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Effect of Gibberellin Biosynthesis Inhibitors on Native Gibberellin Content, Growth and Floral Initiation in *Sorghum bicolor*

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Abstract. CCC, uniconazol, ancymidol, prohexadione-calcium (BX-112), and CGA 163′935, which represent three groups of gibberellin (GA) biosynthesis inhibitors, were applied as a soil drench to *Sorghum bicolor* cultivars 58M (*phyB-1*, phytochrome B-deficient mutant) and 90M (*phyB-2*, equivalent phenotypically to wild type, PHYB, except for small differences in flowering dates). The inhibitors that block steps before GA_{12} (CCC, uniconazol, and ancymidol) lowered the concentrations of all endogenous early-C13α-hydroxylation pathway GAs found in sorghum: GA_{12}, GA_{53}, GA_{44}, GA_{19}, GA_{20}, GA_1, and GA_8. In contrast, the inhibitors that block the conversion of $GA_{20} \rightarrow GA_1$, (CGA 163′935 and BX-112) drastically reduced GA_1 and GA_8 levels, but they either did not change or caused accumulation of intermediates from GA_{12} to GA_{20}. Combinations of pre-GA_{12} inhibitors and GA_3 plus GA_1 strongly reduced GAs other than GA_1 and GA_3. Each of these compounds inhibited shoot growth in both cultivars and delayed floral initiation in 90M. Floral initiation of 58M was also delayed by CCC, uniconazol, and ancymidol but not by CGA 163′935 and BX-112. This separation of shoot elongation from floral initiation in sorghum is novel. Both inhibition of shoot growth and delayed floral initiation were almost completely relieved by a mixture of GA_3 and GA_1 in both 58M and 90M. This observation, plus the much lower levels of endogenous GA_3 than of GA_1 observed in these experiments, implies that GA_1 is the major endogenous GA active in shoot elongation. CGA 163′935 and BX-112 also failed to promote tillering in 58M, whereas inhibitors active before GA_{12} did so. The possibility that the $GA_{20} \rightarrow GA_1$ inhibitors fail to block flowering and promote tillering in 58M because biosynthetic intermediates between GA_{12} and GA_{20} accumulate and/or because 58M is altered in GA metabolism in this same region of the biosynthetic pathway is discussed.

Key Words. Gibberellin biosynthesis inhibitors—Phytochrome B—Flowering—*Sorghum*

GA biosynthesis in the short day (SD) *Sorghum bicolor* appears to follow the early-C13α-hydroxylation pathway that occurs in *Zea mays* (Fujioka et al. 1988, Kobayashi et al. 1996). GA_1 is synthesized through the sequence $GA_{12} \rightarrow GA_{53} \rightarrow GA_{44} \rightarrow GA_{19} \rightarrow GA_{20} \rightarrow GA_1$ in maize (Ingram et al. 1986, Kamiya et al. 1992, Phinney 1984), and these GAs occur in sorghum (Beall et al. 1991, Rood et al. 1986). GA_3, occasionally found in small amounts in sorghum (Beall et al. 1991, Foster and Morgan unpublished data), is synthesized in maize from GA_{20} via GA_5 (Fujioka et al. 1990, Spray et al. 1996). In sorghum, the relatively photoperiod-insensitive cultivars containing the allele ma_3^R flower very early, and seedlings grow taller, contain less chlorophyll and anthocyanins, accumulate more shoot dry matter, develop fewer adventitious roots, and exhibit stronger apical dominance compared with the non-ma_3^R phenotype (Beall et al. 1991, Childs et al. 1991, Foster et al. 1994, Pao and Morgan 1986a). When GA_3 is applied to Ma_3- or ma_3-containing cultivars, they become morphologically like the ma_3^R phenotype, and floral initiation is hastened (Beall et al. 1991, Pao and Morgan 1986b). Cultivars containing the ma_3^R-allele appeared to contain increased GA levels, both of GA_1 and several of its precursors, compared with non-ma_3^R genotypes (Beall et al. 1991, Foster et al. 1994). The apparent elevated GA content of the ma_3^R cultivar was proposed to account for much of its phenotype (Beall et al. 1991). ma_3^R-containing plants

Abbreviations: GA, gibberellin; SD, short day; LD, long day; DAS, days after seeding; GC-MS-SIM, gas chromatography–mass spectrometry-selected ion monitoring; HPLC, high performance liquid chromatography; ABA, abscisic acid.

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also showed abnormal phytochrome control of deetiolation and anthocyanin synthesis (Childs et al. 1991) and failed to exhibit a red light-high irradiance inhibition of hypocotyl elongation (Childs et al. 1995), a response typical of known *phyB* mutants (McCormac et al. 1993). ma_3^R-containing plants were shown to be missing an immunologically detectable light-stable, green plant-abundant phytochrome (Childs et al. 1992, Foster et al. 1994). The ma_3^R allele was shown to be a single-base deletion mutation of the phytochrome B gene, which results in a premature stop codon before the location of the presumed second dimerization site (Childs et al. 1997). The alleles Ma_3 and ma_3^R were redesignated PHYB and *phyB-1*. Another allele, ma_3, which results in plants phenotypically similar to Ma_3 but which flower a little earlier, was redesignated *phyB-2* (Lee et al. 1998). Because both GA and phytochrome B levels are altered in cultivar 58M (*phyB-1*), phytochrome B apparently regulates GA metabolism in sorghum.

GAs are not thought to be highly specific florigenic hormones; however, there is an association between GA treatment or increases in GA content and flowering in a large number of species (Pharis and King 1985, Zeevaart 1983). Application of GA_3 hastens floral initiation in sorghum, but it has never promoted earliness to the degree exhibited by *phyB-1* plants (Beall et al. 1991, Pao and Morgan 1986b, Williams and Morgan 1979). The GA biosynthesis inhibitor tetcyclacis delayed floral initiation and inhibited shoot growth in sorghum (Beall et al. 1991). In contrast, in several LD plants, GA biosynthesis inhibitors reduced shoot growth without noticeably delaying flowering (Talon et al. 1991, Zeevaart et al. 1993). The increased array of GA biosynthesis inhibitors currently available offered the opportunity in this study to search for a link between the content of a specific GA and floral initiation.

