

アカマツおよびスギ針葉におけるスルファミン酸アンモニウム(AMS)の浸透ならびに移行に関する研究

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論文

Studies on the Penetration and Translocation of Ammonium Sulfamate (AMS) in the Needles of Akamatsu and Sugi*

Masaki ITOH**

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To estimate the relationships between the penetration and translocation of AMS in Akamatsu and Sugi and the susceptibility to its phytotoxicity, the penetration of AMS into the needles of the two species and its translocation in the two species were investigated. The penetration of AMS through stomata is greater into the needles of Sugi than into those of Akamatsu, while cuticular penetration is more in Akamatsu than in Sugi. The cuticular penetration is much less than the stomatal penetration in both species. The penetration during twenty days after spraying the AMS solution was measured, showing that AMS continued to penetrate during the experimental period. In all experiments mentioned above, penetration was increased by adding surfactant Tween 20 to the AMS solution. AMS or its metabolites containing ^{35}S was more easily translocated in Sugi than in Akamatsu, as observed with radioautography.

伊藤昌樹: アカマツおよびスギ針葉におけるスルファミン酸アンモニウム (AMS) の浸透ならびに移行に関する研究 日林誌 58: 41~46, 1976 本研究においては, AMS 水溶液がアカマツならびにスギの針葉のなかへ浸透する量を測定するとともに, その移行のしかたについても調べた。そして, これらと両樹種のあいだでの AMS にたいする感受性のちがいについて比較, 考察した。これによれば, 気孔を通しては, アカマツよりもスギのなかに, 反対に, 葉面のクチクラからはスギよりもアカマツのなかに多くの AMS が浸透した。そして, クチクラを通して行なわれる浸透は, 気孔を通してのものに比較してきわめて少量である。また, AMS 水溶液を噴霧処理して 20 日間にわたってその浸透量を測定した結果によれば, AMS は日数の経過とともに浸透し続ける。そして, その浸透量はアカマツよりもスギにおいて多い。上記のいずれの場合にも, 展着剤, Tween 20 は, AMS の浸透量を増加させる。ラジオオートグラフによって AMS の移行について調べた結果によれば, AMS あるいは ^{35}S を含むその代謝物はアカマツよりもスギにおいて移行しやすい。

I. Introduction

The chemicals retained on the surfaces of plant leaves may penetrate them through cuticle and/or stomata. A previous paper(9) reported some morphological factors which affect the cuticular and stomatal penetration of chemicals in the needles of Akamatsu and Sugi.

The amount of the penetration of herbicide may affect the plant's susceptibility to herbicide. Generally, plants which permit easy penetration of herbicides seem to be susceptible(5, 11). However, some reports(13, 15) suggest that the herbicidal penetration does not always coincide with suscep-

tibility, since the degree of susceptibility can be affected by other factors.

It may be supposed that most herbicidal penetration into plants is through stomata(1, 6) or cuticle (5). Stomatal penetration of chemicals is affected by the stomatal aperture which is influenced not only by physiological factors of the plant but also by environmental factors. Cuticular character does not alter the penetration of herbicide as much as stomata.

Chemicals which penetrate a plant move symplastically and/or apoplastically. Herbicides, especially translocated types, may act on cells in the process of translocation across cells. After they migrate

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** Fac. of Agr., Nagoya Univ., Nagoya 464 名古屋大学農学部 Present address: Kameyama Br. St. of the Oji Inst. for For. Tree Imp., Nobono, Kameyama 519-02. 現在勤務先: 王子製紙(株)林木育種研究所亀山育種場

in the vascular systems and accumulate in the tissues, they may also produce toxic effects on plants. It is reported that the susceptibility of plants to herbicides is affected by the rate and the direction of translocation(14).

Contact herbicides act on plants at the site of application. Ammonium sulfamate (AMS) is the contact type but it shows some translocation in plants. There are few reports about the translocation of herbicides in Akamatsu and Sugi(17).

The present paper examines the penetration and translocation of AMS in Akamatsu and Sugi, and compares the susceptibility of the two species to AMS.

II. Materials and Methods

Plant materials used were Akamatsu (*Pinus densiflora* SIEB. et ZUCC.) and Sugi (*Cryptomeria japonica* D. DON.). The details of these materials are given in the previous paper(9). All experiments were performed in August and September, 1973.

