Plant Physiol. (1990) 94, 151–156
0032-0889/90/94/0151/06/$01.00/0

Received for publication February 21, 1990
Accepted May 17, 1990

Comparison of Biological Activities of Gibberellins and Gibberellin-Precursors Native to *Thlaspi arvense* L.[1]

James D. Metzger

U.S. Department of Agriculture, Agricultural Research Service, Biosciences Research Laboratory, State University Station, Fargo, North Dakota 58105

ABSTRACT

Field pennycress (*Thlaspi arvense* L.) is a winter annual weed with a cold requirement for stem elongation and flowering. The relative abilities of several native gibberellins (GAs) and GA-precursors to elicit stem growth were compared. Of the eight compounds tested, gibberellin A_1, (GA_1), GA_9, and GA_{20} caused stem growth in noninduced (no cold treatment) plants. No stem growth was observed in plants treated with *ent*-kaurene, *ent*-kaurenol, *ent*-kaurenoic acid, GA_{53}, or GA_8. Moreover, of the biologically active compounds, GA_9 was the most active followed closely by GA_1. In thermoinduced plants (4-week cold treatment at 6°C) that were continuously treated with 2-chlorocholine chloride to reduce endogenous GA production, GA_9 was the most biologically active compound. However, the three kaurenoid GA precursors also promoted stem growth in thermoinduced plants, and were almost as active as GA_{20}. No such increase in activity was observed for either GA_8 or GA_{53}. The results are discussed in relation to thermoinductive regulation of GA metabolism and its significance to the initiation of stem growth in field pennycress. It is proposed that thermoinduction results in increased conversion of *ent*-kaurenoic acid to GAs through the C-13 desoxy pathway and that GA_9 is the endogenous mediator of thermoinduced stem growth in field pennycress.

Work in this laboratory has been focused on plant responses to environmental stimuli, and specifically on determining the mechanisms by which plants perceive and transduce environmental signals that lead to alterations in growth and development. To study such processes, we have been using cold-induced stem elongation (bolting) in field pennycress (*Thlaspi arvense* L.) as a model system. This species is a cruciferous winter annual weed of the North American Great Plains with a cold requirement for the initiation of reproductive development (12, 14). Plants grown above 15°C remain as vegetative rosettes. However, subjecting plants for several weeks to temperatures in the range of 0 to 10°C initiates events characteristic of reproductive development: the initiation of microscopic flower primordia, followed by stem elongation, and culminating with the production of open flowers (14).

We have been concerned chiefly with the stem elongation response to low temperatures (12–14). Previous work showed that application of GAs[2] to noninduced field pennycress plants evokes stem growth in a manner that closely resembles thermoinductive cold treatments. In addition, thermoinduced stem growth was also shown to require continued GA biosynthesis (12). From a physiological standpoint, the rosette growth habit in field pennycress closely resembles that of many GA-deficient dwarfs. These observations led to the hypothesis that noninduced plants remain as rosettes because of an inability to accumulate a bioactive GA. In this scenario, thermoinduction results in an alteration in GA biosynthesis and metabolism causing the level of the bioactive GA to rise above a certain threshold and initiate stem growth (12).

Implicit in this hypothesis is the existence of a step(s) in the metabolic pathway leading to the bioactive GA that is under thermoinductive control. Thus, a logical experimental approach is to adapt the strategy used to identify blocks in GA metabolism in genetic dwarfs of maize (3, 17) and peas (7–9). These investigations included quantitative analysis, metabolism studies, and a comparison of biological activities of endogenous GAs and GA precursors when applied to plants. This last aspect is the focus of the present paper. The rationale for this line of experimentation is based on the assumption that application of a GA or GA-precursor to noninduced field pennycress plants will not result in bolting if the compound occurs before a thermoinductively regulated block(s) in the metabolic pathway. This situation results presumably because the applied compound cannot be converted to the bioactive GA responsible for initiating stem growth. However, a compound inactive for this reason in noninduced plants should evoke stem growth following thermoinduction. In this paper, a comparison of biological activities of various native GAs (16) and GA precursors (15) when applied to noninduced plants or thermoinduced plants depleted of endogenous GAs by treatment with an inhibitor of GA biosynthesis is reported. The results are used to formulate a hypothesis concerning the biochemical basis for thermoinductive regulation of stem growth in field pennycress.

