Translocation of Radioactive Kinetin¹, ²

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Summary. Kinetin has generally been thought to be immobile in plants. This was confirmed in the case of laminar applications in this study, but not in regard to petiole, vein, or root applications. Radioactivity from kinetin-8-¹⁴C (Kn*) moved freely in the vascular system of several types of leaves. This movement was usually distal to the point of application and seemed to occur with the transpiration stream. Basipetal as well as acropetal translocation of radioactive kinetin was achieved in tobacco leaves. The translocated material was extracted from veinal tissue, shown to be radioactive, and to be able to retard senescence. Similar but less decisive results were obtained from agar blocks inserted into the vascular system of leaves receiving Kn* by petiole uptake.

A bioassay employing disks from primary bean leaves was developed for the qualitative determination of substances like kinetin which possess the ability to retard chlorophyll breakdown and plant senescence. The use of radioactive kinetin provided a refinement in this bioassay because treated non-senescent areas could be correlated with exposed areas on radioautographs made from dried leaf disks.

Root treatments showed that cotton seedlings did not take up Kn* but that similarly treated tobacco seedlings both absorbed and translocated the isotope readily.

The pattern of translocation of kinetin in plants has received only cursory attention because of the difficulties involved in demonstrating that the applied substance rather than a metabolite was translocated. The availability of radioactive kinetin has made such translocation studies more feasible. Kinetin has generally been considered to be immobile in plants because of responses obtained near the site of application (9). Kinetin, applied as a spot to the lamina of the leaf, has prevented senescence as measured by chlorophyll degradation (6,7). Various plant metabolites appear to be mobilized towards such kinetin treated areas (4,5).

Richmond and Lang (8) were first to report the senescence-retarding effect of kinetin. Though not emphasized by them or by subsequent reviewers, they also demonstrated that kinetin could be translocated through cocklebur petioles. They exposed petioles of detached leaves to kinetin solutions and observed senescence retardation in the leaf blade.

The current study was conducted to determine the general patterns of kinetin-8-¹⁴C (Kn*) translocation in selected plants.

Materials and Methods

Radioactive Kinetin. Chromatographically pure Kn* was obtained from the British Radiochemical Center, Amersham, England. It was co-chromatographed with kinetin from Nutritional Biochemicals Corporation and found to have the same R_f value. Whatman No. 1 filter paper was used for ascending chromatography with an n-butyl alcohol: ammonia solvent system. The specific activity of the sample was 9.89 mc/mM from which a kinetin stock solution of 40 mg/l concentration was prepared.

Plant Materials. Two species of tobacco, Nicotiana xanthi and N. tabacum L. var. Samsun were used for the major part of this study along with leaves of cotton, Gossypium hirsutum var. Deltapine 14, cocklebur, Xanthium pensylvanicum L. and bean, Phaseolus vulgaris var. Blue Lake. The

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leaves of these plants do not autolyse rapidly when placed in the dark, and as a result they have been used in studies of senescence as well as translocation.

Methods of Treatment. Kinetin-8-\textsuperscript{14}C was applied to leaves in varying concentrations by several different methods: as spots within lanolin rings, painted on half-leaves, and by petiole uptake. The roots of cotton and tobacco seedlings were placed in a solution of Kn\* at a concentration of 1 mg/l. Both acropetal and basipetal translocation were studied in tobacco leaves. From 2 to 6 replications of each treatment were radioautographed in each experiment.

Maintenance of Detached Leaves. Petioles of treated, detached leaves were inserted in orchid vials of water; the leaves were maintained in clear plastic boxes. These boxes were provided with small holes for ventilation and lined with moist paper towels to retard transpiration (3). The Kn\* treated leaves were placed in continuous darkness to accelerate senescence. Pictures were taken of the fresh leaves prior to killing to show differences in chlorophyll breakdown in treated and untreated areas.

Radioautographic Techniques. Excised leaves were heat killed and dried in a forced draft oven, then radioautographed. Exposed areas on the film were correlated with green areas of the fresh leaf. Replicate samples of leaves were quick frozen and radioautographed as a check on the oven drying procedure. The general distribution of the label was the same with both techniques. Tobacco seedlings which were root-treated with Kn\* had their roots, stems, and leaves dried separately (1). Thus, any radioactivity present in the various parts had to be there at the time of harvest and could not be due to movement during drying.

Kinetin Leaf Disk Bioassay. A bean leaf disk bioassay was developed for the qualitative determination of substances having the ability to retard chlorophyll breakdown. Disks from fully expanded primary bean leaves were spotted with extracts from Kn\* treated leaves or exposed to agar blocks which had been allowed to take up translocated substances. These disks were placed on moist filter papers in Petri dishes with appropriate controls and allowed to senesce in the dark. A positive test for the senescence-retarding effect of kinetin was the presence of a green island in the treated area of an otherwise chlorotic leaf disk. This green island was subsequently matched to an exposed area on a radioautograph. This senescence retarding bioassay was not consistently reproducible with kinetin concentrations below 1 mg/l; however, radioactivity could be detected even when the bioassay was negative.

