzwetzkyana (Dieck) Schneid.] and 'Seckel' pear (*Pyrus communis* L.) were cultured on Murashige and Skoog (MS) medium containing 8.8 μM BA. Media were solidified with either Bacto-agar, Phytagar, or TC agar at concentrations varying from 0.3% to 1.2%. Explant nutrient levels were influenced both by agar brand and concentration. The trends in nutrient composition, although not identical, tended to be similar for both genera. Increasing agar concentrations resulted in increased P, Fe, Zn, and Al in the explant and reduced Ca, Mg, and Mn levels. Although striking variations in many elements occur both in agar brands and in explants cultured on media containing similar concentrations of different agar brands, variations in shoot proliferation and growth of explants cannot be explained on the basis of variations in individual elements. From the nutritional standpoint, the alterations in the elemental composition of the basal medium by the addition of specific agars best explain variations induced by different agar brands. Chemical names used: *N*-(phenylmethyl)-1H-purin-6-amine (BA).**

Tissue culture studies in recent years have demonstrated that agar concentrations have a strong influence on the growth and development of various explants (9, 13, 14, 16). The level of agar in the culture medium also has a marked influence on in vitro shoot proliferation of many plant species (2, 12). Although low agar concentrations generally promote shoot proliferation, the response appears to vary with plant species. In 'Seckel' pear, 0.3% Bacto-agar resulted in poor shoot proliferation, and the explants had large translucent leaves and woody stems, whereas growth and proliferation on 0.6% agar was quite good (12). Globe artichoke explants tended to show a high degree of vitrification on media containing 0.6% agar, and though raising the agar level to 1.1% overcame the problem, it greatly reduced the proliferation rate (2).

Besides agar concentrations, the agar brand also can influence explant growth and development (4, 5, 9, 11). Debergh (1) reported that both agar brand and concentration affect the chemical and physical characteristics of the culture medium. The firmness of the medium varies greatly between similar concentrations of different agar brands. Debergh (1) concluded that "this is a strong argument in urging tissue culturists to state both the brand and type of agar used." Similar conclusions were reached based on the variations in shoot proliferation in crabapple and pear on medium containing different agar brands (11).

Hypotheses proposed to explain agar-induced variations in vitro include the presence of inhibitors in agar (4), the effect on the rate of diffusion of molecules through the medium (9, 13), variations in the availability of water (13), and gel firmness (1). Variations in the nutritional composition of Difco Purified Agar and Difco Bacto-agar have been reported previously (15). The present study was undertaken with the objective of determining a) the mineral nutrient composition of shoots proliferated on media containing varying concentrations of 3 commercial agars, and b) whether nutritional differences would explain the variations in proliferation and growth responses caused by agar brands.

Materials and Methods

Culture initiation. Following surface sterilization, shoot tips of 'Almey' crabapple and 'Seckel' pear were cultured on 20 ml of MS salt mixture (7) supplemented with 555 μM myo-inositol, 1.2 μM thiamine HCl, 87.6 mM sucrose, and 4.4 μM BA. The medium contained 0.6% Phytagar, and the pH was adjusted to 5.7–5.8 with dilute HCl or KOH prior to autoclaving for 15 min at 121°C. The detailed procedure for culture initiation was reported earlier (12). Cultures were maintained under 16 hr illumination provided by a 1:1 combination of daylight and Grolux fluorescent lights (about 60 μmol $s^{-1}m^{-2}$) at 25° ± 1°. 'Almey' and 'Seckel' cultures were initiated about 8 and 30 months, respectively, prior to use for experimentation.

Effect of agar brands on nutritional composition. Following the procedure reported previously (11, 12), shoots produced in vitro were cut to a length of about 1.5 cm and cultured on 20 ml of medium described earlier, but containing 8.8 μM BA. Bacto-agar (Difco Laboratories, Detroit, MI 48232), Phytagar (Grand Island Biological Company, Grand Island, NY 14072), or TC agar (KC Biological, Lenexa, KS 66215) was added at concentrations varying from 0.3% to 1.2%. After adjusting the pH to 5.7–5.8, media were dispensed into 25 x 150 mm culture tubes and autoclaved. A 0% agar treatment (liquid medium) was used to obtain a comparative benchmark for explant nutrient levels. The explants in this treatment were placed on Heller filter-paper supports. All culture tubes were closed with polypropylene caps and sealed with polyvinyl chloride film to reduce desiccation of the medium.

