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Correlative Bud Inhibition and Growth Habit of the Strawberry as Influenced by Application of Gibberellic Acid, Cytokinin, and Chilling during Short Daylength

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Abstract. **In axillary meristems laid down just prior or subsequent to commencement of growth regulator applications, exogenous gibberellic acid (GA) alone promoted runner formation. Strongly-inhibited axillary buds with leaf primordia responded to GA + BA (benzyladenine) with runner formation in the June-bearer 'Fortune'. Few of the inhibited buds of the everbearer 'Geneva' responded to GA or GA + BA. Following application of BA alone, inhibited buds of June-bearing cultivars formed lateral crowns ('Fortune') or runners ('Earlidawn'). Runner formation following chilling of June-bearing 'Fortune' plants exposed to short days (SDs) occurred from meristems initiated after satisfaction of the cold requirement. Comparable buds on plants receiving no chilling during SDs remained inhibited, as did axillary meristems present during SDs. In contrast, runners formed by chilled, everbearing 'Geneva' plants originated from axillary meristems initiated prior to chilling but not from subsequent meristems. Chilling 'Geneva' plants during exposure to SDs removed the inhibition from existing axillary meristems and promoted runner formation. Runnering did not occur in activley growing 'Geneva' plants maintained under long days (LDs).**

The axillary bud of the strawberry plant may become inhibited or develop into either a lateral crown or a runner. Axillary bud inhibition can be an intermediate phase before continued development. In commercial culture, the development of the axillary bud is controlled largely by genotype-environment interaction. June-bearing cultivars are SD plants, and are generally good runner producers and shy lateral crown formers. Everbearing cultivars generally are shy runner producers. Lateral crown formation is an important fruit yield component under the spaced plant culture system, whereas runner plants are important under the matted row system and for propagation. Hence, the ability to direct axillary bud development would be very important commercially.

Strawberry plants enter a state of restricted growth in the fall, largely in response to SDs (2, 3). There is no specialized winter resting bud, and limited growth may occur during periods of moderate temperature. Transfer of plants restricted in growth by SDs without chilling into conditions which normally maintain vegetative growth initially is characterized by suppressed growth and continued flower initiation before normal vegetative growth gradually ensues (5, 8, 10, 15). In contrast, vegetative growth is promoted strongly and floral induction inhibited for several weeks, even under SD inductive conditions, if these same plants are first exposed to several months of chilling (5, 6, 7, 8, 10, 16, 20, 21).

Endogenous hormonal balances and interactions play major roles in plant differentiation and growth. Hormonal interactions have been implicated in the control of flower induction (9), bud dormancy (26), apical dominance (19), control of morphological form of lateral shoots (18) and growth habit in strawberry (12, 17, 25). Plant growth inhibitors may block the gibberellin-mediated runnering response, and cytokinins may be required to elicit that response in the everbearing strawberry 'Geneva' (17). Following the SD-induced growth suppression of strawberry, exogenous gibberellins can replace chilling to some extent, and endogenous gibberellins have been suspected to have a role in the vegetative response to chilling (13, 14, 21).

This study was conducted to investigate correlative inhibition and development patterns of axillary buds of the strawberry plant as influenced by GA, BA, and chilling.

Materials and Methods

Plant material and chemical application — growth regulator experiment. In early March, plants of 'Geneva' (everbearer) and 'Fortune' (a June-bearer) were dug and planted in 15.3 cm polyethylene pots containing 1 loam: 1 peat: 1 perlite (by volume) medium. Plants were maintained in the greenhouse with a minimal temperature of 21°C. Natural day length was extended to 16 hr by supplemental fluorescent and incandescent light added during the morning and evening hours of each day (0500 to 700 and 1700 to 2100 HR). Plants were fertilized once every 2 weeks with a nutrient solution containing 800N–352P–664K ppm.

All plants were deflowered throughout the experiment until observations began. Prior to chemical applications, all plants were reduced to a single crown with 6 to 7 leaves per plant.

On 10 May, after all inflorescences had emerged on the 'Fortune' plants, growth regulator treatments were applied. Plants were treated with either GA or BA at 50 ppm or a combination of GA + BA at 50 ppm each. Aqueous solutions of 5% potassium gibberellate (GA_3) (Gibrel, Merck and Co., Rahway, N.J.) or water-dispersible benzyladenine in propylene glycol (SD 4901, Shell Development Co., Modesto, Calif.) were made. All solutions, including those applied to the controls, contained 0.1% of the surfactant Tween 20 (Atlas Chemical Industries, Inc., Wilmington, Del.) and were applied by spraying until drip-off. Plants were sprayed 5 times at weekly intervals.