When this study was planned, our initial objective was to dissect the early 13α-hydroxylation pathway with several GA biosynthesis inhibitors and observe the effects on growth and floral initiation in the *phyB*-containing cultivar 58M compared, when appropriate, with the non-*phyB-1* cultivar 90M. After experiments were under way, other work in our laboratory revealed that cultivars 90M and 100M vary in levels of GA_{12}, GA_{53}, and GA_{20} and GA_1 in a diurnally rhythmic fashion with peaks occurring from mid day to late afternoon (Foster and Morgan 1995). In 58M, however, peaks in GA_{20} and GA_1 occurred near the beginning of the light period. Thus, with routine early morning sampling, 58M was harvested near its daily peak of GA_{20} and GA_1 content, whereas 90M and 100M were near their daily minima. The reported two- to fivefold difference in GA_1 content (Beall et al. 1991, Foster et al. 1994) did not exist except with respect to the time of day (Foster and Morgan 1995, Lee et al. 1998). As a result, we adjusted our initial objective toward a detailed analysis of effects of GA biosynthesis inhibitors on endogenous GA levels in both 58M and 90M. The results illustrated several roles of GAs in growth and floral initiation.

Materials and Methods

Plants and Growth Conditions

Sorghum [*S. bicolor* (L.) Moench] cultivars 58M and 90M (Quinby 1967) were used in this study. At maturity gene locus 3, 58M is homozygous for ma_3^R (redesignated *phyB-1*, Childs et al. 1997), and 90M is homozygous for ma_3 (redesignated *phyB-2*, Lee et al. 1998). Maturity genes Ma_1, Ma_2, and Ma_4 are homozygously dominant in both 58M and 90M. Seeds supplied by Dr. Fred Miller were germinated and grown in pots (19-cm diameter × 14-cm depth) filled with a fertilized peat-perlite-vermiculite mix (Beall et al. 1991). Plants were grown in growth chambers (EGC, Chagrin Falls, OH) equipped with a mixture of cool-white fluorescent and incandescent sources yielding an intensity of 250–300 μmol m^{-2} s^{-1} (400–800 mn) measured at the pot surface by a LI-COR portable spectroradiometer (LI-800). The photoperiod was 11 h with 31°C days and 21°C nights. Although most of the experiments were conducted in growth chambers, several preliminary experiments were carried out in a greenhouse between October and April. During the greenhouse experiment, which is reported here in detail, day length was approximately 11 h, and temperature averaged about 32°C day and 21°C night with extremes of 40°C and 15°C.

Gibberellin Biosynthesis Inhibitors

The following compounds were used in this study: (1) *ent*-kaurene synthesis inhibitor (Frost and West 1977) CCC (2-chloroethyltrimethylammonium chloride); (2) inhibitors for conversion of *ent*-kaurene to *ent*-kaurenoic acid (Izumi et al. 1985, Rademacher 1989) uniconazol [(*E*)-1-(4-chlorophenyl-4,4-dimethyl-2-(1,2,4-triazol-l-yl)-1-penten-3-ol], ancymidol [α-cyclopropyl-α-(p-methoxyphenyl)-5-pyrimidine methyl alcohol]; and (3) cyclohexanetriones, which block the conversion of GA_{20} to GA_1 (Adams et al. 1992, Adams and Weiler 1991, Griggs et al. 1991, Nakayama et al. 1990a, 1990b), BX-112 known as prohexadione-calcium (calcium 3,5-dioxo-4-propionylcyclohexanecarbonxylate), and CGA 163′935 [ethyl 4-cyclopropyl-(hydroxy)-methylene-3,5-dioxocyclohexanecarboxylate]. Uniconazol, CCC, and BX-112 were dissolved in distilled water. Ancymidol and CGA 163′935 were dissolved in a small amount of acetone and then were added, with stirring, to distilled water. The solvent did not produce observable effects.

To gain information about appropriate concentration ranges and which application method produced the most consistent results, a series of preliminary experiments was conducted with 58M. Observations of shoot growth, floral initiation dates, and tiller growth indicated that application of the inhibitors to roots as a soil drench produced more consistent results than application into the whorl or as a spray. All treatments were applied in 50 mL/pot at 5 days after seeding (DAS). Every 7 days another 50 mL was applied until the experiments were completed. Because results with uniconazol vs ancymidol as well as CGA 163′935 vs BX-112 were very similar, ancymidol and BX-112 were not included in some experiments in which GAs were analyzed; for clarity of presentation, the data for these compounds are not reported in detail. For convenience, CGA 163′935 is referred to as CGA hereafter.

Single Inhibitors. To determine the minimum GA biosynthesis inhibitor concentrations effective in inhibiting both growth and flowering, 10 concentrations of CCC, uniconazol, and CGA were used.

Inhibitor Combinations. Based on results of treatments with single inhibitors, combinations of moderate concentrations of CCC, uniconazol, and CGA were used to test the effect of inhibitor combinations on growth and floral initiation.

Inhibitor and GA_3 Combinations. To test whether or not exogenous GA could overcome the effects of various GA biosynthesis inhibitors on sorghum, GA_3 (70.3% purity, Eli Lilly Co.) was dissolved in 95% ethanol and then diluted to each concentration with distilled water. Analysis of this batch of GA_3 by GC-MS-SIM revealed that it contained 60.9% GA_3, 17.2% GA_1, 18.3% isolactone GA_3 (Foster et al. 1997). Concentrations were calculated based on combined GA_3 plus GA_1 concentrations. Applying a mixture of GA_3 and GA_1 fit the objectives of our experiments, and the commercial grade GA_3 is referred to as GA_3 plus GA_1 in the text hereafter.

Determination of Growth and Floral Status

Measurements of plant height (from soil to tip of tallest leaf), and culm height (from soil to the tallest leaf collar) were taken at 14 and 21 days after seeding. At 2- to 3-day intervals, floral initiation was determined by splitting the shoot of two or three plants and examining the apical meristem under a dissecting microscope. Floral stage 2 (visible flower primordia) was used as the criterion for floral initiation (Lane 1963). After one plant in a population differentiated an inflorescence, several additional plants were examined to ensure that the response was typical. The days after planting to reach floral stage 2 were plotted to give floral initiation data.

Measurement of Endogenous GAs

For GA analysis, 14-day-old plants treated with a single inhibitor or a combination of inhibitors, GA_3 plus GA_1, or inhibitors plus GA_3 plus GA_1 were harvested at 2:00–3:00 p.m. (peak GA levels in wild type sorghum). Plants were cut at the root-shoot junction and at the top of the tallest leaf collar. The resulting shoot samples were frozen in liquid N_2 within 10 min of removal of the plant from the growth chamber. After lyophilization, the three oldest leaf sheaths were removed, and the samples were stored at −20°C until extracted. The extraction of GAs followed the general procedure of Foster et al. (1994), Foster and Morgan (1995), and Lee et al. (1998). After methanolic extraction, GAs were purified using a combination of preparatory chromatography, solvent partitioning and HPLC. GAs were quantified using GC-MS-SIM by calculating the area ratio of individual nondeuterated GAs to their respective deuterated [$^2H_2$]GA standards, which had been added during extraction (Foster et al. 1994). Details for replication of these experiments are given in figure legends.

