Plant Physiol. (1980) 66, 679–683
0032-0889/80/66/0679/05/$00.50/0

Comparison of the Levels of Six Endogenous Gibberellins in Roots and Shoots of Spinach in Relation to Photoperiod[1]

Received for publication January 29, 1980 and in revised form May 27, 1980

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ABSTRACT

This communication describes the distribution of gibberellins (GAs) in roots and shoots of spinach in relation to photoperiod. From previous work (Metzger, Zeevaart 1980 Plant Physiol 65: 623–626) shoots were known to contain GA_{53}, GA_{44}, GA_{19}, GA_{17}, GA_{20}, and GA_{29}. We now show by combined gas chromatography—mass spectrometry that roots contain GA_{44}, GA_{19}, and GA_{29}. Trace amounts of GA_{53} were detected by combined gas chromatography—selected ion current monitoring. Neither GA_{17} nor GA_{20} were detected in root extracts. Analysis by the d-5 corn bioassay also showed no effect of photoperiodic treatment on the levels of GA-like substances in root extracts. Both phloem and xylem exudates had patterns of GA-like activity similar to those found in shoots and roots, respectively. Moreover, foliar application of $[^3H]GA_{20}$ resulted in the transport of label from the shoot to the roots. Over half of the label in the roots represented unmetabolized $[^3H]GA_{20}$, indicating that part of the GA_{20} in the phloem is transported to the roots. Consequently, if GA_{20} is made in, or transported to the roots, it is rapidly metabolized in that organ. This is a clear indication that regulation of GA metabolism is greatly different in roots and shoots.

Photoperiodic control of stem growth and petiole elongation in the LD[3] plant spinach is mediated by GAs (19, 20). Apparently, photoperiod exerts its control by regulating some, as yet unknown, aspect of the GA status of the plant. One aspect of a change in the GA status which appears to be important is the change in levels of the various GAs with LD treatment (19). This, coupled with the recent identification of six GAs in spinach shoots (12), has led to further investigations of photoperiodic regulation of GA levels in spinach.

A priori, the levels of individual GAs in a particular organ could be regulated in at least two ways. Most obvious would be a direct control of the organ's enzymes responsible for GA biosynthesis and metabolism. However, it is quite possible that transport of GAs or GA precursors and intermediates between organs could play a significant role in regulating developmental events. In fact, GAs are known to be present in the phloem and in the xylem and appear to be transported in these tissues over long distances (4, 8). Went (17) originally proposed that substances produced in the root exerted a hormonal control over shoot growth. Later, it was suggested by other investigators that these substances were, at least in part, GAs (14, 15). However, the conclusions reached by these authors are disputed by others (4). Thus, although GAs appear to be transported over long distances, the physiological importance of such movement remains unclear.

This report represents a preliminary study of the possible role of root-shoot interactions in GA-mediated growth responses in spinach. First of all, we have analyzed the GA content of roots and shoots of spinach in relation to photoperiod. Second, we report on the distribution between roots and shoots of the six GAs previously identified in spinach shoots. Finally, we examined the GA content of both phloem and xylem exudates as an indication of the movement of GAs between the shoot and the roots.

MATERIALS AND METHODS

Plant Culture and Photoperiodic Treatment. Spinach seeds (*Spinacia oleracea*, cv. Savoy Hybrid 612, Harris Seed Co., Rochester, N.Y.) were sown on Vermiculite. After 10 days, the seedlings were transferred to 37- × 30- × 22-cm trays outfitted with a cover to hold 20 seedlings. The trays were filled with half-strength Hoagland's solution which was continuously aerated. During the course of an experiment, the medium was changed once a week.

The plants were maintained under SD conditions until ready for experimentation, approximately 6 weeks after sowing. SD treatments consisted of an 8-h period of light from fluorescent and incandescent lamps followed by 16 h darkness. LD treatment consisted of the same 8 h illumination as in the SD treatment, followed by 16 h low intensity illumination from incandescent lamps (19).

At the end of an experiment, whole plants were harvested and divided into roots and shoots. Both parts were frozen in liquid N_2, lyophilized, and stored at −15 C prior to extraction.