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The experimental design was a completely randomized block for each cultivar, with the number of plants evaluated given in Table 1.

Growth habit observation following growth regulator application. At the initial chemical application, the most recently emerged leaf of each plant was tagged. Subsequent emerging leaves were consecutively numbered acropetally on the developing crown. When an inflorescence was formed, consecutive numbering continued on the extension crown, originating from the axillary meristem of the leaf subtending the inflorescence.

Just prior to the 1st chemical application, 5 plants of each cultivar were collected and fixed in a formalin-aceto-alcohol solution. Later, these plants were dissected to identify: (a) the number of unemerged leaf primordia within the apical bud of the primary crown, (b) the node position of unemerged inflorencences and axillary buds subtended by leaf primordia and the 3 youngest emerged leaves, and (c) the node position of undifferentiated axillary meristems. The node position of each organ is presented in growth-habit diagrams (Fig. 1). Axillary meristems with no leaf primordia are considered undifferentiated. Axillary meristems with 1 or more leaf primordia are hereafter referred to as axillary buds.

Eight weeks after the 1st spray applications, the number (Table 1) and node position (Fig. 1) of runners, crowns, and inflorescences formed by treated plants were determined.

A 2nd growth regulator experiment was conducted with the everbearers 'Geneva' and 'Ozark Beauty', and the June-bearing 'Earlidawn' and NY 856. NY 856 is a shy-runnering selection from the New York State breeding program. Dormant plants were potted in early February. Growth regulator treatments were initiated on 18 Mar. and applications made 4 times at weekly intervals. Observations were made 8 weeks after 1st application. Responses by 'Geneva', 'Ozark Beauty', and 'NY 856' were consistent with those of 'Geneva' and 'Fortune' in the 1st experiment. Results are presented for 'Earlidawn' control, GA, and BA treatments (Fig. 2).

Plant material and treatment — chilling experiment. 'Fortune' and 'Geneva' plants were dug from the field on 24 Sept. and placed in 12.5 cm clay pots filled with a 1 loam: 1 peat: 1 perlite (by volume) medium. They were maintained in a greenhouse under natural daylength at a temperature range of 13° to 21°C. As daylength shortened, the SD responses of reduced leaf emergence rate, very short leaf petioles, and small leaves became characteristic of the plants. Inflorescences and lateral crowns were removed as they emerged.

Ten plants of each cultivar were fixed on 19 Jan. in a formalin-aceto-alcohol solution for eventual dissection. Eight plants were transferred to another greenhouse and maintained in an environment promoting vegetative growth. These conditions included: a 16-hr daylength achieved by supplying supplemental cool-white fluorescent and incandescent light from 0500 to 2100 HR, and a minimal temperature of 21°C. This LD-warm forcing environment is hereafter referred to as the after-treatment to distinguish it from the 3 differential environment treatments: SD-cold (chilled), SD-warm (unchilled), and LD-warm (actively growing).

The remaining plants of each cultivar were divided into 2 groups and either chilled or left unchilled. Chilled plants were maintained at -1°C(±1°) in a dark cold-storage room. Unchilled plants were held in a biotron with an 8 hr photoperiod of cool-white fluorescent and incandescent light and a temperature of 18°(±1°). Light energy at plant level was; 800–1100 uW cm^{-2} of blue light in the 400–500 nm band, 690–1050 uW cm^2 of red light in the 600–700 nm band, and 90–170 uW cm^{-2} of far-red light in the 700–800 nm band.

After 91 days of differential treatment, 5 plants from each treatment were fixed on 19 Apr. for later dissection. Remaining plants were transferred to the after-treatment greenhouse. To assess the differences in seasonal solar radiation as a cause of variation in greenhouse plant growth, actively growing plants of 'Fortune' and 'Geneva' had been dug and potted in early August. They were maintained in the after-treatment greenhouse in active growth throughout the entire experiment. These plants are hereafter referred to as actively growing plants and constitute the 3rd differential treatment. Growth observations were made simultaneously for all 3 treatments.

Growth habit observation procedures — chilling experiment. Observations were made on dissected plants and plants following 12 weeks of after-treatment growth in the same manner as described above for the regulator study (Table 2 and Fig. 3). Growth-habit diagrams of dissected unchilled plants were similar to chilled plants and are not shown.

