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The Role of Free Indole-3-acetic Acid (IAA) Levels, IAA Transport, and Sucrose Transport in the High Temperature Inhibition of Primary Root Development in Pea (*Pisum sativum* L. cv. Alaska)

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Abstract

Growth of "Alaska" pea (*Pisum sativum* L.) at high temperature results in general inhibition of the root system. As part of a study on the effects of temperature on developmental timing, we tested the possibility that reductions in free indole-3-acetic acid (IAA) levels, IAA transport, or sucrose transport might be responsible for inhibition of primary roots. Seedlings were grown at 25 and 32°C. For transport studies, pea cotyledons were inoculated with ^{3}H-sucrose and ^{14}C-IAA. Free endogenous IAA in tips of pea primary roots was determined at three stages of development by gas chromatography–mass spectrometry (GC-MS) using a ^{13}C-IAA internal standard. Differences in mean free IAA concentration between temperature treatments were not significant during the early stages of development (28 to 58 ng/g FW). IAA concentration increased significantly in root tips during the late stage at both temperatures (108–165 ng/g FW) when elongation was declining. Differences in sucrose and IAA transport rates between temperatures were minor. Differences at each temperature over time were consistent with stage-specific growth patterns. Neither transport failure nor differences in free IAA level appear responsible for developmental inhibition of pea primary roots. Results suggest that pea primary root inhibition at high temperature is not due to heat-induced stress on transport systems but rather to shifting of developmental timing.

Key words: IAA; Pea root inhibition; *Pisum sativum L. cv. Alaska*; Sucrose; Temperature; Transport

Indole-3-acetic acid (IAA) in its free acid form has been frequently implicated as a phytohormone that regulates the expansion of cells in the elongation zone of primary meristems of leaves, stems, and roots (overview in Taiz and Zeiger 1991). It follows that the concentration of free IAA within growing tissues may positively correlate with the elongation rate of an organ such as the primary root. A previous

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study of endogenous IAA in pea primary roots supported this hypothesis (Pengelly and Torrey 1982). Dramatic red light–induced growth reduction in *Zea* mesocotyl was shown to correlate temporally with a relatively small (about twofold) decrease in endogenous IAA (Barker-Bridgers and others 1998, Jones and others 1991), which shows that large changes in hormone levels may not be required to cause significant effects in changes in growth. Because of some contradictory evidence and the ongoing controversy over the role of hormone sensitivity, the case for this model has remained unclear (Pilet 1991, Trewavas 1991). Changes in hormone sensitivity or concentration, interactions with other growth regulators, and interactions with the environment must be evaluated to fully understand phytohormone regulation of development.

IAA has also long been implicated in the regulation of lateral root development (Blakely and others 1988, Goodwin and Morris 1979, Hinchee and Rost 1986, Hurren and others 1988, Muday and Haworth 1994, Reed and others 1998, Thimann 1936, Torrey 1950). It has also been shown that early lateral root development in pea will be irreversibly interrupted along a primary root region by a temporary change from 25°C to a period at 32°C. Chronic exposure to 32°C will interrupt early lateral root development quasi-reversibly along the entire primary root (Gladish and Rost 1993). Given this information, it seems reasonable that some aspect of auxin metabolism might be involved in this inhibition.

In addition, temperature and other environmental factors have a role in influencing patterns of development. This has been shown in studies of root development that used temperature (Gladish and Rost 1993, Pardales and others 1991, Pritchard and others 1991), nutrient availability (Adalsteinsson and Jensen 1990, Granato and Raper 1989), mechanical impedance (Bengough and Mullins 1990, Dawkins and others 1983), and salt-stress (Reinhardt and Rost 1995, Snapp and Shennan 1992) as tools to study development.

Alaska pea was selected for this study because it has a very large cotyledonary storage capacity that permits seedling development in darkness for long periods of time (Rost and Toriyama 1991, Sutcliffe 1977a), and it has a determinate primary root with distinct elongation kinetics that have been well characterized (Gladish and Rost 1993). On the basis of these previous findings we have arbitrarily defined three stages of primary root development. These stages have specific elongation rate characteristics as follows: after penetration of the testa, the primary root tip accelerates continuously (early stage) to a rate plateau (medial stage) that begins approximately at one third the root's final length, then growth slows exponentially (late stage) until its determinate length is reached. It has also been shown that this pattern is mediated by environmental temperature (15°–32°C) such that developmental activities are kinetically and temporally attenuated at high temperatures, leading to a shorter final length than would occur at cooler temperatures (Gladish and Rost 1993). This might simply be a manifestation of biologic stress leading to a "biologic strain" as described by Levitt (1980). However, many aspects of the development of Alaska pea primary and lateral roots show continuous mathematical relationships when temperatures ranging from 15 to 32°C are used to modulate them, suggesting that they are under coordinated regulation throughout this temperature range (Gladish and Rost 1993), perhaps by variation of IAA concentration. Furthermore, sustainable respiration (O_2 absorption) of pea plants has been demonstrated up to 35°C, the temperature at which the sustainable rate was greatest (Sutcliffe 1977b). Rather than being the result of stress per se, this time-shifting of developmental stages and stunting of the root system at high temperatures may have a closer kinship to the "slipping time frames" of heterochronic developmental mutants (Freeling and others 1992) or the "elastic developmental time scales" described by Bradford and Trewavas (1994).