1. Synthesis of AMS-³⁵S

The labeled-AMS was synthesized by the Dupon method. Five mCi of sulfuric acid-³⁵S was mixed with 5 ml of 30% unlabeled-sulfuric acid. This diluted sulfuric acid was deliberately dropped into 50 ml of fuming sulfuric acid (up 60%). The labeled-fuming sulfuric acid in a tap funnel was dropped slowly and carefully into 30 g of urea. The product was dissolved into 70% sulfuric acid with heating. After one night, crude sulfamic acid was obtained by filtering the product with a glass filter. The sulfamic acid was recrystallized to obtain the refined sulfamic acid. Ammonia water (14%) was added to the sulfamic acid, and the product was filtered and oven-dried. Then, 7.3 g of AMS-³⁵S was obtained. The melting point of the AMS was 123~135°C.

2. Treatment of AMS-³⁵S Solution

As the test solution of the labeled-AMS, 20% aqueous solution with and without 0.1% Tween 20 as a surfactant was used. Each solution of 30 μ l was applied as droplets of 1 μ l with a microsyringe to the five needles of Akamatsu and needles of a twig of Sugi at 10:00 a.m. Application in the light was performed outdoors. And that in the dark was done in a dark room.

3. Extraction and Determination of AMS-³⁵S

After a given treatment period, the treated needles of Akamatsu and the twigs with the treated needles of Sugi were harvested and washed for about 30 seconds in running water to remove the unabsorbed AMS. The extraction of the penetrated AMS was carried out basically in accordance with the procedure developed by PEASE(12). The tissues were

cut into small sections and homogenized with distilled water. Then the homogenate was centrifuged for 30 minutes at 10,000 rpm, and the water extract was transferred to a beaker. Residue was recentrifuged as before, and the extract was collected. Then the extract was evaporated with an infra-red lamp and concentrated to a volume of 5 ml. The concentrated extract was decolorized with 0.2 g of active carbon and filtered to 10 ml of extract of the penetrated AMS-³⁵S.

The radioactivity of ³⁵S in the water extract was measured with a liquid scintillation spectrometer. The counting vial contained 2 ml of the extract and 10 ml of the scintillation solution which had the following composition: *p*-dioxane, 1 l; PPO, 5 g; naphthalene, 100 g. All experiments were repeated twice.

4. Treatment and Determination of Unlabeled-AMS

As applied solutions, a 3% aqueous solution of AMS and one containing 0.1% of Tween 20 were used. The solution of 100 ml was sprayed on the foliage of a seedling. To prevent AMS penetration through the soil surface of a pot, pot and soil surfaces were covered with polyvinyl sheets. After the application plants were kept in a green house. The needles of Akamatsu and the twigs with needles of Sugi were harvested and washed in running water for about 30 seconds. The content of sulfur in the plant tissues was determined by a LECO sulfur determinator. The amount of the penetrated AMS was established from the differences of sulfur content among control and treated plants.

5. Autoradiography in the Needle

As the treated solution, 20% AMS-³⁵S aqueous solution containing 0.1% Tween 20 was used. Treatments were performed outdoors. Two droplets of the 1 μ l aqueous solution were placed on a needle of Akamatsu and two needles of a twig of Sugi. After the treatment periods of five and twenty-four hours, the treated tissues of the two species were harvested. The tissues were washed in running water and blotted, and then were pressed and dried by heated iron. The dried plant tissues were mounted on cardboard, covered with a film of Saran Wrap and exposed to Fuji X-ray film. After exposure time of approximately four months, the film was developed in a usual procedure.

6. Autoradiography in the Seedling

Thirty droplets of the 1 μ l of 20% AMS-³⁵S solution containing 0.1% Tween 20 were placed on the needles of each species. Five days after the application, all the seedlings were harvested. The method used for mounting is described by LUND-HØIE & BAYER(11). The representative needles or

twigs and lateral roots as well as the cut sections of approximately 0.5 mm thickness from stem, main roots and branches were mounted on cardboard in their respective positions. After the mounted specimens were air dried, autoradiographs of them were obtained by the procedures mentioned above.