Results

Growth regulator experiment. Runner production increased significantly following GA application alone (Table 1). 'Earlidawn' plants treated with GA averaged 3.2 runners per plant vs. 0.7 runners per control plant (sig. at $P=0.01$). The major GA runner response by 'Fortune' occurred at axillary meristems laid down by the apical meristem after the 1st GA application (Fig. 1). This response suggests that GA delayed runner formation compared with control plants. Runner formation by 'Geneva' in response to GA occurred predominantly at axillary meristems present at the initial application and at nodes laid down after the 1st application. Assuming that 'Earlidawn' had a comparable number of nodes at the 1st GA application, the majority of runners were formed by undifferentiated and subsequently initiated meristems (Fig. 2).

The number of runners formed following GA + BA was similar to GA alone for 'Fortune' and 'Geneva' (Table 1). The site of 'Fortune' runner formation was quite different, however, with runners forming predominately at axillary buds present at the initial application (Fig. 1). 'Geneva' runners again formed predominately at undifferentiated and subsequently initiated axillary meristems.

The response to BA alone differed among the 3 cultivars. 'Fortune' produced a low but significant number of lateral crowns formed by axillary buds present at initial application (Table 1 and Fig. 1). Runner formation was reduced compared to control plants (Table 1), but most of the reduction did not occur at node positions from which the lateral crowns arose (Fig. 1). In contrast, BA treatment of 'Earlidawn' resulted in runner formation by axillary buds (Fig. 2). BA treated 'Earlidawn' plants averaged 1.7 runners vs. 0.7 per control plant (sig. at $P=0.01$). BA alone had no effect on runner or lateral crown formation by 'Geneva' (Table 1).

No 'Fortune' plants formed inflorescences after initiation of the growth regulator treatments. The total number of inflorenscences formed by 'Geneva' was not affected significantly by the growth regulator treatments (Table 1). However, all of the regulator treatments inhibited inflorescence formation in 'Geneva' at nodes formed after treatments had commenced when compared to control plants (Fig. 1). The average number of inflorescences formed at node 5 and younger nodes was 0 for GA and GA + BA and 0.1 for BA treated plants vs. 0.8 inflorescences for control plants (sig. at $P=0.01$).

