Accumulation of ^{35}Sulfur in Peaches Sprayed with Radiolabeled Dimethyl Sulfoxide

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Peach leaves and fruit sprayed with dimethyl sulfoxide (DMSO) labeled with ^{35}sulfur (^{35}S) accumulated a portion of the ^{35}S. Sprayed leaves accumulated greater radioactivity than fruit. Repeated applications increased the radioactivity at harvest time. Eighty-six to 90% of the radioactivity disappeared in both fruit and leaves within 2 weeks after application. Fruit sprayed one, two, or three times showed, when mature, 7.4, 15.4, and 34.9 p.p.m. of total DMSO residue (determined as ^{35}S), respectively. The addition of the antibiotic (oxytetracycline) to the sprays of DM^{35}SO did not significantly change the radioactivity recovered. Only 1 to 2% of the radioactivity applied to leaves and fruit could be recovered from adjacent unsprayed (bagged) leaves and fruit 2, 3, and 4 weeks after application.

Greenhouse tests and 2 years of field studies demonstrated enhanced control of peach bacterial spot caused by *Xanthomonas pruni* (E. F. Sm.) Dows when the solvent dimethyl sulfoxide (DMSO) was added to sprays of the antibiotic oxytetracycline (Keil, 1965, 1967; Keil and Carroll, 1967; Keil *et al.*, 1965). These studies suggested that the nonbactericidal DMSO may have aided adsorption and translocation of the antibiotic. Studies reported here were conducted to learn more about the role played by the solvent. An abstract covering this work has been published (Keil *et al.*, 1967).

MATERIALS AND METHODS

Eight 6-year-old Shippers Late Red peach seedlings were used as the test plants. Selected branches on six trees were sprayed with the ^{35}sulfur-labeled DMSO alone or in combination with oxytetracycline. Treatments were replicated three times on branches having 25 mature leaves, seven fruit, and either two or four terminals. At the start the terminals, 7 to 13 cm. long, consisted of undeveloped leaves and a tender green shoot. Branches on two trees were used exclusively for unlabeled control sprays with either stable DMSO alone or the combination of stable DMSO and oxytetracycline.

To maintain photosynthetic balance between fruit and leaves two concurrent tests were conducted. In test 1, two terminals and two fruits were protected by polyethylene bags during treatment. In test 2, four terminals were protected by the bags. All bags were removed 1 hour after spraying. Only the fruits (sprayed and unsprayed) from test 1 without removal of leaves were used for ^{35}S assay. Likewise, only the leaves without removal of fruit from test 2 were assayed.

A 2% aqueous solution of DMSO with a specific activity of 31.8 μc. per mmole was sprayed alone and in combination with 132 p.p.m. of oxytetracycline. The control solutions were made with stable DMSO. Sprays were applied three times at weekly intervals at a pressure of 70 p.s.i. and were stopped at the start of runoff. The spray nozzle was held 10 to 15 cm. from the plant part being sprayed.

Treatments were started August 1 and the experiments terminated on August 29, or 4 weeks after the first application. At the beginning of the study the fruits were 2.5 to 3.7 cm. in diameter, and by the end of the study they had reached their maximum size and had ripened.

Samples of three leaves or one fruit were collected at various intervals and digested by gentle heating for 15 minutes under reflux in 4 ml. of concentrated nitric acid. Leaves were weighed and digested without special preparation. The unpeeled fruit was cut into wedge-shaped pieces, each having one outside surface about 3 mm. square which was in contact with the spray. A random 1-gram sample of these pieces was digested. A 1-ml. portion of the digest was used for determination of radioactivity. A Nuclear Measurement Scaler, Model PC-3 thin-window model, was used for counting. Logarithms of counts were analyzed for statistical significance. All fruit weights were based on the flesh without the pit.

The data are presented as mean counts per minute (c.p.m.) per gram of fresh weight. Because no significant difference was found in count from DM^{35}SO alone and those from DM^{35}SO plus oxytetracycline, each figure presented is the mean of six replicates (three from DM^{35}SO alone and three from DM^{35}SO plus oxytetracycline).

RESULTS

Accumulation and Persistence of ^{35}Sulfur in Sprayed Leaves and Fruit. In treatments involving a single spray, similar amounts of ^{35}S were recovered from leaves and fruit (Figure 1). With the second application, the leaves accumulated substantially more ^{35}S than the fruit. There was no significant increase of ^{35}S in either leaves or fruit due to the third spray. Two weeks after the third and final application, a sharp drop in ^{35}S was noted in both leaves and fruit. At that time the residual ^{35}S in fruit and leaves was 14 and 10%, respectively, of that found after the third application. Because of the method of sampling it is not known whether radioactivity found in sprayed fruit and leaves is associated with the surface, interior portions, or both.