MATERIALS AND METHODS

Plant Material

Seeds of the inbred line CR_1 of field pennycress (*Thlaspi arvense* L.) (12) were germinated in Petri dishes in the light at 21°C. After 1 week, seedlings were transferred to 10 cm plastic pots containing moist vermiculite. The plants were continuously subirrigated with one-fourth strength Hoagland solution (2) and grown at 21°C with an 8 h photoperiod of

[1] Supported in part by U.S. Department of Agriculture-Competitive Research Grants Office Grant No. 86-CRCR-1-1967.

[2] Abbreviations: $GA_{(x)}$, gibberellin $A_{(x)}$; CCC, 2-chlorocholine chloride; kaurene, *ent*-kaur-16-ene; kaurenoic acid, *ent*-kaur-16-ene-19-oic acid; kaurenol, *ent*-kaur-16-ene-19-ol.

light (300 μE m^{-2} s^{-1}, 400–700 nm) from fluorescent and incandescent bulbs, followed by 16 h of darkness.

After 6 weeks, half of the plants were transferred to a growth chamber at 6 to 8°C and an 8 photoperiod for 4 weeks. All of the plants were then moved to a growth chamber at 21°C with a daily photoperiod of 8 h of light as described above, followed by 16 h of low intensity illumination (20 μE m^2 s^{-1}, 400–700 nm) from incandescent lamps.

Chemical Treatments

Test compounds were dissolved or suspended in aqueous solutions containing 50% (v/v) acetone and 0.1% (v/v) Tween-20[3]. Ten μL of a solution containing, 0, 0.1, 1, or 10 μg of a test compound were applied to the shoot tips three times a week. To reduce endogenous GA levels in thermoinduced plants, 2 mM CCC was incorporated directly in the nutrient solution at the end of the cold treatment as described before (12). This treatment has consistently reduced endogenous GA levels by 90% as judged by d-5 maize bioassays (our unpublished data). Treatment with the test compounds began 1 week after the first watering with nutrient solution containing CCC. In contrast, test compounds were first applied to noninduced plants 1 week earlier or when they were the same chronological age as the thermoinduced plants were when the cold treatment ended.

Stem lengths were measured from the subtending leaf of the oldest measurably elongated internode to the last true leaf. Each treatment had five replicates and all experiments were repeated twice with similar results.

Sources of Chemicals

The structures of the applied test chemicals are shown in Figure 1. All of these compounds are endogenous to field pennycress (15, 16). Kaurenoic acid was obtained from sunflower florets as described previously (15). Kaurenol was synthesized from kaurenoic acid methyl ester by refluxing the latter with $LiAlH_4$ in tetrahydrofuran for 2 h (18). GA_1 was prepared via selective catalytic reduction of GA_3 methyl ester (10) followed by hydrolysis using lithium thiomethoxide in dry hexamethylphosphoramide (11). GA_9 was synthesized from GA_4 as described by Beale *et al.* (1). GA_{53} was prepared by feeding steviol (Sigma) to cultures of *Gibberella fujikuroi* (strain LM 45–399) in a medium with CCC to inhibit endogenous diterpene synthesis (4). GA_8 and GA_{20} were gifts from Dr. B. O. Phinney, UCLA, and kaurene was provided by Abbott Laboratories. All compounds were greater than 99% pure as judged by gas chromatography using a flame ionization detector.

RESULTS AND DISCUSSION

Biological Activities in Noninduced Plants

In a preliminary study, a 10 μg dose of each compound was applied to noninduced plants three times a week for 4 weeks.

[3] Mention of trademark or proprietary product does not constitute a guarantee or warranty of the product by the U.S. Department of Agriculture and does not imply its approval to the exclusion of other products that may also be suitable.