Effect of Photoperiod on Levels of Extractable GA-like Substances from Roots and Shoots. The details of the extraction and purification procedures have been described (12). Methanolic extracts of roots or shoots were purified by charcoal adsorption chromatography and silicic acid adsorption chromatography. The eluate resulting from silicic acid adsorption chromatography was fractionated by preparative TLC. The chromatogram was divided into 10 equal zones and each zone was analyzed for the presence of GA-like substances by the d-5 corn bioassay.

Identification of GAs in Root Extracts. Methanolic extracts of the roots from 500 plants (about 100 g dry weight) were purified as described (12) and then fractionated via preparative reverse phase (Bondapak C_{18}/Porasil B) HPLC (6, 12). Fractions known to contain spinach shoot GAs in this system (12) were purified further by analytical reverse phase (μBondapak C_{18}) HPLC. Appropriate fractions resulting from analytical HPLC were methylated with ethereal diazomethane. The trimethylsilyl ethers of the methyl esters were prepared by adding 50 μl of a solution containing pyridine/hexamethyldisilazane/trimethylchlorosilazane (9:3:1, v/v) to dry samples in Reactivials (Pierce). The derivatized samples then were subjected to GLC-MS under the conditions

[1] This work was supported by the United States Department of Energy Contract EY-76-C-02-1338.

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[3] Abbreviations: LD, long day(s); HPLC, high-performance liquid chromatography; MeTMS-GA, methyl ester trimethylsilyl ether of gibberellin; SD, short day(s); GLC-SICM, combined gas liquid chromatography/selected ion current monitoring.

described (12).

GA Content of Phloem Exudate. Phloem exudate was collected from detached spinach leaves by the method of King and Zeevaart (9). Leaves from 100 plants were detached and placed in beakers containing a 20 mM solution of EDTA (pH 7.0). The beakers contained enough solution so that only the cut surfaces and a few mm of the petioles were exposed to the EDTA solution. After 2 h, the treated ends of the petioles were rinsed with distilled H_2O, and phloem exudate then was collected in double-distilled H_2O over the next 10 to 12 h. The phloem exudate was frozen, lyophilized, and the dry weight of the residue was determined. The residue was taken up in 50 ml 0.1 M phosphate buffer that had been adjusted to pH 2.5 with 6 N HCl and partitioned four times with equal volumes of ethyl acetate. The acidic ethyl acetate fraction was concentrated, subjected to preparative TLC, and the GA content was determined as described (12).

GA Content of Xylem Exudate. Spinach plants were individually grown hydroponically in 4-liter bottles that were wrapped in aluminum foil to keep the root systems in darkness. Plants were maintained under SD for 4 weeks and then were given 7 LD. The plants were decapitated and latex tubes were placed over the cut ends. Xylem exudate was collected via the latex tubes in flasks packed in ice over a 12-h period. The xylem exudate from 100 plants was pooled, adjusted to pH 2.5 with 6 N HCl, and partitioned four times with equal volumes of ethyl acetate. The GA content of the acidic ethyl acetate fraction was determined after fractionation by TLC as described (12).

Transport of [^{3}H]GA_{20}. Two plants were grown hydroponically under SD for 4 weeks as described earlier and then given 7 LD. Each plant received a foliar application of 4.0×10^5 dpm of [2,3-^{3}H]GA_{20} (original specific radioactivity, 3.3 Ci/mmol, diluted with cold GA_{20} to a specific radioactivity of 25 mCi/mmol) dissolved in an aqueous solution of 0.05% Tween 20 and 10% ethanol. After 24 h, the roots were harvested, frozen in liquid N_2, and lyophilized. The freeze-dried root systems were extracted, purified, and fractionated by TLC as described earlier. The chromatogram was divided into 10 zones and each zone eluted. The resulting eluates were dried on cellulose powder in Packard combustocones. Each of these then was combusted in a Packard model 306 Tri-Carb sample oxidizer for 45 s and then counted for 5 min in vials containing 15 ml Packard Monophase-40 and 2 ml Permafluor V in a Packard model 3255 Tri-Carb liquid scintillation spectrometer. The counting efficiency was determined and the data were converted to dpm.