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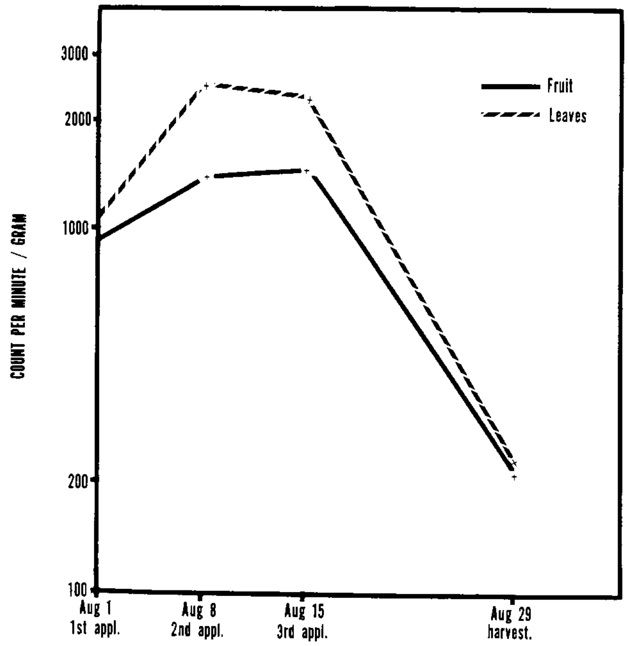

Figure 1. Accumulated ^{35}S in sprayed peach leaves and fruit

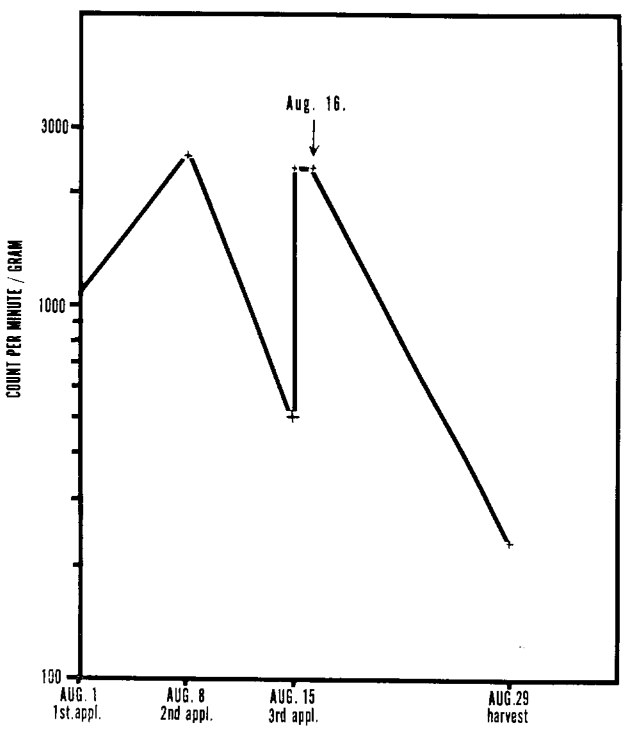

Figure 2. Loss of ^{35}S from sprayed peach leaves based on actual count per minute per gram found

Sample assayed August 16 or 24 hours after third application but none just before application of second spray

Absorption, persistence, and loss of radioactivity in leaves sprayed with ^{35}S-labeled DMSO plus oxytetracycline and radiolabeled DMSO alone were about the same. The radioactivity increased substantially with the second application, but 80% was lost within 1 week (Figure 2). The third application again significantly increased ^{35}S, or about as much as the second application effected; however, 90% of the ^{35}S was lost by the end of 2 weeks. While the loss increased with time, little occurred immediately after spraying, as indicated by measurement 24 hours after the third application (Figure 2).

Radioactivity in fruit decreased after a time. To determine whether this loss was due to the relatively short half life of ^{35}S, or to metabolism of $DM^{35}SO$ by fruit, a theoretical value for count per minute was calculated based on the half life of ^{35}S (Table I). Relation of the theoretical count per minute per gram to the actual count per minute per gram found indicated that the decrease in radioactivity after a time is not primarily due to the half life of ^{35}S in the $DM^{35}SO$. Since radioactivity disappears from tissues far more rapidly than can be accounted for by decay, it appears that much of the DMSO might decompose into a volatile compound.