At 25°C lateral roots emerge in an acropetal sequence, creating a morphologically "normal" root system. At 32°C the primary root usually develops rather vigorously for about 4 d as it follows the typical determinate kinetic pattern, but few lateral root primordia form, and emergence of lateral roots is almost totally inhibited at the same time, resulting in profound inhibition of the root system (Gladish and Rost 1993). A reduction in IAA level could be responsible (Reed and others 1998), or nutrient availability could be differentially limited at the higher temperature by some aspect of nutrient transport or assimilation (Smith and McCully 1977).

As part of an ongoing study on the influences of temperature on developmental timing, we thought it important the possibility that other factors besides timing mechanisms, such as heat stress–induced inhibition of transport of IAA or nutrients or alterations of the availability of free IAA, be investigated. Therefore, the goals of this study were fivefold: (1) to determine endogenous concentrations of free IAA in primary root tips of pea (*Pisum sativum* L. cv. Alaska) at different stages of development under standard conditions and to ascertain whether free IAA concentrations at different stages of development correlate with corresponding elongation kinet-

ics of the roots; (2) to determine whether a higher temperature causes differences in free IAA concentration as primary roots develop; (3) to ascertain what effect high temperature has on IAA export from the cotyledons and subsequent distribution within the seedling axis; (4) to determine whether high temperature inhibition of pea primary roots can be reversed by exogenous application of auxin or nutrients; and (5) to determine the effect of elevated temperature on phloem transport of sucrose and meristem sink strength.

We considered two contradictory hypotheses, one in which the reduction in root growth was attributed to high temperature–induced stress and one in which temperature was thought to affect the timing of development, and this would be reflected in changes in IAA levels over time.

Alternative 1: We hypothesized that IAA concentration in pea primary root tips would be low and correlate with the growth rates of the organs during the early stages of development. We thought that high temperature might cause IAA levels to be reduced in proportion with the associated reduction in growth rate. Consistent with this, we predicted significant interference with the mobilization of sucrose and IAA from the cotyledons into the developing regions of 32°C–grown roots by comparison to roots grown at 25°C. Furthermore, if high temperature conditions reduce IAA or sucrose transport, exogenous application of these in the root media should reverse the high-temperature suppression.

Alternative 2: Because previous research has shown that high levels of IAA can be inhibitory to roots (Chadwick and Burg 1970, Evans 1984, Meuwly and Pilet 1991, Pilet and Saugy 1986), for this hypothesis we expected the endogenous levels of IAA to increase when the primary root growth rate decreased and that at high temperature this would occur when primary roots were shorter than at control temperature. We further argued that if Alternative Hypothesis 1 failed to be supported by our results and Alternative Hypothesis 2 was sustained, the inhibition in root development at high temperature is probably the result of acceleration of the timing of development.

We have quantified the free IAA levels in two tip regions as primary roots developed at 25 and 32°C and compared these data with previously published kinetic data gathered from seedlings grown in an identical manner (Gladish and Rost 1993). We have also quantified the export of ^{14}C-IAA and ^{3}H-sucrose (or their metabolites) from the cotyledons of developing Alaska pea seedlings and their distribution through the developing seedling axis at different stages of development.

Materials and Methods

Indole-3-acetic Acid Quantitation

Plant materials. Seeds of *Pisum sativum* L. cv. Alaska were germinated under axenic conditions against the sides of glass beakers (to permit collection of kinetic data) in growth chambers as previously described (Gladish and Rost 1993). Three stages of development (early, medial, and late) were chosen for IAA quantitation. To ensure uniformity, seedlings were selected when the postgermination ages, root lengths, and root elongation rates of their primary roots fell within specific narrow criteria for each stage of development. Three trials each were conducted at 25 and 32°C. The apical 5-mm and subtending 10-mm segments were quickly excised from the roots, weighed, and frozen in liquid nitrogen. The segments collected from each trial (n = 36–54 roots each) were pooled, lyophilized, and stored on liquid nitrogen until extracted.

IAA extraction. Extraction procedure was modified from Chen and others (1988). Lyophilized root segments were weighed, then ground with a glass rod in an Eppendorf tube under liquid nitrogen. $^{13}C_6$-indole-3-acetic acid (Cambridge Isotope Laboratories, Andover MA) and ^{3}H-indole-3-acetic acid (25.4 Ci/mmol specific activity; Amersham Corp., Arlington Heights IL) internal standards were then added (100 ng and 10^5 dpm per sample, respectively) and each sample extracted for 2 h at 4°C in 1 ml of 35% (w/v) 200 mM imidazole (pH7.0)/2-propanol extraction buffer. The supernatant was decanted after centrifugation and the pellet resuspended in extraction buffer twice. The combined supernatants were reduced to the aqueous fraction by rotary evaporation in vacuo and diluted 10-fold with distilled water.

IAA purification. Extract purification was by the three-step method of Chen and others (1988) with some modifications.

1. Solid phase extraction—The diluted extract was drawn through a conditioned amino column (Fisher Scientific, Pittsburgh, PA) by vacuum. After sequential washing with a solvent series, the sample was eluted with 2% (v/v) acetic acid in methanol, dried in vacuo, and redissolved in 30% (v/v) ACN.