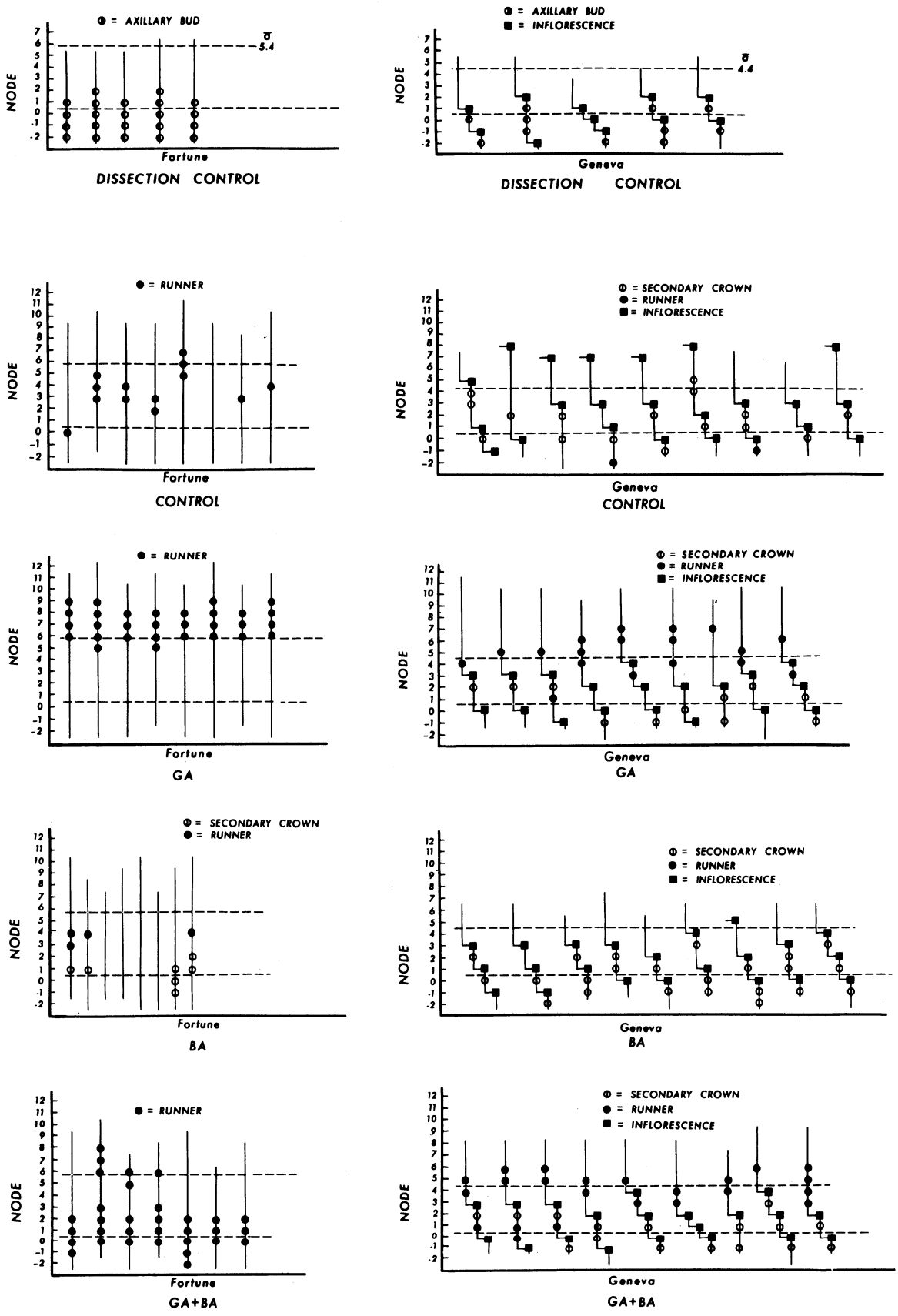

Fig. 1. Growth-habit of 'Fortune' and 'Geneva' following treatment with GA, BA, and GA + BA. Node 1 possessed the 1st leaf to emerge after commencement of spray applications (lower dotted line). The average number of enclosed nodes present at commencement of the 1st spray applications (upper dotted line) was derived from the dissected plants.

Table 1. Effects of GA and BA applied alone and in combination at 50 ppm each on the number of runners, lateral crowns and inflorescences formed by 'Fortune' and 'Geneva' strawberry plants. Number of lateral crowns and inflorescences include only those formed at or above node 1.

Cultivar and treatment	No. plants	No. runners/plant: At nodes 0 and below[z]	No. runners/plant: At nodes 1 and above[z]	No. inflorescences/ plant	No. lateral crowns/ plant
'Fortune'					
GA	8	0.0 b[y]	3.8 a	0.0 a	0.0 b
GA + BA	7	1.4 a	3.1 a	0.0 a	0.0 b
BA	8	0.0 b	0.5 c	0.0 a	0.6 a
Control	8	0.1 b	1.5 b	0.0 a	0.0 b
'Geneva'					
GA	9	0.0 a	2.0 a	1.2 b	0.7 a
GA + BA	9	0.1 a	2.4 a	1.3 ab	1.0 a
BA	9	0.0 a	0.0 b	1.7 ab	1.2 a
Control	9	0.2 a	0.0 b	1.9 a	1.2 a

[z]Node 0 possessed youngest emerged leaf at 1st application, node 1 possessed 1st leaf to emerge after initiation of applications.
[y]Mean separation within each cultivar by Duncan's multiple range test, 5% level.

Chilling experiment. Runner formation by plants exposed to a SD-warm environment with no chilling was negligible during 12 weeks of subsequent growth in a LD-warm after-treatment greenhouse (Table 2). Plants chilled during the final 91 days of SD exposure exhibited significant runner production.

Actively growing 'Fortune' plants from the LD-warm environment did not demonstrate any runnering during the 1st after-treatment period (0-days), but exceeded that of chilled plants during the 2nd after-treatment period (91-days) (Table 2). In contrast, actively growing 'Geneva' plants did not produce runners during either after-treatment growth period.

The pattern of axillary bud/meristem development in dissected 'Fortune' plants collected after 91 days of chilling, compared to the pattern following 12 weeks of growth (Fig. 3), demonstrates that chilled 'Fortune' plants formed most runners at nodes formed subsequent to chilling. Runners produced by chilled 'Geneva' plants originated from axillary meristems and the youngest axillary buds present during chilling.

The number of lateral crowns formed by chilled and unchilled plants within each cultivar was similar (Table 2).

