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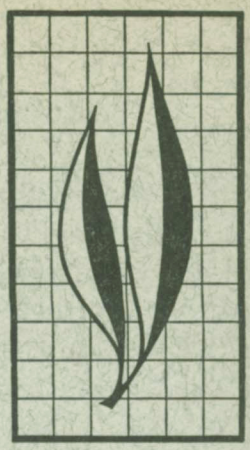
Translocation of Eight C^{14}-labeled Amino Acids and Three Herbicides in Two Varieties of Barley

Shogo Yamaguchi and A. S. Islam

UNIVERSITY OF CALIFORNIA DIVISION OF AGRICULTURAL SCIENCES



Translocation and distribution of eight C^{14}-labeled amino acids were studied in two closely related barley varieties, Atlas and Atsel. The compounds were: L-valine; DL-valine; DL-lysine; L-histidine; DL-phenylalanine; DL-tryptophan; DL-arginine; and glycine. For purposes of comparison, similar experiments were also conducted with labeled amitrole, 2,4-D, and monuron. Labeled compounds were applied either to leaves or roots of eight-day-old plants. For leaf experiments, 0.1 μmole at 10^{-2} M was applied; for root studies, 0.1 μmole at 10^{-6} M in 100 ml of nutrient solution. Autoradiographs were made after one, four, and 14 days.

The amino acids were accumulated in the bud tissues following application to either leaf or root, with a tendency for greater accumulation from leaf treatment.

Extraction and chromatography of labeled L-valine showed greatest extractibility and radioactivity 11 hours following application, and very little after that time. All amino acids tested showed greatest accumulation in bud tissues and root tips at the one-day time interval, indicating that these compounds must have nearly stopped translocating after one day. Results with tryptophan were not entirely satisfactory because of its low solubility. Root uptake was very slightly greater with the Atsel variety than with Atlas—a finding apparently not related to the presence of a greater amount of endogenous tryptophan in bud tissues of Atlas.

In comparison with the amino acids, 2,4-D showed an even more limited phloem distribution pattern. Translocation of 2,4-D from the treated leaf possibly lasted less than 11 hours. Amitrole, on the other hand, continued translocating from the leaf application to the extent that each new leaf emerging from the main axis in the 14-day interval was heavily labeled, and roots also became more heavily labeled with increasing time. Monuron exhibited apoplastic mobility.

THE AUTHORS

Shogo Yamaguchi was formerly Assistant Research Botanist, Davis. He is now Research Associate, Carver Research Foundation, and Associate Professor of Biology, Tuskegee Institute, Tuskegee, Alabama.

A. S. Islam is Professor of Botany, Sind University, West Pakistan.

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and A. S. Islam

Translocation of Eight C^{14}-labeled Amino Acids and Three Herbicides in Two Varieties of Barley[1,2]

INTRODUCTION

A COMPARISON of translocation of arginine-C^{14} in California Mariout barley with that in its Agropyroides mutant, by means of histo-autoradiography of the shoot apex, showed translocation to be much more pronounced in the mutant (Wijewantha and Stebbins, 1964).

Preliminary work by Islam with two barley varieties—a parent, Atlas, and its mutant, Atsel—showed that tryptophan was the only free amino acid that differed quantitatively in the stem apices of the two strains on a plant-number basis. The Atlas variety showed greater abundance at the eight-day stage than did Atsel. All other amino acids were equal with the two strains. From these data we could not be certain that the difference in tryptophan level had influenced development in these strains of barley. Translocation studies were therefore conducted with several labeled amino acids to determine, by autoradiography, whether there might be some distinct varietal differences. Similar studies were also conducted with three labeled herbicides, for purposes of comparison.

MATERIALS AND METHODS

Translocation studies

Plants. The two barley varieties used, Atlas and its mutant, Atsel, are known to differ in one gene pair. Atlas, which carries the dominant gene, is a long-day plant that requires a 13- to 16-hour photoperiod for flowering. Atsel carries the recessive allele, and is a day-neutral plant that requires a photoperiod minimum of 10 hours for normal development. It also has a more rapid growth rate when young. Johnson and Paul (1958) showed that the early development in Atsel may be accounted for on the basis of additive-increaser alleles at two loci. These workers, however, did not include Atlas among the long-day strains crossed with Atsel. Both long- and neutral-day strains contain awned and hooded types. We used awned types in the major part of the studies reported here.

Seeds were planted in sand. On the sixth day, individual plants were transferred to pint jars containing half-strength Hoagland's nutrient solution. (Some roots were broken during transfer.) Volume of the culture solution was reduced to 100 ml at time of treatment.

[1] Submitted for publication February 19, 1965. (Publication was delayed because the original, edited manuscript was lost in the mail.)

[2] The research reported in this paper was supported by A.E.C. contract AT (11–1)–34 Project No. 39.

TABLE 1
SPECIFIC ACTIVITIES OF AMINO ACIDS AND HERBICIDES, AND FILM-EXPOSURE TIMES

Compound	Specific activity	Activity dosage	pH of treatment solution	Film-exposure time
	mc/mmole	*μc*		*days*
L-valine-1-C^{14}	5.73	0.57	6.0	2.5
DL-lysine-1-C^{14}	8.95	0.89	5.0	1.5
L-histidine-UL-C^{14}	9.3	0.93	5.0	1.5
DL-phenylalanine-3-C^{14}	1.3	0.13	5.0	11.0
DL-valine-1-C^{14}	6.05	0.60	6.0	2.3
DL-tryptophan-3-C^{14}	6.64	0.66	6.5	2.1
DL-arginine-guanido-C^{14}	2.2	0.22	7.5	6.4
glycine-2-C^{14}	3.0	0.30	5.0	4.7
2,4-dichlorophenoxyacetic acid-1-C^{14}	12.3	1.2	2.0*	1.1
2,4-dichlorophenoxyacetic acid-1-C^{14}	1.24	0.12	5.0	11.0
3-amino-1,2,4-triazole-5-C^{14}	0.94	0.094	6.0	15.0
3(p-chlorophenyl-1-C^{14})-1,1-dimethyl urea	0.385	0.038	6.0	36.0

* Not intentionally low; strong contact injury at treated spot on leaf.

Plants were supported in the jars by cork stoppers. The root crowns were kept about 1 inch above the solution level to prevent entrance of solution by capillary action, which would have interfered with evaluation of uptake of labeled amino acid by the root. There was no forced aeration of the culture solution.

Labeled amino acids. We used DL-tryptophan, DL-lysine, L-histidine, DL-phenylalanine, DL- and L-valine, DL-arginine, and glycine. Their specific activities, together with the film exposure times, are given in table 1.

The labeled amino acids were dissolved in 50 per cent ethyl alcohol and 0.10 per cent Tween 20, a nonionic surfactant of low phytotoxicity. Concentration of the labeled compounds was 0.10 μmole in 10 μl of solution (10^{-2} M). To aid in dissolving the tryptophan, a small amount of ammonium hydroxide was added to the solution. The pH was adjusted to about 6.5, and the solution was heated to about 120°F. The specific activity levels of the materials as they were received by the laboratory were retained except in the case of L-histidine, which arrived with a specific activity 50 times higher than those of the other amino acids. It was brought within range of the others by dilution with unlabeled L-histidine.

Herbicides. Three herbicides with special mobility characteristics were selected: (1) 2,4-D (2,4-dichlorophenoxyacetic acid), fully mobile in phloem, but highly toxic to the plant, thus limiting its own transport; (2) amitrole (3-amino-1,2,4-triazole), fully mobile in phloem, also slightly mobile in xylem; and (3) monuron (3-(p-chlorophenyl)-1, 1-dimethylurea), fully mobile in xylem. Their specific activities are given in table 1. They were applied at the same concentration and in the same manner, and were evaluated on the same basis as were the amino acids. 2,4-D was used at two specific activities, the higher for leaf treatments, the lower for root treatment.

Application of amino acids. Plants were treated two days after being transferred to the jars, at which time the first leaf had reached almost full size, and could be used as the photosynthetic source for translocation studies. The leaf was supported by a strip of cardboard and some masking tape, to hold it in position for treatment. A ring of thick, invert emulsion of lanolin was applied near the base of the leaf blade, around the spot to be treated. For as-

sessment of translocation by phloem, 10 μl of treatment solution were applied and confined inside the ring (Crafts and Yamaguchi, 1964).

For determining upward translocation by xylem, 10 μl of treatment solution were added to the 100 ml of culture solution, thus changing the concentration to 10^{-6} M.

All applications of solutions were apparently absorbed in two hours, with the exception of tryptophan, which left a deposit. Application of additional solvent failed to improve absorption.

Autoradiographs were made one, four, and 14 days after treatment. During the 14-day period following treatment, water was occasionally added to the culture solution to prevent drying out. None of the plants showed symptoms of phytotoxicity after any treatment.

Extraction and chromatography of L-valine

Labeled L-valine was used to test whether the applied amino acid translocated in its original chemical form.

Plants. Plants of both varieties of barley, Atlas and Atsel, were used.

Application. Plants were treated on the ninth day after seeds were planted. L-valine was applied to the plants at a rate of 0.4 μmole in 40 μl of the same treatment solution (table 1) as that used for the autoradiography studies. Solution was applied to the first leaf of each of five uniform plants of Atlas and Atsel. Plants were harvested at 11 hours and at one, two, three, and four days following application.

For extraction, only the bud was used, including the basal elongating region of the second leaf and the enclosed bud leaves, and a short segment of the stem above the first leaf. Buds were 1 inch long at the time of the 11-hour and the one-day treatments, and about 2 inches after four days. Each bud was stored in dry ice immediately following harvest, and held until all buds had been harvested.

For extraction, an individual bud was placed in a small glass tube similar to a medicine dropper. The small opening of the tube was plugged with glass wool. Crushed dry ice was added to the tube to keep the bud frozen and to aid in grinding. The bud was ground in the tube by being jabbed with small, stiff wires of different sizes. After the buds were ground, the tubes were allowed to come to room temperature. Eight to 12 drops of 80 per cent ethyl alcohol were used for extraction of any one tube. Extractions were spotted directly on the chromatography paper. The solvent system, n-butanol : acetic acid : water (4:1:5) was used in ascending direction for eight hours. The paper was dried and autoradiographed.