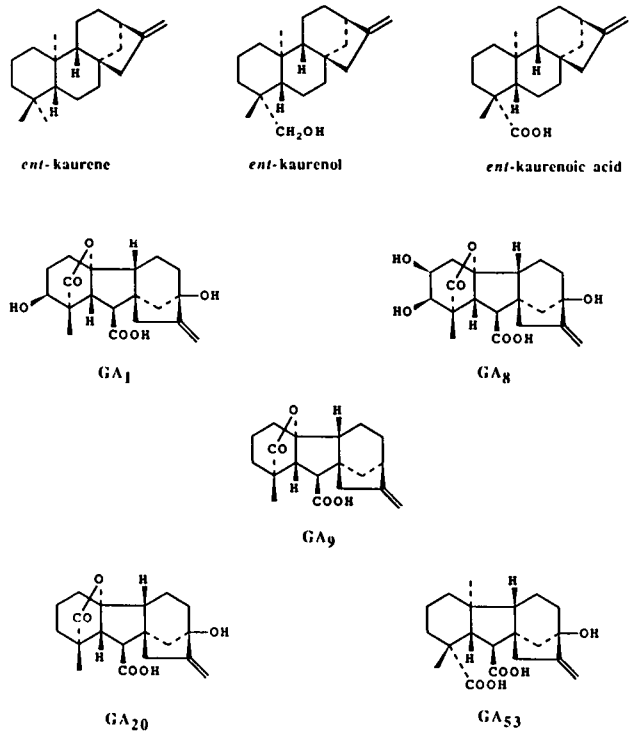

Figure 1. Chemical structures of the compounds used in the bioassay experiments on field pennycress. All of the test compounds occur naturally in field pennycress (15, 16).

Stem lengths were measured 5 weeks after the first application. No stem growth was observed in plants treated with the three kaurenoid precursors of GAs, nor with GA_{53} and GA_8 (Table I). Of the three biologically active compounds, GA_9 was the most active, followed closely by GA_1; GA_{20} was substantially less active than either GA_1 or GA_9 (Table I).

The relative biological activities of the three active GAs were compared in a dose-response experiment. Noninduced plants were treated with 0.1, 1.0, or 10.0 μg of GA_1, GA_9, or GA_{20} three times a week for 4 weeks. Stem lengths were measured weekly from 4 to 8 weeks after the first application (Fig. 2). The relative biological activities observed in the preliminary study (Table I) were confirmed in the dose-response experiment. The GA_9-treated plants had higher growth rates and greater final stem lengths than plants treated with either GA_1 or GA_{20}, although GA_1 was much more biologically active than GA_{20} (Fig. 2). Only plants treated with 10 μg of GA_9 attained final heights typical of plants receiving a 4-week thermoinductive cold treatment (12).

The flowering behavior of the noninduced plants treated with the three GAs was also observed. Open flowers appeared 4 weeks after the first application in noninduced plants treated with 10 μg of GA_1 and GA_9. No open flowers were observed in plants treated with 10 μg of GA_{20} until 8 weeks after the first application. None of the treatments with the lower concentrations of any of the three GAs resulted in the appearance of open flowers (data not shown).

Growth continued for several weeks after the last GA application, particularly for the higher concentrations of GA_1

Table I. *Comparative Effects of GAs and GA-Precursors on Stem Height of Thermo- and Noninduced Field Pennycress Plants*[a]

Compound	Mean Stem Growth[b]	
	Noninduced	Thermoinduced
	mm ± SD	
Control	0 ± 0 a	323 ± 15 a
Kaurene	0 ± 0 a	190 ± 12 b
Kaurenol	0 ± 0 a	220 ± 15 b
Kaurenoic acid	0 ± 0 a	215 ± 14 b
GA_{53}	0 ± 0 a	49 ± 10 c
GA_{20}	127 ± 12 b	229 ± 17 b
GA_1	300 ± 21 c	330 ± 18 d
GA_8	0 ± 0 a	36 ± 9 c
GA_9	381 ± 15 d	367 ± 19 d
CCC	—	34 ± 8 c