2. HPLC—A Dynamax HPLC system with a reverse-phase Microsorb-MV column (Rainin Instruments, Woburn, MA), was used with acidified (constant 0.1% acetic acid) ACN/H_2O as solvent. The system was programmed with a two-stage gradient that ran from 30 to 80% (v/v) ACN in 6 min at 0.75 mL/min, then 80 to 90% ACN in 7 min at 0.50 mL/min. The retention time for IAA was 11.2 min.

3. GC—The IAA fraction was identified by scintillation counting, dried in vacuo, then methylated with ethereal diazomethane (Cohen 1984). The sample was dried under N_2, redissolved in 100% ACN and run, in splitless mode, on a model 5790 or 5890 Hewlett-Packard gas chromatograph equipped with a 30 m DB-1 fused silica capillary column (J & W Scientific, Folson, CA). The GC temperature gradient was 10°C/min from 140–240°C after a 2-min initial period at 140°C. The retention time for IAA was 6.9 min.

IAA quantification. The amounts of IAA methyl ester in purified and derivatized samples were determined on a VG Trio or Hewlett-Packard 5970 Series mass spectrometer in SIM. For both mass spectrometers the GC source temperature was 180°C and the ionizing voltage was 70 eV. The ions monitored by SIM were m/z = 130, 136, 189, and 195. The amount of free IAA in original samples was determined by isotope dilution relying on $^{13}C_6$-IAA as the stable internal standard (Cohen and others 1986).

Exogenous Application of Supplements

Seeds of *Pisum sativum* cv. Alaska were prepared in beakers as for IAA quantitation, except that half-strength Hoagland's Solution (Hoagland and Arnon 1950), 2% sucrose solution, White's complete culture medium (White 1943), and a 10^{-8} to 10^{-6}M naphthylacetic acid series were each substituted for deionized water. The beakers were placed in growth chambers at 32°C, and the progress of the roots was marked on the outside of the glass beakers as described previously (Gladish and Rost 1993) until they became arrested.

Transport Analysis

Seeds of *Pisum sativum* cv. Alaska were prepared as for IAA quantitation, except that 20 × 3 cm culture tubes were substituted for beakers. Four seeds were placed along the sides of each tube. Ten tubes each were prepared for the 2-d and 5-d experiments. The tubes were placed in dark growth chambers at 25 and 32°C along with beakers prepared as previously for comparison to ensure that similar kinetics prevailed in the culture tubes. Progress of primary roots was marked on the outside of the tubes and beakers daily until the onset of the test period (2 d or 5 d after imbibition) when the "start" position of each root tip was marked. While taking care that the root position was not disturbed, the testa of each seedling was gently peeled away, and the exposed epidermis of both cotyledons were scored lightly with the tip of a scalpel. Using a 10-µL syringe, 5 µL of mixed-isotope radioinoculant was applied by a series of about 10 shallow injections spaced over the scored area; 55,700 dpm of [6,6′(n)-^{3}H]-sucrose (2 Ci mmol^{-1} specific activity; Amersham Corp.) and 64,000 dpm ^{14}C-indole-3-acetic acid (6 mCi mmol^{-1} specific activity; Sigma Chemical Co., St Louis MO) were applied to each seedling; therefore, the amount of sucrose and IAA applied to each seedling was less than 13 pmol and 5 pmol, respectively. The culture tubes were then returned to their original temperature condition to continue development.

Samples (one tube for each temperature) were removed from the growth chambers at intervals starting at 6 h with the 2-d plants and 4 h with the 5-d plants, and the "stop" positions of the root tips were marked. The seedlings were then removed from the tubes and segmented into cotyledon, epicotyl, and root. The roots were cut into 10-mm segments except new growth at the tips (tissue added to the root tip between inoculation and harvest), which varied in length with sampling time. Segments were extracted separately in 95% ethanol for at least 48 h. Extractable radiolabel from each segment was determined using a Tri-Carb 2200CA automated liquid scintillation counter (Packard Instrument Co., Downers Grove, IL) in dual-isotope mode.

Statistics

Computer statistical analyses were performed using Statview II+, Superanova (Abacus Concepts, Inc., Berkeley CA), and JMP (SAS Institute, Cary NC) software for Macintosh. Data validity was checked by the method of Harris (1982), and *t* test for regression slopes was performed as per Zar (1984; $p \leq 0.10$, $R^2 \geq 0.90$). Student's *t* test was used to compare pairs of means ($p \leq 0.10$, except as noted). ANOVA was performed on data series with similar variances ($F \geq 3.00$ and $p \leq 0.10$, except as noted). Welch ANOVA was performed on data series with dissimilar variances ($F \geq 4.50$ and $p \leq 0.10$). Regressions reported have $R^2 \geq 0.90$, except as noted.