Results and Discussion

CCC, CGA, and uniconazol each inhibited shoot growth in 58M. The height to the tallest leaf at 14 DAS was reduced progressively by increasing the concentrations

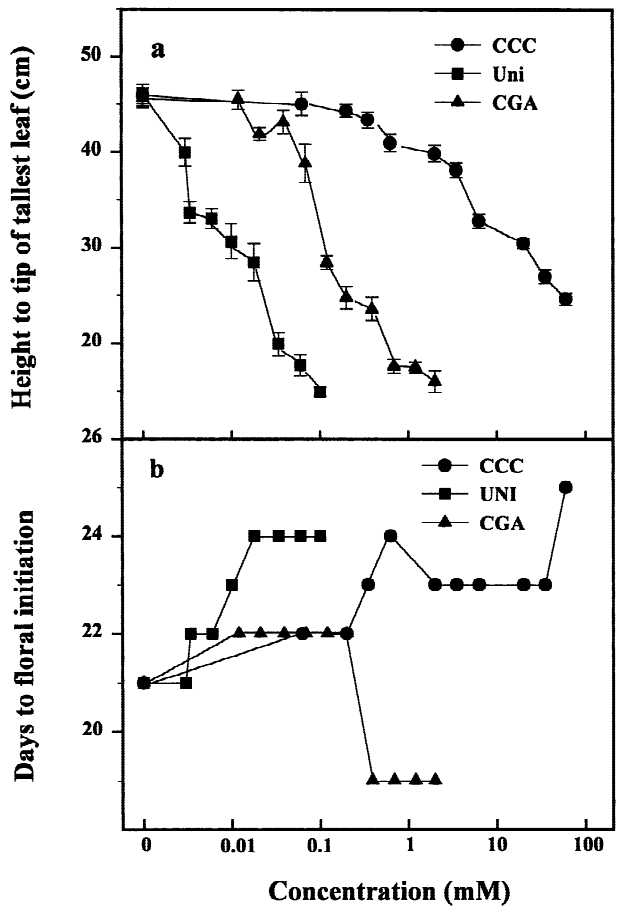

Fig. 1. Effect of GA biosynthesis inhibitors on (*a*) the height to the tip of the tallest leaf and at 14 days after seeding and (*b*) on the days from planting to floral initiation of 58M in the growth chamber. Inhibitors were applied as a soil drench at 5 and 12 days after seeding. Data are the means of three replications with five plants per replication. *Error bars* show standard deviations. Uni, uniconazol.

of each inhibitor (Fig. 1*a*). The dose-response curve for the inhibitors showed that the relative effectiveness for inhibition of shoot and leaf growth was uniconazol > CGA > CCC. Activity of ancymidol was similar to uniconazol, and activity of BX-112 was similar to that of CGA (data not given). The inhibition of the height of the tallest leaf sheath (culm height) also showed the same trend as the height of the tallest leaf tip (data not shown). These same compounds also inhibited total height and culm height of 90M to a similar degree at similar concentrations (data not shown).

Although the pattern of inhibition of growth by the different inhibitors was similar, the effect on floral initiation was different (Fig. 1*b*). Floral initiation of 58M was delayed as the concentration of CCC and uniconazol increased, but initiation was not delayed by CGA. The effect of ancymidol was similar to uniconazol, whereas BX-112 duplicated the effect of CGA (data not given).

The 3β-hydroxylation inhibitor CGA appeared to promote floral initiation slightly (1–2 days), and the promotive effect was consistent at several concentrations (Fig. 1*b*, data not shown for BX-112, which showed floral initiation at day 20 at the same concentrations tested for CGA). Cultivar 90M also exhibited a delay of floral initiation of 2–6 days by CCC and uniconazol (data not given). The comparison of the effect of CGA on floral initiation of 58M vs 90M is presented later.

58M is known to produce fewer preanthesis tillers than 90M (Beall et al. 1991). During the preliminary experiments, an inconsistency in the response of tillering to GA biosynthesis inhibitor treatments was noted in 58M, and subsequently, detailed observations were made. The pre-GA_{12} biosynthesis inhibitors CCC, uniconazol, and ancymidol all reduced height while promoting tillering (data not shown). In contrast, inhibitors of the GA_{20} to GA_1 step, CGA and BX-112, also inhibited height growth but failed to promote tillering (data not shown). Untreated 90M plants tillered prolifically, and tillers were produced by inhibitor-treated plants. Tillering in sorghum and other grasses is known to be responsive to GA treatment (Cline 1991, Morgan et al. 1977). In addition to the effects on plant height and tillering, the shorter leaves of inhibitor-treated plants were much darker green in color and wider than those of control plants.

Combinations of CCC + uniconazol, uniconazol + CGA (data not given) and CCC + CGA (data not given) inhibited growth of genotype 58M at low concentrations (Fig. 2) and delayed flowering, especially at the two highest concentrations (Fig. 3). Reduction of culm length was more severe than total plant height. Simultaneous application of representatives of all three groups of GA biosynthesis inhibitors (CCC, uniconazol, and CGA) inhibited shoot growth (Fig. 4), with no genotype differences (90M data not shown). Combinations of inhibitors reduced height to a greater degree than did the individual inhibitors, indicating more complete depletion of endogenous GAs. Analysis of endogenous GA levels confirmed this conclusion (see below). With either two or three inhibitors, both growth inhibition and the delay of floral initiation were almost completely relieved by simultaneous application of 7.1×10^{-5} M GA_3 plus 2.0×10^{-5} M GA_1 (Figs. 3 and 4 and other data not given). Further, floral initiation was hastened by all treatments containing GA_3 plus GA_1 (Fig. 3). Delays of floral initiation in 90M by CCC, uniconazol, and CGA were also reversed by the applied GAs, usually to cause initiation 1 or 2 days before the control (data not given).