RESULTS

Translocation and distribution

Although the pattern of distribution and accumulation of all the amino acids tested was well defined one day after treatment, there was no evidence of vigorously continued translocation of any of them during the next three days. Furthermore, whether the amino acids themselves were translocated was questionable. Since L-valine came nearest to the midpoint in mobility—equally effective translocation and distribution from either leaf or root applications—the other amino acids were compared with it on the basis of distribution and translocation. L-valine is therefore discussed in some detail.

L-valine distribution. The distribution of L-valine occurred in two major areas. The first was the apoplast region, comprising only the areas fully expanded or elongated at time of treatment and including the fully expanded treated leaf, the short, but fully elon-

gated portion of the stem through which the amino acid translocated, the full length of the root (except the tips), and the fully expanded region distal to the intercalary meristem in the second leaf. Absence of a large accumulation of label at the leaf tip indicated that there was no strong xylem or apoplastic mobility. The second major distribution was in the symplastic area, characterized by active growth at the root tip, the bud, and the elongating regions in the leaves and stem. In the autoradiograph made one day after treatment, these regions showed decidedly heavier concentration of label than did those of the apoplast (figs. 1 through 8). In addition, radioactivity in the mounted plant, as assayed with a portable GM ratemeter, was 20,000 cpm in and near the area of leaf treatment, and 4,500 cpm in the rest of the plant (fig. 1, Atlas leaf, one day)—that is, a ratio of about four parts remaining at the treatment site to one part moving beyond it. At first glance, the autoradiographs one day after treatment and those four days after treatment appeared similar, since the distribution pattern had not greatly changed in that time. Closer examination with the ratemeter, however, showed that, for the first day, activity in the intercalary meristem region, with its enclosed bud, in the first internode, and in the root tips was twice (or more) that shown after four days. Close comparison of the autoradiographs revealed differences in image density. Apparently, translocation from the 0.1 μmole of amino acid applied to the leaf and from that added to the culture solution had either diminished or almost stopped in one day. Growth during the four days (plants had developed another leaf) apparently had a diluting effect on activity concentration. Image density distribution of the second leaf (to which translocation was strongest on the first day) showed no evidence of redistribution of activity after the first day.

Autoradiographs 14 days after treatment still show a very small accumulation of L-valine in the growing bud, in both barley varieties, but it is doubtful that the activity was carried in the original compound. The pattern shows very limited distribution of activity in the later-developing leaves and internodes. Internodes that were elongating, and that were assimilate sinks at time of application did not accumulate as much activity originally as did the elongating leaves, and what did accumulate was diluted by the fourth day. By the fourteenth day, activity in this region was no more concentrated than it was in the later-developing internodes and leaves. Because the internode is a passage area for the flow of assimilates, not a terminal, its accumulative activity at the one-day time interval could have been carried away before much incorporation was possible.

The radioactivity accumulated in the second leaf, which was rapidly enlarging at the time of treatment and was a major assimilate sink, was not lost, and at 14 days was still carrying the highest concentration of activity except for that in the treated leaf. Presumably the amino acid had been incorporated in synthesis in the second leaf and rendered immobile.

L-valine activity. That L-valine translocates seems beyond question. An autoradiograph of the chromatograph of the extract showed some radioactivity at the rf value of L-valine-1-C^{14} in all extracts of both barley varieties. The extract at the 11-hour time interval carried by far the greatest L-valine activity, but with great activity in some compound remaining at the origin. This indicates the presence of some compound intermediate between the amino acid and nonextractable compounds carrying the label of the applied L-valine. Very little of this unknown intermediate was present in the extracts at the one-day period. A sharp break shows between the 11-hour and the one-

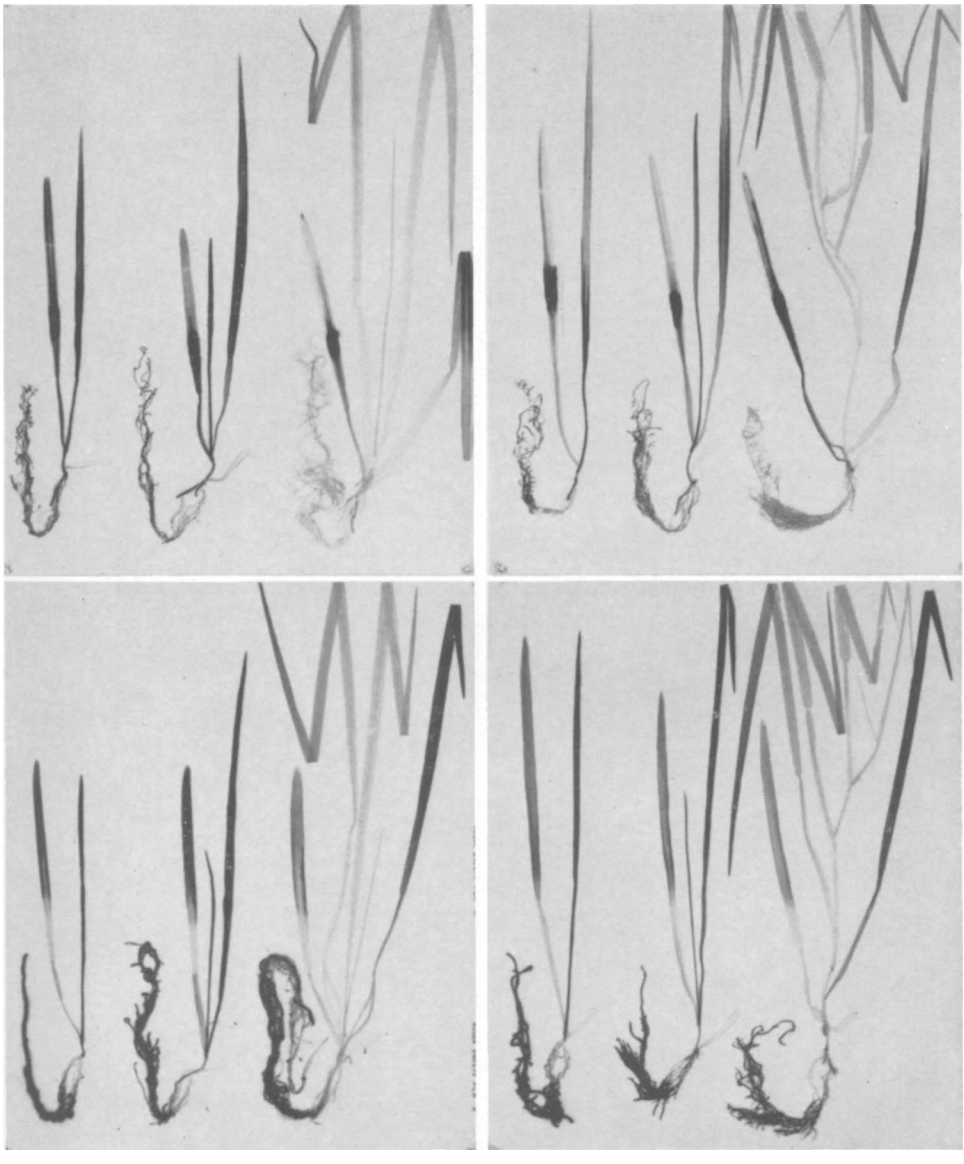

Fig. 1. Autoradiographs of barley varieties Atlas (left) and Atsel (right) following application of L-valine-1-C^{14} to first leaf (top photos) and root (bottom photos). Treatment time, left to right in each group: one, four, and 14 days.

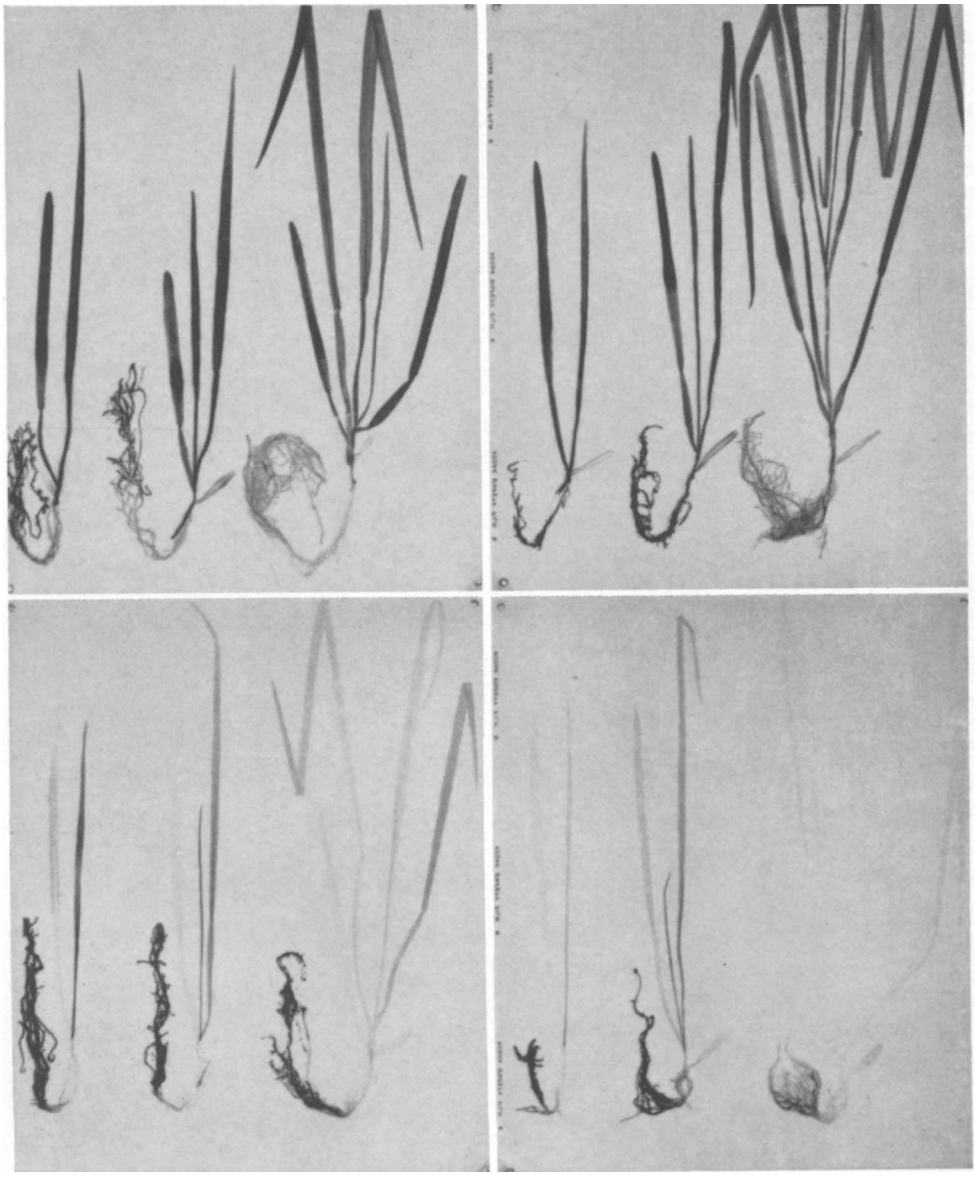

Fig. 2. Autoradiographs of barley varieties Atlas (left) and Atsel (right) following application of DL-lysine-1-C^{14} to first leaf (top photos) and root (bottom photos). Treatment time, left to right in each group: one, four, and 14 days.

