

Infusion and Translocation of Systemic Fungicides Applied to Seeds in Acetone

N. R. O'Neill, G. C. Papavizas, and J. A. Lewis

Soilborne Diseases Laboratory, Plant Protection Institute, Agricultural Research, Science and Education Administration, U. S. Department of Agriculture, Beltsville, MD 20705.

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ABSTRACT

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Systemic fungicides were applied to acid-delinted cottonseed and soybean seed either directly (direct fungicide application) or by organic solvent (acetone) infusion. Seed coats of treated and untreated seeds were removed and the concentrations of the fungicides in seed coats and embryos (cotyledons + embryo) were estimated either indirectly by bioassay or directly by the use of radioisotopes. With either method of application, most of the fungicide remained in the seed coats. However, bioassays revealed more fungicide within or upon the embryo in seeds treated with acetone infusion

than in those that received it by direct application. The accumulation of fungicide in seeds exposed to acetone infusion increased as the seeds began to imbibe water. Fungicides in cotton and soybean seeds treated with acetone infusion were not leached out by water or acetone as readily as were those that had been applied by direct application. The translocation patterns and distributions of ^{14}C -ethazol and ^{14}C -carboxin were similar in cotton and soybean plants produced from seeds treated by either method.

The technique of applying chemicals such as plant hormones, insecticides, and fungicides to dry seeds in organic solvent carriers has been described (1,3,4,6,7,9). In greenhouse and field experiments, Papavizas et al (9-11) demonstrated the effectiveness of this technique for reducing seedling diseases in cotton, soybean, and snapbean crops and detected no adverse effects of the solvents alone on seedling development.

The infusion of growth regulators in solvents through seed coats and into the cotyledons of various seeds was demonstrated quantitatively by Tao and Kahn (13). They found that the amount of growth regulator that penetrated seeds depended on the chemical, seed type, permeation time, and solution concentration. Meyer and Mayer (6) reported that dichloromethane could be used as a carrier to introduce chemicals into lettuce seeds without apparent effects on germination or respiration. Royce et al (12) detected antibiotic activity in soybean seeds immersed in a mixture of potassium penicillin G and dichloromethane. Ellis et al (3) reported fungicidal activity in dormant soybean seeds after immersion in methyl 2-benzimidazolecarbamate and thiabendazole in dichloromethane, but not when captan, thiram, or carboxin were applied similarly.

Although there are a few reports on the uptake of systemic fungicides applied as conventional seed dressings, the extent of the permeation and translocation of fungicides applied in organic solvents has not been investigated. The present research was undertaken to study the infusion of systemic fungicides into seeds and to compare the translocation of such fungicides in dormant cotton and soybean seeds treated by an organic solvent (acetone) infusion technique (OSI) and by direct fungicide application (DFA). A preliminary report has been published (8).

MATERIALS AND METHODS

Seed treatment. 5-Ethoxy-3-(trichloromethyl)-1,2,4-thiadiazole (ethazol, ETMT; Olin Corp., Agri Division, Little Rock, AR), 2-iodobenzanilide (BAS 3170F; BASF Wyandotte Corp., Parsippany, NJ), and 5,6-dihydro-2-methyl-1,4-oxathiin-3-carboxanilide (carboxin; Uniroyal Chemical, Bethany, CT) were used for seed

treatments. The radioactive fungicides ^{14}C -carboxin (3- C^{14}) and ^{14}C -ethazol (uniformly labeled in the phenyl moiety) were provided by Uniroyal Chemical and Olin Research Center, respectively. Fungicides were applied to seeds of cotton (*Gossypium hirsutum* L. 'Stoneville 213') and soybean (*Glycine max* [L.] Merr. 'Amsoy') either by the direct fungicide application method or by the organic solvent infusion (acetone infusion in these experiments) technique described previously (9,11). Direct application of commercially formulated fungicides to small batches of seed was done by tumbling them with the chemical in glass jars for 5 min and allowing them to dry. With the OSI technique, the fungicides were dissolved in acetone (nearly all experiments) or dichloromethane (only initial trials) in amounts indicated in each particular experiment and the seeds were immersed in the solutions for various lengths of time. Unless otherwise stated, the seeds were separated from the solvent and the solvent was allowed to evaporate in a fume hood. Controls included untreated seed and seed treated with solvent only. In some experiments, treated seeds were rinsed in water or acetone before analysis.