The effect of CGA on floral initiation was contrasted directly in 58M and 90M over a range of concentrations. The inhibition of shoot growth of CGA was very similar in both cultivars (Fig. 5*a*), whereas effects on floral initiation differed significantly (Fig. 5*b*). Floral initiation of 58M was not delayed by CGA, but that of 90M was delayed progressively as the CGA concentration increased (Fig. 5*b*). Rapid shoot growth occurs before flowering in sorghum. CGA separated these effects; it was possible to inhibit growth without delaying floral initiation in 58M but not in 90M. In fact, at the highest concentrations of CGA tested, floral initiation of 58M

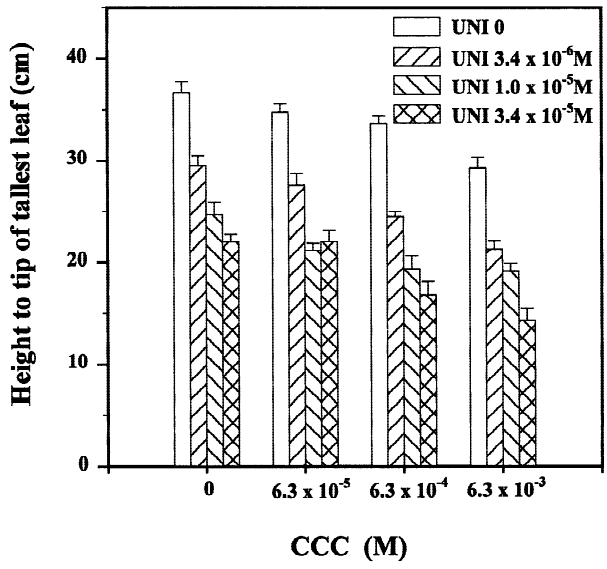

Fig. 2. Effect of combinations of CCC and uniconazol (Uni) on the height to the tip of the tallest leaf of 58M in the greenhouse at 14 days after seeding. Inhibitors were applied as a soil drench at 5 and 12 days after seeding. Replication is as in Fig. 1.

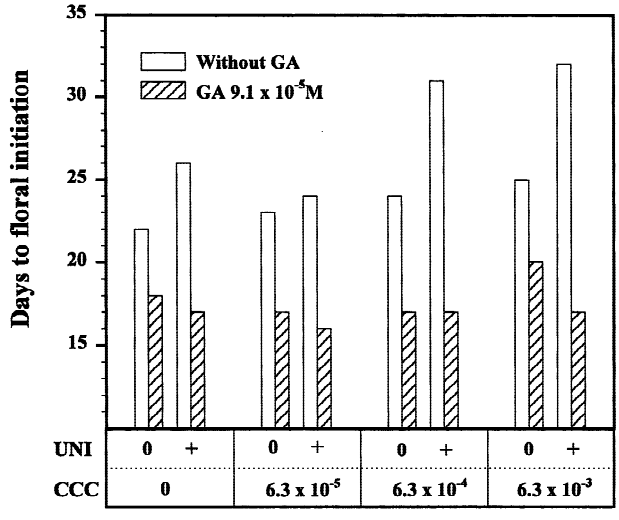

Fig. 3. Effect of CCC (concentrations shown in M) and uniconazol (Uni) at 0 or 3.4×10^{-5} M with or without GA_3 plus GA_1 on floral initiation of 58M. Inhibitors and GA_3 plus GA_1 were applied as a soil drench at 5 and 12 days after seeding. Data are means of three replications with five plants per replication.

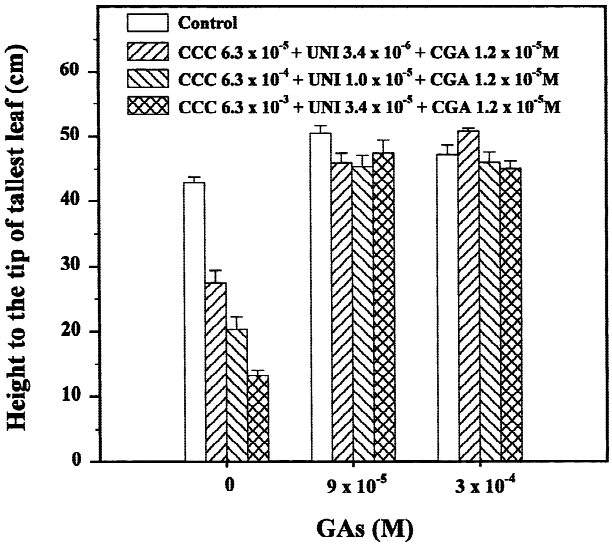

Fig. 4. Effect of combinations of three different types of GA biosynthesis inhibitors (CCC, uniconazol, and CGA) and with or without GA_3 plus GA_1 on the height to the tip of the tallest leaf of 58M in the growth chamber at 14 days after seeding. Inhibitors were applied as a soil drench at 5 and 12 days after seeding. Replication is as in Fig. 1.

appeared to be hastened slightly while the degree of growth inhibition was maximal (Fig. 5*b*, see also Fig. 1*b*).

Up to this point 58M and 90M had responded similarly to most of the treatments reported and to others omitted for the sake of brevity. The exceptions to this generalization were: (1) the early pathway inhibitors initiated tillering in 58M, but tillering occurred normally in untreated 90M; (2) the 3β-hydroxylation inhibitors (CGA and BX-112) did not promote tillering in 58M; (3) the 3β-hydroxylation inhibitors delayed flower initiation in 90M but did not do so in 58M. To determine whether these differences are caused by the absence of a phytochrome B effect on GA metabolism in 58M or by differences in the effects of early (CCC, uniconazol) vs late (CGA) inhibitors, GA biosynthesis inhibitors were applied to both 58M and 90M, and the content of endogenous GAs was measured. The pre-GA_{12} inhibitors (CCC and uniconazol) lowered the concentrations of all the early-C13α-hydroxylation pathway GAs, including GA_{12}, GA_{53}, and GA_{44}, as well as lowering concentrations of the GAs later in the pathway, GA_{19}, GA_{20}, GA_1 and GA_8 (Figs. 6 and 7) (ancymidol had similar effects; data not shown). Concentrations of 2×10^{-3} M CCC and 1×10^{-5} M uniconazol lowered GA_1 levels in 58M to around 20% of control levels. This reduction of GA_1 was consistent with the growth retardation caused by these compounds (Fig. 1*a*). There was no apparent difference in the response of 58M and 90M to these inhibitors.