After SD exposure, 'Geneva' plants produced a significant number of lateral crowns in contrast to 'Fortune' (Table 2). The pattern of axillary bud/meristem development in both chilled and unchilled 'Geneva' plants (Fig. 3) indicates that the lateral crowns were formed by the youngest axillary meristems present at the start of LD-warm after-treatment and by meristems laid down during after-treatment. Axillary buds, and all but perhaps the youngest meristems of 'Geneva' present during unchilled SDs, remained inhibited during the subsequent after-treatment. Actively growing plants of both cultivars formed significantly more lateral crowns than either group of SD plants during after-treatment.

Unchilled 'Fortune' plants produced more inflorescences during after-treatment than chilled plants (Table 2). The mean numbers of enclosed nodes found by dissection of unchilled plants suggest that inflorescence formation occurred only at apical meristems laid down prior to after-treatment (Fig.3).

'Geneva' plants chilled 91 days formed one additional

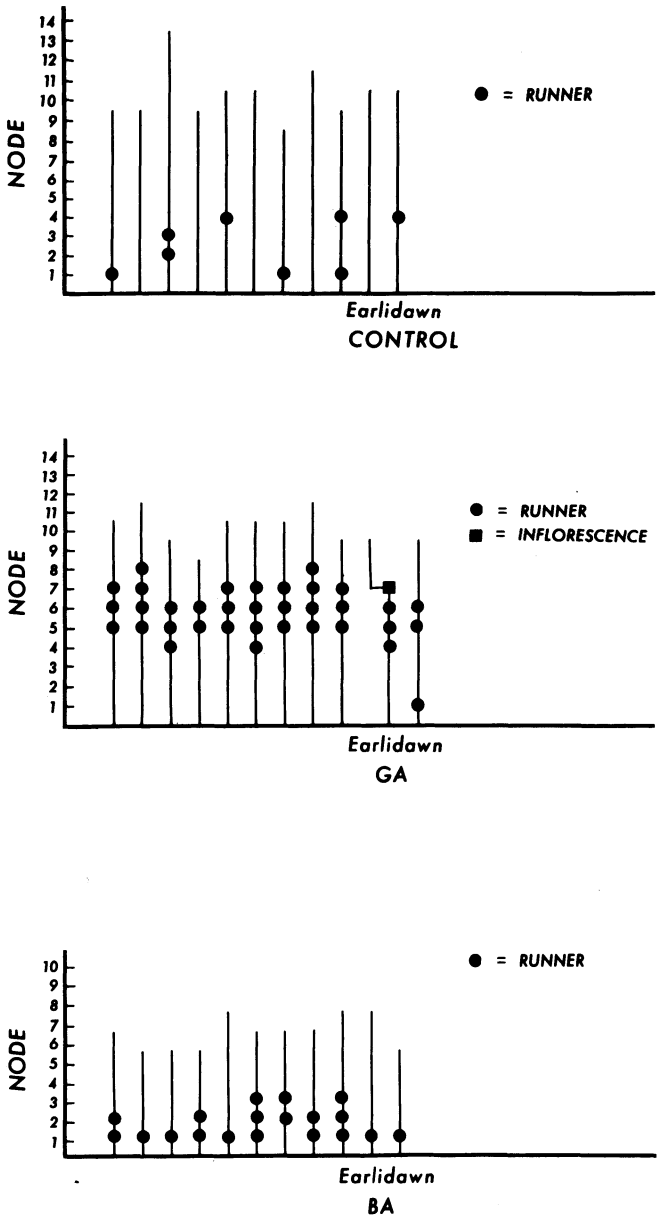

Fig. 2. Growth-habit of 'Earlidawn' plants following treatment with GA and BA. Node 1 possessed the 1st leaf to emerge after commencement of spray applications.

inflorescence per plant compared to unchilled plants (Table 2). These additional inflorescences were initiated by the apical meristems following transfer to after-treatment (sig. at $P=0.01$) (Fig. 3). Actively growing 'Geneva' plants developed more inflorescences than either SD treated group (Table 2).

Discussion

Following release from correlative inhibition, the morphological form of lateral shoots of intact *Solanum andigena* L. (18) has been regulated by application of GA and cytokinin. A similar release has been noted for excised strawberry buds (25). In both studies, GA application resulted in stolon formation whereas cytokinin produced leafy shoots.