The relationship of one, two, and three spray applications to the actual loss of ^{35}S in peach fruit is shown in Figure 3. All three curves are more or less parallel, suggesting the same loss pattern. On August 29 (4 weeks after one application) only 5% of the initial radioactivity remained. Three weeks after two applications 7% remained, and 2 weeks after the third application 14% remained.

Calculated DMSO Residue in Mature Fruit. The authors calculated the dimethyl sulfoxide in mature fruit (August 29) on the basis of the ^{35}S determined, assuming that all ^{35}S was present as $DM^{35}SO$. In this calculation the decay of ^{35}S as well as the specific activity of $DM^{35}SO$ applied was taken into consideration. Four weeks after a single application, 7.4 p.p.m. of the DMSO remained (Figure 4). Three weeks after two applications 15.4 p.p.m. remained, compared with 34.9 p.p.m. 2 weeks after three applications. Most of the DMSO disappeared from the fruit soon after each spray, but the small portion that remained increased with each additional application. Four weeks after a single application, only half as much DMSO was present as found 3 weeks after two applications (lines *A* and *B*). However, the proportionality does not exist between treatments involving the residues 3 weeks after two applications and 2 weeks after three applications (lines B and C).

Translocation of ^{35}S to Unsprayed Leaves and Fruit. Fruit and terminal leaves protected by polyethylene bags during spray applications were assayed for radioactivity on August 29 when the fruit was ripe. Only 1 to 2% of the ^{35}S deposited on exposed sprayed plant parts was translocated to the untreated bagged fruit or leaves. This radioactivity was not significantly increased by a second or third application.

DISCUSSION

More ^{35}S was recovered from $DM^{35}SO$ sprayed leaves than from sprayed fruit. There was also a more rapid loss of radioactivity from leaves than from fruit. Both facts may be explained by the greater area to weight ratio of leaves.

Because only selected branches were sprayed, possibly

Table I. Mean[a] Radioactivity of Sprayed Peach Fruit, Test 1

No. of Appl.	Date and Sampling Time	Actual C.P.M./Gram[b]	Theoretical C.P.M./Gram[c]
1	Aug. 1, 2 hours after 1st application	937 ab	937
	Aug. 8, 1 week after 1st application	665 b	900
	Aug. 15, 2 weeks after 1st application	63 de	862
	Aug. 22, 3 weeks after 1st application	28 fg	825
	Aug. 29, 4 weeks after 1st application (harvest time)	45 ef	785
2	Aug. 8, 2 hours after 2nd application	1393 a	1837
	Aug. 15, 1 week after 2nd application	258 c	1690
	Aug. 22, 2 weeks after 2nd application	92 d	1543
	Aug. 29, 3 weeks after 2nd application (harvest time)	99 d	1396
3	Aug. 15, 2 hours after 3rd application	1465 a	2627
	Aug. 22, 1 week after 3rd application	269 c	2497
	Aug. 29, 2 weeks after 3rd application (harvest time)	210 c	2207

[a] Consists of composite of 6 counts (3 from DM^{35}SO alone and 3 from DM^{35}SO plus oxytetracycline).
[b] Corrected for background. Counts followed by same letter not significantly different at 5% level (Duncan multiple range test).
[c] Calculations based on ^{35}S half life of 87 days.

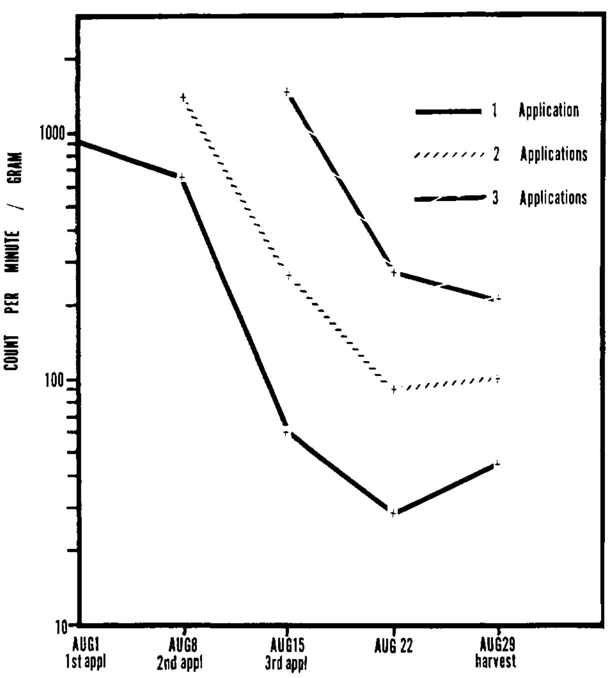

Figure 3. Loss of ^{35}S from sprayed peach fruit based on actual count per minute per gram found