CGA reduced GA_1 and GA_8 levels to amounts ranging from 70 to 10% of the control, but, in contrast to the early

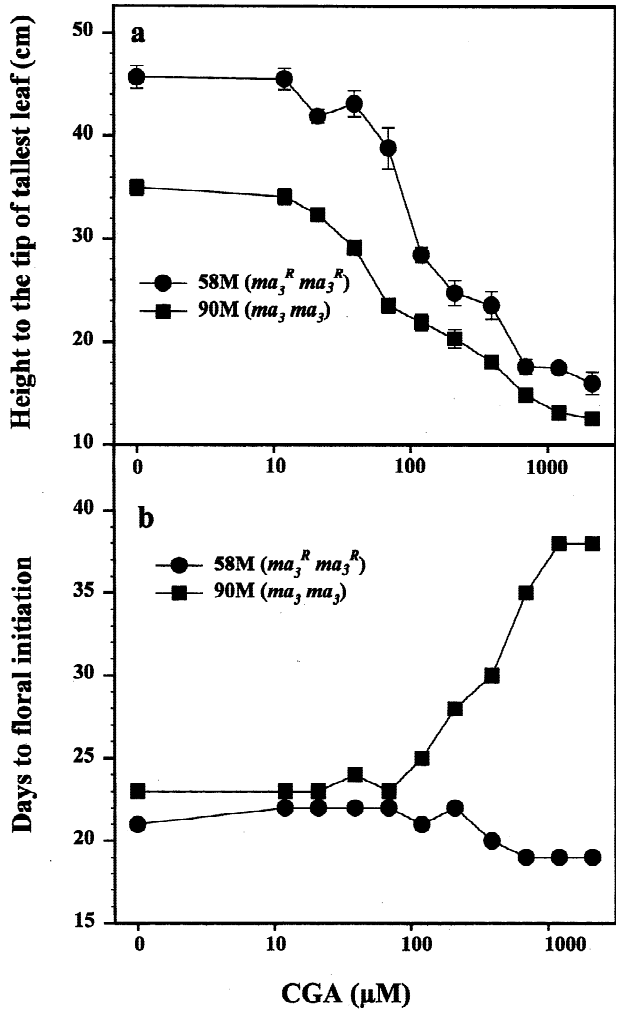

Fig. 5. Effect of CGA on growth (*a*) and floral initiation (*b*) in 58M and 90M in the growth chamber. CGA was applied as a soil drench at 5 and 12 days after seeding. Replication is as in Fig. 1.

pathway inhibitors, it often caused accumulation of intermediates from GA_{12} to GA_{20} (Fig. 8) (BX-112 effects were similar; data not shown). Some concentrations of CGA resulted in the accumulation of about twice as much GA_{20} in treated plants as was present in control plants for both 58M and 90M. In contrast to 58M, 90M showed no tendency to accumulate GA_{53} and GA_{19} in response to CGA treatment (Fig. 8). GA_{53} pool sizes showed a unique response to CGA treatment; in 58M GA_{53} increased progressively with increasing inhibitor concentration, but it decreased in 90M.

The effect of exogenous GA_3 plus GA_1 on the content of endogenous GAs was determined. The response of GA pool sizes was similar in 58M and 90M, with the main effect being an increase in GA_1 pool sizes (Fig. 9). The treatments with lower concentrations of GA_3 plus GA_1 increased GA_1 levels in plants by approximately the

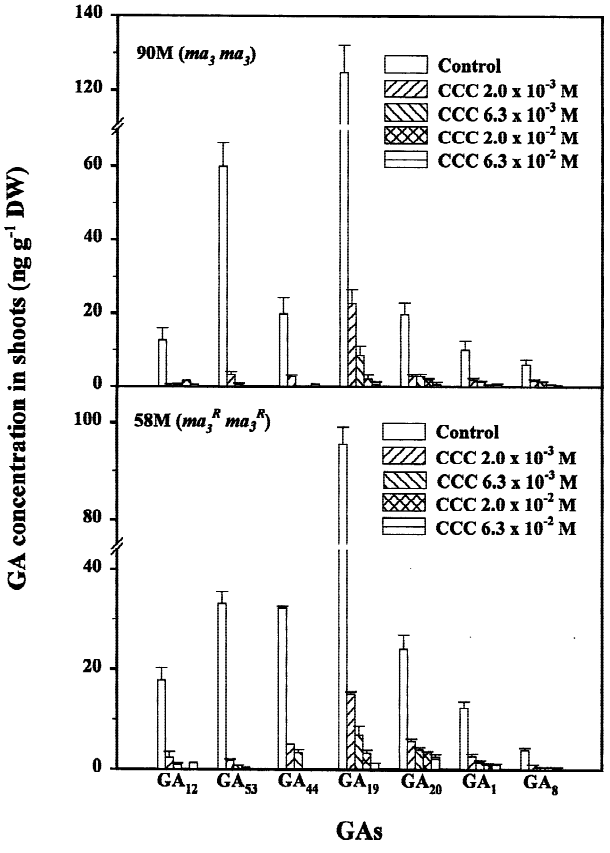

Fig. 6. Effect of CCC on native GA content in shoots of 90M (*top*) and 58M (*bottom*) at 14 days after seeding. CCC was applied as a soil drench at 5 and 12 days after seeding. GA levels were measured by GC-MS-SIM using deuterated internal standards. Data are the means of three replicate samples from two experiments. *Error bars* show standard deviations. DW, dry weight.

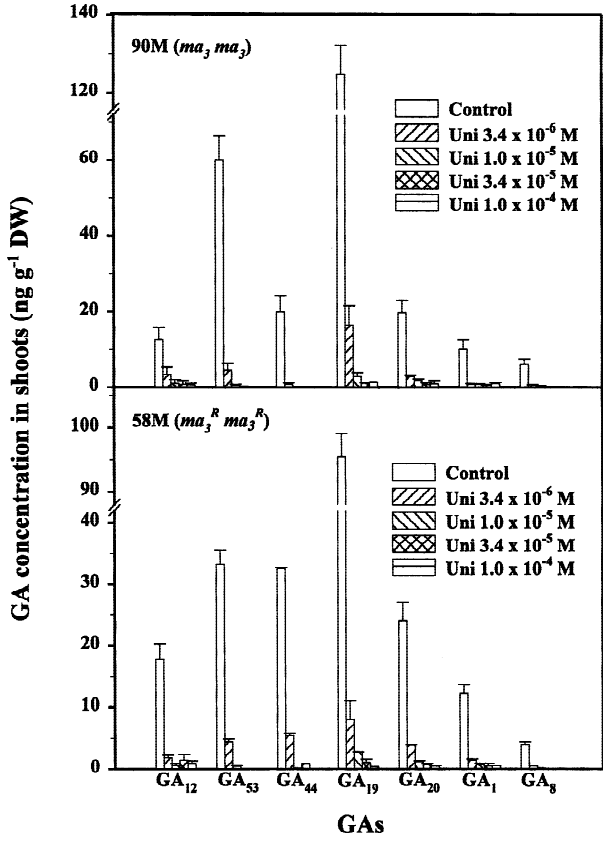

Fig. 7. Effect of uniconazol (Uni) on native GA content in shoots of 90M (*top*) and 58M (*bottom*) at 14 days after seeding. Uniconazol was applied as a soil drench at 5 and 12 days after seeding. GA levels were measured by GC-MS-SIM using deuterated internal standards. Replication is as in Fig. 6. DW, dry weight.