[a] Compounds (10 μg) were applied to the shoot tips of noninduced plants in 10 μL of an aqueous solution containing 50% and 0.1% (v/v) acetone and Tween-20, respectively, three times a week for 4 weeks. Stem heights were measured 5 weeks after the first application. For the thermoinduced plants, compounds were applied identically, except that the application period was for 3 weeks, and stem heights measured 4 weeks after the first application. [b] Means in the same column followed by a different letter are significantly different (P = 0.05) as determined by the Newman-Kuels multiple range test.

and GA_9. Growth rates gradually declined, but did not cease until 4 weeks after the last application. Therefore, in order to more accurately compare the dose-response relationships between the three GAs, the final heights measured 4 weeks after the last application were used. Regression lines fitted to the dose-response data for each GA are shown in Figure 3. A high positive correlation was found between final stem height and log of the applied dose for each GA (r = 0.98, 0.96, and 0.97 for GA_9, GA_1, and GA_{20}, respectively). In addition, statistical analysis (20) showed that all three regression lines were linear (P = 0.05).

Visually, regression lines for GA_1 and GA_{20} appear roughly parallel, *i.e.* have similar slopes, while the slope of the line for GA_9 is steeper (Fig. 3). This notion was confirmed through analysis of covariance followed by separation of statistically different (P = 0.05) slopes with the Newman-Keuls multiple range test (20). The slope of the regression line for GA_9 was significantly greater than the slopes of the lines fitted to the dose-response data for GA_1 and GA_{20}, while the slopes for the latter two were not statistically different.

Although the data in Table I and Figure 2 clearly show the relative biological activities of the applied compounds, a more quantitative comparison of the biological activities of the three GAs in noninduced plants was made by comparing the x-intercept of the respective regression lines. This value represents the theoretical minimum amount of a given GA necessary to elicit measurable stem growth. The calculated x-intercepts were 0.09, 0.11, and 0.93 μg for GA_9, GA_1, and GA_{20}, respectively. Thus, GA_9 was 18% more active than GA_1, and greater than an order of magnitude more active than GA_{20}.

Biological Activities in Thermoinduced Plants

The six compounds were also tested in thermoinduced plants in which endogenous GA levels were reduced by continuous treatment with CCC. In a preliminary experiment, thermoinduced plants were treated with 10 μg of each test compound three times a week for 3 weeks beginning 1 week after the end of the thermoinductive cold treatment. Stem lengths were measured 4 weeks after the first application. As in noninduced plants, GA_9 caused the greatest amount of stem growth, followed closely by GA_1 (Table I). In contrast to the response by noninduced plants, the three kaurenoid compounds evoked almost as much stem growth in thermoinduced plants as GA_{20}. No significant increase in stem growth over the CCC-treated controls was observed in the GA_{53}- or GA_8-treated plants (Table I). Only GA_9 and GA_1 were able to cause stem growth comparable to that observed in non-CCC-treated control plants (Table I).

The dose dependence of the response of thermoinduced plants to GA_1, GA_9, and GA_{20} was investigated. Thermoinduced plants, depleted of endogenous GAs, received treatments of 0.1, 1.0, or 10 μg of each GA three times a week for 3 weeks. Stem heights were measured 4 weeks after the first application. Regression analysis of the dose-response curves (Fig. 4) showed a highly positive linear (P = 0.05) relationship between stem growth and log amount of applied GA (r = 0.93, 0.94, and 0.96 for GA_1, GA_9, and GA_{20}, respectively). As was the case for noninduced plants, the slope of the regression line for GA_9 was significantly greater (P = 0.05) than both GA_1 and GA_{20} which had parallel regression lines.