Results

Quantitation of IAA

The mean concentrations of IAA found in pea root tips and subtending mature primary tissue were determined at three stages of development (Figure 1). The concentration of IAA in comparable tissues at each stage was usually slightly higher at 25 than at

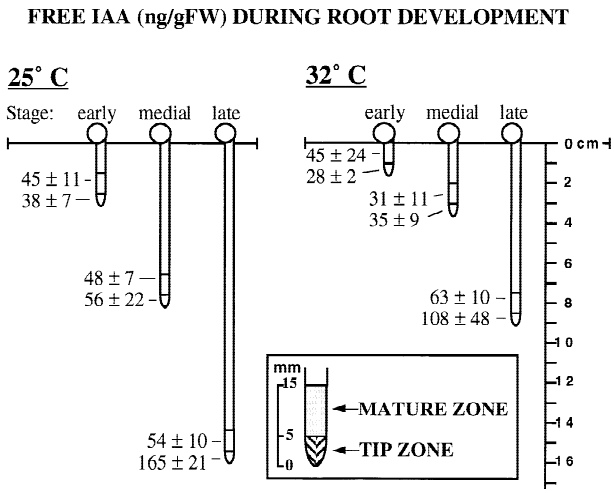

Figure 1. Schematic diagram of pea primary roots grown at control (25°C) and high (32°C) temperature shown at the time of IAA extraction of tip and mature primary regions (tip zone and mature zone, respectively; *inset*). Comparable stages of development (early, medial, and late) were determined by evaluating and normalizing previously published kinetic data (Gladish and Rost 1993). Endogenous IAA concentration in ng/g FW ± SD as determined by GC-SIM-MS is indicated adjacent to the tissue segment it represents in the diagram (n = 36–54 roots each).

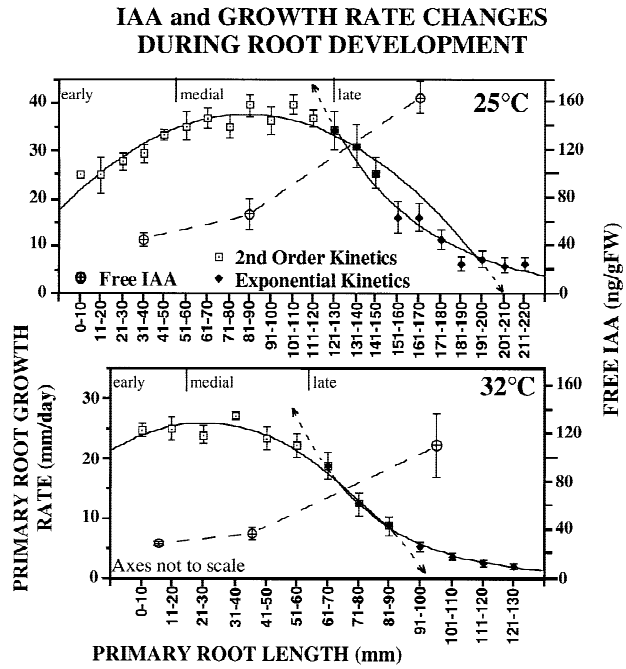

Figure 2. IAA concentration (ng/g FW) in the tip zone as a function of primary root length by comparison to length-dependent growth rate changes (biphasic kinetic data redrawn from Gladish and Rost 1993). 1AA concentration rose insignificantly between the early and medial stages of development at both temperatures, then increased 3-fold during the late stage, a very significant change (Welch Anova, F = 19.0). Error bar = SD. Note that the axes are not to scale.

32°C, but growth at different temperatures did not result in significant differences in IAA concentration at each stage of development in comparable tissue zones (Figures 1, 2). Slight rises in IAA concentration from early to medial stages were not significant (Figures 1, 2), but from the medial to late stage IAA concentration rose threefold as root growth slowed.

Exogenous Application of Nutrients and Auxin

Seedlings grown at 32°C in the presence of supplemented solutions rather than deionized water were not saved from attenuation by the exogenous application of mineral nutrients, auxin, sucrose, or a complete culture medium. Except for half-strength Hoagland's solution and 10^{-8}M naphthylacetic acid, which had no significant effect, all supplemental treatments inhibited growth slightly by comparison to 32°C controls (data not shown).

Transport and Distribution of IAA and Sucrose

Loss of radiolabel by diffusion into the surrounding medium was not uniform across the populations of pea seedlings. From 38 to 72% of total label per seedling was lost to the surrounding medium. Therefore, transport of IAA and sucrose from cotyledons into developing pea primary roots was quantified proportionally and analyzed at 25 and 32°C with respect to the following parameters:

1. Export from the cotyledons into the seedling axis. The percent of total extractable seedling ^{3}H (or ^{14}C)-label that was found in the axis (root + shoot excluding cotyledons) after the prescribed sampling period.

2. Root allocation. The percent of the total ^{3}H (or ^{14}C)-label extracted from the seedling axis that was found in the root after the sampling period.

3. Accumulation by new primary root tip tissue that developed during the sampling period. These data are given as percent of ^{3}H (or ^{14}C)-label found in new root tip tissue compared with the amount extracted from the entire root.

4. Intensity of labeling of new root tip tissue. These data are the percents of the total root ^{3}H or ^{14}C -label that were extracted from new root tip tissue, divided by the length of the new root tip tissue (primary

Table 1. The influence of a temperature difference on primary elongation, polar IAA transport, and sucrose and IAA (or IAA metabolite) allocation patterns in pea seedling primary roots.

