Acta Botanica Neerlandica **10** (1961) 99–104

TRANSLOCATION OF 3-AMINO-1, 2, 4-TRIAZOLE IN PLANTS

II. INHIBITION STUDY

P. MASSINI

(Philips Research Laboratories)

Eindhoven

(received December 10th, 1960)

INTRODUCTION

In a previous paper (MASSINI, 1958) the uptake and translocation of 3-amino-1, 2, 4-triazole (AT) and 3-hydroxy-1, 2, 4-triazole (OT) had been studied. It was concluded that AT was translocated by both phloem and xylem vessels, whereas OT was only mobile within the xylem system. The present paper reports a study of the translocation and its inhibition by light-starvation and by cyanide of amino-triazole.

MATERIALS AND METHODS

Seedlings of tomato and French dwarf bean have been used as described earlier (MASSINI, 1958). AT, labelled with C^{14} in the 5-position with a specific activity of 2 mc/mmole was used. For foliar uptake, 10 μl of a 0.05 M solution of AT was applied to one leaf either by smearing it on within a ring of lanoline that had a diameter of 12 mm, or by the leaf injection method of BIDDULPH (1941). For uptake through the stem, the roots of bean plants were cut off under water and the plants placed in Erlenmeyers flasks containing a 5.10^{-5} M solution of AT.

The translocation of AT was inhibited by keeping a section of the stem or of a petiole in an atmosphere containing HCN prior to and during the treatment with AT. Small cylindrical gas chambers were constructed from perspex. The chambers consisted of two halves of a hollow cylinder with a length of 30 mm and a diameter of 16 mm (internal dimensions). One half-cylinder was lined with filter paper which was soaked with 0.1 cc of cyanide buffer (ROBBIE, 1948). The buffer contained 0.5 M KOH and KCN at a concentration such that a solution of physiological pH which was in equilibrium with the buffer via the gas phase acquired a certain constant concentration of HCN. The concentrations of KCN used and the corresponding equilibrium concentrations of HCN are listed in Table 1.

TABLE 1

Composition of gas chamber fluid according to ROBBIE (1948)

Concentration of KCN (M) in 0.5 M KOH	Equilibrium concentration of HCN at 20° C (M)
0.50	4.6×10^{-5}
2.0	1.8×10^{-4}
5.5	4.6×10^{-4}

A section of petiole or stem was enclosed between two half-cylinders and a gas-tight seal made with lanoline. The chamber was supported in an appropriate way, so that the stem was not loaded by its weight. Fig. 1 shows a gas chamber mounted on the petiole of the third leaf of a tomato plant. The lateral leaflets were cut off in order to expose a sufficient length of petiole. In this experiment AT was applied by the leaf smear method.

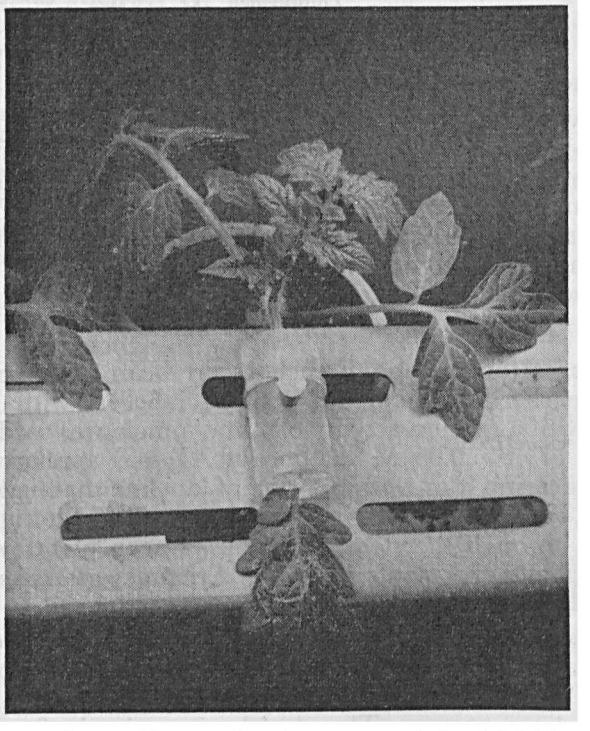

Fig. 1. Tomato plant with gas chamber mounted for inhibition experiment. AT was applied to the leaflet protruding from the gas chamber.