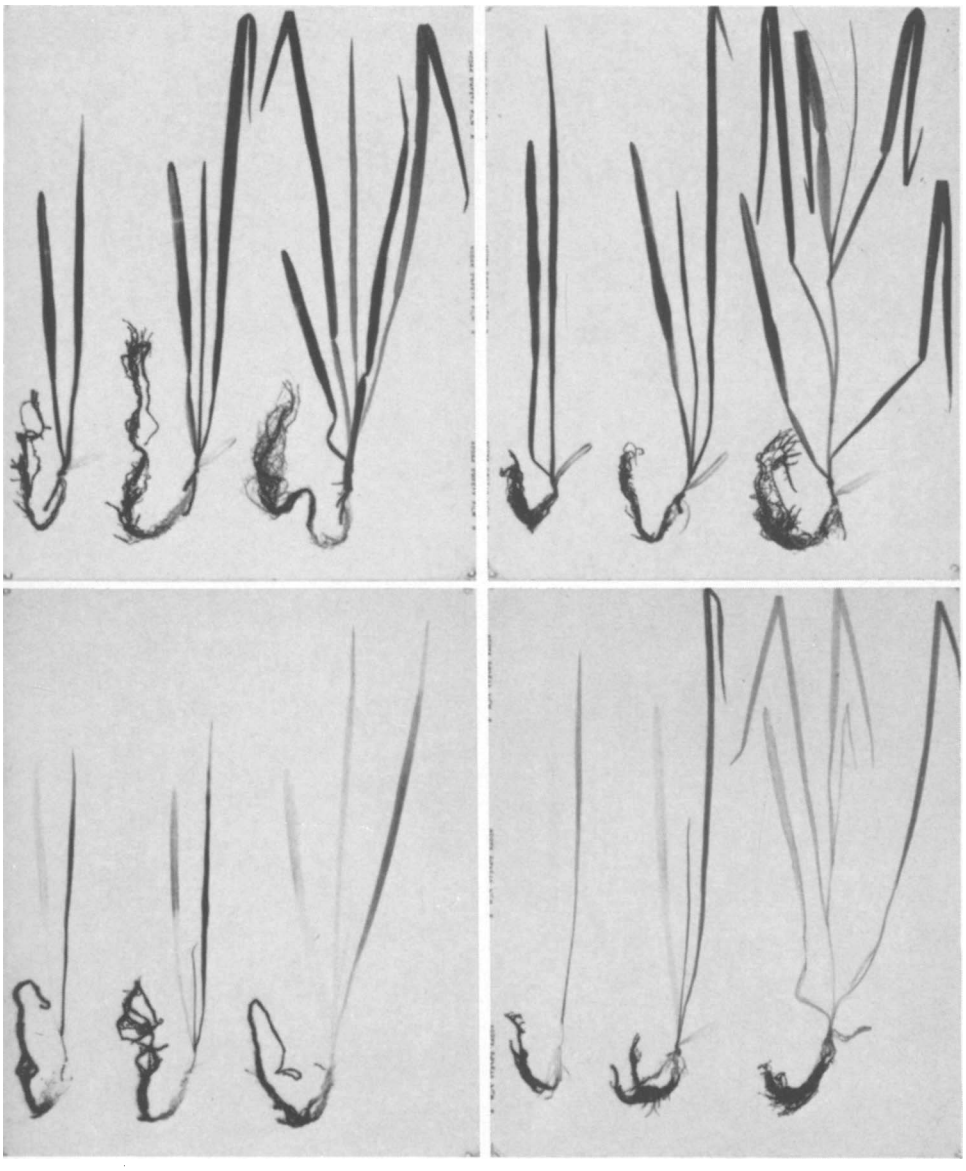

Fig. 3. Autoradiographs of barley varieties Atlas (left) and Atsel (right) following application of L-histidine-UL-C^{14} to first leaf (top photos) and root (bottom photos). Treatment time, left to right in each group: one, four, and 14 days.

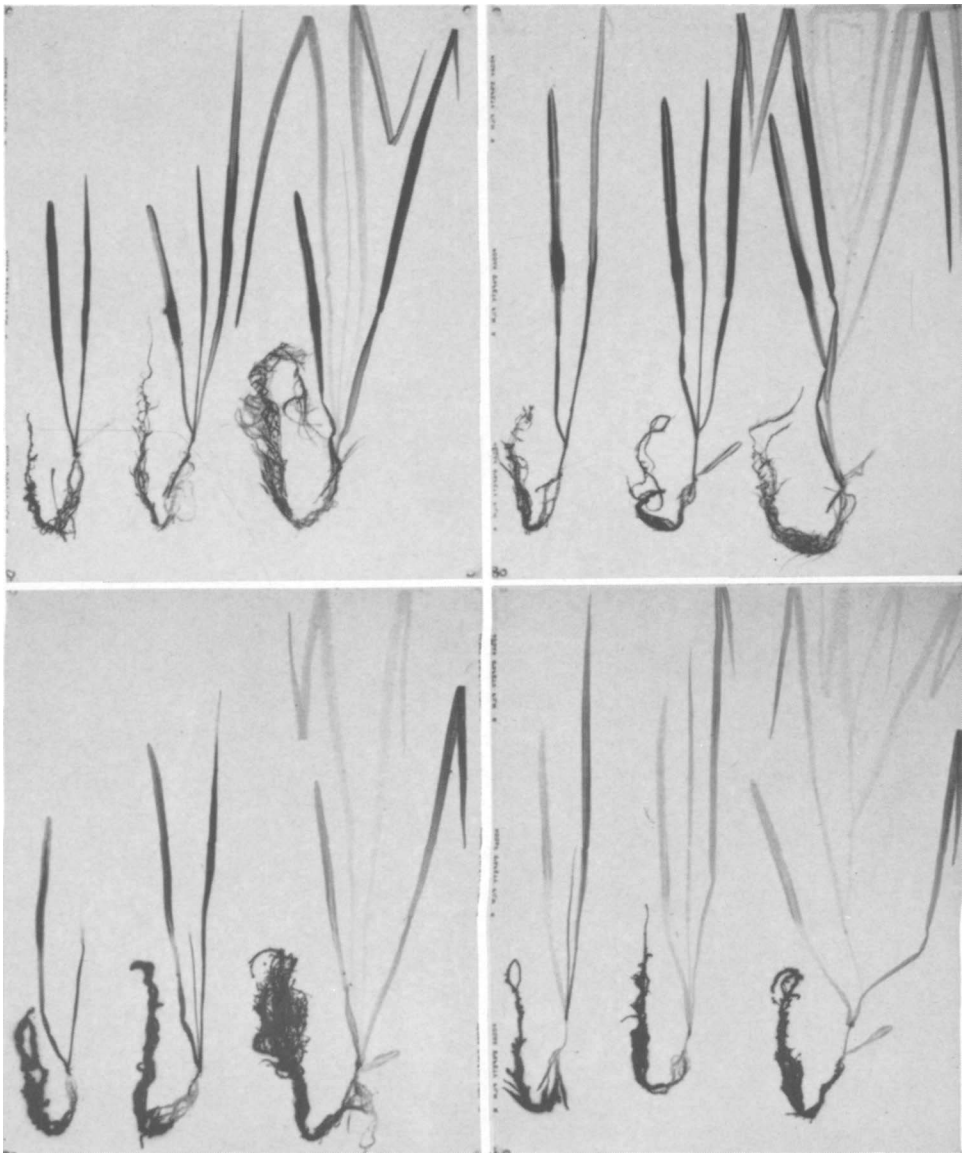

Fig. 4. Autoradiographs of barley varieties Atlas (left) and Atsel (right) following application of DL-phenylalanine-3-C^{14} to first leaf (top photos) and root (bottom photos). Treatment time, left to right in each group: one, four, and 14 days.

