ing caryopses would likewise be a fraction of the total number of perfect pedicellate florets. One count showed that one-third of the florets of spikelets containing caryopses were pedicellate. Another count yielded 25.2 per cent pedicellate florets with caryopses and represented a lot of more than 1,000 pounds of commercial seed. Detailed examinations are being continued.

Other species of the genus containing such florets are the native *C. latisquamea* Nash and *C. verticillata* Nutt., of the subgenus *Euchloris*. While no indication of perfect pedicellate florets has been found to date in *C. cucullata* Bisch., *C. ciliata* Swartz, *C. canterai* Arech., *C. divaricata* R. Br., and *C. virgata* Swartz, all indigenous to or naturalized in Texas, it is probable that insufficient material was examined. *C. verticillata*, a prolific seeder and a very common species, contained 6 per cent of spikelets with mature or maturing caryopses in the pedicellate spikelets, all of which also contained caryopses in the basal florets.

In view of the above findings, it is proposed that the generic description of *Chloris* should include wording somewhat as follows: "Spikelets 3- to 4-flowered, the florets reduced progressively upward; perfect florets, 1 to 2; the first, basal and sessile; the second, pedicellate on the prolonged rachilla and perfect staminate, or neuter; the third staminate, or reduced to lemma and palea or only the lemma; and the fourth, when present, reduced to an empty lemma, or represented only by the rachilla apex, the two uppermost florets forming a club-shaped rudiment."

Specimens of the three species found to have perfect pedicellate florets have been submitted to Agnes Chase, agrostologist of the Smithsonian Institution. The writer would appreciate further confirmation of the facts presented and will be glad to examine other species of *Chloris* for additional information.

References

1. Hitchcock, A. S. *Manual of the grasses of the United States.* (U. S. Dept. of Agriculture Misc. Publ. 200.) Washington, D. C.: Government Printing Office, 1935.
2. Mauldin, M. P. Seed statistics of grasses, legumes, and other forbs, 1937–1943. S. C. S. Seed Lab. Memo. No. 7, 1946.
3. Silveus, W. A. *Texas grasses.* 1933.

Translocation of a Radioactive Plant-Growth Regulator in Bean and Barley Plants

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Previous experiments have shown that the application of a nonradioactive growth regulator (2,4-D) to a leaf or the roots of a young bean plant causes a stimulus to be translocated to the stem, where it brings about an easily detected growth response (curvature). A study of this type of response has revealed that the translocation of such a growth-regulating stimulus from a leaf to the stem is associated with the translocation of sugars along the same course and that it moves mainly in living cells (phloem); but when the stimulus is translocated from the roots, it can apparently move within the stem in nonliving cells (xylem) (*3*). However, neither the amount of stimulus translocated and its entire course of movement throughout a plant nor the absorption and translocation of such a stimulus in grasses can be detected readily on the basis of curvature measurements.

The present investigation, in which a radioactive growth-regulating substance was used, was undertaken (a) to determine whether or not the radioactive component was absorbed and translocated by representative dicotyledonous and monocotyledonous plants, and (b) to measure the amount of the radioactive component translocated and accumulated in various parts of the treated plant.

Experimental Method

The compound, 2-iodo-3-nitrobenzoic acid (INBA), used in this study causes form changes in leaves of bean seedlings when a few micrograms are applied to one primary leaf of each seedling. The response to INBA was found to be similar in character to that elicited by 2,3,5-triiodobenzoic acid (*4*). Radioactive iodine^{131} was used as the tracer atom because of the relatively high energy of its beta radiation (0.67 Mev), its availability in experimental quantities,[1] its convenient half-life (8.0 days), and the ease with which it could be incorporated into INBA. Synthesis was accomplished by replacing the mercury by iodine in anhydro-2-hydroxymercuri-3-nitrobenzoic acid (*1*). With this method the synthesis can be completed in less than three hours, and no isomers or troublesome by-products are formed. The method has the disadvantage, however, that mercuric iodide, a by-product of the reaction, contains an appreciable fraction of the radioactive iodine used in the synthesis. This disadvantage can be minimized by employing an iodine solution of high specific activity or might be circumvented by using a synthesis involving the diazotization of 3-nitroanthranilic acid (*2*).