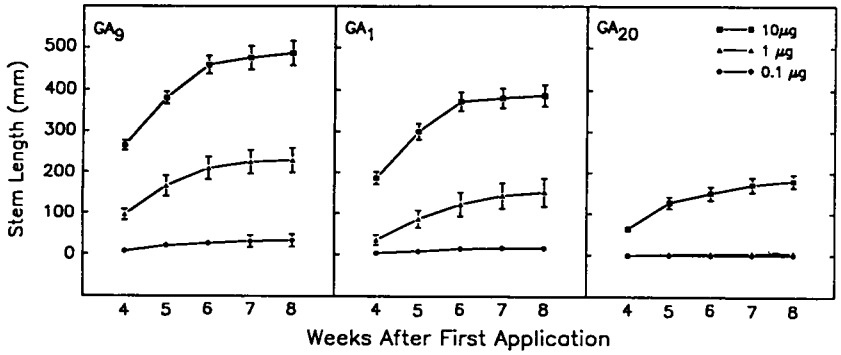

Figure 2. Effects of GA_1, GA_9, and GA_{20} on stem growth of noninduced plants. Ten μg of each GA was applied to the shoot tips three times a week for 4 weeks. Stem lengths were measured weekly from 4 to 8 weeks after the first application. Data points are the mean of five replicates. Vertical bars represent the standard deviations.

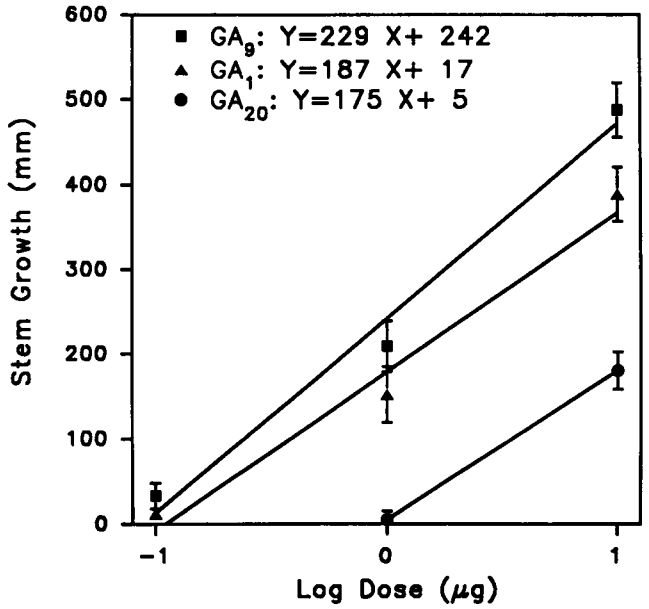

Figure 3. Regression lines and equations fitted to the dose-response data presented in Figure 2. Data points are the means of stem lengths measured 8 weeks after the first application. Vertical bars represent standard deviations.

Points of Thermoinductive Regulation in GA Biosynthesis and Metabolism

When the three kaurenoid precursors to GAs were applied to noninduced field pennycress plants, no stem growth was observed. However, thermoinduction resulted in the ability of plants to respond to these compounds to a level almost as great as that obtained from GA_{20} (Table I). One interpretation of these results is that noninduced plants cannot respond to the kaurenoids because they cannot metabolize the compounds to GAs. The kaurenoids elicited substantial stem growth in thermoinduced plants possibly by thermoinductive activation of specific biochemical steps responsible for their conversion to the GA responsible for biological action. Such regulation of GA metabolism may form the biochemical basis for thermoinduced stem growth in field pennycress.

The formation of GA_{12}, the first true GA in the biosynthetic pathway, involves the reaction sequence: kaurene → kaurenol → kaurenal → kaurenoic acid → 7β-OH-kaurenoic acid → GA_{12} aldehyde → GA_{12} (5). Although the bioassay data indicate that the conversion of kaurenoids to GAs is rate limiting in noninduced plants, it is impossible from these results alone to determine which of the step(s) from kaurene to GA_{12} are under thermoinductive control. However, the detection of kaurene, kaurenol, and kaurenoic acid in shoots of noninduced plants (J. D. Metzger, unpublished results), coupled with the observation that the endogenous content of kaurenoic acid in shoot tips is 40 times greater in noninduced plants than in thermoinduced plants (6) indicates that the biosynthesis of kaurenoic acid from kaurene is not limiting in noninduced plants. This suggests that thermoinduction activates one or more steps in the conversion of kaurenoic acid to GAs. In the accompanying paper, evidence from metabolism experiments is presented indicating that thermoinductive regulation of GA metabolism is probably limited to, at most, the two steps involved in the conversion of kaurenoic acid to GA_{12}-aldehyde (6).