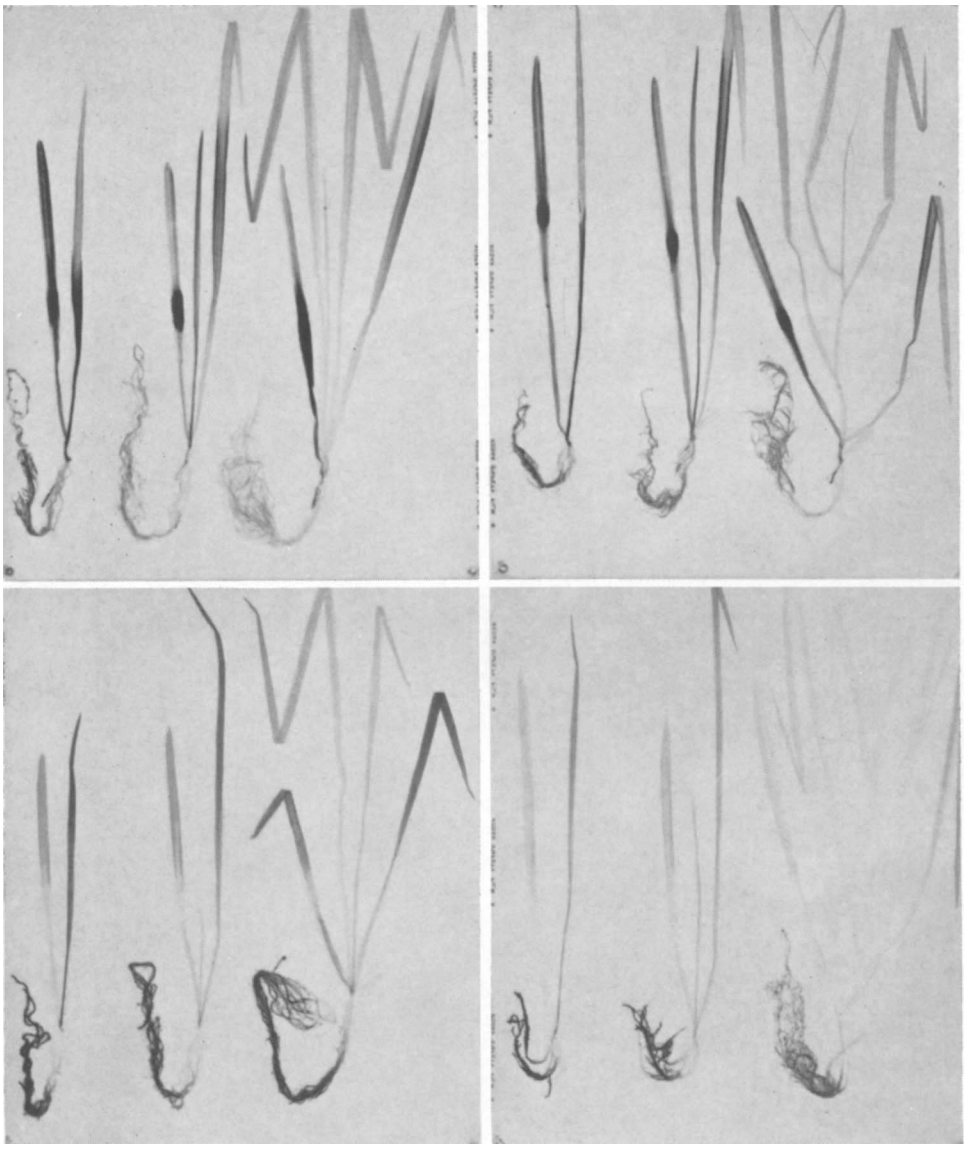

Fig. 5. Autoradiographs of barley varieties Atlas (left) and Atsel (right) following application of DL-valine-1-C^{14} to first leaf (top photos) and root (bottom photos). Treatment time, left to right in each group: one, four, and 14 days.

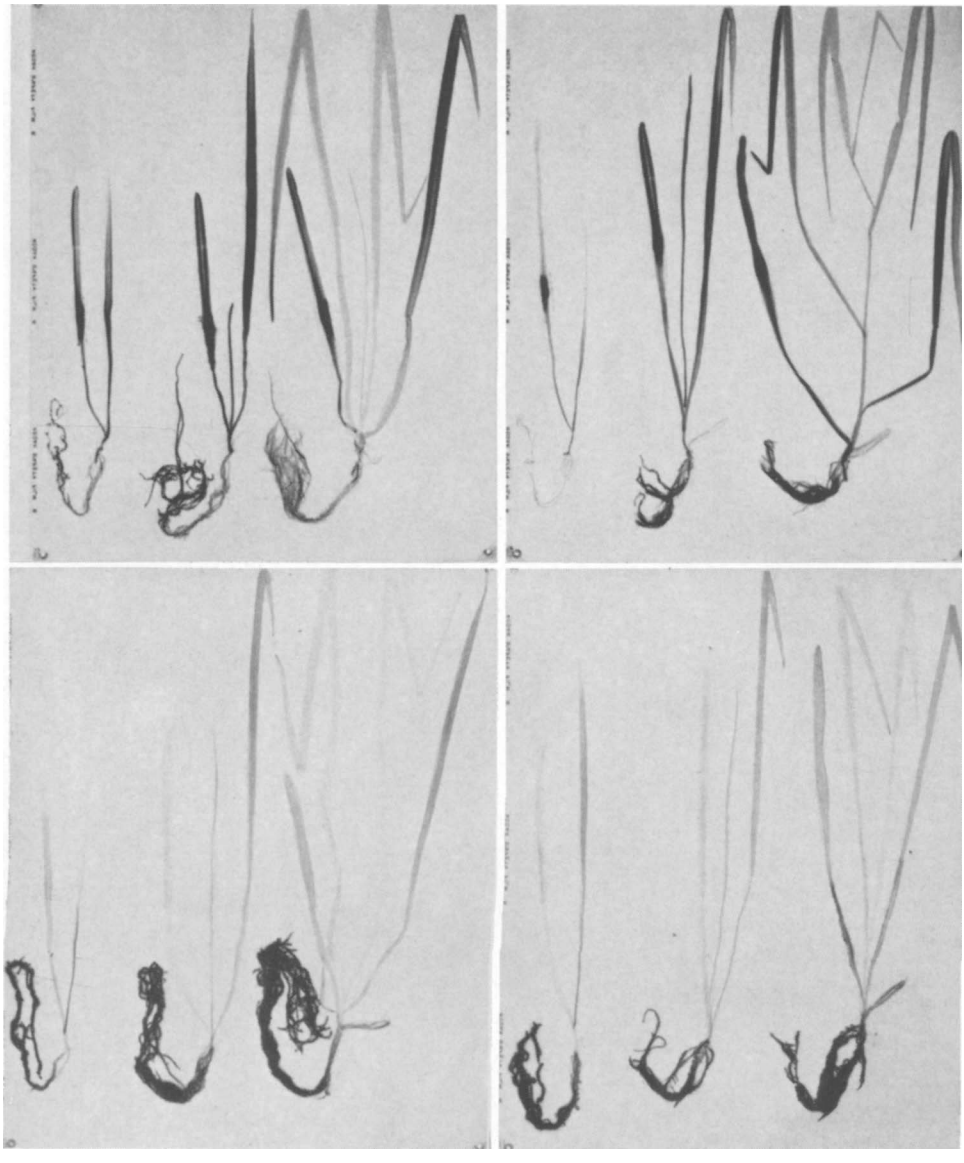

Fig. 6. Autoradiographs of barley varieties Atlas (left) and Atsel (right) following application of DL-tryptophan-3-C^{14} to first leaf (top photos) and root (bottom photos). Treatment time, left to right in each group: one, four, and 14 days.

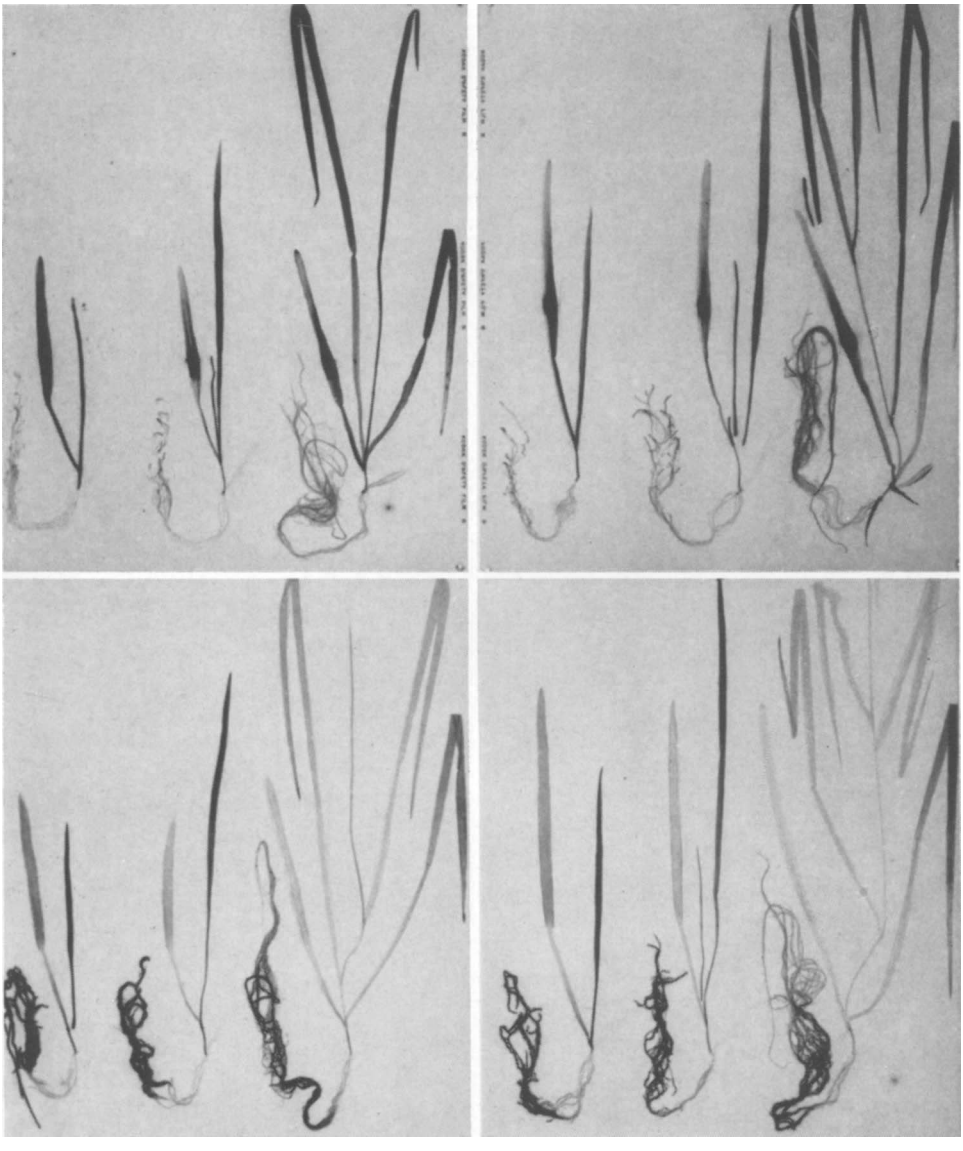

Fig. 7. Autoradiographs of barley varieties Atlas (left) and Atsel (right) following application of DL-arginine-guanido-C^{14} to first leaf (top photos) and root (bottom photos). Treatment time, left to right in each group: one, four, and 14 days.