Radioactive INBA was synthesized on a submacroscale as follows: The reaction was conducted in a round-bottomed pyrex vessel of 15-ml. capacity having two necks fashioned from interchangeable ground-glass joints. A pyrex Liebig condenser was inserted in one outlet and a ground-glass stopper in the other. Stirring of the reaction mixture was accomplished by means of a small, hollow, sealed, glass capsule containing several lengths of soft iron wire, which was externally rotated by means of a motor-driven permanent magnet. An iodine solution was prepared by dissolving 200 mg. of potassium iodide in 7.5 ml. of radioactive iodine solution[2] and adding a solution of 230 mg. (1.39 mM) of potassium iodide and 360 mg. (1.42 mM) of stable iodine in 0.35 ml. of water. To a boiling solution of alkali (70 mg. of NaOH in 2.1 ml. of water) in the reaction flask was added portionwise, with stirring, 460 mg. (1.26 mM) of anhydro-2-hydroxymercuri-3-nitrobenzoic acid. Boiling and stirring were continued during the dropwise addition of 0.12 ml. of concentrated HCl. Heating was stopped and 0.04 ml. of glacial acetic acid was added. To this rapidly stirred mixture the iodine solution was added rapidly from a glass, 10-ml. hypodermic syringe equipped with a capillary

[1] Obtained through the Isotopes Branch, Manhattan District, Oak Ridge, Tennessee (*5*).

[2] Radioactive iodine was received on August 26, 1946, in the form of a N/10 sulfuric acid solution having a specific activity of 1 mc./ml.

delivery tube attached to the barrel. The reaction mixture was stirred and refluxed for 5 minutes and was then made alkaline (30 mg. NaOH), centrifuged, and the supernatant solution acidified to Congo red (ca. pH 4) by adding concentrated HCl. The precipitated INBA was collected by centrifugation and washed successively with a small volume of water and a solution of 0.1 gram potassium iodide in 2 ml. of water. After centrifuging, the acid was dissolved in 2 ml. of hot 50 per cent ethanol centrifuged hot and cooled to crystallize. The acid was recrystallized, using the same procedure, to yield 206 mg. of product (49 per cent of theory based on the 3-nitrophthalic acid used in preparing the anhydro-2-hydroxymercuri-3-nitrobenzoic acid) melting at 204.5–207.5° C. (cor.)—reported melting point (*1*), 204–205.5° C. Analysis calculated for $C_7H_4INO_4$: C, 28.69; H, 1.37; I, 43.31; N, 4.78; neutral equivalent, 293. Found: C, 28.83, 28.69; H, 1.84; 1.75; I, 44.13, 43.99; N, 4.27, 4.28; neutral equivalent, 295.5, 297.1.

Two series of reference standards for the radioactivity measurements, one of bean leaf tissue and one of bean petiole tissue, were prepared by adding known amounts of radioactive INBA to known amounts of the dried, ground (20 mesh) plant material. The acid was in the form of a dilute aqueous solution prepared by dilution of an alcoholic solution of the acid. At least 10 ml. of the aqueous solution was added for each gram of dried plant material to insure adequate distribution of the acid. The watery suspension of plant material which resulted was thoroughly mixed in a ball mill, dried (80° C.), and reground to 20 mesh in a Wiley mill. Standards of both leaf and petiole tissue were prepared to contain 1 part of the radioactive acid in 5000, 1 in 10,000, 1 in 100,000, and 1 in 1,000,000 parts of tissue.

For tracing the movement of radioactivity through bean

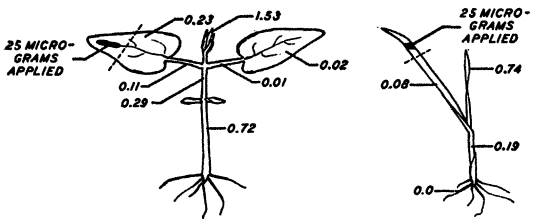

FIG. 1. Diagram showing distribution of radioactive INBA (μg.) in various parts of bean (left) and barley plants 3 days after treatment. Distal ends of treated leaves were severed as indicated by broken line and discarded prior to sectioning of plants.