RESULTS

Effect of Photoperiod on Levels of GAs in Shoots and Roots. The dry weight ratio of shoot to root material from hydroponically grown plants was approximately 5:1. Therefore, it was necessary to extract the roots from 80 plants as compared to shoots from 12 plants. Figure 1 shows the GA content of extracts from roots and shoots as affected by length of LD treatment. In agreement with previous work, extracts of shoots showed the presence of two GA-like substances which changed in level with LD treatment (19). The level of a polar GA (R_F, 0.1–0.3), which has been identified as GA_{19} (12), declined with LD treatment, whereas the level of GA_{20} (R_F, 0.5) increased during the same period. Roots, on the other hand, contained only one zone with GA-like activity and with chromatographic properties identical to that of the shoot GA_{19}. The level of this substance(s) remained constant with different durations of the LD treatment. The root extracts showed no GA-like activity in the zone where GA_{20} chromatographs, regardless of photoperiodic treatment. The shoots had a higher GA content than roots, whether expressed on a per unit weight basis (three times) or a per plant basis (20 times).

Identification of Root GAs. It was of interest to see which of the six GAs previously identified in spinach shoots (12) were present in root extracts. For this purpose, it was necessary to extract and purify roots from about 500 plants. Various fractions were derivatized and then analyzed by GLC-MS. Similar preparations from shoots of the same plants were used for analysis by GLC-MS. Full repetitive scans of various root fractions indicated the presence of three substances which had retention times identical to, and mass spectra similar to, those of three GAs previously found in shoots (12): GA_{44}, GA_{19}, and GA_{29} (Table I). Neither GA_{53}, GA_{17}, nor GA_{20} (Fig. 2), all of which were found in the shoot extracts, was detected in root extracts by repetitive scanning MS. However, the lower limit of detection by this technique was 100 ng GS_{20}/injection. Presumably, this figure would be similar for other GAs as well. Thus, it is possible that GA_{53}, GA_{17}, and GA_{20} were present in only minute quantities and, therefore, escaped detection by GLC-MS with repetitive scanning. A more sensitive, albeit less definitive detection technique was GLC-SICM which increased sensitivity 100 times to 1 ng GA_{20}/injection. Using this technique, a compound was detected with the same retention time and with percentages for four *m/e* values similar to those of MeTMS-GA_{53} (Table II). We conclude from these data that GA_{53} is present in trace amounts in the roots. No evidence was obtained by GLC-SICM for the presence of either GA_{20} or GA_{17} in root extracts.

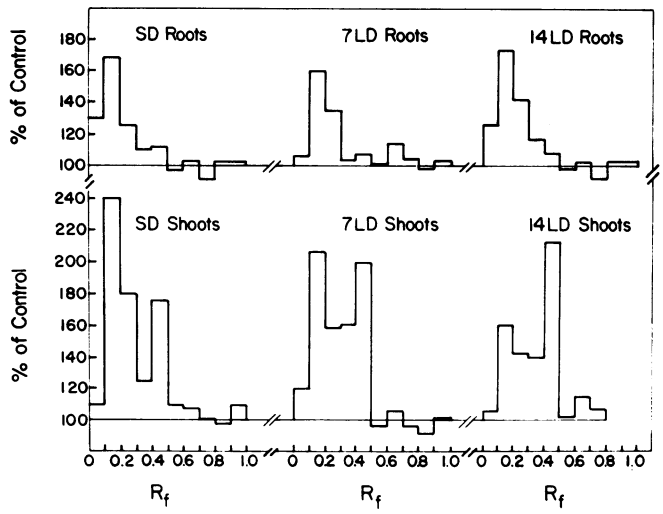

FIG. 1. The effect of photoperiod on GA-like activity in roots and shoots. Partially purified acidic extracts were fractionated by TLC and the resulting chromatograms were divided into 10 equal zones. Each zone was assayed for the presence of GA-like substances using the d-5 corn bioassay. Roots from 80 plants (20 g dry weight), shoots from 12 plants (15 g dry weight).