After 8 weeks, 4 replicate samples from each treatment were analyzed for nutrient composition. Based on previous growth responses (11, 12), the samples were a composite of 3 cultures in all treatments, except the 0.9% and 1.2% agar concentrations for 'Almey', and 1.2% agar concentration for 'Seckel'. Due to the lowered growth at these concentrations, the samples were a

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composite of 5 cultures. Shoots were removed from culture tubes, carefully washed in a stream of distilled water and placed in an ultrasonic cleaner containing distilled water for about 30 sec. They were rinsed with double distilled water and dried for 48 hr at 60°. Samples were dry ashed at 450°, digested with nitric acid, and the nutrients analyzed with a Jarrell-Ash ICP (inductively coupled argon plasma) spectrograph.

Agar nutrient analysis. Three replicate determinations were made of the nutrient composition of each agar brand. Samples were wet ashed using a 5 nitric acid:1 perchloric acid mixture, and the nutrients were determined with an ICP spectrograph.

Results

Agar nutrient analysis. Wide variations existed in the elemental composition of the three agar brands (Table 1). With a few exceptions, the results from the analysis of Bacto-agar were in general agreement with those reported previously (15). Although Al is not a plant nutrient, it was included in this study due to its toxic effects on plant growth (3, 6). One of the most striking differences between the 3 agar brands was the variation in Na, with Batco-agar having a very high level. Besides Na, Bacto-agar contained the highest amounts of K, Mg, B, and Zn, whereas levels of P, Mn, Fe and Al were highest in Phytagar.

Explant nutrient analysis. The nutrient composition of *Malus* 'Almey' shoots cultured on MS medium was influenced greatly both by agar brand and concentration (Fig. 1).The comparative values in liquid medium, obtained to derive a benchmark of explant nutritional status on MS medium, were either higher or lower than those obtained with the addition of 0.3% agar. In the absence of standard nutrient values for crabapples, comparisons of explant nutrient levels in liquid medium to those of leaves from apple trees (10) indicate that the Ca, Mg, and Cu values were below the normal range. Calcium concentrations in callus cultures of carrot and other plants also have been reported to be lower than in intact plants (15). Agar-containing media behaved differently from the liquid medium. Whereas certain trends in explant nutrient composition are apparent with increasing concentration of all agars, these cannot be related to the liquid medium (Fig. 1). The relationship between agar concentration in the medium and nutrient level in the explant was not consistent. There generally was an inverse relationship between agar concentration and explant K, Ca, Mg, and Mn values, which was different from the trend seen with P, Fe, Zn, and Al. The trends for Cu, B, and Na were dependent both on agar brand and concentration.

Table 1. Spectrographic analysis of the nutrient composition of Bacto-agar, Phytagar, and TC agar.[z]

Element	Concentration (ppm)		
	Bacto-agar	Phytagar	TC agar
K	317 ± 8	86 ± 5	24 ± 3
P	42 ± 1	331 ± 4	51 ± 1
Ca	1997 ± 31	2097 ± 57	2542 ± 64
Mg	1002 ± 25	635 ± 8	478 ± 13
Mn	0.25 ± 0.03	46 ± 1	2.2 ± 0.1
Fe	8.3 ± 0.1	226 ± 1	25 ± 0.5
Cu	0.84 ± 0.01	0.80 ± 0.02	0.21 ± 0.02
B	109 ± 5	57 ± 1	80 ± 11
Zn	6.6 ± 0.1	4.5 ± 0.1	5.7 ± 0.2
Al	6.2 ± 0.5	75 ± 1	16 ± 0.2
Na	7194 ± 62	1244 ± 12	596 ± 40

[z]Means of 3 determinations ± SE.