		^{3}H-Sucrose root allocation (% axis total)			New growth (mm)			^{3}H-Sucrose in new growth (% root total)		
		Sampling time			Sampling time			Sampling time		
Plant age	Temperature	6 h	13 h	38 h	6 h	13 h	38 h	6 h	13 h	38 h
2 d	25°C	55(8)	44(7)	42(5)	5(2)	12(2)	36(3)	13(1)	22(5)	41(3)
	32°C	59(5)	37(2)	38(3)	5(2)	15(2)	34(8)	15(3)	30(6)	37(5)
			***		**	***		***	*	
5 d	25°C	52(6)	75(4)		9(2)	21(2)		6(2)	11(2)	
	32°C	48(11)	45(6)		6(1)	12(4)		2(1)	8(3)	

		^{14}C-IAA root allocation (% of axis total)			Polar IAA transport rate	^{14}C-IAA in new growth (% root total)		
		Sampling time				Sampling time		
Plant age	Temperature	6 h	13 h	38 h	(mmh^{-1})	6 h	13 h	38 h
		***	*					
2 d	25°C	73(8)	76(6)	65(4)	8(2)	4(1)	10(2)	13(4)
	32°C	89(4)	65(14)	65(9)	8(2)	4(1)	7(4)	15(4)
			**					
5 d	25°C	81(3)	91(3)		12(4)	2(1)	3(1)	
	32°C	74(13)	69(13)		12(2)	2(1)	4(3)	

**Significantly different (*t *test) at* $p < 0.10$; **$p < 0.05$; ***$p < 0.01$.
Pea seedlings were grown in culture tubes. Two d or 5 d after imbibition cotyledons were inoculated with ^{3}H-sucrose and ^{14}C-IAA. The positions of the root tips were marked on the glass. At the indicated time after inoculation a sample was selected at random and root tip positions were marked. The seedlings were segmented and extracted for 2+ d. Extractable radiolabel was measured by scintillation counter. Results are given as mean % (SD) of total extractable label for axis or root and mean length (SD) in mm of new primary root tip tissue (new growth); $n = 4$ *sample*$^{-1}$. *Polar IAA transport rate was determined from roots in first samplings of each experiment; given as* mmh^{-1}.

tissue added to the root tip between inoculation and harvest). They represent an estimate of how vigorously label was incorporated into each new millimeter of tissue added by the apical meristem.

5. Rate of phloem transport and polar IAA transport. The appearance of radiolabel at high levels in root tip tissue separated from the point of inoculation by a distinct intervening region of very low labeling was taken as evidence of phloem transport.

Growth. As determined from regression analysis, the increases in primary root length were linear and nearly identical for 2-d-old seedlings grown at 25 or 32°C over a 38-h sampling period ($Rate_{25°} = 1.02$ mmh^{-1}, $Rate_{32°} = 0.95$ mmh^{-1}). The 5-d-old seedlings displayed linear, but different, increases in primary root length over a 13-h sampling period ($Rate_{25°} = 1.63$ mmh^{-1}; $Rate_{32°} = 0.92$ mmh^{-1}, $R^2 = 0.84$). The kinetics of growth before and during the sampling periods were the same as controls.

^{3}H-Sucrose labeling patterns. The cotyledons of 2-d and 5-d-old seedlings in all cases rapidly transported applied ^{3}H-sucrose (or subsequent metabolites) into developing seedling axes (Table 1).

In 2-d-old seedlings, temperature did not make a significant difference in the percent of ^{3}H-label that was transferred to the seedling axis over time (Figure 3) or in the percent allocated to roots (Table 1). At both temperatures the highest percent of ^{3}H-label was found after 31 h, although second order regressions suggested that the maximum was earlier at 32 than at 25°C (Figure 3). Allocation of sucrose within 2-d-old seedling axes initially favored roots, but root allocation dropped below 50% after 6 h. Although the decline appeared marked, because of high variability at 25°C, it was statistically significant only for the 32°C treatment. In contrast, 5-d-old seedlings at 25°C strongly increased the root allocation between 6 and 13 h, but it was constant at 32°C (Table 1).

New root tip tissue accumulated increasing amounts of ^{3}H-sucrose during the course of the experiments at both temperatures (Table 1). Temperature made no difference in this regard in 2-d-old plants, but labeling was significantly greater at 25°C

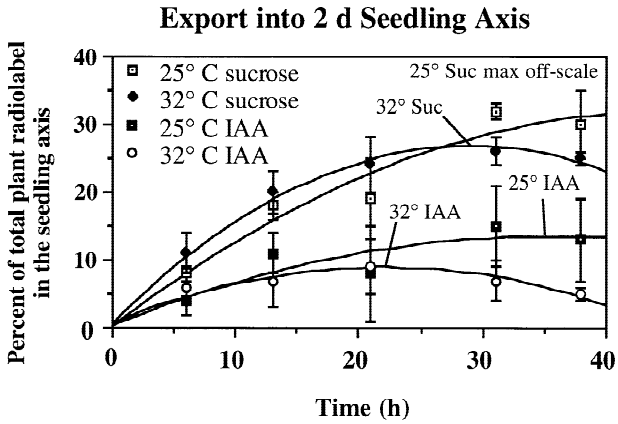

Figure 3. Transport from the cotyledons of 2-d-old seedlings into the seedling axis. Second-order regressions of export data for ^{3}H-sucrose and ^{14}C-IAA from 2-d-old seedlings after inoculation of the cotyledons are shown. The regression maxima of sucrose and IAA axis fractions (f′[x] = 0) are indicated by pointers. The theoretical maximum for ^{3}H-sucrose at 25°C is off chart. Error bar = SD.