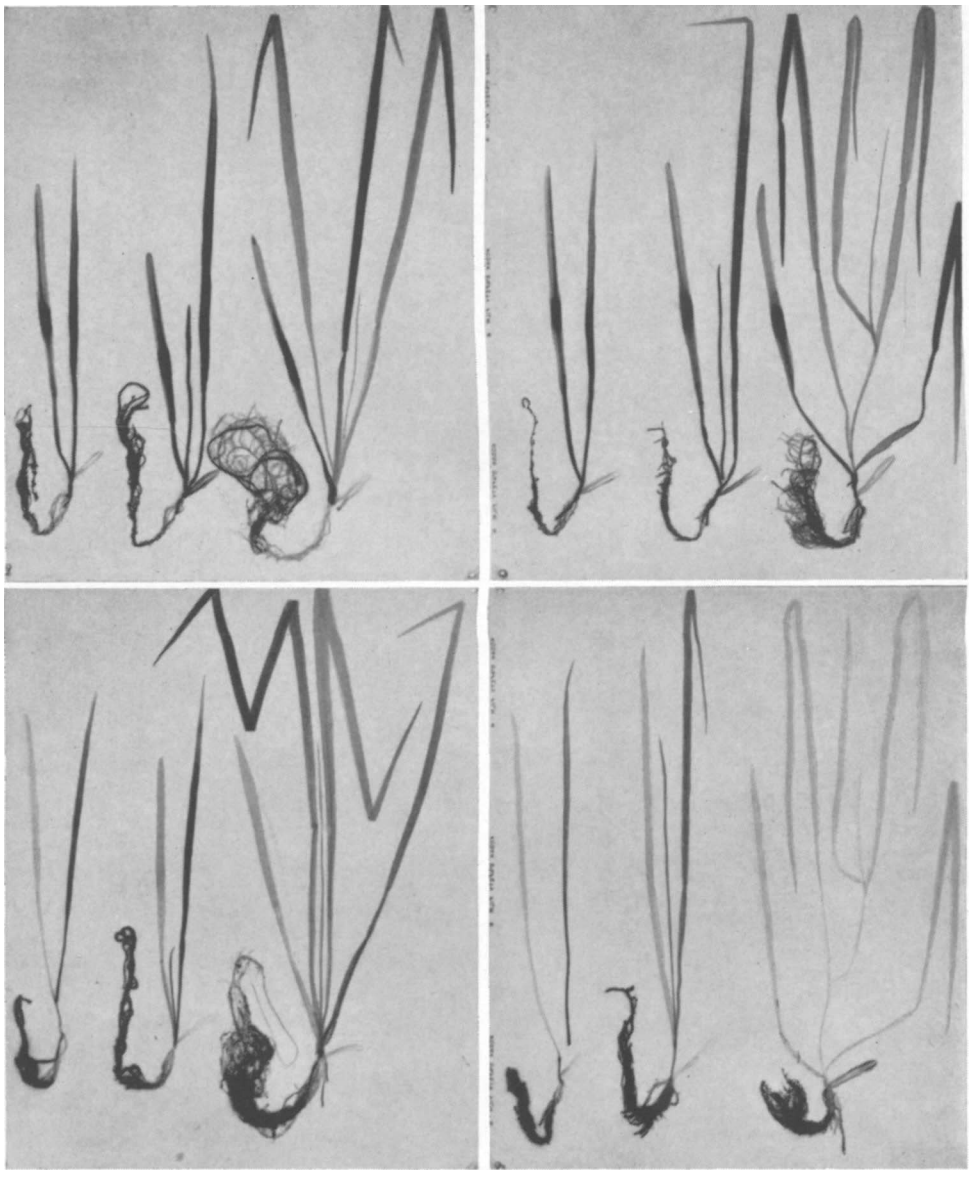

Fig. 8. Autoradiographs of barley varieties Atlas (left) and Atsel (right) following application of glycine-2-C^{14} to first leaf (top photos) and root (bottom photos). Treatment time, left to right in each group: one, four, and 14 days.

day intervals, apparently the result of cessation or inhibition of translocation. Trace amounts of activity did show, however, at the rf value of L-valine in the one-, two-, three-, and four-day treatments (fig. 9).

Other amino acids. In comparing the other amino acids with L-valine, only the larger differences are considered; statistical treatment is not attempted here. The major emphasis is not on quantity transported, but on relative mobility by the phloem and xylem—that is, a comparison of distribution following leaf treatment (phloem transport) with that following root treatment (xylem transport).

Activity was determined in the key distribution regions of the mounted plants that were used for the autoradiographs. Here again, only those for the one-day time interval were used. The end window of a GM tube of a Tracerlab Scaler was placed directly over the tip, the mid, and the basal regions of the second leaf, to determine phloem translocation and accumulation from treatment to the first leaf (table 2, column A). These three regions were assayed because of some variability in the distribution of activity in the second leaf, and the total of the three assays was used for an estimate of the activity in that leaf.

Data in table 2, column B, show activity estimates obtained in the same way for plants that received root treatment. Accumulation from root treatment can occur in the second leaf only when there is some phloem mobility in a substance that is otherwise xylem-mobile, because the young second leaf is at a growth stage in which transpiration is just becoming functional, but normal phloem transport of sugar to the leaf is maximal. Movement of a substance up the roots is a xylem function, but accumulation, in the bud leaves, of a substance absorbed by roots can result from its transfer from xylem to phloem in some region of the vascular tissue connecting the first and the bud leaves. The value of B in table 2 is zero when a given compound absorbed by roots does not accumulate in the young developing leaves, but enters the first leaf only. In our case the tip region of the second leaf seemed fully expanded and able to transpire, and the B values are therefore a little higher than they would be for phloem movement alone. The value of B is also zero when a phloem-mobile compound, such as 2,4-D, does not come up the root. If a compound is absorbed into the sieve tube of the root, the phloem movement is normally downward, not upward. On the other hand, a compound that comes up the xylem freely and enters the first leaf (fully developed, with full transpiration), but does not accumulate in the second leaf (young, developing, and with very limited transpiration), would be considered entirely xylem-mobile because it follows the path of transpirational water and accumulates in terminal areas of transpirational water movement, not in the young, developing leaf, to which water movement is very limited. With a compound of this nature, the value for both A and B would be zero. The major advantage of B is apparent in those compounds that are intermediate in mobility and exhibit it in both xylem and phloem; here the amount of activity that enters the second leaf will show a value for B which can be used for lessening the phloem mobility rating A, as obtained from first-leaf treatment, to arrive at a relative mobility value, A/B.

An A/B value of 1 means equal accumlation in the bud and bud leaves from leaf treatment and root treatment. Higher values mean greater accumulation from leaf treatment; in other words, greater phloem mobility than xylem mobility. The ratios in table 2 show that 0.6 (L-valine) to 10 (DL-lysine) times as much of most of the amino acids accumulated in the second leaf from leaf treatment as from root treatment, and that most of the amino

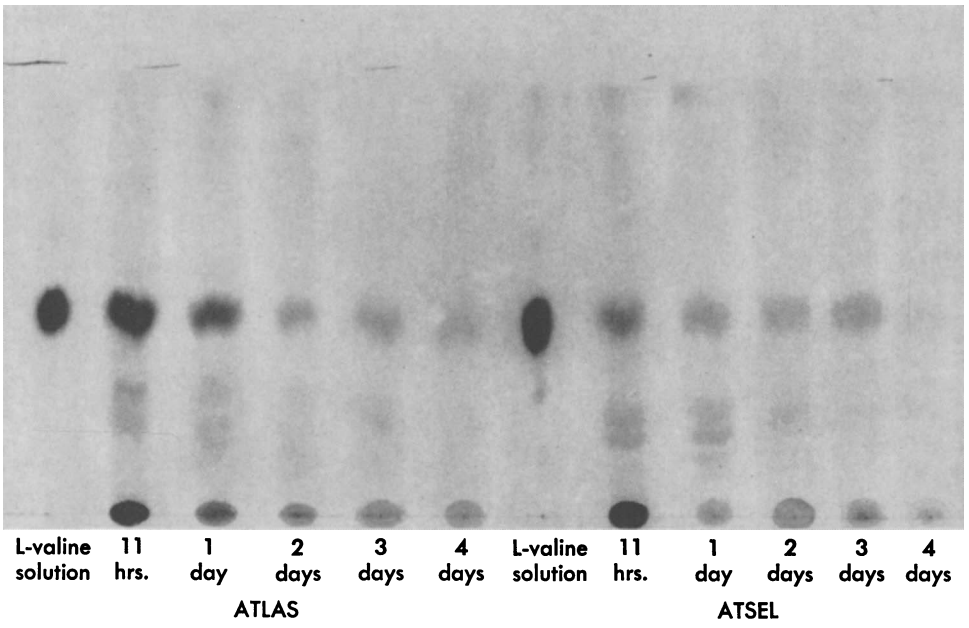

Fig. 9. Chromatograph of 80 per cent ethly alcohol extract from bud of Atlas and Atsel barley varieties following application of L-valine-1-C^{14}. Time intervals as shown.

acids used were more phloem-mobile than xylem-mobile by this test. A low A/B value was generally associated with greater xylem mobility.

Activity was estimated for 1⅛ inches of the tip of the first leaf in the one-day root treatment (table 2, column C). This column would provide a substantial rating for xylem movement when B rating is low or dubious.