III. Results

1. Penetration of AMS

The penetration of AMS in the light is shown in Fig.1. AMS penetrated more into the needles of Sugi than into those of Akamatsu whether the sur-

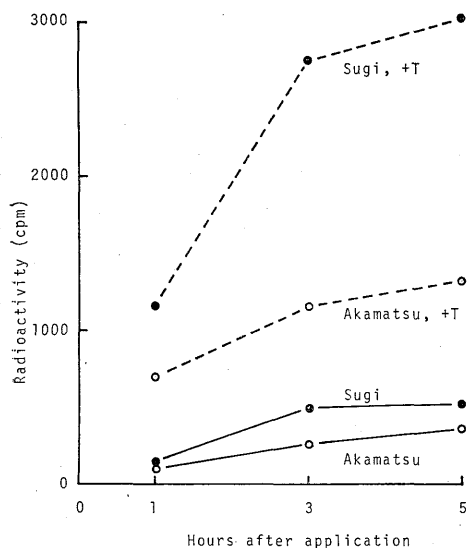


Fig.1. The penetration of AMS- ^{35}S into the needles of Akamatsu and Sugi treated in the light +T indicates that the AMS solution contains Tween 20.

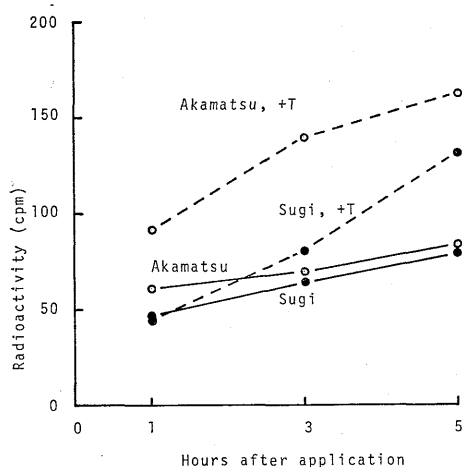


Fig.2. The penetration of AMS- ^{35}S into the needles of Akamatsu and Sugi treated in the dark +T indicates that the AMS solution contains Tween 20.

factant was present or not. When the surfactant was added to the AMS solution, the penetration accelerated in both species. Consequently, the addition of the surfactant to the AMS solution made the difference of the penetration between the two species noticeable.

The penetration of AMS in the dark was significantly less than that in the light as shown in Fig.2. AMS penetrated more into the needles of Akamatsu than Sugi, which showed opposite results in the light. The effect of the surfactant on increasing the penetration was apparent in both species, and the resultant increment of the penetration was higher in Akamatsu than in Sugi. The penetration increased during the treatment period of five hours.

Measurements of the penetration in the outdoors were made for five days (Fig.3). One day after the application of AMS the penetration into the needles of Sugi was more than Akamatsu. The surfactant made the penetration of AMS increase in both species. Three days after the application the radioactivity in the needles in all treatments reached the maximum and then decreased.

The penetration of the sprayed unlabeled-AMS solution was measured for twenty days after the application (Fig.4). The figures show that AMS continued to penetrate into the needles of both species for the experimental period. When the

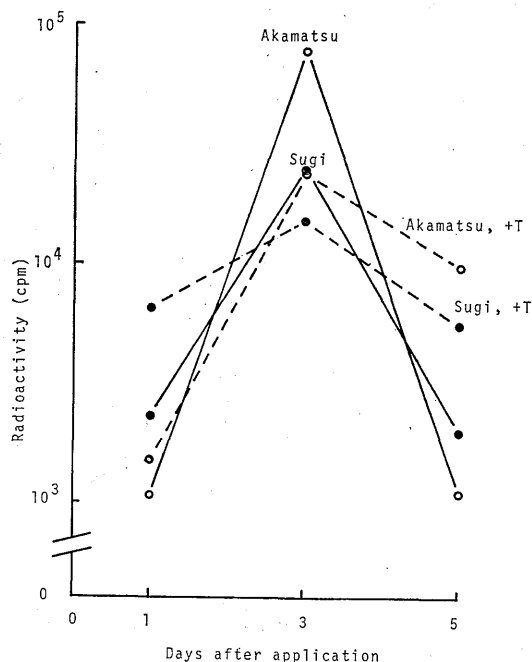


Fig.3. The penetration of AMS- ^{35}S into the needles of Akamatsu and Sugi during five days +T indicates that the AMS solution contains Tween 20.