The control plants were treated in the same way except that the lining of the gas chambers was soaked with 0.5 M KOH.

The plants were mounted in a hood under a row of fluorescent lamps (light intensity 2000 $\mu W/cm^2$). After 5 hours of pretreatment with cyanide the solution of AT was applied, and after 17 to 19 hours of translocation the plants were harvested and cut into parts. These parts were extracted at room temperature, first with 10 cc of 70 % alcohol containing 5 % formic acid, then with 10 cc of water. Previous experiments had shown that with this procedure 80 to 90 % of the radio-activity was extracted. An aliquot of the collected extracts was evaporated on an aluminium planchet and assayed for radioactivity.

The influence of cyanide on the respiration was studied manometrically in a Warburg respirometer, at 20° C in the dark. Each

vessel contained: a disc of filter paper, soaked with 0.2 cc of water; some 600 mg of petiole sections (tomato) or 400 mg of stem sections (bean), cut to pieces of 5 to 10 mm; 0.6 cc cyanide buffer or 0.5 M KOH in the side-arm. The atmosphere was air. The measurement of the respiration was started 15 minutes after closing the vessels and continued for 3 hours.

For the starvation experiments bean plants grown in a gravel culture were used. The dark plants were kept in the dark for 48 hours, then treated with AT under a red safelight, then put back in the dark for another 24 hours. The light plants were grown in 16 hours light per day and kept in 24 hours of continuous light after the treatment with AT. 42 μg of AT was applied to a primary leaf, either by the leaf smear or by the leaf injection method. After the translocation period, the treated leaf was cut off and the treated area punched out and discarded. The treated leaf and the rest of the plant were extracted separately and the extracts assayed for radioactivity.

Results

The results of the inhibition experiments are compiled in Table 2. In experiment 1 the plants were mounted and treated as shown in Fig. 1. After the translocation period the treated leaf, including the petiole section enclosed in the gas chamber, was discarded and the rest of the plant assayed for radioactivity. The translocated amount is expressed in this experiment as a percentage of the amount applied. With 4.6×10^{-5} M HCN the inhibition was not significant, but 4.6×10^{-4} M HCN inhibited the translocation almost completely. The lower concentration inhibited the respiration of petioles by 50 %; with the higher concentration the inhibition was 75 %.

After one experiment the respiration of some non-treated leaves of a plant that had been treated with 4.6×10^{-4} M HCN (cf Fig. 1) was measured manometrically; it did not differ from the value for untreated plants. It seems thus that the action of the HCN is localized to the treated leaf.

In experiments 2 and 3 bean plants were treated in an analogous manner by applying AT to a primary leaf by the two methods indicated. The gas chamber was mounted around the stem above the node of the primary leaves, and after the translocation period the treated leaf (without the treated area in experiment 2), the top (above the chamber) and the rest of the plant were assayed separately. The translocation past the gas chamber is expressed as activity in the top in percent of the total activity in the plant. In both experiments 4.6×10^{-4} M HCN inhibited the translocation of AT almost completely. This concentration also inhibited the respiration of stem sections almost completely, whereas with 1.8×10^{-4} M HCN the respiration was almost normal, although the effect on the translocation of AT was still very pronounced. The uptake of AT and the translocation to parts other than the top were not affected by the treatment with HCN.

TABLE 2

HCN inhibition of translocation

Exp. no.	Plant	Place of application of AT	Method of application	Place of gas chamber	Part assayed for translocation	Concentration of HCN, M	Translocation past chamber, % of amount taken up	Inhibition %	Ns. of plants
1	tomato	terminal leaflet 3rd leaf	leaf smear	Petiole 3rd leaf	whole plant except treated leaf	0	2.5	—	4
						4.6×10^{-4}	0.055	98	4
						0	3.7	—	4
						4.6×10^{-5}	3.2	14	4
2	bean	primary leaf	leaf smear	internode above primary leaf	top	0	13	—	2
						1.8×10^{-4}	6	50	3
						4.6×10^{-4}	1	92	3
3	bean	primary leaf	leaf injection	internode above primary leaf	top	0	3.0	—	3
						1.8×10^{-4}	0.21	93	3
						4.6×10^{-4}	0.22	93	3
4	bean	through stem		internode above primary leaf	top	0	46	—	3
						1.8×10^{-4}	43	—	3
						4.6×10^{-4}	57	—	3

In experiment 4 the AT was fed through the stem. The gas chambers were mounted on the same place and the plants were assayed in the same manner as in the other experiments. In this experiment the translocation past the treated section was not inhibited by 4.6×10^{-4} M HCN (the apparent slight stimulation was not significant).