(*Phaseolus vulgaris*) and barley (*Hordeum vulgare*) seedlings, the radioactive INBA was applied to a leaf of these plants as a paste made by first dissolving 25 mg. of the acid in 1 gram of melted polyethylene glycol (Carbowax 1500), followed by thorough mixing with 4 grams of melted lanolin. In treating leaves, 5-mg. aliquots of the paste (25 μg. of INBA) were applied to the upper surface and along the midrib of one primary leaf of each bean plant and as a band about 0.5 cm. wide across the upper surface and near the tip of the first true leaves of barley seedlings (Fig. 1). The bean plants used had well-developed primary leaves, but the trifoliate leaves were still folded tightly in the terminal buds at the time the plants were treated. The barley seedlings had one well-developed true leaf and a second leaf rapidly expanding at the time the plants were treated.

Three days after treatment the plants were carefully divided into parts, and the corresponding parts were combined, dried (forced draft oven at 80° C.), and ground (20 mesh) in a Wiley mill. One hundred mg. of each sample was spread uniformly over the surface of a shallow plastic cup, 3.5 cm. in diameter, and the radioactivity of each was measured by means of an Edelmann electrometer-ionization chamber.[3] Measurements of the radioactivity of the tissue standards and unknown samples were made in the same manner on the same day. Background measurements (natural radioactivity) were made at intervals during each series of measurements.

RESULTS AND CONCLUSIONS

The marked inhibitory action of INBA on the growth of the dicotyledonous bean plant and its slight action on the monocotyledonous barley plant are illustrated in Table 1.

TABLE 1

EFFECT OF RADIOACTIVE INBA ON GROWTH OF PARTS OF BEAN AND BARLEY PLANTS

Part	Untreated* control (mg./plant)	Treated* (mg./plant)	Wt. reduction (%)
Bean			
Primary leaf	218	193	11.5
Bud	15	9†	40.0
First internode	33	28‡	15.2
Hypocotyl	94	78†	17.0
Total above-ground portion	360	308‡	14.4
Barley			
First leaf	130	116	10.8
Second leaf	120	112	6.7
Stem§	122	110	9.8
Total plant	372	338	9.1

* Figures represent average fresh weight per plant 3 days after treatment, based on pooled samples of corresponding parts of 15 bean and 12 barley plants.

† Difference between corresponding parts from treated and untreated plants highly significant.

‡ Difference between corresponding parts from treated and untreated plants significant

§ Includes the stem and the section of leaves that sheathes the growing point.

In the bean plant the compound is most effective in inhibiting development of the bud. It should be pointed out that there is no apparent difference in the growth responses resulting from application of stable INBA and of radioactive INBA when equal amounts of the two compounds are applied separately to the leaves of bean seedlings.

The measurements given in Table 2 indicate that the compound was apparently absorbed by the leaves of bean

[3] The authors wish to express their appreciation to Dean Cowie, P. H. Abelson, and Charles Ksanda, Department of Terrestrial Magnetism, Carnegie Institution, for permitting them to use an electrometer and for guidance and assistance during these investigations.

seedlings and that it was translocated from treated leaves to the stems of the plants, where it accumulated mainly in the terminal buds and in the hypocotyls (Fig. 1). Greatest reduction in growth was observed in those parts of bean plants which became most highly radioactive as a result of the treatment.

Radioactive INBA was also apparently absorbed by the leaves of barley plants and accumulated mainly in the second leaf, which developed rapidly during the period of treatment (Table 3, Fig. 1).[4] In contrast to the results obtained with the bean plants, the presence of radioactive INBA in barley plants was not associated with a statistically significant decrease in their rate of growth. Since INBA appears to be absorbed by both bean and barley plants in these experiments, it must be inferred that the growth-inhibiting effects of INBA in the bean plant and its failure to produce significant inhibition in the barley plant must be due to differences either in INBA concentrations in the two plant types or in the manner in which INBA reacts with the plant constituents in each case.

The appearance of radioactivity in the plants following treatment with radioactive INBA does not prove, of course, that the intact INBA molecule actually entered the plant and was translocated as such. In subsequent experiments it has been shown, however, that the application of elemental iodine, 3-nitrobenzoic acid, or 3-nitrosalicylic acid fails to bring about responses in the bean plant similar to those resulting from application of INBA. Although these data do not constitute conclusive proof, it appears, in the case of the bean plants at least, that INBA enters the plants in molecular form and is translocated as such.