GA Content of Phloem Exudate. Figure 3 shows the GA content of phloem exudate from plants subjected to either SD or 10 LD. The pattern of GAs detected by bioassay was similar to that in extracts from leaves. The concentration of GA-like substances can be calculated, assuming that sugar constitutes about 15% (w/v) of the phloem contents and that sugar comprises almost all of the dry weight (13, 21). The volume of phloem exudate then can be calculated from the dry weight of the exudate (Table III). The total GA-like substances found in the phloem exudate can be determined by interpolation of bioassay data from a GA_3 standard curve. The total concentration of GA-like substances for both SD and 10 LD phloem exudate was quite similar: 86 and 81 $\times 10^{-4}$ μg ml^{-1} for SD and 10 LD phloem exudate, respectively (Table III). These numbers compare quite well with determinations made for concentrations of GA-like substances in the phloem exudate from other species, which ranged from 11 to 230 $\times 10^{-4}$ μg GA_3-eq ml^{-1} (3, 10).

Unfortunately, the logistics of obtaining sufficient exudate for analysis by GLC-MS precluded identification of the GAs present.

Table I. *GLC-MS Data Obtained with Samples from Spinach Shoots and Roots*

Sample	Time of Scan	Peaks in Mass Spectrum (*m/e* values)[a]						
	min							
MeTMS-GA_{19}								
Root	11.6	462(M^+,7)	447(4)	434(100)	402(30)	374(50)	208(39)	207(36)
Shoot	11.6	462(M^+,8)	447(4)	434(100)	402(27)	374(50)	208(47)	207(40)
MeTMS-GA_{44}								
Root	13.2	432(M^+,24)	373(12)	251(6)	238(38)	208(42)	207(100)	
Shoot	13.2	432(M^+,30)	373(17)	251(6)	238(42)	208(53)	207(100)	
MeTMS-GA_{29}								
Root	12.6	506(M^+,100)	491(18)	477(5)	447(15)	389(20)	208(20)	207(40)
Shoot	12.6	506(M^+,100)	491(12)	477(5)	447(9)	389(10)	208(12)	207(41)

[a] Relative abundances are given in parentheses.

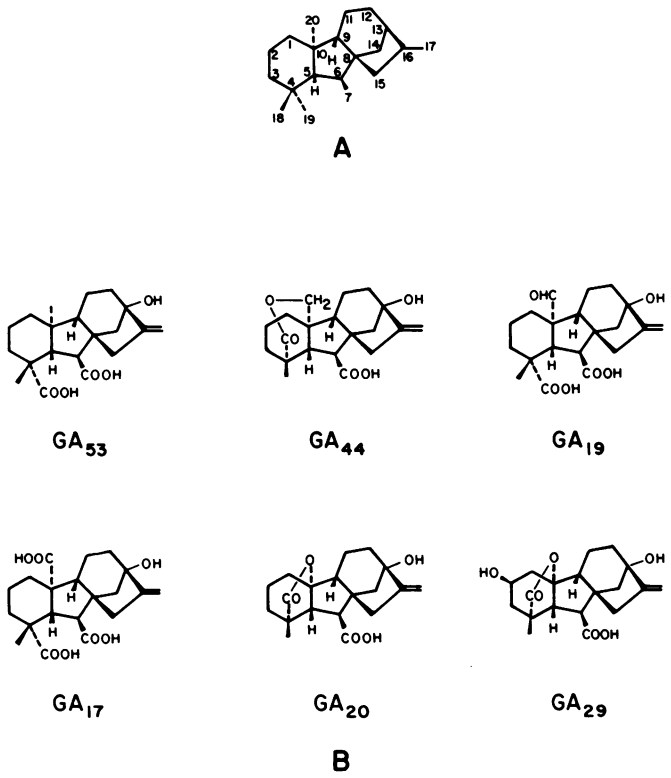

FIG. 2. Numbering system of the *ent*-gibberellane skeleton (A) and structures of the six GAs identified in spinach (B).

Table II. *Identification of GA_{53} in Spinach Roots*

Comparison of relative abundances of 4 ions obtained by GLC-MS of MeTMS-GA_{53} from shoot extracts with the same four ions obtained by GC-SICM of a MeTMS-derivatized extract from spinach roots. Retention time for all ions was 11.2 min.