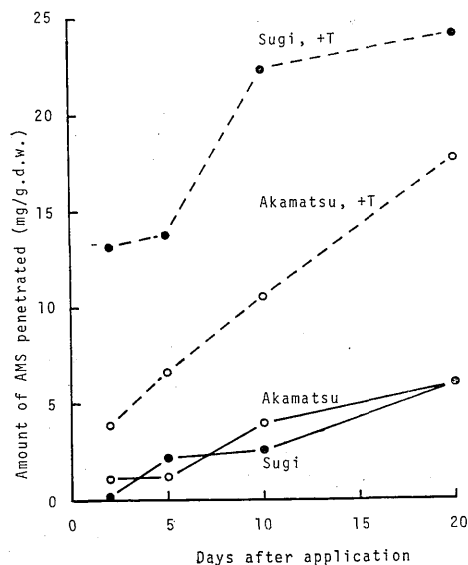


Fig. 4. The penetration of AMS into the needles of Akamatsu and Sugi during twenty days
+T indicates that the AMS solution contains Tween 20.

spraying solution did not contain the surfactant, there was no apparent difference of penetration between the two species. However, when the surfactant was added to the spraying solution, the difference of the penetration became more distinct than when it was not added.

2. Translocation of AMS

The translocation of ^{35}S in the needle of the two species in the light is shown in Figs. 5 and 6 respectively. ^{35}S translocated in the needle of Akamatsu upward and downward from the applied points, but had not extended throughout the needle

within twenty-four hours after the application. ^{35}S translocated in the treated needle of Sugi five hours after the application, and twenty-four hours after the application, it had extended throughout in the twig.

Translocation of ^{35}S in the seedlings of Akamatsu and Sugi during five days is shown in Figs. 7 and 8 respectively. The autoradiographs of each species differ remarkably. In Akamatsu, translocation of ^{35}S was limited to the treated needle. In Sugi, ^{35}S translocated not only in the twig with the treated needles but also to the upper and lower twig. Moreover, it moved downward in the phloem of the lateral branch and the stem.

IV. Discussion

Many studies have been made on the stomatal and cuticular penetration of chemicals(1, 6, 7, 16), but very few reports have yet been published on the penetration of AMS. On the basis of the present results, the stomatal penetration of AMS is greater in Sugi than in Akamatsu, while the cuticular penetration is greater in the latter species than in the former. In the previous paper(9), some morphological factors affecting the penetration of chemicals were reported in the needles of the two species. Based on the results obtained, the sunken stomata in Akamatsu, the difference of the thickness of the cuticle and the leaf surface wax and that of the stomatal density between the two species may be supposed to contribute effectively towards the difference of the stomatal and cuticular penetration of AMS between them.

The results in Figs. 1 and 2 are obtained from the short-term experiments carried out in the light and in the dark. Long-term study of the penetra-

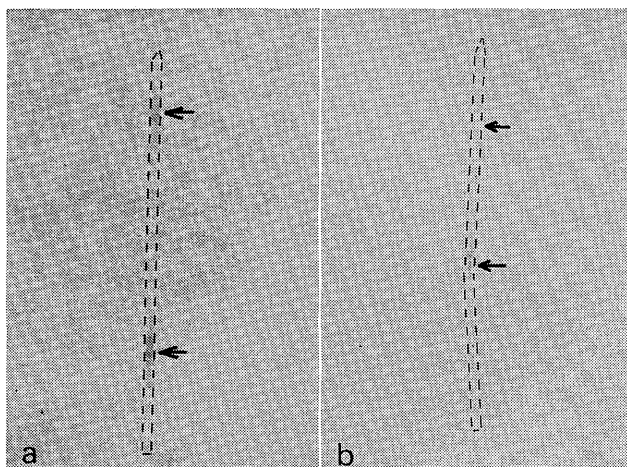


Fig. 5. Radioautographs of the needles of Akamatsu 5 hours (a) and 24 hours (b) after the application of AMS- ^{35}S (arrows)

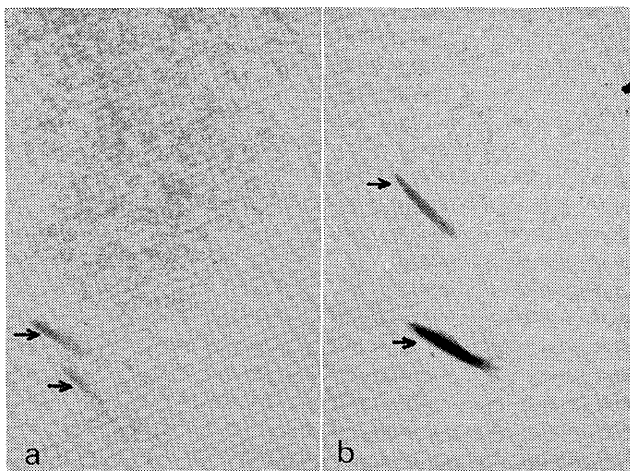


Fig. 6. Radioautographs of the twigs containing the treated needles of Sugi 5 hours (a) and 24 hours (b) after the application of AMS-³⁵S (arrows)