Obtaining Translocated Substances. The translocated substance was obtained by 2 methods. Blank agar blocks prepared with water were temporarily inserted into a notch cut into the main vein of tobacco leaves actively taking up Kn\* through their petioles. Some of these agar blocks were checked for radioactivity in a liquid scintillation counter and others were bioassayed with bean leaf disks and subsequently radioautographed. The second method of obtaining the translocate was by extraction of main and lateral veins of tobacco leaves following petiole uptake of Kn\*. The veins were sectioned and then extracted 3 times with absolute alcohol. Chlorophyll was removed by partitioning with petroleum ether which also removed some radioactivity. The 3 volumes of extract were pooled, evaporated to about 5 ml, and acidified. The extract was made aqueous and concentrated to 1 ml by evaporation. The pH was adjusted to 5 with diibasic sodium phosphate as determined by pH 3orion paper. Drops of extract were applied to bean leaf disks to determine the presence of senescence-retarding substances.

Results

Spot Application of Kn\* to Leaf Lamina. Figure 1a shows a tobacco leaf spot-treated with Kn\* at a concentration of 20 mg/l. Two spots were made on this leaf with 100 \( \mu l \) of Kn\* placed in each of 2 lanolin rings. The green areas remaining in the leaf after 9 days in the dark illustrate the ability of kinetin to retard chlorophyll breakdown and leaf senescence. Figure 1b is a radioautograph of the same leaf. The exposed areas in the radioautograph correlate with the treated areas in figure 1a and show no spread of radioactivity from the applied spot.

Spot Application of Kn\* to Leaf Veins. Figure 2 shows a radioautograph of 3 tobacco leaves following spot applications of Kn\* at proximal, middle, and distal positions on the main vein. The leaves were killed 24 hours after treatment, thus retardation of senescence was not determined. The radioautograph indicates that the radioactivity readily enters the leaf veins and spreads almost completely in the area distal to the applied spot, presumably with the transpiration stream. Spotting treatments on cotton leaves produced similar results.

Figures 3a and 3b represent radioautographs from detached tobacco leaves which were spotted with Kn\* at the center of the main vein and maintained in the dark for 2 and 14 days prior to killing. The 2 figures indicate very little redistribution of radioactivity after 2 days, but after 14 days considerable redistribution had occurred. The identity of the redistributed \( ^{14}C \) was not determined.

Surface Application of Kn\* to Detached Leaves. The distal or proximal halves of tobacco leaves were painted with Kn\* at a concentration of 20 mg/l and placed in the dark. After 5 days in the dark, the treated areas exhibited very little chlorophyll breakdown (figs 4a and 5a). This was especially striking in figure 4a because leaves
Fig. 1. a) Spot applications of kinetin-8-14C at a concentration of 20 mg/l show retardation of chlorophyll breakdown in the treated areas of an old tobacco leaf maintained in the dark for 9 days. b) Radiocautograph of the leaf in 1a.

Insert
Fig. 2. Translocation of radioactivity from spots of kinetin-8-14C applied in proximal, middle and distal positions on the main vein of tobacco leaves. Leaves were killed 24 hours after treatment.

Inset:
Fig. 3. a) Translocation of radioactivity from a spot of kinetin-8-\textsuperscript{14}C applied to the center of a main vein of a tobacco leaf. Very little basipetal movement of \textsuperscript{14}C is apparent 2 days after treatment. b) Similarly treated leaf 14 days after treatment. A redistribution of the radioactivity is apparent.
Fig. 4. a) Tobacco leaf showing senescence-retarding effect of kinetin-$\text{R}$.\textsuperscript{14}C when applied on the distal half at a concentration of 20 mg/l. The leaf was maintained in the dark for 5 days. b) Radioautograph of the leaf in 4a. It shows no basipetal translocation of $\textsuperscript{14}$C when the distal half of the leaf was treated.

Insert
Fig. 5. a) Tobacco leaf showing senescence-retarding effect of kinetin-8-\textsuperscript{14}C when applied on the proximal half at a concentration of 20 mg/l. The leaf was maintained in the dark for 5 days. b) Radioautograph of the leaf in 5a. It indicates that the leaf veins treated in proximal applications, absorbed some radioactivity and translocated it acropetally.

Insert
Fig. 6. Small orbial vial with treatment solution of kinetin-8-\(^{14}\)C placed on the main vein of a tobacco leaf for basipetal translocation experiment.
Fig. 7. Radioautograph of the leaf treated with 0.5 ml kinetin-8-\(^{14}\)C, and the leaves above it. Translocation of the isotope was basipetal in the leaf and acropetal in the stem. Little lateral movement of the isotope appeared to have occurred in the stem since only leaves in the orthostichy were radioactive.
usually become chlorotic at the distal end first. Figures 4b and 5b show that the radioactive portions of the leaf were correlated with the green areas shown in figures 4a and 5a.