Fungicide infusion and penetration. Bioassays. The seed coats of dry, treated and untreated seeds were removed carefully with tweezers and the embryos were arranged in the center of petri dishes containing potato dextrose agar (PDA) or V-8 juice agar. One disk of the assay fungus was placed on each side of the embryos. *Rhizoctonia solani* Kühn was used as the assay organism for carboxin and 2-iodobenzanilide. The dishes were incubated at 25 C for 3-5 days and the inhibitory effect of fungicides in embryos was estimated by measuring the zones of inhibition of fungal colony growth.

Seed coats were removed both from the dry, fungicide-treated seeds and from seeds immersed in water. For this approach, treated and untreated seeds were kept dry in the laboratory for 1 wk and then either washed five times with acetone and 45 min with running sterile water or washed with water only to remove the fungicide from the seed surface. The seed coats were aseptically removed, and fungicide residues in the embryos were assayed as before.

To determine which of the two seed treatment methods (direct application or acetone infusion) resulted in more carboxin accumulation in embryos, cottonseed was treated so that both methods added approximately the same quantity of carboxin to the seeds. Carboxin was dissolved in acetone so that 20 ml of solution added to

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20 g of seed in a petri dish delivered the same amount of the chemical to the seeds as that provided by direct application (2 g active ingredient [a.i.] per kilogram of seed). The petri dishes were covered for 30 min and then uncovered for an additional 30 min to allow the acetone to evaporate. The seeds were subdivided into two lots for each treatment; those in one lot were washed vigorously for 1 min with running tap water and the seeds in the second lot were not washed. Seed coats were removed from all seeds, and the embryos were bioassayed on PDA against *R. solani*. A similar experiment was done with 2-iodobenzanilide.

Radioactivity assays. Assays with ^{14}C -carboxin and ^{14}C -ethazol were performed to determine whether method of seed treatment, fungicide concentrations, or immersion time influenced fungicide infusion and penetration into cotton and soybean seeds. Seeds were immersed up to 3 hr in acetone solutions containing from 1 to 10% a.i. of ^{14}C -ethazol or ^{14}C -carboxin.

Equal amounts of the active ingredient (2.0 mg a.i./g seed, 25.0 $\mu\text{Ci/g}$ a.i.) were added to the seeds by direct fungicide application. The seeds were dried and the seed coats were removed either immediately or after a 30-min wash with water. In all experiments, concentrations of the two radioactive fungicides in seeds and seed parts were determined by counting radioactivity in samples that had been oxidized in a Packard Tri-carb oxidizer. The $^{14}\text{CO}_2$ evolved during combustion was trapped in a scintillation vial in 8 ml of carbo-sorb (Packard Instrument Co., Inc., Downer's Grove, IL). Ten milliliters of a toluene-based scintillation solution was added to the vials and the samples were counted for 50 min in a Beckman LS-3100 liquid scintillation system with a 2.0% standard deviation.

The ability of cotton and soybean seeds to retain fungicides applied by direct application and acetone infusion was measured as rate of fungicide leakage from treated seeds. Cottonseed was treated by the direct application method with ^{14}C -carboxin (2.3 mg a.i./g seed, 6.0 $\mu\text{Ci/g}$ a.i.) or by the acetone infusion technique by immersing the seed for 1 hr in an acetone solution of ^{14}C -carboxin calculated to give the same amount of active ingredient per gram of seed. Soybean seeds were similarly treated with ^{14}C -ethazol to add 1.0 mg a.i./g seed (5.0 $\mu\text{Ci/g}$ a.i.) with either method. Treated and untreated seeds were immersed in acetone (or water) that was subsequently replaced by acetone (or water) after 2, 6, 12, 24, and 48 min for cottonseed and 2, 5, 10, 20, and 40 min for soybean seed. The rinsing solutions were evaporated and the radioactivity was determined in the residues.