Which GA is the Endogenous Bioregulator of Stem Growth?

Previously we reported the identification of 13 endogenous GAs in field pennycress (16). Based on hydroxylation patterns of the *ent*-gibberellin skeleton, two distinct families of GAs were shown to exist: an early C-13 hydroxylation pathway starting from GA_{53} and culminating in GA_1 and its C-2β hydroxylated inactivation product, GA_8, and the C-13 desoxy pathway leading to GA_9 and GA_{51} (C-2β OH GA_9). GA_{12} represents the branch point at which these two pathways diverge. In the vegetative tissues of species such as maize, peas, and *Silene armeria*, the early C-13 hydroxylation pathway predominates, and GA_1 is generally believed to be the main endogenous bioregulator of stem growth (9, 17, 19). It is therefore logical to suggest that GA_1 controls stem growth in field pennycress as well. However, members of the C-13 desoxy pathway compose a large proportion of the total endogenous GA content in this species (16). Thus, it is also possible that a member of this pathway such as GA_9 is responsible for regulating thermoinduced stem growth.

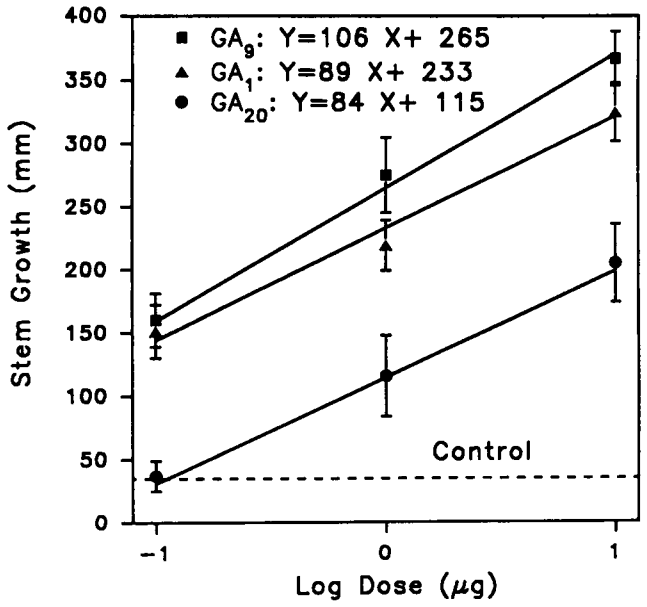

Figure 4. Effects of GA_1, GA_9, GA_{20} at three concentrations on stem growth of thermoinduced plants with reduced endogenous GA levels. Plants were continuously subirrigated with 2 mM CCC beginning at the end of a 4-week cold treatment. First application of each GA began 7 d after the end of the cold treatment and continued three times a week for 3 weeks. Stem lengths measured 4 weeks after the first GA application. Each line represents the regression curve fitted to the dose-response data obtained for each GA. The regression equation for each line is shown in the upper left hand corner. Data points and vertical bars represent the mean stem lengths and standard deviations, respectively for each GA concentration.

Indeed, support for this suggestion can be inferred from two observations. First, exogenous GA_9 was more active than GA_1 in eliciting stem growth in both thermo- and noninduced plants (Figs. 2 and 3). Second, the observation that GA_{53} was much less active in thermoinduced plants than the three kaurenoids (Table I) is more readily explained by the preferential metabolism of these compounds via the C-13 desoxy pathway to GA_9. The formation of GA_{53} represents the first committed step in the early C-13 hydroxylation pathway leading to GA_1 (5). If it were necessary for the kaurenoids to be metabolized to GA_1 for the expression of a biological response (stem growth), then GA_{53} would be a key intermediate in that conversion. If this were the case, then GA_{53} should be at least as active as the three kaurenoids.