Ion(*m/e*)	Shoot	Root
207 (base peak)	100	100
448 (M^+)	31	25
416 (M^+-32)	23	16
389 (M^+-59)	44	31

However, the close similarity of the GA patterns found in phloem exudate and in leaf extracts indicates that GA_{19} and GA_{20} are the major GAs present in the exudate.

GA Content of Xylem Exudate. Figure 4 shows the GA content of 1 liter xylem exudate collected from 100 plants treated with 7 LD. It also shows, for comparison, the GA content of a shoot extract derived from the plants used for obtaining xylem exudate.

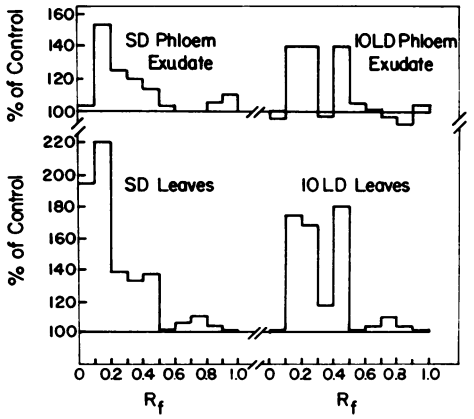

FIG. 3. The effect of photoperiod on the GA-like activity in phloem exudate and leaves. Acidic extracts were fractionated by TLC and the resulting chromatogram analyzed for the presence of GA-like substances using the d-5 corn bioassay. Phloem exudate derived from the leaves of 100 plants; leaf extracts were from 10 plants.

Table III. *Comparison of GA Concentration in Phloem and Xylem Exudates from Spinach Plants under SD or LD Conditions, as Determined by d-5 Corn Bioassay*

Photoperiodic Treatment	Exudate	Dry Wt	Volume	GA Content	GA Concentration
		mg	*ml*	*μg GA_3-eq*	*μg GA_3-eq ml^{-1}*
SD	Phloem	552	3.7[a]	3.2×10^{-2}	86×10^{-4}
10 LD	Phloem	882	5.9[a]	4.8×10^{-2}	81×10^{-4}
7 LD	Xylem		1,000	10.0×10^{-2}	10^{-4}

[a] Calculated on the assumption that phloem sap contains 15% (w/v) solute.

The pattern of GA-like substances was similar for both the xylem exudate and root extracts (compare Figs. 1 and 4) in that both lack a zone of biological activity associated with the region where GA_{20} chromatographs in the TLC system used in this study. Not enough xylem exudate was available to identify the GA-like substances by GLC-MS. It is likely that at least part of the biological activity in spinach xylem exudate is due to the presence of GA_{19} since GA_{19} is present in the roots and the chromatographic behavior of the GA-like substance(s) found in the xylem exudate was similar to that of GA_{19}. The total GA content in the xylem exudate was calculated to be 0.1 μg GA_3-eq in 1 liter xylem exudate. This is in the same range as found in other species (8). Clearly, the concentration of GA-like substances is much higher in phloem exudate than in xylem exudate (Table III).

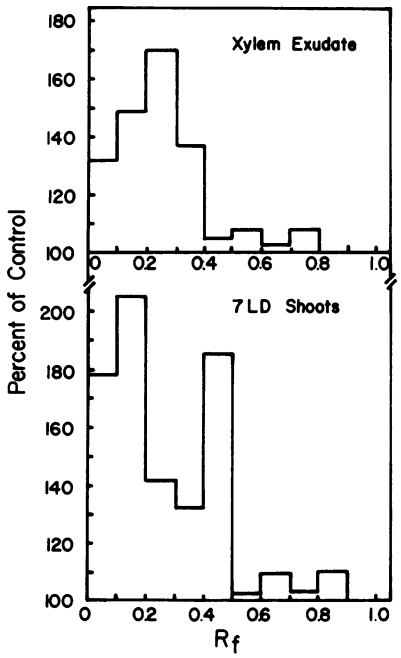

FIG. 4. GA-like activity in xylem exudate and shoots from plants that were used for obtaining the xylem exudate. Xylem exudate from 100 plants; shoots were from 10. All plants exposed to 7 LD prior to use. Extracts were fractionated by TLC and analyzed by the d-5 corn bioassay.