The A/B ratio is not particularly useful for rating compounds of high apoplastic mobility and low symplastic mobility. For those compounds, C/B will give a high positive value and will be more realistic in showing up the tendency for accumulation at the tip of the first leaf as against what little amount might show up in the bud leaves. The function of B here is to reduce the rating of C by the degree of phloem mobility exhibited by a compound that is otherwise xylem-mobile. By this ratio, DL-phenylalanine had the highest value, 0.5, in Atlas, but in Atsel it was 0.2. DL-phenylalanine, DL-valine, and L-valine seem to be within range of each other. Although L-valine rated the lowest on the A/B basis for phloem mobility, and might therefore be expected to have been highest on the C/B basis for xylem mobility, this was not so. These differences or inconsistencies did occur within narrow limits of mobility. One contributing factor may have been the degree of immediate absorption, and also adsorption, characteristic of each compound when it comes in contact with the plant tissue.

Phloem mobility could not be readily assessed for tryptophan, because its low solubility interfered with leaf penetration. Therefore, an indirect means of assessing xylem mobility was attempted. In a one-day dosage series, 0.01, 0.02, 0.05, and 0.1 μmole of tryptophan were added to 100 ml of culture solution. Plants were uniform and entirely intact, and included both hooded and awned types of Atlas and Atsel. The Atsel types showed an activity in the leaves only very slightly greater than that in the corresponding types of Atlas (figs. 10, 11). The translocatability and

TABLE 2

RELATIVE MOBILITY OF CERTAIN C^{14}-LABELED AMINO ACIDS AND HERBICIDES IN PHLOEM AND XYLEM OF ATLAS AND ATSEL BARLEY VARIETIES ONE DAY AFTER APPLICATION TO LEAF OR ROOT

Compound	Barley variety and treatment area	Activity in sampled areas: A: second leaf (leaf treatment)	Activity in sampled areas: B: second leaf (root treatment)	Activity in sampled areas: C: tip of first leaf (root treatment)	Ratios: A/B Phloem mobility	Ratios: C/B Xylem mobility
		cpm	*cpm*	*cpm*		
L-valine	Atlas:					
	Tip...	331	1,028			
	Mid...	2,518	725	540	$\frac{3,980}{2,280} = 1.78$	$\frac{540}{2,280} = 0.24$
	Base..	1,131	527			
		3,980	2,280			
	Atsel:					
	Tip...	77	1,057			
	Mid...	884	508	353	$\frac{1,292}{2,023} = 0.639$	$\frac{353}{2,023} = 0.17$
	Base..	331	458			
		1,292	2,023			
DL-lysine	Atlas:					
	Tip...	401	184			
	Mid...	5,436	474	111	$\frac{10,361}{987} = 10.5$	$\frac{111}{987} = 0.11$
	Base..	4,524	329			
		10,361	987			
	Atsel:					
	Tip...	342	20			
	Mid...	2,281	44	14	$\frac{5,524^*}{112} = 49.3$	$\frac{14}{112} = 0.12$
	Base..	2,901	48			
		5,524	112			
L-histidine	Atlas:					
	Tip...	560	269			
	Mid...	5,493	416	52	$\frac{11,252}{1,225} = 9.19$	$\frac{52}{1,225} = 0.042$
	Base..	5,199	540			
		11,252	1,225			
	Atsel:					
	Tip...	889	137			
	Mid...	6,104	114	6	$\frac{14,988^*}{359} = 41.7$	$\frac{6}{359} = 0.017$
	Base..	7,995	108			
		14,988	359			
DL-phenyl-alanine	Atlas:					
	Tip...	77	14			
	Mid...	606	53	54	$\frac{954}{108} = 8.83$	$\frac{54}{108} = 0.50$
	Base..	271	41			
		954	108			
	Atsel:					
	Tip...	19	17			
	Mid...	203	40	14	$\frac{322}{68} = 4.74$	$\frac{14}{68} = 0.21$
	Base..	100	11			
		322	68			
DL-valine	Atlas:					
	Tip...	94	281			
	Mid...	1,121	224	191	$\frac{1,601}{657} = 2.44$	$\frac{191}{657} = 0.29$
	Base..	386	152			
		1,601	657			
	Atsel:					
	Tip...	108	104			
	Mid...	544	74	66	$\frac{1,167^*}{225} = 5.19$	$\frac{66}{225} = 0.29$
	Base..	515	47			
		1,167	225			

TABLE 2—*continued*

Compound	Barley variety and treatment area	Activity in sampled areas: A: second leaf (leaf treatment)	B: second leaf (root treatment)	C: tip of first leaf (root treatment)	Ratios: A/B Phloem mobility	Ratios: C/B Xylem mobility
		cpm	*cpm*	*cpm*		
DL-tryptophan	Atlas:					
	Tip...	51	7			
	Mid...	691	112	31	$\frac{1,082}{239} = 4.53$	$\frac{31}{239} = 0.13$
	Base...	340	120			
		1,082	239			
	Atsel:					
	Tip...	0	50			
	Mid...	72	66	23	$\frac{126}{173} = 0.728$	$\frac{23}{173} = 0.13$
	Base...	54	57			
		126	173			
DL-arginine	Atlas:					
	Tip...	258	172			
	Mid...	493	176	80	$\frac{1,186}{454} = 2.61$	$\frac{80}{454} = 0.18$
	Base...	435	106			
		1,186	454			
	Atsel:					
	Tip...	66	140			
	Mid...	124	147	32	$\frac{474}{396} = 1.20$	$\frac{32}{396} = 0.081$
	Base...	284	109			
		474	396			
Glycine	Atlas:					
	Tip...	125	88			
	Mid...	2,344	187	10	$\frac{3,764}{396} = 9.51$	$\frac{10}{396} = 0.025$
	Base...	1,295	121			
		3,764	396			
	Atsel:					
	Tip...	82	57			
	Mid...	1,391	84	8	$\frac{2,557}{290} = 8.82$	$\frac{8}{290} = 0.028$
	Base...	1,084	149			
		2,557	290			
2,4-D	Atlas:					
	Tip...	29†	0‡			
	Mid...	233†	1‡	0‡	$\frac{382}{3} = 127$	$\frac{0}{3} = 0$
	Base...	120†	2‡			
		382	3			
Amitrole	Atlas:					
	Tip...	60	7			
	Mid...	109	3	7	$\frac{313}{17} = 18.2$	$\frac{7}{17} = 0.41$
	Base...	144	7			
		313	17			
Monuron	Atlas:					
	Tip...	2	41			
	Mid...	6	8	244	$\frac{10}{56} = 0.18$	$\frac{244}{56} = 4.36$
	Base...	2	7			
		10	56			

* These values seem abnormally high, apparently the result of low absorption by broken roots (see figures 2b, 3b).

† Cpm of the higher specific activity leaf treatment was reduced here for comparison with that of the lower specific activity root treatment so that the A/B expression would be valid.

‡ Based on total of 2,000 counts, where the background was 40 cpm.

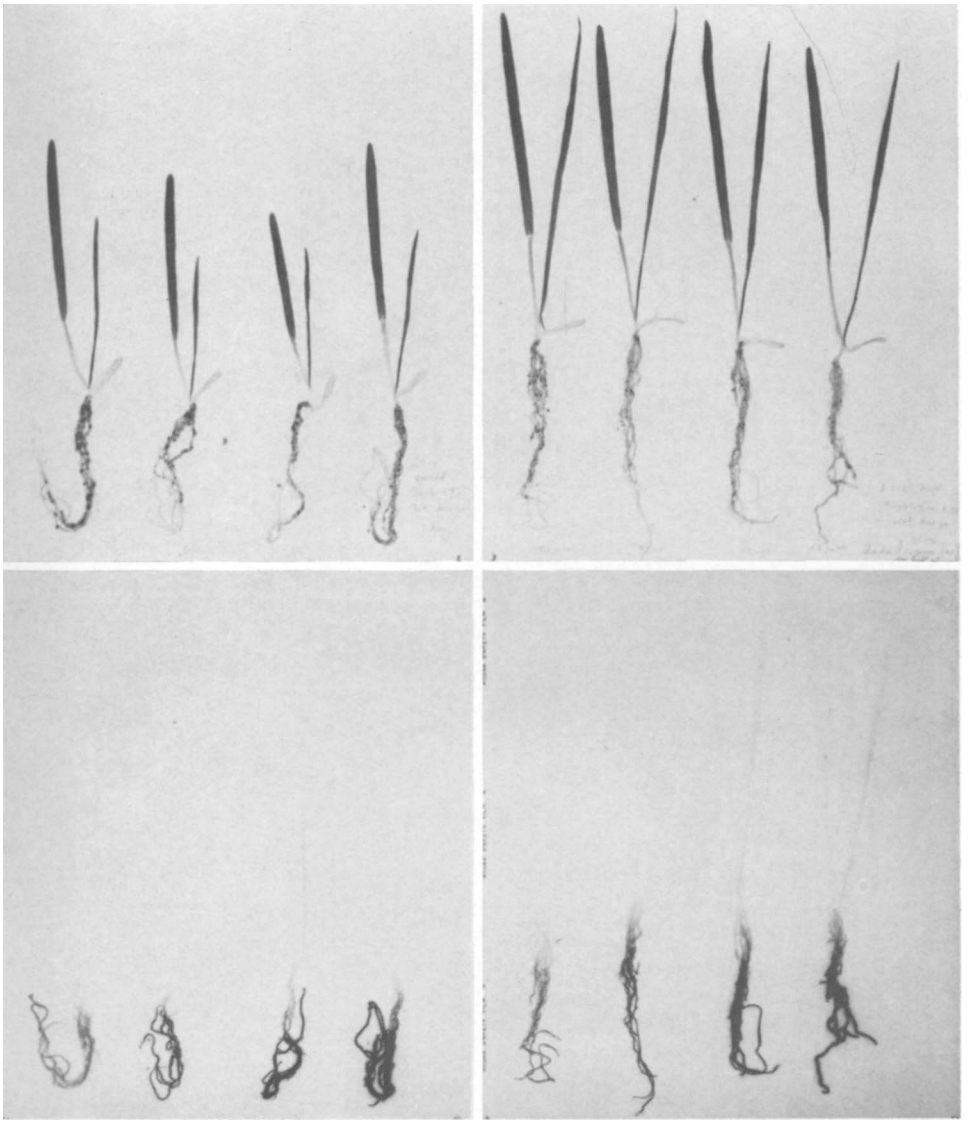

Fig. 10. Root uptake of labeled tryptophan in awned barley varieties Atlas (left) and Atsel (right). Above, intact plants; below, autoradiographs. Left to right in each group received tryptophan in 100 ml of half-strength Hoagland's solution at the following concentrations: 10^{-7}, 2×10^{-7}, 5×10^{-7}, and 10^{-6} M. All autoradiographs at one day following treatment.