in 5-d-old plants. In general, percents accumulated were higher for 2-d-old plants than 5-d-old plants. The intensity of ^{3}H-labeling in new primary root tissue was also the same at both temperatures in 2-d-old seedlings, and only slightly higher at 25°C in 5-d-old plants (Table 2). This suggests that temperature did not make a difference in sink strength. The minimum time required for the primary root tip to become significantly labeled by ^{3}H was less than 4 h in 5-d-old plants at both temperatures. Only the tip and the basal 1 cm of the roots were labeled 4 h after inoculation. It was assumed that this was due to phloem transport activity. The calculated mean phloem velocity was at least 27 ± 5 mmh^{-1} at 25°C and 26 ± 2 mmh^{-1} at 32°C in roots of 5-d-old plants. By the time of the first sampling of 2-d-old plants (6 h), root tips were heavily labeled at both temperatures, consequently we can say only that the value was larger (probably much larger) than 8 ± 2 mmh^{-1} at both temperatures. Furthermore, on the basis of the new tissue-labeling patterns of 2-d-old seedlings described previously, at both temperatures the rates of phloem transport are very likely the same in these younger plants.

^{14}C-IAA labeling patterns. The cotyledons of 2-d and 5-d-old seedlings in all cases transported applied ^{14}C-IAA (or subsequent metabolites) into developing seedling axes, but to a significantly lesser degree than applied ^{3}H-sucrose. The transfer of ^{14}C-label from cotyledons to the seedling axis was significantly faster than for ^{3}H-label at both temperatures, however (Figure 3). The ^{14}C-label maximum was earlier at 32 than at 25°C by at least 10 h (Figure 3).

Transport of ^{14}C-label into the root was favored strongly over transport into the shoot system in all samples tested (Table 1). This is evidence that ^{14}C-label remained substantially in the form of a free auxin during the experiment. Root IAA allocation in 2-d-old seedlings had very high initial ^{14}C-label values, but these declined significantly in later samplings. In 5-d-old seedlings grown at 25°C the root allocation of IAA increased significantly between 6 and 13 h. At 32°C root allocation of IAA was constant (Table 1).

Seedlings accumulated ^{14}C-label in new primary root tissue in a similar manner at both temperatures, but accumulation was greater in 2-d-old plants (Table 1). The intensity of ^{14}C-labeling in new root tip tissue, however, declined with time. The intensity of ^{14}C-labeling in new root tip tissue developed by 5-d-old seedlings was twofold higher at 32 than 25°C, in spite of (or perhaps because of) a much higher growth rate at 25°C (Table 1, 2).

Phloem transport of ^{14}C-IAA (or IAA metabolites) could usually be distinguished from polar IAA transport in the early samplings. Phloem-borne ^{14}C-label appeared in root tips simultaneously with ^{3}H-label, and it could be detected appearing in a root tip while a separate and distinct "leading-edge" of ^{14}C-label could be detected moving acropetally from the base of the same root. The intervening region had much lower levels of radiolabeling than the root base or tip (Figure 4). The rate of polar IAA transport was not faster at 32°C (Table 1). The observed difference between the age groups may not be significant either, because the resolution of the length measurement was coarse. A value of approximately 10 mmh^{-1} is probably reliable in this temperature range as an overall estimate of the rate of polar IAA transport during primary root development.

Discussion

Although it is true that the morphology of the meristematic zone of pea primary roots changes over developmental time, in general becoming narrower and shorter with time (Rost and Baum 1988), and root tissues are not homogeneous in their abilities to transport and metabolize auxin (Taiz and Zeiger 1991, Mitchell and Davies 1975), our data show that mean auxin levels across all tissues are in general similar in the mature primary and meristem/growth zones during most of root development (Figure 1). Therefore, morphological change of the meristem should not have had a significant effect on the results during the early and medial stages of develop-

Table 2. Relative labeling intensity of new root tip tissue at 25° and 32°C as a measure of the sink strength of developing primary root tissues with respect to sucrose and IAA.

	^{3}H-Sucrose				
	6-h sample		13-h sample		38-h sample
Temperature	2-d-old plants	5-d-old plants	2-d-old plants	5-d-old plants	2-d-old plants
25°C	3.3(0.6)	0.6(0.1)	1.9(0.2)	0.5(0.1)	1.3(0.4)
32°C	3.3(0.5)	0.5(0.1)	2.0(0.5)	0.7(0.1)	1.1(0.1)
	^{14}C-indole-3-acetic acid				
	6-h sample		13-h sample		38-h sample
Temperature	2-d-old plants	5-d-old plants	2-d-old plants	5-d-old plants	2-d-old plants
		*	**	**	
25°C	1.1(0.3)	0.2(0.0†)	0.8(0.2)	0.1(0.0[a])	0.5(0.2)
32°C	0.9(0.2)	0.4(0.2)	0.4(0.2)	0.3(0.1)	0.4(0.1)

**Significantly different (*t *test) at* $p < 0.10$; **$p < 0.05$.
[a]*SD value < 0.05.*
Relative labeling intensity was calculated as the percent of total root label in tip tissue that grew during the sampling time, divided by the length of that new growth. Values are given as mean % (SD) mm^{-1}, $n = 4$.