It has been found that cytokinins affected the release of axillary buds from correlative inhibition in several species (23). Subsequent GA application often produces continued shoot growth rather than the transitory growth caused by cytokinin alone (19,

Table 2. Effect of SD-cold (chilled) and continued SD-warm (unchilled) treatment of 'Fortune' and 'Geneva' strawberry plants for 0 and 91 days on number of lateral crowns, runners and inflorescences formed during subsequent 12 weeks of LD-warm after-treatment growth and compared to actively growing plants receiving no prior exposure to SDs.

Cultivar and treatment	No. lateral crowns/plant		No. runners/plant		No. inflorescences/plant	
	0–days	91–days	0–days	91–days	0–days	91–days
'Fortune'						
Unchilled	0.0 c[z]	0.3 c	0.0 a	0.3 cd	1.4 c	1.1 d
Chilled		0.0 c		2.3 b		0.3 e
Actively-growing	0.9 b	2.1 b	0.0 a	6.2 a	0.0 d	0.0 e
'Geneva'						
Unchilled	0.6 bc	1.9 b	0.0 a	0.1 d	2.4 b	2.5 c
Chilled		1.8 b		1.7 bc		3.7 b
Actively-growing	3.3 a	4.7 a	0.0 a	0.0 d	3.3 a	4.6 a

[z]Mean separation within columns by Duncan's multiple range test, 5% level.