After the translocation period the treated stems had often lost their turgescence or were even shrivelled completely. The inhibition of translocation was not reversible; after a treatment sufficient to produce inhibition the stems did not recover again.

The results of the starvation experiments are listed in Table 3. It is shown that the translocation out of the treated leaf is strongly dependent on light. In the leaf smear experiment the darkened plants absorbed more AT than the light plants, possibly because in the former the treated area stayed wet for a longer time due to the lower surface temperature.

TABLE 3

Influence of light on uptake and translocation of AT by bean plants. (2 plants per treatment)

Method	Treatment	µg of AT taken up [1]	Translocated [2]	Translocation %
Leaf smear	dark	4.6	0.03	0.7
„ „	light	2.4	0.5	23
Leaf injection	dark	8.2	0.04	0.5
„ „	light	8.8	1.0	11

[1] Without treated area.
[2] Out of the treated leaf.

Attempts were made to replace the light by sucrose: starved bean plants were painted with a 0.3 M solution of sucrose two hours and one hour before the treatment with AT. However, the translocation out of the treated leaves was the same as in the starved control plants.

DISCUSSION

Dependence of translocation in plants on photosynthesis or some other source of carbohydrates had been found earlier. Several authors found that the translocation of (2,4-dichloro-phenoxy) acetic acid was stimulated by light or by application of sucrose (MITCHELL and BROWN, 1946; ROHRBAUGH and RICE, 1949; and others). HAY and THIMANN (1956) found the same dependence for the translocation of phosphate. In our experiments the light could not be replaced by sucrose, but this failure may be caused by an experimental difficulty rather than by a fundamental difference.

The inhibition of the translocation of phosphate by respiratory poisons has been described by KENDALL (1955). WILLENBRINK (1957)

made a thorough investigation on the inhibition of phloem transport of various inorganic and organic substances. He found that the translocation from a leaf of Pelargonium zonale of C^{14} labelled assimilates, of fluorescein, of N and of P compounds could be inhibited reversibly by incubating the phloem tissue of the petiole in an atmosphere containing HCN. The atmosphere was in equilibrium with a 10^{-3} M solution of KCN. It is difficult to compare the concentrations of HCN, on the one hand because Willenbrink's KCN solution was not buffered, and on the other hand because his technique—exposure of the central phloem tissue directly by cutting away the surrounding tissue—greatly facilitated the action of the poison.

In our experiments the action of the HCN was not reversible, and the wilting of the treated stems suggests that the poison may have killed the tissue. So it is not possible to decide if inhibition of the respiration alone is sufficient for a blocking of the translocation. The concentration of HCN which blocked the translocation also inhibited the respiration of the stem sections, but the degree of inhibition of both processes was different at different concentrations.

Comparison of experiments 3 and 4 indicates that in the bean plant the translocation of AT from a leaf to the top requires the presence of living tissue, and presumably occurs in the sieve tubes, whereas the same substance taken up by the transpiration stream passes through xylem tubes by a mechanism which is independent of living tissue. In this respect the conclusions arrived at in the previous paper and which were based on the distribution of AT in the plant after application to different parts, could be confirmed by the experiments described here.

SUMMARY

The translocation of AT out of leaves of tomato and bean plants has been found to be dependent on light and to be inhibited by HCN. The transport of AT taken up by the transpiration stream was not inhibited by the poison. The concentration of HCN which blocked translocation also inhibited the respiration of stem sections.

REFERENCES

BIDDULPH, O. 1941. Am. Jour. Bot. **28**: 348.
HAY, J. R. and K. V. THIMANN. 1956. Plant Physiol. **31**: 446.
KENDALL, W. A. 1955. Plant Physiol. **30**: 347.
MASSINI, P. 1958. Acta bot. Neerl. **7**: 524.
MITCHELL, J. W. and J. W. BROWN. 1946. Botan. Gaz. **107**: 393.
ROBBIE, W. A. 1948. Methods in Medical Research **1**: 307.
ROHRBAUGH, L. M. and E. L. RICE. 1949. Botan. Gaz. **111**: 85.
WILLENBRINK, J. 1957. Planta **48**: 269.