same proportion in both 58M and 90M. At 2.9×10^{-4} M applied GAs, GA_1 increased almost fourfold in 58M and more than ninefold in 90M; however, because the effects of GA treatment on growth and flowering are achieved at the lower concentrations, this apparent difference between 58M and 90M is probably not important. The increased GA_1 pool presumably resulted from the approximately 20% GA_1 present in the applied GAs. When compared across concentrations, the applied GA_3 plus GA_1 had little consistent effect on the concentrations of any other GA except GA_8. The exception is presumably because of the increased conversion of GA_1 to GA_8. These data indicate that there is no obvious feedback mechanism from GA_3 and GA_1 to earlier pathway GAs in 58M and 90M. Thus, applications of GAs apparently do not promote floral initiation by altering content of endogenous GAs (Morgan and Quinby 1987, Pao and Morgan 1986b, Williams and Morgan 1979).

Because application of GA biosynthesis inhibitors and GA_3 plus GA_1 restored growth and eliminated the delay of flowering caused by inhibitors alone (Figs. 3 and 4), such treatments should have lowered the endogenous GAs, and the applied GA_3 plus GA_1 should have been absorbed and used by the plant. An experiment was carried out to verify this supposition. GAs other than GA_3 and GA_1 were strongly reduced by the combination of pre-GA_{12} inhibitors and GA_3 plus GA_1 (Fig. 10), whereas GA_1 levels were over 12- to 15-fold higher. The treatment with CGA and GA_3 plus GA_1 did not reduce levels of the GAs before the GA_{20} to GA_1 step, but the levels of GA_1 and GA_3 were elevated in a fashion similar to the other treatments. CGA and GA_3 plus GA_1 showed extremely low levels of GA_8 compared with other treatments (Fig. 10); the levels of GA_1 available for conversion to GA_8 were high in this treatment as in the others. This is evidence that CGA blocks the 2β-hydroxylation of GA_1 to GA_8 in the early-C13α-hydroxylation pathway as observed in other plants (Adams et al. 1992, Adams and Weiler 1991, Nakayama et al. 1990a).

Although an original goal of this study was to find where the *phyB-1* allele (phytochrome B mutant gene)

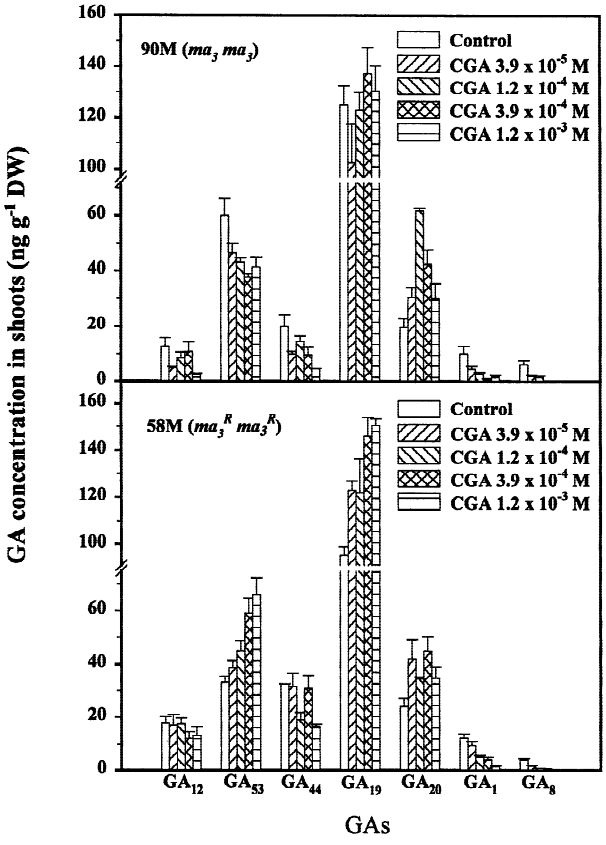

Fig. 8. Effect of CGA on native GA content in shoots of 90M (*top*) and 58M (*bottom*) at 14 days after seeding. CGA was applied as a soil drench at 5 and 12 days after seeding. GA levels were measured by GC-MS-SIM using deuterated internal standards. Replication is as in Fig. 6. DW, dry weight.

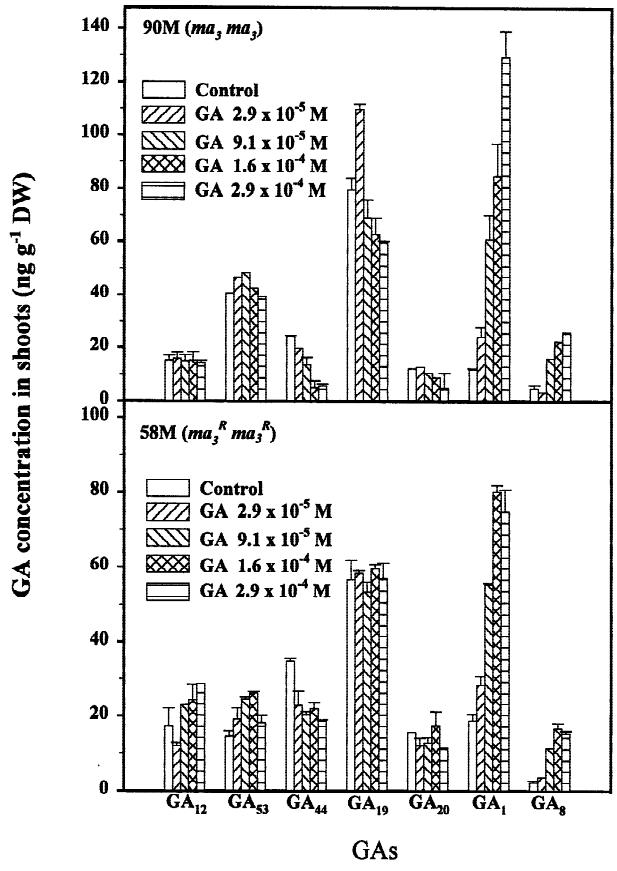

Fig. 9. Effect of GA_3 plus GA_1 on native GA content in shoots of 90M (*top*) and 58M (*bottom*) at 14 days after seeding. GA was applied as soil drench at 5 and 12 days after seeding. GA levels were measured by GC-MS-SIM using deuterated internal standards. Data are the means of three replicate samples. *Error bars* show standard deviations. DW, dry weight.

modifies GA metabolism, there was no strong indication in the data as to the step(s) it actually influences. GA biosynthesis inhibitors that block steps in three different parts of the pathway failed to demonstrate major differences in GA metabolism between the two cultivars. However, the three classes of inhibitors did not block every step of GA biosynthesis. Thus, one possible explanation for the absence of a response that identifies the site of the *phyB-1* allele effect is that it does not affect the biosynthetic steps blocked by the inhibitors used in this study.