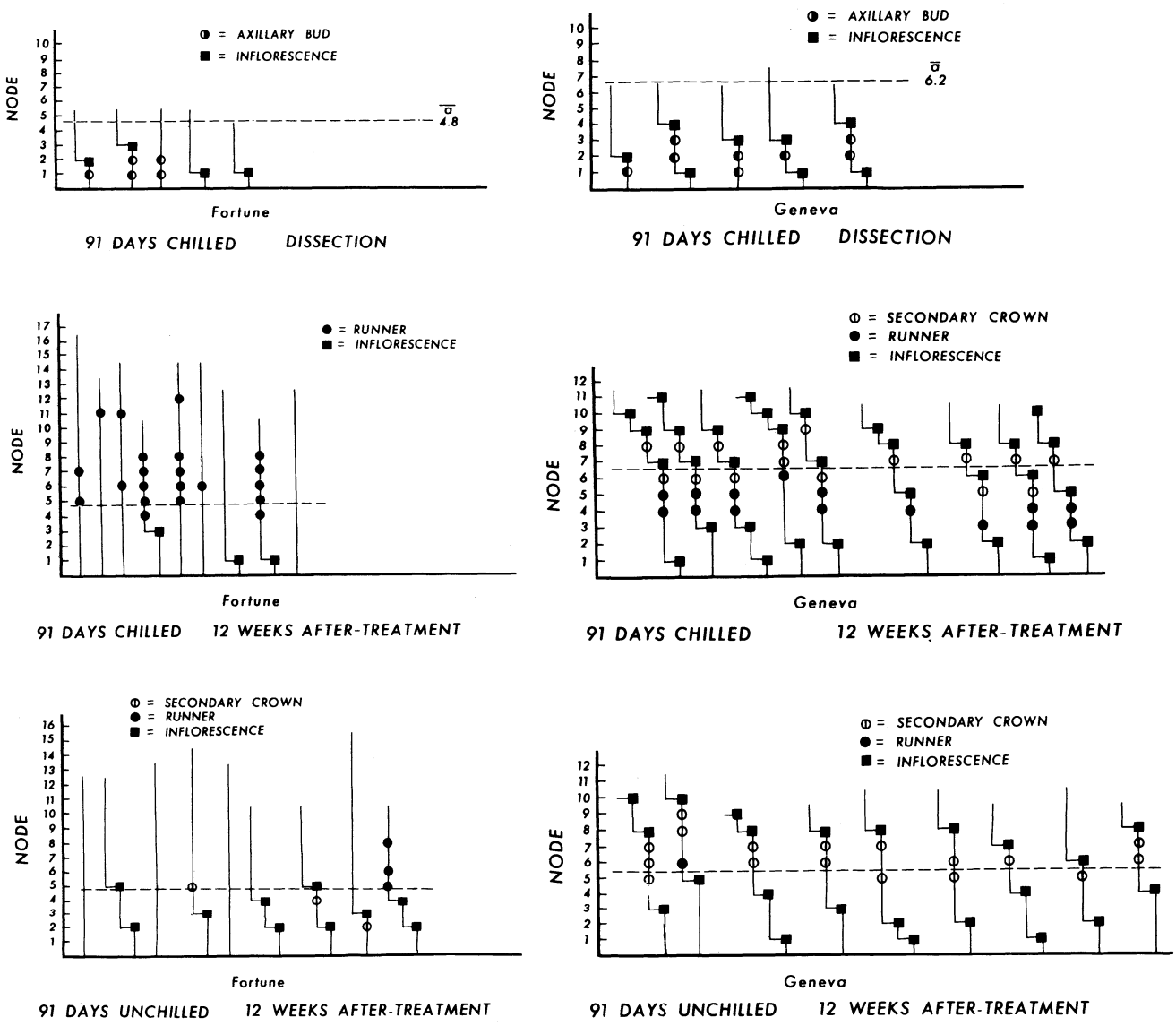

Fig. 3. Growth-habit of 'Fortune' and 'Geneva' after 12 weeks of LD-warm after-treatment growth following SD-cold (chilled) and continued SD-warm (unchilled) treatment. Node 1 possessed the 1st leaf to emerge during after-treatment growth. The average number of enclosed nodes present at commencement of after-treatment growth (dotted line) was derived from the dissected plants. Diagrams of dissected unchilled plants are not shown.

22). GA application alone has been found to promote the growth of meristems still in an active state of mitosis (1).

An analogous response mechanism seems apparent in the strawberry. Axillary meristems laid down at commencement of chemical application or formed subsequently were promoted to form runners by exogenous GA alone. Older, more strongly-inhibited axillary buds of 'Fortune' responded to exogenous GA + BA with runner formation.

Very few of the older 'Geneva' buds responsed to GA + BA with runner formation. Although a slightly increased response was found for 'Ozark Beauty' (Braun, unpublished data), a substantial number of the axillary buds or meristems of these everbearers are released early in development without the benefit of exogenous BA. Apparently, the majority of these buds quickly reach a developmental and/or hormonal balance which leads to their eventual development as lateral crowns. Once this course of development is initiated, it shows little response to exogenous GA and/or BA.

In the case of 'Fortune', BA application alone resulted in lateral crown formation by axillary buds present at the 1st application. Existing axillary buds of 'Earlidawn', however, responded with the runner formation. The response of these 2 June-bearers to exogenous BA may depend on endogenous gibberellin levels.

As has been shown repeatedly (6, 10, 11, 12, 14, 20), chilling dormant strawberry plants increases subsequent vegetative growth. Chilling 'Fortune' (June-bearer) and 'Geneva' (everbearer) plants during SDs increased subsequent runner production during LD-warm conditions. Runner production by chilled 'Geneva' plants exceeded that of plants kept actively growing in a LD-warm environment, unlike 'Fortune' plants which formed fewer runners. This performance by chilled 'Fortune' plants is confounded by the increased runner production of actively growing 'Fortune' plants during the course of the experiment.

This increased runner production by actively growing 'Fortune' plants indicates that vegetative growth was increased during the late growth period. Lack of runner formation during the 1st growth period may have been due to lower greenhouse temperatures in February than in July. Strawberry plants have been shown to exhibit less runnering at lower temperatures (14° and 20°C) compared to a higher temperature (26°) (24).

Runner formation by chilled 'Fortune' plants occurred at nodes laid down sebsequent to chilling. During the same period, the axillary meristems of unchilled 'Fortune' remained inhibited. Axillary meristems present during SDs remained inhibited regardless of SD temperature conditions. Actively growing plants were runnering at the start of the 2nd after-treatment period (91-day), and continued runnering throughout this growth period. These observations suggest that chilling of SD plants removed a block to runner formation in 'Fortune' meristems which was not present in LD-warm actively growing plants.

The runnering response of chilled 'Geneva' plants differed in several respects from that of 'Fortune'. Runners formed by chilled 'Geneva' plants originated from axillary meristems present during chilling. This runner formation occurred under conditions in which the axillary meristems of actively growing plants formed lateral crowns and not runners. Chilling in 'Geneva' not only removed the block responsible for inhibiting existing plant meristems, but also promoted their subsequent formation of runners. The chilling effect was transitory and did not elicit runner formation by axillary meristems laid down during after-treatment. This result reflects the poor runner production of 'Geneva', as previously reported (17). In contrast, chilled everbearing plants in Smeets' study (24) continued to form runners from new axillary meristems through several weeks of growth.