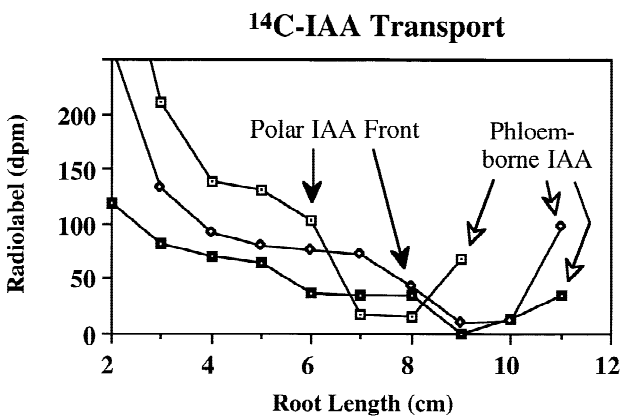

Figure 4. An example of data that permitted the distinction of phloem-borne IAA (or IAA metabolites) from IAA moved by polar transport in pea primary roots. Note the region of low radiolabel between the tip and the polar IAA. These three typical 32°C-grown seedlings were 2 d old, and were sampled 6 h after radioinoculation.

ment. Because the meristem zone is smallest at the late stage, our use of the apical 5 mm of the root tip to represent the meristem/growth zone may have caused our values for endogenous auxin to be an underestimate of what they really were in the meristem zone at the late stage. Regardless of whether the endogenous IAA was in the epidermis or vascular cylinder and regardless of the local direction of transport in the root tips (Mitchell and Davies 1975), our IAA quantitation data show that, as far as free IAA levels are concerned, pea primary roots growing at 25 and 32°C experienced similar changes in free IAA levels in a common, developmentally specific sequence (early, medial, and late). In absolute temporal terms, these stages of development occurred in more rapid succession at 32°C (Figure 2). But if it is assumed that the time to root determinacy represents 100% of the developmental time available, then it can be seen that significant changes in IAA level occurred in the same proportion of developmental time. Consequently, after 2 d 25°C primary roots were in the early stage, but roots at 32°C were nearing the medial stage. These were stages in which we found IAA levels to be similar (Figure 2). These data support Alternative Hypothesis 2. Growth, transport, and sink strength were also similar in these roots (Table 1, 2; Figure 3). In contrast, 5-d-old roots were in the medial stage at 25° but in the late stage at 32°C, stages in which IAA concentrations differed (Figures 1, 2). Growth and transport parameters also differed in many respects between 5-d-old seedlings at the two temperatures (Table 1). Although we did not attempt to discriminate between the original inoculants and possible radiolabeled metabolites, the results from this study suggest that at 32°C mobilization and transport of sucrose and IAA from the cotyledons into the seedling axis and their relative distribution therein were not impaired in 2-d-old plants, nor were these parameters significantly affected in 5-d-old plants.

These latter data do not support Alternative Hypothesis 1.

Figure 2 shows the effect temperature has on the growth kinetics of pea roots (kinetic data redrawn from Gladish and Rost 1993), and it can be seen that growth is biphasic. Pengelly and Torrey (1982) reported that endogenous free IAA concentration over time was positively correlated to growth rate of pea primary roots (cv. Little Marvel; 24°C). They also reported a biphasic growth pattern for pea roots but did not give data beyond 7 d after imbibition. Because Little Marvel, like Alaska, is a determinate pea cultivar, it probably has determinate primary roots as well. (Alaska and many other pea cultivars are known to have determinate primary roots; Gladish and Rost 1993). We believe that data from our study can be directly compared with the results of Pengelly and Torrey (1982), but we note that they probably were not aware that just as they terminated their experiment their subjects were beginning a rapid growth rate decline. The root tip–specific IAA quantitation of Pengelly and Torrey (1982), which was only performed on 7-d-old seedlings, very likely was a late-stage determination. Our late-stage IAA tissue concentration results are identical to theirs. Therefore, we disagree with their generalization that free IAA concentration is highest in the root tip, because this appears only to be true during late stage. We disagree also with their conclusion that IAA concentration corresponds positively with growth rate and suggest that Pengelly and Torrey (1982) would probably have obtained results similar to ours had they used root tips, rather than whole roots, for their time-course IAA quantitations.

Our results are consistent with those showing that increases in endogenous IAA levels in maize roots or exogenous application are associated with a decrease in growth rate (Meuwly and Pilet 1991; Pilet and Saugy 1985; Saugy and Pilet 1987). Saugy and Pilet (1987) showed that after germination maize primary root tips maintained a constant free IAA concentration and growth rate for 6 d, followed by a marked growth rate decline correlated with an abrupt increase in endogenous free IAA concentration. The slight rise in pea root tip IAA concentration we observed during the early and medial stages of development at both temperatures did correlate with a slight rise in their growth rates, but means testing suggested that the differences were not significant. Because pea and maize possess rather different genetic lineage, morphology, and environmental requirements, this concurrence of results suggests a general mechanism for the regulation of root growth.