This method of leaf treatment substantiates results obtained in the leaf spotting treatments. The distal application of radioactive kinetin (figs 4a and 4b) exhibited no basipetal movement of the label. The proximal application (figs 5a and 5b) indicated that some of the radioactivity moved into the veins and extended into the untreated area.

Acropetal Translocation of Kn*. Several experiments with detached tobacco leaves have shown that Kn* is readily taken up in the transpiration stream when petioles are immersed in the radioactive solution. Radioautographs of these leaves exhibit acropetal movement of the 14C in most of the veinal system of the leaf when leaves were killed after one-half hour of petiole uptake. Uniform distribution of the label into the leaf lamina was usually evident 24 hours after uptake.

Basipetal Translocation of Kn*. Basipetal translocation of Kn* was accomplished by treating the lower leaf of intact tobacco plants and manipulating the environment to accelerate transpiration. One-half ml of Kn* at concentrations of 20 and 40 mg/l was applied to the distal end of the main vein of a basal leaf. This was done by cutting away some of the leaf blade and placing a small orchid vial containing the treatment solution over the end of the exposed main vein (fig 6). By illuminating the top of the plant, the solution was taken up by the apical end of the leaf vein in 30 to 60 minutes.

The radioautograph in figure 7 shows the treated leaf and the leaves above it. The radioactivity moved basipetally in the main vein of the treated leaf. It also moved acropetally in the lateral veins of that leaf. Further presence of the isotope is indicated in one-half of the fourth and in all of the ninth leaf above the treated leaf. This shows that the isotope also moved acropetally in the stem. The fourth and ninth leaves were located partly and directly above the treated leaf.

Free-hand sections cut from the stem and radioautographed verified that little lateral movement of the isotope occurred. Cellular details were not distinguished with this technique, so the identity of the translocating tissue was not established.

Root Treatments of Kn*. Split root treatments with cotton seedlings using Kn* at 1 mg/l for 24 hours showed no uptake of isotope. Only the treated lateral root exhibited radioactivity when exposed to film.

Tobacco seedlings were placed in a solution containing 1.7 mg/l Kn* for 24 hours or 1 mg/l for 48 hours. In each experiment 2 seedlings with intact roots and 2 seedlings with half of the root system removed were used. Excising half of the root system made no difference in uptake by tobacco seedlings as the isotope was readily absorbed and moved throughout the plant whether the roots were cut or not.

Verification of Translocated Kn*. Agar blocks which were temporarily inserted into petiole notches for collecting the translocated solute always yielded radioactivity but usually failed to give a positive kinetin bioassay. A weak but positive kinetin bioassay could be obtained by increasing the concentration of kinetin, the size of the agar block, the depth of the notch, and the length of time the block was exposed to the translocated solute.

Tissue extracts applied as a concentrated drop produced positive results in the kinetin bioassays and correlated with areas of radioactivity.

Discussion

The areas of senescence retardation shown in figures 1a, 4a, and 5a were caused by applied kinetin-8'-14C and could subsequently be verified as being radioactive. Thus laminar applications of Kn* applied as spots appeared to be immobile. However, if such spots covered main or lateral veins some of the radioactivity would penetrate the vein and move distally. On the basis of 90 radioautographs resulting from various spotting treatments with radioactive kinetin it was concluded that these results were consistent for cotton and cocklebur leaves as well as for tobacco, for attached as well as detached leaves and for leaves kept in the dark or in diffused light. Leaf age, however, had a marked influence on the rate of chlorophyll breakdown and thus influenced the appearance of the kinetin response in the fresh state. The most striking results or contrasts were obtained with old leaves which had a tendency to senesce more rapidly.

The correlation of green leaf areas with exposed areas in radioautographs would indicate that the exposed areas correctly depict the location of Kn*. Radioactivity seen in leaf veins distal to the point of application is also indicative of Kn*, but the redistributed 14C in the detached leaf shown in figure 3b could be a breakdown product of kinetin.

Bollard (2) has indicated that the uptake of solutes by cut petioles is believed to proceed in the xylem via the transpiration stream. Experiments involving spotting treatments across leaf veins and petiole uptake both verify that the radioactivity of Kn* moves acropetally. The combination of bean leaf disk bioassays and radioautographs have verified that the translocated material possessed the senescence retarding property of kinetin and radioactivity. Since no other radioactive substances possessing cytokinin activity were used in these tests, the translocated radioactive substance would appear to be Kn* or a metabolite that retained both the cytokinin activity and the 14C label.

It is well recognized that differences exist between plant species in regard to root absorption.
and transport of exogenous substances (2). Such differences in absorption of radioactivity from \( {\text{Kn}}^* \) were observed for root-treated cotton and tobacco seedlings. It was not established that the radioactivity in the tobacco stem and leaves was the actual \( {\text{Kn}}^* \) applied to the roots.

**Literature Cited**