As in crabapple, the nutrient concentration in pear shoots was influenced both by the agar brand and its concentration (Fig. 2). Based on standard nutrient levels for pears, explant Ca, Mg, and Cu levels in liquid medium were again below the normal range (10). Also, as with crabapple, nutrient composition of pear explants on liquid medium were either higher or lower than those on media containing 0.3% agar. However, whereas there was an increase in explant Mg level on 0.3% agar media compared to the liquid medium in both genera, the situation was reversed for P, Fe, and Zn (Fig. 1 and 2). The general patterns for nutrient trends were, and with some exceptions, similar for both genera.

Discussion

Elemental analysis of the agar brands shows that they contain variable levels of a number of nutrients (Table 1). The incorporation of increasing agar concentrations, depending on the agar brand, would result in the addition of significant amounts of nutrients to the basal medium. In the extreme situation, with Bacto-agar, the amount of Na in MS medium would increase from 202 μM to 1141, 2080, and 3958 μM at 0.3%, 0.6%, and 1.2% agar, respectively; whereas corresponding levels following the addition of TC agar would be 280, 357, and 512 μM. This variation in Na, and other nutrients, among these agars was reflected in the explants (Fig. 1K, 2K). In species with a low tolerance to exchangeable sodium (8), the growth response on the same basal medium containing the same concentration of different agars could conceivably be different. However, it is not possible to explain variations in shoot proliferation or growth in either crabapple or pear based solely on an individual element (like Na or Al).

Though proliferation and growth rates were not measured in this study, visual ratings of various treatments were similar to responses reported earlier (11, 12). In crabapples, explant proliferation and growth were reduced as Bacto-agar and TC agar concentrations were increased from 0.3% to 1.2%, the reduction being especially severe with Bacto-agar (11). Whereas explant proliferation and growth were similar at the 0.3% concentration of both agars, those on Bacto-agar contained 800 ppm Na compared to 347 ppm on TC agar (Fig. 1K). Although explant Na content increased with increasing concentrations of Bacto-agar, no increase occurred on TC agar. Therefore, the reduction in explant proliferation or growth cannot be attributed to inhibitory levels of Na.

A number of factors related to nutrition that could influence explant growth include nutrient diffusion, nutrient interactions, and the total nutrient concentration in the medium. The rate of diffusion of molecules is reduced with increasing agar concentrations (9, 13). A negative linear relationship was observed between Bacto-agar concentration ranging from 0.8% to 1.5% and the amount of ^{14}C kinetin per gram dry weight in globe artichoke (1). Based solely on diffusion being a limiting factor, nutrient levels would be expected to decline at increased agar concentrations.

Whereas levels of K, Ca, Mg, and Mn in the explant showed a decline, those of P, Fe, Zn, and Al increased with increasing concentrations of all 3 agar brands (Fig. 1 and 2). This is confounded further by the fact that in 'Almey' explants the level of P, Fe, and Zn actually declined from those in liquid medium to those on 0.3% agar and then increased at higher agar concentrations. This variation in nutrient levels between 0.3% agar