Transport processes can be very important factors in development because they can limit the availability of important endogenous regulatory and structural substances. Export of labeled growth regulators and nutrients from the cotyledons would be expected to have a passive (e.g., diffusion) and an active component (e.g., phloem loading, polar IAA transport), and these can usually be differentiated by their temperature sensitivity (Nobel 1983). Q_{10} is defined as the factor by which a 10°C increase in temperature increases the rate of a chemical process. Q_{10} for simple diffusion is small, near unity, whereas Q_{10} for enzyme-catalyzed reactions is large (Nobel 1983). Consistent with this, based on regression analysis, the arrivals of the maximum amounts of ^{3}H-sucrose and ^{14}C-IAA (or their metabolites) from the cotyledons into the seedling axes in our study were separated by temperature by over 10 h in 2-d-old seedlings (Figure 3). This indicates that transport involved active processes, as one would expect. Furthermore, the axis import maxima for IAA preceded those for sucrose at each temperature (Figure 3), suggesting that IAA is mobilized from the cotyledons more rapidly than sucrose under these conditions. On the other hand, temperature difference did not result in a difference in the rate of polar IAA transport (Table 1). Because one would predict a more rapid polar transport rate at the higher temperature, this suggests some heat-induced impairment was occurring. However, the experimental result was that there was no significant difference in rate. We conclude from this that differences in polar transport of IAA are not implicated in the inhibited development of the root system that occurs at high temperature.

The lower proportion of ^{3}H-sucrose among 5-d-old seedlings grown at 32°C may have been due to low sink strength in these slow-growing roots, and this was reflected in the lower percent of total root label that was found in new tip tissue as well (Table 1). The high allocation of sucrose to 5-d-old roots at 25°C coincided with the medial stage of development when root elongation was most rapid and when lateral roots had begun growing, and it is unlikely that this was coincidence. The intensity of assimilation of ^{3}H-sucrose (or its metabolites) among 2-d-old seedlings was the same at both temperatures (Table 2), therefore there was no evidence from this procedure that sink strength was diminished at 32°C. The elevated intensity of ^{3}H-label in 5-d-old roots at 32°C demonstrates that there was assimilation at the root tip even when primary root elongation was markedly less. These root tips were still metabolically active.

The lower proportion of ^{14}C-IAA (or its metabolites) allocated to 5-d-old, 32°C roots vs. stems is

difficult to explain considering the results of our quantitation of endogenous IAA at the late stage of development and the rapid rate of transport we observed (Figure 1, 2; Table 1). These data may say more about how much of the ^{14}C-IAA became conjugated in the cotyledons and the relative sink strength in the shoot system, because polar transport of IAA is not acropetal in stems. The values for the allocation of ^{14}C-label to the root were much higher than 50% in all cases (Table 1), however. Clearly this was a result of polar transport activity and suggests that chemical modifications other than some conjugation of the applied ^{14}C-IAA were probably minimal. The higher intensity of ^{14}C-label observed at 32°C in 5-d-old seedling root tips is consistent with the high levels found by late stage endogenous IAA quantitation at 32°C (Figure 1, 2), and it suggests that transport is a major contributing factor to those high levels.

As for lateral root initiation and emergence, this study does not support the idea that a change of free IAA level or sucrose availability is associated with high temperature inhibition of secondary roots; but it must be kept in mind that in most of the tissues involved in initiation processes (which are located farther than 15 mm from the primary root tip in pea) IAA was not specifically quantified, and these tissues may also regulate IAA metabolism locally. Although indirectly drawn, our conclusion is consistent with critical evaluations of the IAA-induction model for lateral roots (Charlton 1991, Golaz and Pilet 1987, Muday and Haworth 1994, Reed and others 1998). Unlike the cases described by Levitt (1980), in this study exogenous applications of auxin and nutrients did not alleviate attenuation of pea root systems because of high temperature exposure, neither with regard to lateral root initiation nor primary root elongation. Although they have not addressed the issue of target cell/tissue sensitivity, our experiments suggest that auxin or carbohydrate "starvation" is not the cause of high temperature inhibition of root system development. It is clear, however, that lateral root initiation and emergence are more sensitive to temperature differences than primary root elongation.

The most important result of this study was that the difference in temperature did not result in significant differences in root tip IAA concentration or putative IAA and sucrose transport when assessed at comparable stages of development. One would expect that a 7°C difference would significantly affect respiration, enzyme kinetics (including transport processes), and sensitivity in a manner that would result in differences in IAA level or phloem conduction, especially if the higher temperature was truly stressful. The differences in growth kinetics and morphology between pea roots grown at 25 and 32°C is quite dramatic (Gladish and Rost 1993). An acceptable reconciling explanation is that pea seedling development is shifted in real time when they are grown at different temperatures, and the developmental reference frame within the plant remains fairly coordinated because of the integrated effect of the temperature difference on all of their metabolic and regulatory systems. When normalized on the basis of developmental markers (Figure 2), our data suggest that the two populations were not very different physiologically at 25 and 32°C with respect to transport systems and auxin metabolism. Clearly, the relative kinetics of growth of these roots was not altered by the temperature difference. Confirmation of sustainable respiration in pea roots at 32°C would rule out impairment of metabolic systems as the cause of attenuation of root development. Should this prove to be the case, time-shifting of regulatory systems would be implicated as the cause.

What has emerged as a result of these studies is a new idea about what occurs as development proceeds at temperatures near the upper adaptive limits of a plant. In this case, in contrast to Levitt's proposal of "biologic strain" (Levitt 1980), we suggest that the inhibition of root development that occurs at 32°C in pea roots is the result of accelerated completion of a normal genetic program, rather than competition with heat-shock responses or general, thermally induced dysfunction of metabolism. Our stage-specific IAA data independently supports the "biotime" model for any developmentally influential factor and empirically substantiates the concept of "a matrix of elastic developmental time scales" (Bradford and Trewavas 1994).

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