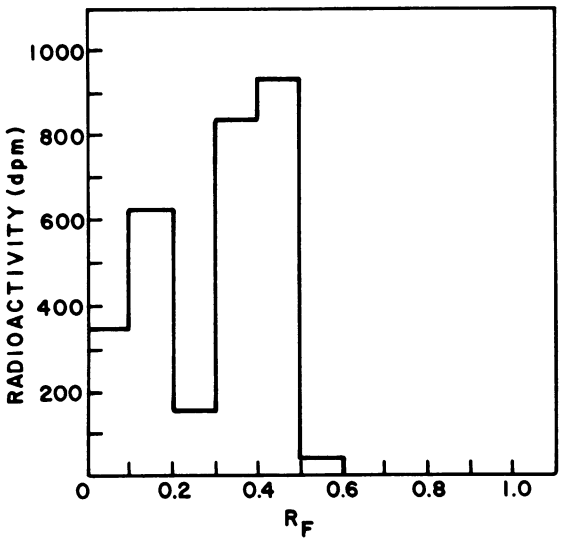

FIG. 5. Distribution of radioactivity on a chromatogram of a root extract from two plants 24 h following a foliar application of 4×10^5 dpm [2,3-^{3}H]GA_{20} to each plant.

Transport of [^{3}H]GA_{20} from the Shoot to the Roots. Figure 5 shows the distribution of radioactivity on a chromatogram of a root extract 24 h following foliar application of [^{3}H]GA_{20}. Two distinct zones of radioactivity are apparent: a polar zone at R_F = 0.1 to 0.2 and one which co-chromatographs with authentic GA_{20} (R_F, 0.4–0.5) and probably represents unmetabolized [^{3}H]GA_{20}. Although the total radioactivity found in the roots was only 0.35% of the total [^{3}H]GA_{20} applied to the plant, this result demonstrates that exogenous GA_{20} does move from the shoot to the root. This supports the idea that at least part of the GA_{20} present in the phloem moves to the root system.

DISCUSSION

Distribution of GAs between Roots and Shoots. Spinach shoots were previously shown to contain GA_{53}, GA_{44}, GA_{19}, GA_{17}, GA_{20}, and GA_{29} (12). In this investigation, only four of the six GAs found in spinach shoots were detected in extracts of roots by GLC-MS or GLC-SICM: GA_{53}, GA_{44}, GA_{19}, and GA_{29} (Fig. 2). Neither GA_{17} nor GA_{20} was detected in root extracts. The physiological significance of the striking qualitative difference in the spectrum of GAs found in shoots and roots is unclear. Because GA_{20} is present in the phloem exudate and [^{3}H]GA_{20} applied to the leaves moves to the roots, it seems that GA_{20} is continuously translocated from the shoot to the roots. This suggests that, if GA_{20} is made in, or is transported to, the roots, it is rapidly metabolized in that organ. Thus, it seems that regulation of GA metabolism is very different in roots and shoots.

The very presence of [^{3}H]GA_{20} in root extracts (Fig. 5) seems to contradict the idea of rapid GA_{20} metabolism in the roots. This paradox can be explained in the following manner. The total radioactivity associated with the region of the chromatogram where GA_{20} ran was 1872 dpm. Since the specific radioactivity of the applied [^{3}H]GA_{20} was 25 mCi/mmol, the radioactivity present as [^{3}H]GA_{20} in the root extracts was equivalent to 11.3 ng GA_{20}, or 5.65 ng GA_{20}/plant. However, there was only about 0.15 ng GA_3-eq/plant associated with the GA_{20} region of the chromatogram from the LD phloem exudate. Thus, the applied [^{3}H]GA_{20} contributed 35 times more substrate to the roots than is normally transported via the phloem. This could mean that the enzymes in the roots responsible for metabolizing [^{3}H]GA_{20} were saturated.

Possible Sites of GA Synthesis. The pattern of GA-like substances detected by bioassay in xylem and phloem exudate was remarkably similar to that found in roots and shoots, respectively (Figs. 3 and 4). In general, such data cannot normally be used to ascertain the sites of GA synthesis (4). But since GA_{20} was not detected in either xylem exudate or root extracts, the GA_{20} detected in shoot extracts and phloem exudate must have been synthesized in the shoots. Previously, we have suggested that in the shoots GA_{19} is converted to GA_{20} and that this conversion is under photoperiodic control (12).