The data in this study are remarkably consistent with the theory that GA_1 is the dominant GA for shoot growth in sorghum as well as many other plants (Ingram et al. 1986, Nakayama et al. 1992, Phinney 1984, Zeevaart et al. 1993). Inhibitors of both pre-GA_{12} and $GA_{20} \rightarrow GA_1$ biosynthetic steps inhibited growth (Figs. 1*a* and 5*a*) and markedly lowered GA_1 levels (Figs. 6–8). Combinations of inhibitors of different biosynthetic steps were even more effective than individual compounds (Figs. 2 and 4). Sorghum contained very small amounts of endogenous GA_3, if any, compared with GA_1 (data not given). In maize, GA_3 is present at only 1/20 to 1/50 the level of GA_1 (Fujioka et al. 1988, 1990). Application of GA_3 plus GA_1 elevated levels of those compounds in shoots (Fig. 10) and reversed the inhibition of shoot growth achieved by all of the inhibitors (Fig. 4). There were no substantial differences in the responses of phytochrome B-deficient 58M and phytochrome B-normal 90M to any of the treatments relative to growth and GA_1 levels. Thus the collected data indicate that GA_1 is the major or primary regulator of shoot growth in sorghum. This same conclusion was supported by other recent work in our laboratory in which ring-D modified GA_5 (exo and endo isomers of C16,17-dihydro GA_5 and C16,17-dichloromethano-dihydro GA_5) inhibited shoot growth and significantly reduced GA_1 levels (Foster et al. 1997).

How the data in this study relate to flowering is not clear. First, in both 58M and 90M the pre-GA_{12} inhibitors delayed floral initiation (Fig. 1*b*) and lowered GA_1

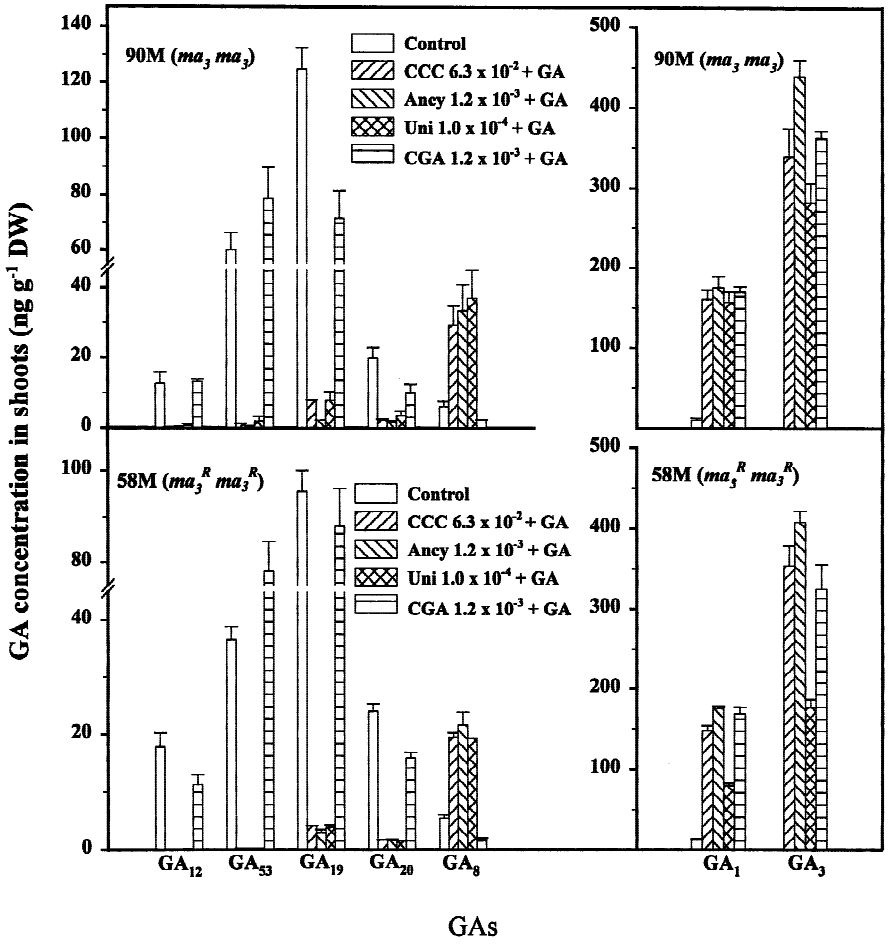

Fig. 10. Effect of combinations of GA biosynthesis inhibitors and GA_3 plus GA_1 on native GA content in shoots of 90M (*top*) and 58M (*bottom*) at 14 days after seeding. Inhibitors and GA were applied as soil drench at 5 and 12 days after seeding. GA levels were measured by GC-MS-SIM using deuterated internal standards. Replication is as in Fig. 9. DW, dry weight; Ancy, ancymidol; Uni, uniconazol.

levels (Figs. 6 and 7), whereas application of GA_3 plus GA_1 eliminated the inhibitor-mediated delays of floral initiation (Fig. 3). The $GA_{20} \rightarrow GA_1$ inhibitor CGA also delayed floral initiation and decreased GA_1 levels in 90M (Figs. 5*b* and 8), and treatment with GA_3 plus GA_1 reversed both effects (data not shown). These results suggest that GA_1 is necessary for floral initiation. However, the $GA_{20} \rightarrow GA_1$ inhibitors failed to delay floral initiation in 58M (Figs. 1*b* and 5*b*), although they did lower GA_1 levels markedly (Fig. 8). Effects on tillering in 58M appear parallel; pre-GA_{12} inhibitors promoted tillering, but the $GA_{20} \rightarrow GA_1$ inhibitors did not (see text above). Our growth room conditions were set to hasten floral initiation (11-h photoperiods), but even under those conditions there was an apparent tendency for the $GA_{20} \rightarrow GA_1$ inhibitors to hasten floral initiation in 58M slightly (Figs. 1*b* and 5*b*). Perhaps related to this is the observation that the exo and endo isomers of C16,17-dihydro-GA_5 hastened both floral initiation and floral development in 58M, but they strongly inhibited shoot growth, lowered GA_1 levels, and failed to promote tillering (Foster et al. 1997). Thus, in 58M there are several cases where $GA_{20} \rightarrow GA_1$ step inhibitors have produced effects inconsistent with a GA_1 role in flowering and tillering.