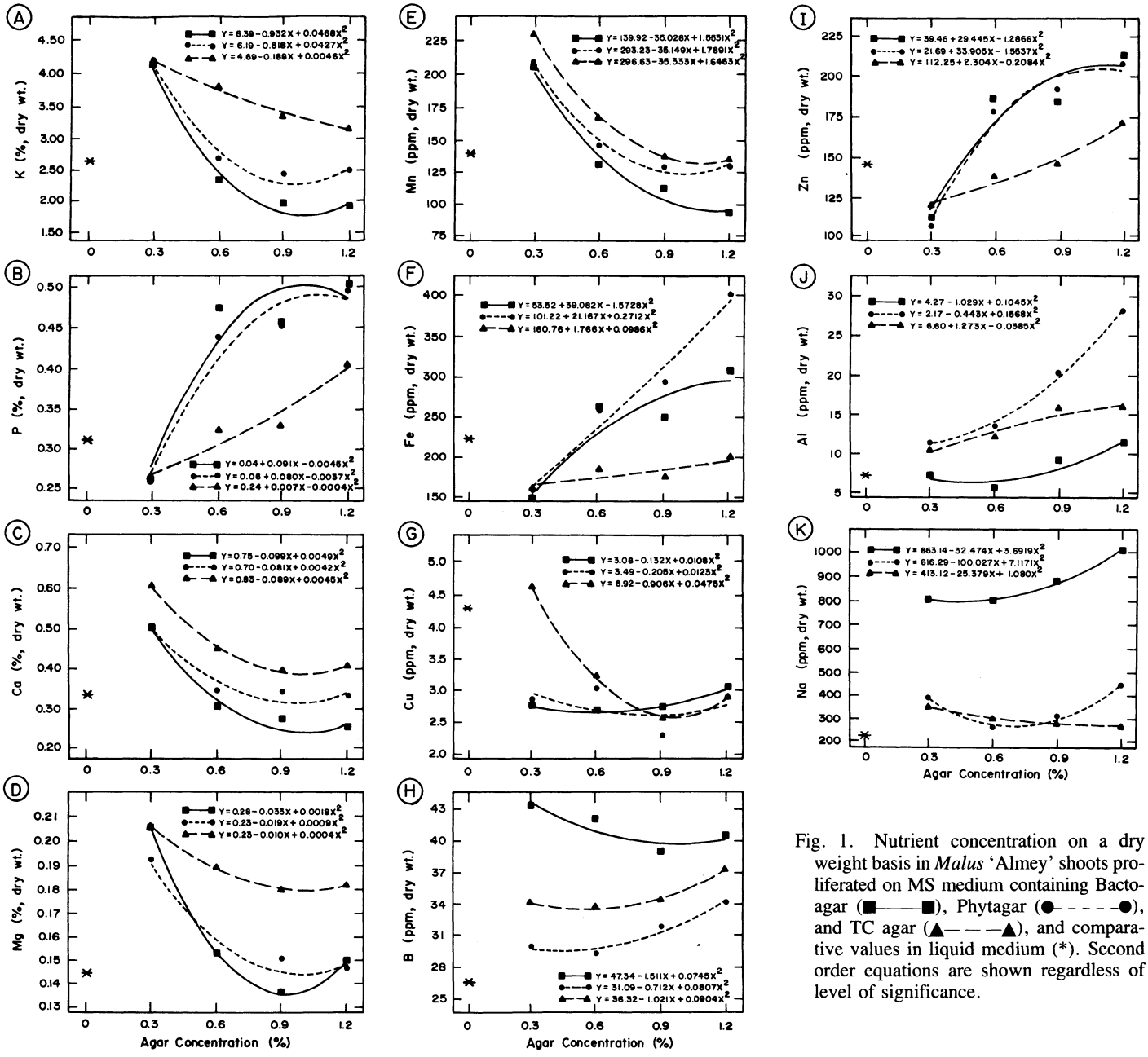

Fig. 1. Nutrient concentration on a dry weight basis in *Malus* 'Almey' shoots proliferated on MS medium containing Bacto-agar (■———■), Phytagar (●- - - -●), and TC agar (▲— — —▲), and comparative values in liquid medium (*). Second order equations are shown regardless of level of significance.

media and liquid medium, and the inability to relate explant nutrient trends observed on agar containing media to that in liquid medium, leads to the conclusion that agar-containing media behave differently from liquid media, in a manner not explainable either by diffusion rates or the simple addition of agar-borne nutrients to the culture medium. Attempts to explain nutritional variations in explants based on interactions between explant nutrient levels have been unsuccessful.

From a nutritional standpoint, the alteration of the elemental composition of the basal medium by the addition of agar best explains the agar effects. Assuming that a portion of the nutrients in the agar are in an available form, the addition of the same concentration of different agars would results in significant changes in the basal medium composition. Different basal media can exert a strong influence on shoot proliferation (17). Changes in the basal medium by the addition of agar-borne nutrients should elicit a response similar to that obtained with different basal media. Kohlenbach and Wernicke (4) have reported that water-soluble inhibitors of anther growth were present at much higher levels in Difco Bacto-agar than in the more purified Difco Noble agar. The aqueous fraction would be expected to contain many mineral elements, including Na and Cl.