Translocation of fungicides. The uptake and translocation of carboxin and ethazol was studied in cotton and soybean seedlings grown from treated and untreated seed. No attempt was made to apply equal amounts of ethazol to soybean seed with the two methods. With the direct application method, ethazol was applied to soybean seed at 1.5 mg a.i./g seed (32.20 $\mu\text{Ci/g}$ a.i.). With the acetone infusion technique, the applied ethazol was equivalent to 11.69 $\mu\text{Ci/g}$ a.i. In a similar experiment with cottonseed, ^{14}C -carboxin was applied to give 2.3 mg a.i./g seed (equivalent to 5.8 $\mu\text{Ci/g}$ a.i.) with both methods. Soybean seed was planted in pasteurized soil in the greenhouse, whereas cottonseed was planted in unsterilized field soil. Soybean seedlings were harvested at 0, 5, 8, 11, and 14 days and cotton seedlings at 5, 9, 12, 19, 26, and 33 days. Six replications of 10 plants were used. Radioactivity was determined in dried plant parts and seed coats recovered from soil. No attempt was made to distinguish between the fungicides and their breakdown products.

RESULTS

Fungicide infusion and penetration. *Bioassays.* More carboxin reached cottonseed embryos after immersion in a carboxin solution in acetone than when the fungicide was applied to the seed directly (Table 1). The results of the assay were not influenced by the method of seed coat removal (seed coat removal from dry seeds or after washing with water for 1 min).

Similar results were obtained in a test in which cottonseed was washed five times with acetone and 45 min with running water after the application of the fungicide 2-iodobenzanilide by the direct application method or by the acetone infusion technique. More fungicidal activity against *R. solani* was detected in dormant cot-

tonseed embryos following seed immersion in 2-iodobenzanilide solution in acetone for 40 min than when this fungicide had been applied by DFA.

In another experiment, untreated cottonseed and seed treated with carboxin (direct application or acetone infusion) were washed with running tap water for 45 min, but not with acetone. Two concentrations of acetone (70 and 100%) and two immersion times (1 and 2 hr) were used. More carboxin reached the embryos of dormant cottonseed after immersion in fungicide solutions in acetone than when the fungicide was applied to the seed directly; the 70% aqueous solution of acetone was more effective than the 100% acetone (Fig. 1). With the 70% acetone solution of carboxin, more inhibitory activity was observed after 2 hr than after 1 hr of immersion. Seed germinability in soil did not change as a result of the treatments.

Radioactivity assays. The amount of ethazol infused into soybean seed after 1 hr of immersion was directly proportional ($r = .99$) to the ethazol concentration in the acetone treatment (Fig. 2). Longer infusion times, however, did not transfer greater quantities of ethazol into the seeds. Most of the ethazol in soybean seeds treated by either method was deposited upon or within the seed coats (Table 2). There were no differences in the rate of permeation of ethazol into cotyledons of seeds treated by either method before the imbibition of water. After imbibition, however, ethazol had moved deeper into the cotyledons of seeds treated by acetone infusion than into those treated by direct application.

Cottonseed treated with carboxin by direct application and rinsed successively five times with water retained only 13% of the deposited fungicide (Fig. 3). Seeds treated with acetone infusion, however, retained 60% of the carboxin following water rinses. Acetone rinsing removed more of the carboxin than did water rinsing. Nevertheless, seeds treated with acetone infusion retained 34% of the fungicide versus only 1% retained by seed that had been treated directly. Soybean seeds treated with ethazol by the direct application method retained 15% of the fungicide, and those treated with acetone infusion retained 65% following five successive rinses in acetone.