The structure of GAs necessary for florigenic activity in the LD grass species *Lolium temulentum* has been studied in detail, and GA_1 has low or neglible florigenic activity but very high shoot growth-promoting activity (Evans et al. 1990, 1994). This raises the possibility that if SD sorghum follows LD *Lolium*, GA_1 or at least high levels of GA_1 should inhibit floral initiation. This possibility plus the failure of the $GA_{20} \rightarrow GA_1$ inhibitors to delay floral initiation in 58M although severely inhibiting shoot growth and lowering GA_1 levels, suggests either that: (1) GAs have no significant role in floral initiation or that (2) a GA(s) other than GA_1 is (also) involved. Explanation (1) is unsatisfactory in three ways. First, it does not explain how GA_3 and GA_1 reverse the delay of flower initiation in both 58M and 90M by early pathway inhibitors (Fig. 3). Second, in two experiments CGA actually caused a small hastening of floral initiation at higher concentrations (Figs. 1*b* and 5*b*). Third, the noninvolvement idea gives no explanation for the failure of CGA to delay flowering in 58M. This explanation is also not favored by the demonstration in LD *Lolium* that some GAs are highly florigenic but have little shoot growth-promoting activity (Evans et al. 1990, 1994, Mander et al. 1995). Additionally, the requirement for

applied GA for flowering under SD in the LD *Arabidopsis* mutant defective in *ent*-kaurene synthesis also argues for a GA role in flowering (Okada and Shimura 1994, Wilson et al. 1992).

Hypothetically some aspect of GA metabolism between *ent*-kaurenoic acid and GA_{20} may influence floral initiation and tillering. Such a possibility is compatible with the data in this study for several reasons. First, the inhibitors used all had primary effects outside of the *ent*-kaurenoic acid to the GA_{20} segment of the biosynthetic pathway. Second, there are differences between effects of the pre-GA_{12} inhibitors and the $GA_{20} \rightarrow GA_1$ inhibitors, namely the general depletion of GAs between GA_{12} and GA_{20} with the former compounds (Figs. 6 and 7) and accumulation with the latter (Fig. 8). Third, there were some differences between 58M and 90M in the way the $GA_{20} \rightarrow GA_1$ inhibitor effected pool sizes of the GAs between GA_{12} and GA_{20}. Specifically, although GA_{20} accumulated in both cultivars, accumulation of GA_{53} and GA_{19} only occurred with CGA treatment in 58M (Fig. 8). In another study, 58M and 90M were compared, as well as the near isogeneic cultivar 100M, which is dominant for *PHYB-1* (Childs et al. 1997), and data from nine sampling times per day for 10-, 14-, 16-, and 18-h photoperiods were presented as averages (Lee et al. 1998). GA levels in 90M and 100M were remarkably consistent, and both cultivars contained higher levels of GA_{53} and GA_{19} than 58M but less GA_{20}. This suggests that the step from GA_{12} to GA_{53} is retarded in 58M compared with 90M and 100M, and the step from GA_{19} to GA_{20} is retarded in 90M and 100M compared with 58M. These findings are consistent with the data of Figs. 6–8. Although differences in GA pool sizes can have many explanations other than variations in synthetic enzyme activity, it is possible that the apparent differences in GA metabolism between *phyB-1* and the *PHYB* and *phyB-2* genotypes at the $GA_{12} \rightarrow GA_{53}$ or the $GA_{19} \rightarrow GA_{20}$ steps might be related to the failure of the $GA_{20} \rightarrow GA_1$ inhibitor to delay floral initiation in 58M (Fig. 5*b*) and to promote tillering in 58M.

If alteration of the metabolism of GAs between GA_{12} and GA_{20} in 58M causes it not to be delayed in floral initiation by $GA_{20} \rightarrow GA_1$ inhibitors, perhaps some alternate pathway GA is produced from GA_{53} or GA_{19}, and/or perhaps there is a change in GA sensitivity which is associated with the phytochrome B gene lesion in 58M. Examples support either possibility. For example, Pharis et al. (1987) found that exposure of *L. temulentum* to a single LD induced flowering and caused a transient increase in endogenous GA activity, especially an unidentified, putative polyhydroxylated GA. A similar compound would not have been detected in the present study. Also, Reed et al. (1996) found that a phytochrome B gene lesion in *Arabidopsis thaliana* altered responsiveness to GAs endogenous to that species.

One final disclaimer should be stated. It is well known that cytokinins, ABA, GAs, and sterols are synthesized all or in part in the terpenoid pathway. Recently an *Arabidopsis* mutant was recognized to be blocked in brassinosteriod biosynthesis, to exhibit altered floral induction, and to be rescued by application of brassinolide (Li et al. 1996). Also, two dwarf mutants of garden pea, shown to be deficient in brassinosteriods and growth, were rescued by brassinolide and its precursors (Nomura et al. 1997). Levels of all four groups of the above mentioned substances are known to be altered, especially in cell cultures, by various growth retardants/GA biosynthesis inhibitors, depending upon the concentration and species (Grossman 1988, Grossman et al. 1983, Hedden 1990, Izumi et al. 1988). Thus, the differences in effects of pre-GA_{12} inhibitors vs $GA_{20} \rightarrow GA_1$ inhibitors between 58M and 90M may be caused by complex interactions with levels of a number of plant growth and development regulators and completely beyond analysis by this study. We do not think this is the case because we were careful to work with several inhibitors affecting three different GA biosynthetic steps and to use a wide range of concentrations. The consistency of the results plus the fact that ring-D modified GAs produce similar effects on flowering, tillering, and GA_1 levels in 58M (Foster et al. 1997) as do the $GA_{20} \rightarrow GA_1$ inhibitors (this study) make us beleive that the data here relate primarily to GA levels rather than cytokinins, ABA, or brassinosteriods.

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