Gibberellin-like activity in strawberry has been found to remain low during 4 months of chilling, and to increase rapidly during growth following chilling (4). In the present study, promotion of 'Fortune' runner formation occurred at meristems initiated during the period when weekly applications of GA were made or following chilling. Presumably, meristems initiated in the presence of high GA levels were responsible for runner formation in both instances. With 'Geneva' plants, only meristems existing during chilling formed runners. Exogenous GA also promoted meristems existing at the initial application to form runners and BA enhanced this effect. Meristems initiated during the period of exogenous regulator application also were promoted. This suggests that an adequate promotive level of gibberellin or gibberellin plus cytokinin was present in chilled 'Geneva' plants for a very short time, and decreased to inadequate levels prior to subsequent meristem initiation.

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Simplified Method for Rooting Apple Cultivars in Vitro

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Additional index words. micropropagation, tissue culture, *Malus domestica*

Abstract. **Apple cultivar cuttings, prepared from shoots proliferated in vitro, initiated roots readily when placed for 3–7 days in the dark in a liquid medium containing only 43.8 mM sucrose and 1.5 μM indolebutyric acid (IBA). Raising the temperature during dark treatment from 25° to 30°C improved rooting for several cultivars. The addition of half-strength Murashige and Skoog (MS) salts or of phloroglucinol (PG) to the rooting medium did not improve rooting. IBA was the most effective auxin tried and indoleacetic acid (IAA) the least effective. Maintaining the cultures in the dark for more than 6 days improved rooting of only 1 cultivar and after 9 days rooting percentages sometimes decreased. More than 80% rooting was obtained with 'McIntosh' and 'Mutsu' after only 4 subcultures (4–5 months in culture), whereas some other cultivars in culture for a much longer time rooted no better or not as well. Roots that were initiated in liquid medium elongated after cuttings were inserted into preformed peat plugs. The resulting plantlets acclimated easily for transfer to greenhouse conditions.**

An economical and reliable propagation technique is a prerequisite for using self-rooted apple trees as a replacement for trees on seedling rootstocks. Although a number of apple cultivars have been propagated successfully in vitro (3, 7, 10), certain ones, such as 'Delicious', have remained difficult to root. Rooting in vitro of 'Delicious' and 'Jonathan' increased with increasing numbers of subcultures to a maximum value and at a rate characteristic of the cultivar (6). Recently, the importance of temperature, dark treatment, and phloroglucinol (PG) on rooting micropropagated shoots of 'Delicious' and several of its strains was reported (8).

Typically, rooting shoots of apple cultivars in vitro has meant using an agar solidified medium with single shoots in tubes or multiple shoots in larger containers (7), although successful rooting of 'Granny Smith' in agitated liquid medium has been reported (5). Using the information developed in earlier studies (8), we evaluated several factors affecting root initiation using a simplified stationary liquid medium.

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Materials and Methods

Cultures were initiated from actively growing shoot tips collected from field or greenhouse-grown stock plants or from meristem-tips dissected from dormant buds. Details of the culture establishment procedures have been published (9, 10). Shoots were proliferated on a medium containing MS salts (4) supplemented with 0.56 mM myo-inositol, 1.2 μM thiamine HCl, 4.4 μM benzylaminopurine (BA), 0.5 μM IBA, 1.4 μM gibberellic acid (GA_3), 87.6 mM sucrose and 7 g/liter Difco Bacto-agar. Iron was supplied at the appropriate concentration using (ethylenedinitrilo) tetraacetic acid, ferric sodium salt. The basal medium for rooting contained only 43.8 mM sucrose and 1.5 μM (0.3 mg/liter) IBA. Half-strength MS salts, or 1 mM PG, were added to the basal medium to determine if they were necessary for rooting. For other tests, 1.7 μM (0.3 mg/liter) IAA or 1.6 μM (0.3 mg/liter) naphthaleneacetic acid (NAA) replaced IBA in the basal rooting medium. All media were autoclaved for 15 min at 121°C and 1.1 kg/cm^2.

For shoot proliferation, cultures were grown at 25° ± 2°C with 16 hr photoperiods provided by warm white fluorescent lights at a photon flux density of 40–60 μmol s^{-1} m^{-2}. The same conditions were used for rooting in light. Dark treatment at 25° was obtained by wrapping culture vials in aluminum foil and placing them in the growth room; dark treatment at temperatures higher than 25° was obtained by placing vials in darkened growth chambers.

Ten terminal shoot cuttings 15 to 20 mm long, prepared from proliferating cultures and retaining the leaves only at the distal 1 cm of the cutting, were placed upright in 3 ml of liquid medium in 25 × 95 mm shell vials (Fig. 1). At the end of the