As stated earlier, crabapple shoot proliferation and growth were reduced as Bacto-agar and TC agar concentrations in the medium were increased from 0.3% to 1.2%, with the reduction being especially severe with Bacto-agar (11). At 0.6% agar, the total addition of nutrients to the MS medium would be far greater with Bacto-agar than with TC agar that has a lower nutrient content (Table 1). Continued addition of TC agar would gradually change the composition of the basal medium and cause a decrease in proliferation and growth. A similar explanation also would account for growth variations observed in pear on similar concentrations of these agars (11). The decline in growth at increased levels of Bacto-agar is not as severe in pear as in

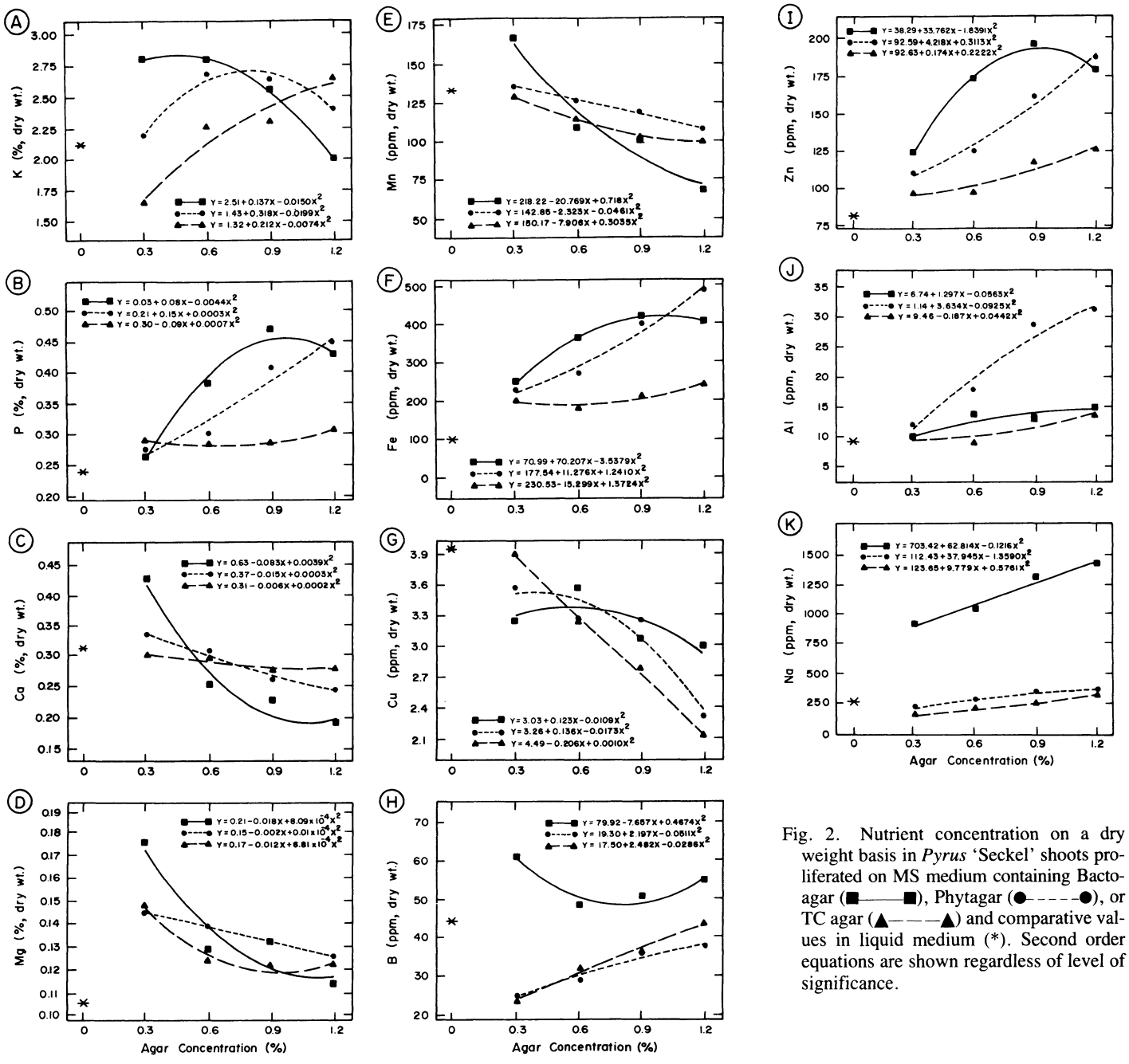

Fig. 2. Nutrient concentration on a dry weight basis in *Pyrus* 'Seckel' shoots proliferated on MS medium containing Bactoagar (■———■), Phytagar (●- - - -●), or TC agar (▲— — —▲) and comparative values in liquid medium (*). Second order equations are shown regardless of level of significance.