TABLE 2

RESULTS OF RADIOACTIVITY MEASUREMENTS ON PARTS OF BEAN PLANTS AFTER TREATMENT WITH RADIOACTIVE INBA

Plant part	Fresh wt. 46 plant parts (mg.)	Dry wt. 46 plant parts (mg.)	Net activity*	INBA/100 mg. dry plant part (γ)	INBA/single plant part† (γ)	INBA/unit net activity (γ)
Terminal bud	4,610	544	2.23	12.9	1.53	
Treated leaf section	34,700	3,820	0.048	0.277	0.230	
" petiole	7,940	526	0.162	0.935	0.107	
Untreated leaf	46,200	5,080	0.003	0.017	0.019	
" petiole	8,180	554	0.018	0.014	0.001	
First internode	17,400	1,274	0.184	1.06	0.294	
Hypocotyl	32,400	2,876	0.200	1.15	0.719	
Leaf standard: 1/10,000			1.59	10.0		6.29
Petiole standard: 1/10,000			1.82	10.0		5.49
			1.67	10.0		5.99
Leaf standard: 1/100,000			0.114	1.00		8.77‡
Petiole standard: 1/100,000			0.188	1.00		5.32
Leaf standard: 1/1,000,000§						
Petiole standard: 1/1,000,000§						
Average						5.77

* Corrected for background.

† Calculated from the relationship, $\frac{a \times b/100}{46}$, where a = dry weight (mg.) of 46 plant parts and b = INBA (γ)/100 mg. of plant part.

‡ Not included in average.

§ Radioactivity too weak to detect.

TABLE 3

RESULTS OF RADIOACTIVITY MEASUREMENTS ON PARTS OF BARLEY PLANTS AFTER TREATMENT WITH RADIOACTIVE INBA

Plant part	Fresh wt. 37 plant parts (mg.)	Dry wt. 37 plant parts (mg.)	Net activity*	INBA/100 mg. dry plant part (γ)	INBA/single plant part† (γ)	INBA/unit net activity (γ)
Treated first leaf	3,600	275	0.139	1.08	0.080	
Second leaf	3,440	384	0.919	7.16	0.743	
Stem and sheath	3,290	220	0.419	3.26	0.194	
Root	8,800	750	Nil			
Untreated whole barley			Nil			
Leaf standard: 1/5,000‡			2.42	20.0		8.26
Petiole standard: 1/5,000‡			2.76	20.0		7.25
Leaf standard: 1/5,000‡			2.38	20.0		8.40
Petiole standard: 1/5,000‡			2.63	20.0		7.60
Leaf standard: 1/10,000‡			1.04	10.0		9.62§
Petiole standard: 1/10,000‡			1.34	10.0		7.46
Average						7.79

* Corrected for background.

† Calculated from the relationship, $\frac{a \times b/100}{37}$, where a = dry weight (mg.) of 37 plant parts and b = INBA (γ)/100 mg. of plant part.

‡ These standards were prepared from parts of the bean plants and are the same ones reported in Table 2. A comparative analysis made at a later date between unit net activities of the bean plant standards and whole barley plant standards of the same concentration failed to show a difference greater than the 10–15 per cent which exists between duplicate analyses.

§ Not included in average.

[4] The fact that no radioactivity could be detected in the large, composite barley root sample means only that an insignificant portion of the applied INBA is present in the roots three days after application. This does not preclude the possibility, however, that a relatively high concentration of INBA might have been found in the actively growing root tips if it had been feasible to sample and measure the radioactivity of this extremely small fraction of the plant. No measurements were made on the roots of treated bean plants.

References

1. CULHANE, P. J. In *Organic syntheses*. (Coll. Vol. 1.) New York: John Wiley, 1932. Pp. 120–122.
2. JAMES, C. W., KENNER, J., and STUBBINGS, W. V. *J. chem. Soc.*, 1920, **117**, 773–776.
3. MITCHELL, J. W., and BROWN, J. W. *Bot. Gaz.*, 1946, **107**, 393–407; WEAVER, J. R., and DEROSE, H. R. *Bot. Gaz.*, 1946, **107**, 509–521.
4. ZIMMERMAN, P.W., and HITCHCOCK, A. E. *Contr. Boyce Thompson Inst.*, 1942, **12**, 321–343; 491–496.
5. ———. Availability of radioactive isotopes. *Science*, 1946, **103**, 697–705.