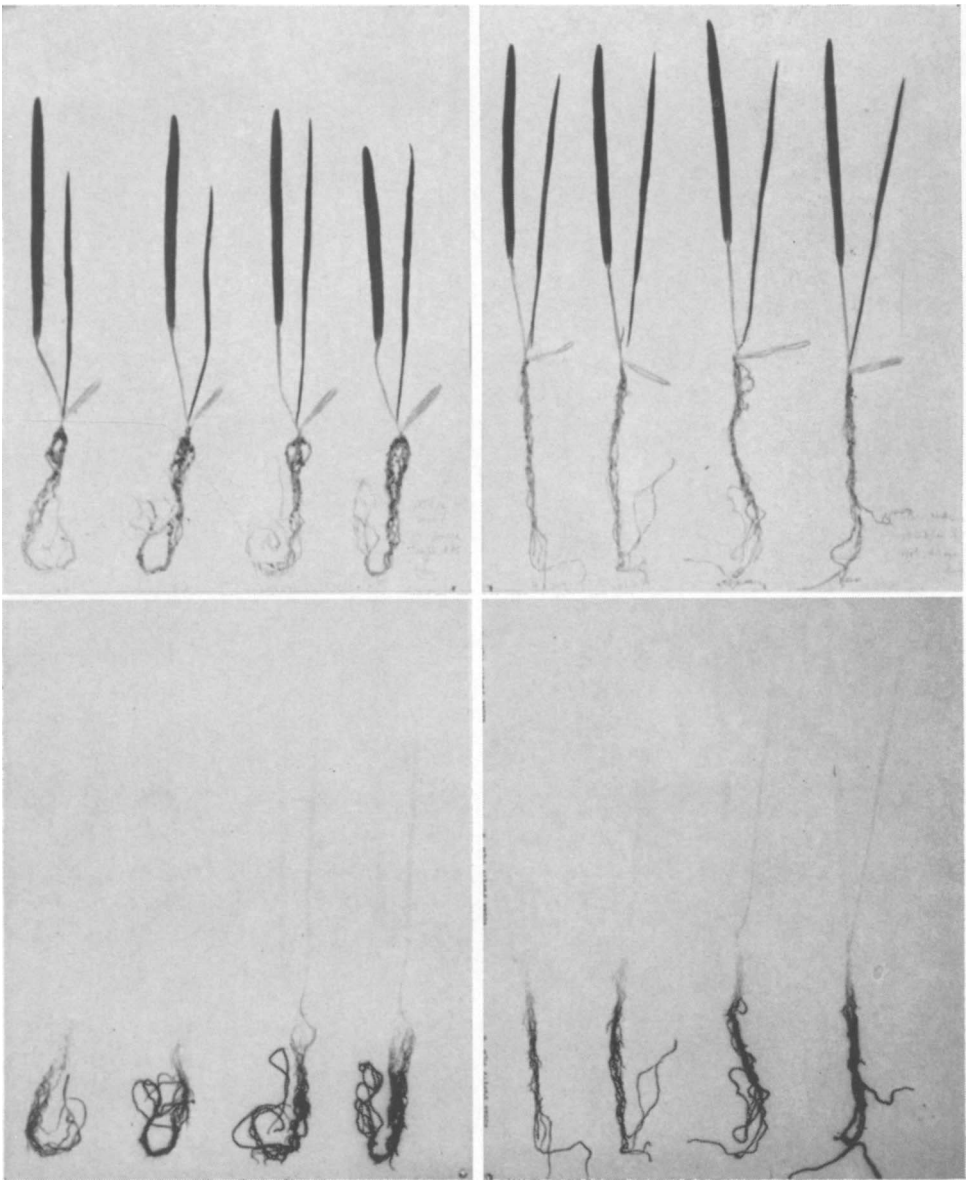

Fig. 11. Root uptake of labeled tryptophan in hooded barley varieties Atlas (left) and Atsel (right). Above, intact plants; below, autoradiographs. Left to right in each group received tryptophan in 100 ml of half-strength Hoagland's solution at the following concentrations: 10^{-7}, 2×10^{-7}, 5×10^{-7}, and 10^{-6} M. All autoradiographs at one day following treatment.

mobility of amino acids did not correlate with the earlier finding that tryptophan occurred in greater amounts in the bud of Atlas at eight-day stage of growth. Naturally-occurring tryptophan in the bud can be of different origin from that translocating to the bud.

Herbicides. The A/B value for 2,4-D was 127, which is many times greater than those for the amino acids (0.6 to 10); amitrole's value of 18 was nearly twice that of the highest amino acids; monuron was 0.18, much lower than the lowest of the amino acids. On the other hand, xylem mobility, by the C/B ratio, was zero for 2,4-D; for amitrole and the amino acids, 0.17 (L-histidine with Atsel) to 0.5 (DL-phenylalanine with Atlas); and for monuron, 4. Monuron is distributed almost entirely by the apoplast system; its A/B ratio was much lower than that for any of the amino acids. The value is even lower if considered in light of the fact that the second leaf (bud leaf), as explained earlier, had a mature region toward its tip at the time of treatment and a transpirational movement apparently carried some monuron activity toward that tip (see table 2, column B, monuron, tip). The high apoplastic mobility of monuron shown by the C/B ratio of 4 could have been more than twice as high if the factor of accumulation at the tip of the second (bud) leaf had been deleted from the denominator, because most of the activity in this leaf was at its tip (see table 2, column B). The amino acids tested are therefore quite different, in mobility, from monuron.

The amino acids, as compared with 2,4-D, appeared to have a little longer period of phloem translocation, as evidenced by the greater amount of tracer in the tip of the third leaf (figs. 1 through 8, and 12). The pattern of translocation and distribution of the labeled amino acids closely approximated that of labeled sugar from C^{14}-urea feeding of similar plants (Clor, 1959). Both exhibited the most intense accumulation in the bud and root tips after one day, and intense and large accumulation only in the second leaf and at the tip of the third leaf (figs. 1 through 8, and 13). (The urea-treated plants were nine days old, whereas the amino acid-treated plants were eight days old.) The distribution patterns of the amino acids and of sugar in the time series (one, four, and 14 days) show very close similarity, not only in mobility characteristics, but also in ready metabolism, incorporation, and exhaustion of the exogenous supply.

In comparison with the amino acids and 2,4-D, amitrole is much less readily metabolized or incorporated. Amitrole-5-C^{14} results at one, four, and 14 days after treatment are shown in figure 14. The first leaf was treated by a spot application of 0.1 μmole toward the base of the lamina, but thorough dispersal made the entire leaf appear black in the autoradiograph. The fact that root image was darker in the four- and 14-day treatments and that the image of every leaf emerging from the main axis after application was dense (fig. 14), suggests effective translocation over a period of several days, not just 11 hours or less, as with amino acids and 2,4-D.

DISCUSSION

The amino acids tested had both phloem and xylem mobility, with some showing greater phloem mobility than others. Within narrow limits, the greater mobility by phloem did not necessarily mean that there was less by xylem although over a wide range of compound types, phloem and xylem mobility tended to be mutually exclusive at the extremes.