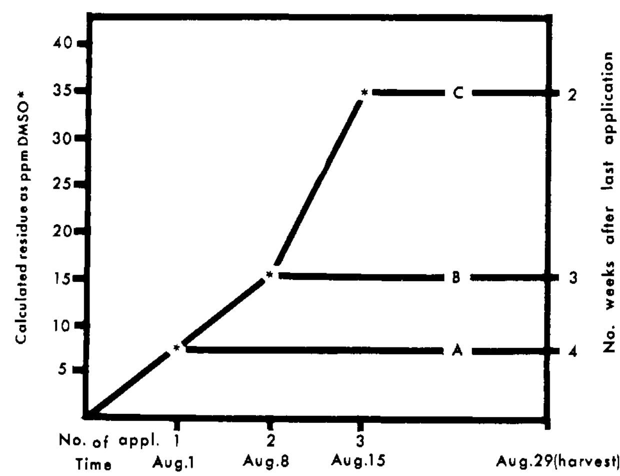

Figure 4. Calculated DMSO residue in mature fruit as affected by time and number of applications

* Calculation based on ^{35}S determinations

the nonvolatile part of the radioactive DMSO was transported into the unsprayed parts of the tree which could act as a sink. This might also explain the rapid loss of radioactivity from sprayed leaves and fruit with time. With each additional application sprayed fruit showed about as much radioactivity added back as lost during the previous week. This is shown by the lack of significance in the count made two hours after one, two, or three applications. Another possible explanation suggested by the data is that sprayed leaf and fruit tissues become saturated with the second DM^{35}SO application. These findings, along with the data on radioactivity loss, suggest the possibility of an induced metabolism triggered by the DMSO as it penetrates the leaf and stem tissues and enters the cells. This system reaches maximum efficiency within 1 week after the first spray. The hypothesis would account for the plateau in leaf and fruit curves in Figure 1 and the loss patterns illustrated in Figures 2 and 3.

The addition of oxytetracycline to DM^{35}SO sprays did not significantly alter the persistence or recovery of radiolabeled sulfur from fruit or leaves. The influence of dimethyl sulfoxide on uptake of antibiotic cannot be determined from the present work, but studies with DMSO-oxytetracycline sprays on control of bacterial leaf spot of peach suggest that antibiotic absorption is enhanced by DMSO (Keil, 1967).

Four, 3, and 2 weeks after the first, second, and third spray applications (August 29), the authors calculated fruit residues of 7.4, 15.4, and 34.9 p.p.m. of dimethyl sulfoxide, respectively (Figure 4). Relating these residues to the percentage loss of radioactivity, apparently the residue approximately doubles with each weekly application. The data may indicate that some of the sulfur portion of $DM^{35}SO$ is retained as a nonvolatile compound for at least 2 weeks. The small amount of radioactivity remaining suggests, however, that if there is metabolism of the sulfur from the $DM^{35}SO$ molecule, it does not involve any more than 5 to 7% of the applied $DM^{35}SO$. The amount of sulfur retained in the plant can build up with more than one application. Since the determinations were not intended to characterize the compounds containing the ^{35}S, the authors do not know whether the whole dimethyl sulfoxide molecule or only the S portion is retained.

Garren (1967) applied approximately 33% stable DMSO containing $DM^{35}SO$ to young pear trees through a reservoir around the trunk and demonstrated the absorption and systemic distribution of radioactivity. The authors' observations with sprays of 2% aqueous mixtures of DMSO containing radiolabeled $DM^{35}SO$ also show the translocation of radioactive sulfur. It was thought, however, on the basis of demonstrated enhanced bacterial spot control (Keil and Carroll, 1967), that more than 1 to 2% of the applied ^{35}S would be translocated. Also, these studies gave no evidence of increased radioactivity in the unsprayed leaves or fruit with additional applications. These data possibly suggest that DMSO aids only in the absorption, and not in the translocation of the oxytetracycline.

Experiments reported were begun late in the season because the radiolabeled $DM^{35}SO$ was not available for earlier studies. During most seasons, sprays for control of peach bacterial spot are not necessary within 40 days of harvest. If DMSO is approved as a carrier for oxytetracycline, time of application would not be important if a normal spray schedule was followed, in that the major portion of the DMSO residue disappears from the plant in a much shorter period than 40 days. However, based on these data, the number of applications may play an important role in the residue found at harvest time and should be investigated more fully.

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