crabapple, and growth is even less influenced by varying concentrations of TC agar. This response could be attributed to differences in the plants, with pear being more tolerant to higher nutrient levels in the medium. Unlike crabapple, where the greatest shoot proliferation occurs at the 0.3% concentration of all 3 agars, shoot proliferation in pear is better at higher agar concentrations (11, 12), indicating that it proliferates better under conditions of nutrient or water stress.

This study demonstrates that there are marked differences in nutrient composition between different agar brands and in explants cultured on media containing varying concentrations of these brands. These differences, however, are not the only explanations for the variations in growth induced by the 3 brands. The variations in the solidity of gels among similar concentrations of different agar brands (1) would greatly affect the contact surface between explant and the culture medium and influence growth, as would variations in water potential among brands. These results provide further evidence to reinforce previous conclusions (1, 11) that both the agar brand and concentration exert a strong influence on the explant and should be considered as more than simply a means of solidifying the culture medium.

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Chill Unit and Growing Degree Hour Requirements for Vegetative Bud Break in Six Apple Rootstocks

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Abstract. **Estimates were determined for chill unit (CU) and growing degree hour (GDH) requirements for vegetative bud break in 6 apple (*Malus* ×*domestica* Brokh.) rootstocks: Antonovka 313, MM 111, MM 106, M.7a, M.26, and M.9. Rooted layers were lifted in the fall, potted, and kept in a cold room at 4°C for various lengths of time. plants then were moved to a greenhouse, and the percentage of bud break was determined for various GDH intervals. Prediction equations were determined for the percentage of bud break vs. chill unit accumulation and growing degree hour accumulation. M7a had the lowest chill unit and growing degree hour requirements for 50% bud break (590 CU and 4278 GDH). MM 106 required the most chilling (1220 CU), and M.26 the highest number of growing degree hours (6138 GDH) for 50% bud break.**

Crop losses due to spring freezes are significant in most fruit production areas, and avoidance through delayed bud break seems to be the best solution (11). Numerous studies have examined relative cold hardiness of clonal apple rootstocks (6, 9, 13). These reports have established hardiness levels during early, mid- and late-winter, but have not determined the duration of true dormancy associated with cold hardiness. Holubowicz et al. (6) reported that there was no relationship between hardiness in late winter and timing of spring bud break for the apple rootstocks studied. Although timing of spring bud break appears to be determined primarily by the scion (5), rootstock influences on scion bud break have been reported in pear (12), peach (14), and apple (4). Chandler (2) has shown that the rest influence can move across graft unions in apple. Work with Douglas-fir (10) has shown that a dehardening stimulus can move from a nondormant branch into a dormant one. They also found that a nondormant branch can stimulate cambial activity in the dormant branch, even when it is maintained under cold conditions. This evidence suggests that rootstocks with low chilling and heat requirements may influence a dormant scion to deharden and begin activity early.

The objective of this study was to determine the chill unit (CU) and growing degree hour (GDH) requirements for vegetative bud break of the 6 commonly used clonal apple rootstocks.

Materials and Methods

This study was conducted in 2 years, 1982 and 1983, using 1-year-old rooted layers of Antonovka 313, MM 111, MM 106, M.7a, M.26, and M.9 from the same commercial stool beds. Trees were lifted in late fall, after less than 100 hr of temperatures below 12°C. All trees were pruned to 70 cm above the root collar, leaving about 20 nodes per shoot with 10 to 15 apparently viable buds. Trees then were planted in 3.5 liter containers with a 2 sand :1 perlite (v/v) media, and placed in a cold room maintained at 4° ± 0.5°C. Trees were watered in the cold room with half-strength complete nutrient solution as needed.

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