GA_{19} was detected in both root and shoot extracts and is probably present in xylem and phloem exudate as well. It is possible that either roots or shoots (or both) have the capacity to synthesize GA_{19}. Since LD treatment causes a decline in the level of GA_{19} in the phloem exudate, one would expect a concomitant decrease in the level of GA_{19} in extracts of roots, if the shoots are the major source of GA_{19} found in the roots. However, no such decline in levels of GA_{19} in root extracts was observed (Fig. 1). The maintenance of a constant level of GA_{19} can be interpreted to mean that the root system is a major source of GA_{19}, with contributions from the shoot via the phloem as a minor addition to the total GA_{19} content in the roots. However, other interpretations are also possible since the collection technique used for phloem exudate may not give an accurate picture of the flux of substances moving in the phloem from the shoot to the roots (8, 13). Perhaps there is a compensatory increase in the rate of movement of substances in the phloem when plants are transferred from SD to LD that accounts for the steady levels of GA_{19} found in root extracts. Indeed, different light intensities are known to affect the translocation rate of assimilates in the phloem (5).

Butcher (1) has shown that a clone of excised tomato roots maintained in culture for 5 years contained GA-like substances, indicating that roots are able to synthesize GAs. Excised spinach roots cultured for 2 weeks, as well as the medium in which they were grown, contained a GA-like substance with chromatographic properties identical to those of GA_{19} (unpublished results). This is consistent with the notion that GA_{19} is synthesized in the roots. However, the possibility cannot be ruled out that carry-over from the germinating seed was responsible for GAs present in the

cultured spinach root tips.

Physiological Significance of GA Transport in Phloem. Knott (11), as well as Withrow *et al.* (18), found that stem elongation in spinach occurred when the leaves were exposed to LD while the bud remained under SD. When only the bud was subjected to LD and the leaves exposed to SD conditions, stem elongation did not occur. This led Knott (11) to hypothesize that a substance was produced in the leaves and transported to the growing point, causing stem growth. Since stem growth is correlated with an increase in the level of GA_{20} in shoot extracts (12, 19) and phloem exudate (Fig. 3), it is suggested that GA_{20} is the substance which is synthesized in the leaves and transported via the phloem to the tip where it exerts control over stem growth.

Physiological Significance of GA Transport in Xylem. Skene (16) has calculated that there was enough GA in the xylem exudate of grapevines to account for all of the GA content in the leaves of the plant simply by transport in the transpiration stream from the roots to the leaves during a 24-h period. Since GA_{19} is apparently present in the xylem exudate of spinach, it is tempting to suggest that the roots supply the shoot with GA_{19}. Assuming a transpiration rate of 100 ml day^{-1}, approximately 0.01 μg GA_3-eq will pass from the roots to the shoot each day (Table III). The SD shoots in Figure 1 contained about 0.04 μg GA_3-eq/plant in the GA_{19} region of the chromatogram. It seems that transport of GAs from the roots to the shoot can contribute a significant portion of the GA present in the shoot. Obviously, since the site(s) of GA_{19} synthesis is (are) not definitively known, the possibility also exists that GA_{19} is synthesized in the shoot, transported to the roots via the phloem, and then recycled back to the shoot in the xylem.

It is generally assumed that both root and shoot tips are sites of GA synthesis, and many workers feel that GAs are produced near the sites of GA utilization or action (4). It has also been suggested that the roots and the shoot are responsible for different steps of the total GA biosynthetic and metabolic pathway (2, 7). Although the data presented here are consistent with the idea of integration of GA biosynthesis and metabolism in the plant as a whole, they do not provide proof. For a more definitive resolution of these questions, *in vitro* systems must be developed to study the regulation and location of the enzymes responsible for GA biosynthesis and conversion.

Acknowledgments—We wish to thank Dr. Richard Pharis, Department of Biology, University of Calgary, Alberta, Canada, for the generous gift of [2,3-^{3}H]GA_{20} as well as the staff of the Mass Spectrometry Facility of Michigan State University for aiding us in the use of the mass spectrometer.

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