Atsel was the more vigorous grower, but translocation of the applied amino

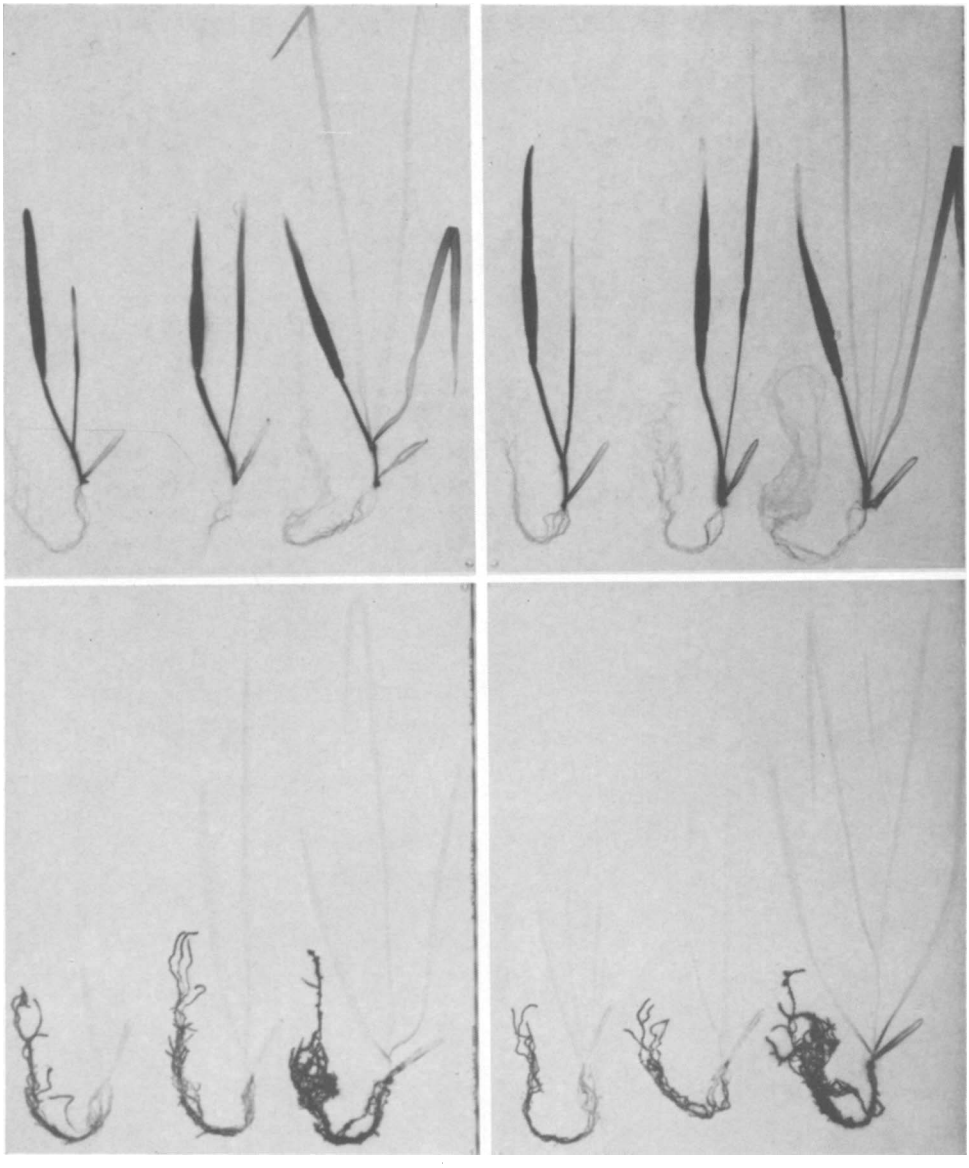

Fig. 12. Treatment time series with labeled 2,4-D, 1.24 μc/μmole. Left, Atlas barley; right, Atsel. Upper autoradiographs are of leaf treatment, 0.1 μmole, spot applied toward base of lamina of first leaf. Lower autoradiographs are of root treatment with 10^{-6} M in 100 ml of half-strength Hoagland's solution. Treatment time, left to right in each group, one, four and 14 days.

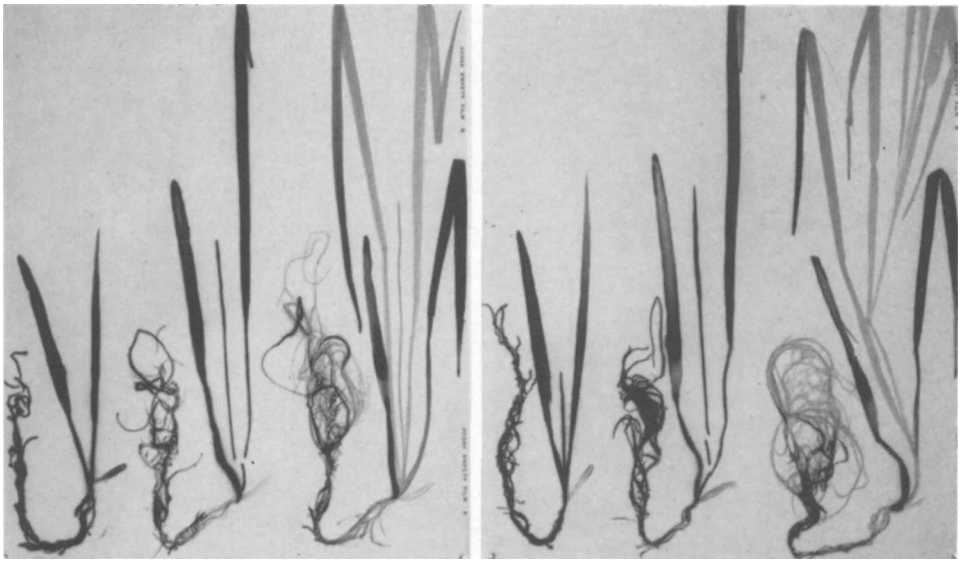

Fig. 13. Time series of spot-treatments with 0.1 μmole of urea-C^{14} toward base of lamina of first leaf. Autoradiographs are of nine-day-old plants of Atlas barley (left) and Atsel (right). Left to right in each group, treatment times, one, four, and 14 days.

acids was not necessarily more rapid in this strain. Since it is the faster growing, it would be expected to transport assimilates more rapidly, and thereby, the amino acids. GM counting, however, showed that DL-phenylalanine, L-valine, and DL-arginine were accumulated to twice the extent in the bud leaf of the slower-growing Atlas; on the other hand, L-histidine apparently accumulated to a greater extent in the bud leaf of Atsel (table 3). Uptake of tryptophan and distribution into leaves from low concentrations in the culture solution were only slightly greater in Atsel than in Atlas (figs. 10, 11).

In considering translocation from treated leaf on a quantitative basis, there is always the problem of the limiting factor of penetration. A greater transport of an amino acid may reflect no more than greater ease of penetration from the cuticular surface to the sieve tube. It is not certain whether the differences of translocation between the two barley varieties were related to structure or to function of the plant. To be sure, within any given plant variety, a greater rate of growth must support greater transport of assimilates and any exogenous mobile substances.

Extraction and chromatography of the bud tissues of Atlas barley, foliar-treated with four times the dosage used for autoradiography, showed maximum extractible L-valine-1-C^{14} and radioactivity after 11 hours rather than after one, four, or 14 days. With all amino

TABLE 3
COMPARISON OF ACTIVITY ACCUMULATED IN THE BUD LEAF OF ATLAS AND ATSEL BARLEY VARIETIES FOLLOWING ONE-DAY LEAF TREATMENT WITH LABELED AMINO ACIDS

Amino acid	Activity in barley variety:		Ratio Atlas/ Atsel
	Atlas	Atsel	
	cpm	*cpm*	
DL-lysine	10,361	5,524	1.88
L-histidine	11,252	14,998	0.75
DL-phenylalanine	954	322	2.96
DL-valine	1,601	1,167	1.37
L-valine	3,960	1,292	3.06
DL-arginine	1,186	474	2.50
Glycine	3,764	2,557	1.47

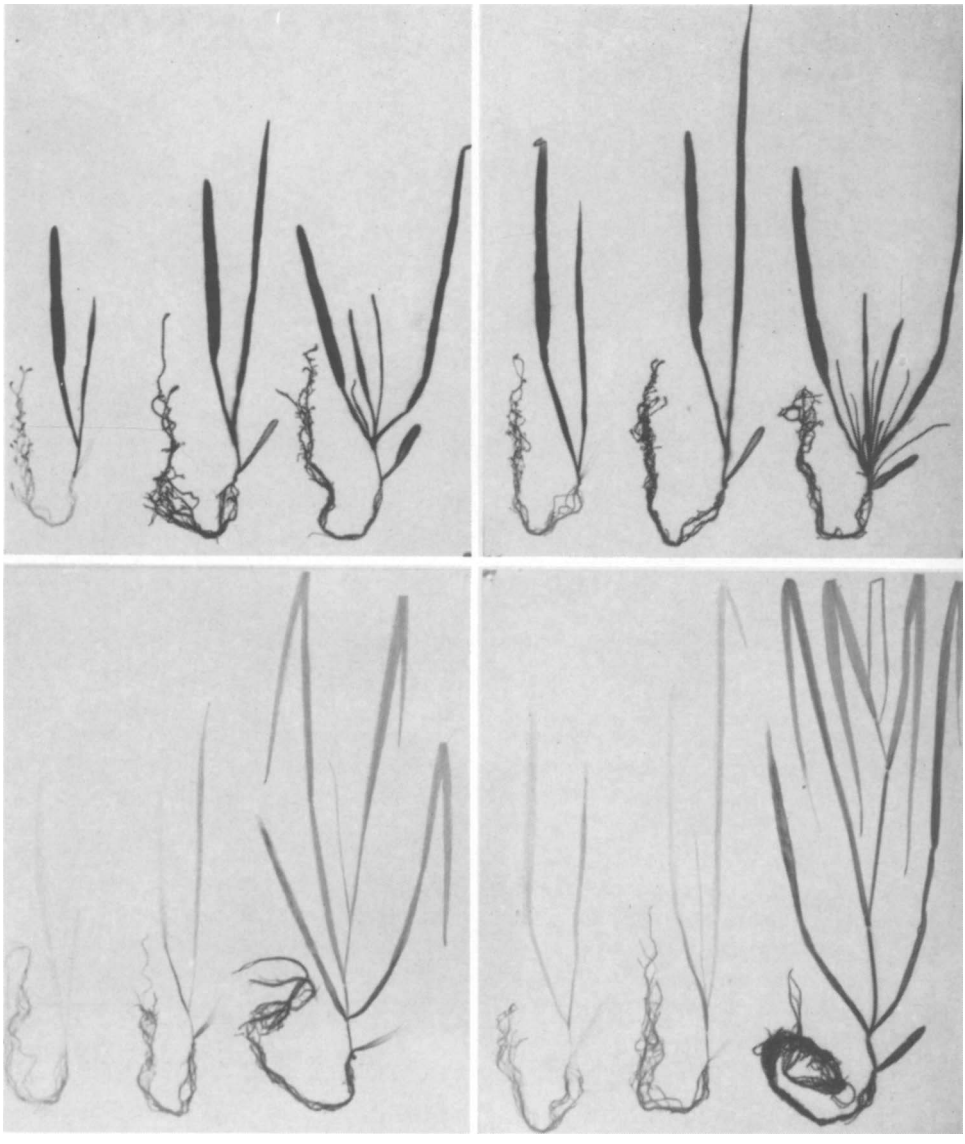

Fig. 14. Above: autoradiographs show eight-day-old barley plants spot-treated toward base of lamina of first leaf with 0.1 μmole labeled amitrole. Below: eight-day-old barley plants treated through roots with 10^{-6} M labeled amitrole in 100 ml Hoagland's solution. Plants on left are Atlas varieties; on right, Atsel. Left to right in each group, treatment times, one, four, and 14 days.

acids, the major fraction of the material applied to the leaf remained either in the leaf or on its surface at the applied spot, and after one day apparently very little was translocated.

The uptake from root treatment was also of brief duration. On close examination of the autoradiographs, the root image showed fine granular distribution of radioactivity on the root surface even after one day. This phenomenon possibly indicated absorption of radioactivity by bacterial colonies, which may have been responsible for inhibition after a brief period of uptake. The internal parts of the roots do not seem to have been highly radioactive according to some of the autoradiographs.

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