It can be argued that GA_9 is biologically active only by virtue of its metabolism to GA_1. Although this possibility has not yet been tested directly through metabolism studies, the following indirect lines of evidence indicate that GA_9 has intrinsic biological activity.

GA_9 should not be more active than GA_1 if metabolism of the former to the latter is a prerequisite for the expression of biological activity. However, GA_9 was significantly more active than GA_1 in both thermo- and noninduced plants at all three concentrations (Table I).

In field pennycress a logical route of metabolism for the conversion of GA_9 to GA_1 would be via the sequence $GA_9 \rightarrow GA_{20} \rightarrow GA_1$. However, both GA_9 and GA_1 are considerably more active than GA_{20} (Figs. 2 and 3) suggesting that this scenario is unlikely. Another possibility is that GA_9 is converted through an initial hydroxylation at C-3 to form GA_4, which has never been detected in extracts of field pennycress shoots (16), followed by another hydroxylation at C-13. This also appears unlikely since exogenous GA_4 was less active in evoking stem growth in noninduced plants than either GA_1 or GA_9, although it was more active than GA_{20}. Stem heights (mm ± SD) of noninduced plants measured 8 weeks after the first application of 10 μg of each of these four GAs three times a week for 4 weeks were as follows: GA_9, 482 ± 17; GA_1, 418 ± 8; GA_4, 374 ± 11; GA_{20}, 128 ± 16.

Another line of evidence that GA_9 has intrinsic biological activity is based on analysis of the dose-response curves for the promotion of stem growth by GA_1, GA_9, and GA_{20} (Figs. 3 and 4). If it is necessary for a compound to be converted to GA_1 before a biological response is initiated, then, assuming penetration does not become limiting at higher concentrations, its dose-response curve should parallel the dose-response curve for GA_1, *i.e.* the two lines will have the same slope. For example, when various endogenous precursors to GA_1 were applied to GA-deficient mutants of maize in dose-response experiments, a series of parallel lines were generated when stem length was plotted as a function of log amount of applied compound (17). Likewise, in field pennycress the slopes of the dose-response curves for GA_1 and GA_{20} were not significantly different (Figs. 3 and 4) suggesting GA_{20} must be converted to GA_1 for the expression of biological activity. In contrast, the slope of the dose-response curve for GA_9 was statistically greater than for GA_1 (Figs. 3 and 4).

Biochemical Basis for the Thermoinductive Control of Stem Growth in Field Pennycress: A Hypothesis

The primary objective in performing the bioassay experiments presented in this paper was to gain enough information to form a basis for reasonable and testable hypotheses about the relationship between the regulation of GA metabolism and thermoinduced stem growth. From the data reported herein it is proposed that thermoinduction results in an increased ability to convert kaurenoic acid to C-13 desoxy GAs. As a result, the level of GA_9 rises above a certain threshold and initiates stem growth. These processes are most likely restricted to the apical portions of the stem since this is where thermoinductive temperatures are perceived (13). This hypothesis is presently being tested through *in vivo* metabolism studies and quantitative analyses of endogenous GAs and GA-precursors as a function of thermoinductive treatments. The subject of the accompanying paper (6) is the thermoinductive regulation of kaurenoic acid and GA_{12}-aldehyde metabolism.

ACKNOWLEDGMENTS

I wish to thank Amy Hassebrock and Marcia Mardaus for their help in the maintenance of the fungal cultures and the production of GA_{53}. I am also indebted to J. George Pomonis for expert technical advice in the chemical syntheses and Jan Hazebroek for the isolation of kaurenoic acid from sunflowers. The preparation of the figures by Brenda Jacobson is gratefully acknowledged.