Fungicide translocation. The translocation and distribution patterns of ethazol in soybean plants from seed treated with direct application and with acetone infusion were generally similar during a 14-day growth period (Fig. 4). After 5 days, 85–90% of the ^{14}C -ethazol in soybean plants was located in cotyledons. Small, but equal amounts were detected in the roots and shoots. From 5 to 14 days, ethazol began to move into leaves and shoots. After 8 days, about 20% of the ethazol associated with the seedlings was located in the roots. Upon termination of the experiment (14 days), about

TABLE 1. Inhibition of *Rhizoctonia solani* on potato dextrose agar by carboxin in dormant cottonseed embryo (cotyledons + embryo) as affected by the method of fungicide application

Mode of seed coat removal and treatment	Concentration (g active ingredient per kilogram of seed)	Inhibition zone (mm)
Wash seeds for 1 min to remove coats:		
None (control)		0.0 a ^y
Carboxin flowable applied directly	2	0.1 a
Carboxin technical 1.5% in acetone ^z	2	6.4 b
Remove dry seed coats:		
None (control)		0.0 a
Carboxin flowable applied directly	2	0.8 a
Carboxin technical 1.5% in acetone ^z	2	6.9 b

^yNumbers followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

^zSeeds were immersed in the solution for 1 hr.



Fig. 1. Inhibition of *Rhizoctonia solani* on potato dextrose agar by carboxin in cottonseed embryos. Upper row (left to right), untreated seed, seed treated with carboxin flowable applied to seed directly, and seed immersed for 1 hr in 1.5% solution of carboxin technical in 100% acetone; lower row (left to right), seed immersed in 1.5% carboxin in 100% acetone for 2 hr, in 1.5% carboxin technical in 70% acetone for 1 hr, and in 1.5% carboxin technical in 70% acetone for 2 hr.

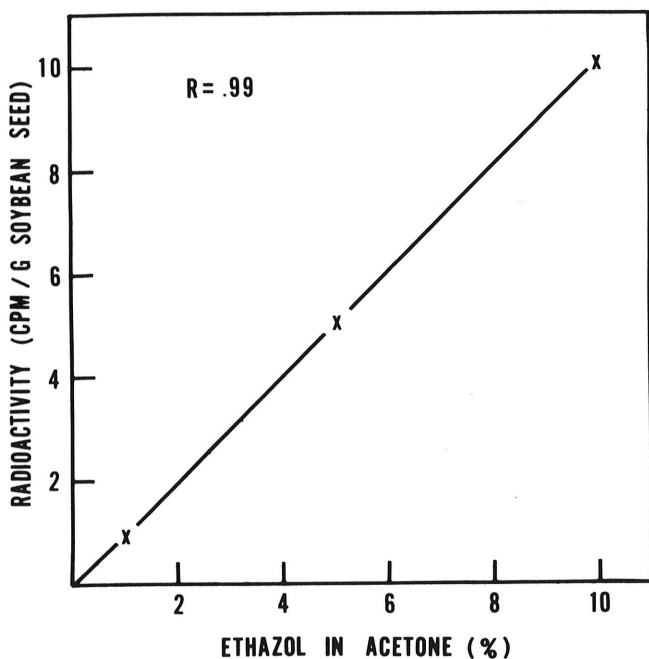


Fig. 2. Infusion of ethazol into soybean seed by immersion for 1 hr in acetone containing 1, 5, and 10% ^{14}C -labeled ethazol. Radioactivity is given in counts per min.

equal amounts of ethazol were detected in cotyledons and shoots. Total counts per min (CPM) from ^{14}C -ethazol (applied directly) in plant tissues from the 5th to the 14th day decreased from 1,506 to 906 CPM per plant. Total CPM ^{14}C -ethazol (applied in acetone) in plant tissues on these sampling dates decreased from 427 to 356 CPM per plant. Because the amounts of ^{14}C -ethazol added to seed with direct application and with acetone infusion were not equal, no comparisons could be made on the effect of the method of application on the rate of translocation.