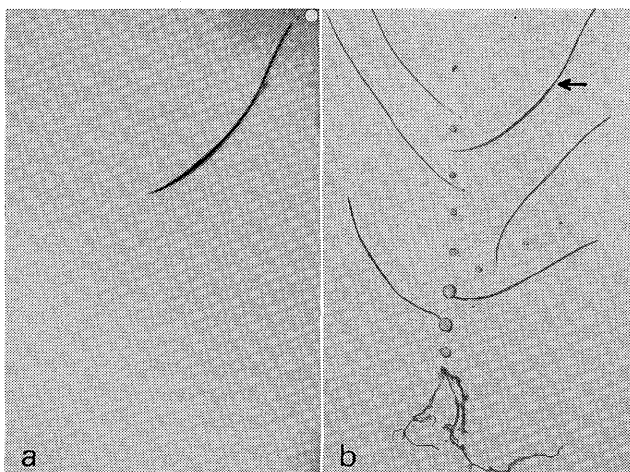


Fig. 7. Radioautograph (a) of the seedling (b) of Akamatsu 5 days after the application of AMS-³⁵S (arrow)

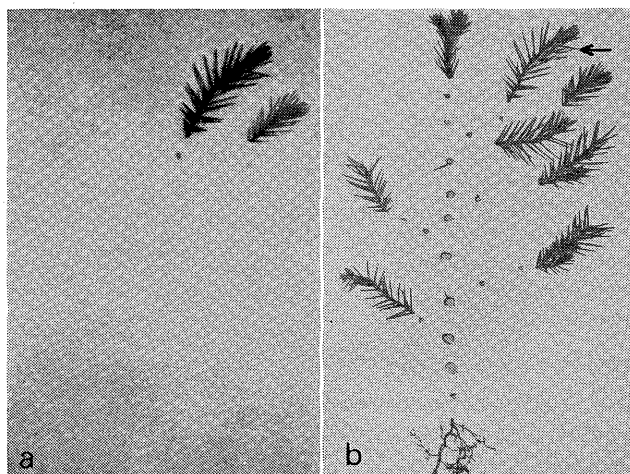


Fig. 8. Radioautograph (a) of the seedling (b) of Sugi 5 days after the application of AMS-³⁵S (arrow)

tion of AMS is necessary when the environmental factors vary considerably. Under natural conditions, it may be possible to assume that the penetration of AMS into the needles is greater in Sugi than in Akamatsu (Fig. 4). The stomatal penetration of AMS into Akamatsu and Sugi is found to be more than the cuticular penetration (Figs. 1 and 2). Accordingly, the penetration of AMS into the needles of the two species in the natural environmental conditions depends more on the stomatal penetration than on the cuticular penetration.

Figure 3 shows that the radioactivity in the needles first increases and then decreases. The decrease of the radioactivity may be caused by the translocation of ^{35}S from the treated needles to the other tissues, especially in Sugi, and by the change of AMS to water-insoluble substances.

Although AMS is a herbicide of the contact type, it is translocated in plant body after the penetration. TAKEMATSU(18) reports the slight translocation of AMS from the aerial parts to the roots in rice plant by observing the phytotoxicity. CARVELL(3) reports the upward and downward translocation of AMS in *Carya tomentosa* NUTT. Based on the present experimental results, AMS or its metabolites containing ^{35}S move in Akamatsu and Sugi and they are more easily translocated in the latter species than in the former.

Akamatsu is more susceptible than Sugi not only to AMS but also to the other foliar applied herbicides(8, 10). In the previous paper(8), it is reported that the retained amount of the sprayed AMS solution is lesser on the needles of Akamatsu than on those of Sugi. The penetration of AMS into the needles of the former species is lesser than into those of the latter. Accordingly, the differences of the retention and the penetration of AMS between the two species do not cause the difference of the susceptibility between them.

It is reported that the susceptibility to the phytotoxic herbicides results from the ability of metabolizing it to the non-toxic substances(2, 4). In Sugi, it may be supposed that AMS is easily metabolized to non-toxic substances and these substances are mobilized. However, this assumption is not ascertained from the present report. A further study should be conducted to determine the factors that cause the difference of susceptibility between Akamatsu and Sugi.

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