LITERATURE CITED

1. **Beale MH, Gaskin P, Kirkwood PS, MacMillan J** (1980) Partial synthesis of gibberellin A_3 and [3α- and 3β-$^2H_1$] gibberellin A_9; gibberellin A_5 and [1β,3-$^2H_2$] gibberellin A_5; and gibberellin A_{20} and [1β,3-$^2H_2$] gibberellin A_5; and gibberellin A_{20} and [1β, 3α-$^2H_2$ and -$^3H_2$] gibberellin A_{20}. J Chem Soc Perkin Trans 1 **1980:** 885–891
2. **Blankendaal M, Hodgson RH, Davis DG, Hoerauf RA, Shimabukuro RH** (1972) Growing plants without soil for experimental use. USDA Misc Publ 1251
3. **Fujioka S, Yamane H, Spray CR, Gaskin P, MacMillan J, Phinney BO, Takahashi N** (1988) Qualitative and quantitative analyses of gibberellins in vegetative shoots of normal, *dwarf-1*, *dwarf-2*, *dwarf-3*, and *dwarf-5* seedlings of *Zea mays* L. Plant Physiol **88:** 1367–1372
4. **Gianfagna T, Zeevaart JAD, Lusk WJ** (1983) Synthesis of [^{2}H] gibberellins from steviol using the fungus *Gibberella fujikuroi*. Phytochemistry **22:** 427–430
5. **Graebe JE** (1987) Gibberellin biosynthesis and control. Annu Rev Plant Physiol **38:** 419–465
6. **Hazebroek JP, Metzger JD** (1990) Thermoinductive regulation of gibberellin metabolism in *Thlaspi arvense* L. I. Metabolism of [^{2}H]-*ent*-kaurenoic acid and [^{14}C]gibberellin A_{12}-aldehyde. Plant Physiol **94:** 157–165
7. **Ingram TJ, Reid JB, Potts WC, Murfet IC** (1983) Internode length in *Pisum*. IV. The effect of the *Le* gene on gibberellin metabolism. Plant Physiol **59:** 607–616
8. **Ingram TJ, Reid JB, Murfet IC, Gaskin P, Willis GL, MacMillan J** (1984) Internode length in *Pisum*. The *Le* gene controls the 3β-hydroxylation of gibberellin A_{20} to gibberellin A_1. Planta **160:** 455–463
9. **Ingram TJ, Reid JB, MacMillan J** (1985) The quantitative relationship between gibberellin A_1 and internode growth in *Pisum sativum* L. Planta **168:** 414–420

10. **Jones DF, McCloskey P** (1963) Selective reduction of gibberellic acid. J Appl Biochem **13:** 324–328
11. **Kelly TR, Dali HM, Tsang W-G** (1977) Lithium thiomethoxide: a convenient mercaptide reagent. Tetrahedron Lett **44:** 3859–3860
12. **Metzger JD** (1985) Role of gibberellins in the environmental control of stem growth in *Thlaspi arvense* L. Plant Physiol **78:** 8–13
13. **Metzger JD** (1988) Localization of the site of perception of thermoinductive temperatures in *Thlaspi arvense* L. Plant Physiol **88:** 424–428
14. **Metzger JD** (1989) *Thlaspi arvense* L. *In* A Halevy, ed, CRC Handbook of Flowering, Vol 4. CRC Press, Boca Raton, FL, pp 601–609
15. **Metzger JD, Hazebroek JP** (1989) Isolation of gibberellin precursors from heavily pigmented tissues. Plant Physiol **91:** 1488–1493
16. **Metzger JD, Mardaus MC** (1985) Identification of endogenous gibberellins in the winter annual weed *Thlaspi arvense* L. Plant Physiol **80:** 396–402
17. **Phinney BO, Spray C** (1982) Chemical genetics and the gibberellin pathway in *Zea mays* L. *In* PF Wareing, ed, Plant Growth Substances 1982. Academic Press, New York, pp 101–110
18. **Pyrek JST** (1970) New pentacyclic diterpene acid, trachyloban-19-oic acid from sunflower. Tetrahedron **26:** 5029–5032
19. **Talon M, Zeevaart JAD** (1990) Endogenous gibberellins and stem growth as related to photoperiod in *Silene armeria* L. Plant Physiol **92:** 1094–1100
20. **Zar JH** (1974) Biostatistical Analysis. Prentice-Hall, Englewood Cliffs, NJ, pp 1–599