Movement of ^{14}C -carboxin in cotton seedlings also was similar during a 33-day growth period in plants grown from seeds treated by either method but not during the first 12 days of growth (Fig. 5). On an equal application rate by either method (2.3 mg a.i./g seed), after 5 days, there was almost twice as much carboxin in cotyledons from seeds treated with ^{14}C -carboxin in acetone than from seeds treated directly. These differences were significant, $P = 0.05$. Even after 12 days, uptake by acetone infusion exceeded direct application by about 10^3 counts per min. Concentrations of carboxin in roots did not change significantly up to the end of the experiment, but levels in shoots increased for both methods between 19 and 33 days of growth (33-day data not shown). High carboxin concentrations were found in cotyledons; carboxin levels increased up to 19 days. For the first 12 days, the majority of the ^{14}C -carboxin in treated seeds was still associated with seed coats recovered from soil. Concentrations in seed coats were gradually reduced as they disintegrated.

DISCUSSION

Seeds took up and retained more fungicides inside the seed coat when treated by immersion in solvent solutions than when treated by conventional methods. The treatment of dormant seeds with systemic fungicides in solvents is a method of introducing the fungi-

cides into seed tissue before water imbibition and germination begin.

In our experiments with fungicides applied in acetone, the amount of ethazol that reached soybean embryos was proportional to the concentration of ethazol used. Permeation time had a minimal effect on uptake. The solvent and fungicide reached maximum concentration under the seed coat in approximately 15 min; little further penetration occurred with longer immersion periods. Cottonseeds, which have thicker seed coats than soybean seeds, continued to accumulate carboxin for at least 2 hr, particularly when the fungicide solution contained 30% water. The quantity of fungicide penetrating into seed can be controlled to some extent by adjusting the permeation time and solution concentrations. Similar conclu-

sions were reached by Tao and Khan (13), who reported that the amounts of hormones that penetrated into seeds depended on the hormone used and the seed type as well as on permeation time and solution concentration.

Infusion of seeds with chemicals dissolved in solvents containing small quantities of water or briefly immersing seeds in water following solvent infusion may be even more effective than nonaqueous solvent solutions alone for introducing chemicals deep into seeds. The seeds could then be dried leaving the fungicide deposited inside the seed. Seed viability following such treatments, however, may be a problem with some types of seeds and chemicals (G. C. Papavizas, unpublished).

Although dry seeds treated by either technique contained about the same quantities of fungicide inside the seed coat, imbibed seeds treated by the acetone infusion technique contained significantly more fungicide in embryos than similar seeds treated directly. The presence of small amounts of fungicide inside seed coats of seed treated by the direct application method probably is not due to contamination during separation, but to partial absorption through the seed coat. Berggren and Pinckard (2) also reported that small amounts of carboxin were absorbed by cottonseed directly through the seed coat. Our results agree with those of others (6,12,13) who reported that the solvent facilitated movement of chemicals through the seed coat into tissues of the seed embryo.

Fungicides introduced with an organic solvent to surfaces inside the seed coats are retained even when washed with water or acetone. Fungicides in seeds treated by direct application were more easily removed by washing than those applied in an organic solvent. The fact that the infusing solvent removed little fungicide from the seeds suggests a physical or chemical binding of the fungicide in the seed. This method of treating seeds would be useful for keeping water-soluble as well as water-insoluble fungicides from being washed or leached out readily in the field.

That carboxin is translocated upward into cotton seedlings, even when applied to dormant cottonseed directly, has already been

TABLE 2. Distribution of ^{14}C -ethazol in soybean seeds treated by direct fungicide application or organic solvent infusion

Seed part	Radioactivity (% in seed part) ^w			
	Dry		Imbibed ^x	
	Acetone infusion ^y	Direct application	Acetone infusion	Direct application
Whole seed	100	100	100	100
Embryo (cotyledons + embryo)	10 a ^z	16 ab	42 c	25 b
Seed coat	90 f	84 ef	58 d	75 e

^wThe quantity of fungicide in a seed part is expressed as a percentage of the radioactivity found in whole, treated seeds.

^xSeeds were allowed to imbibe water for 30 min prior to separation.

^ySeeds were immersed in ethazol in acetone to give a final concentration of 2 mg a.i./g seed. Seeds treated by direct application received ethazol directly at 2 mg a.i./g seed.

^zNumbers followed by the same letter do not differ significantly ($P = 0.05$) according to Duncan's multiple range test.

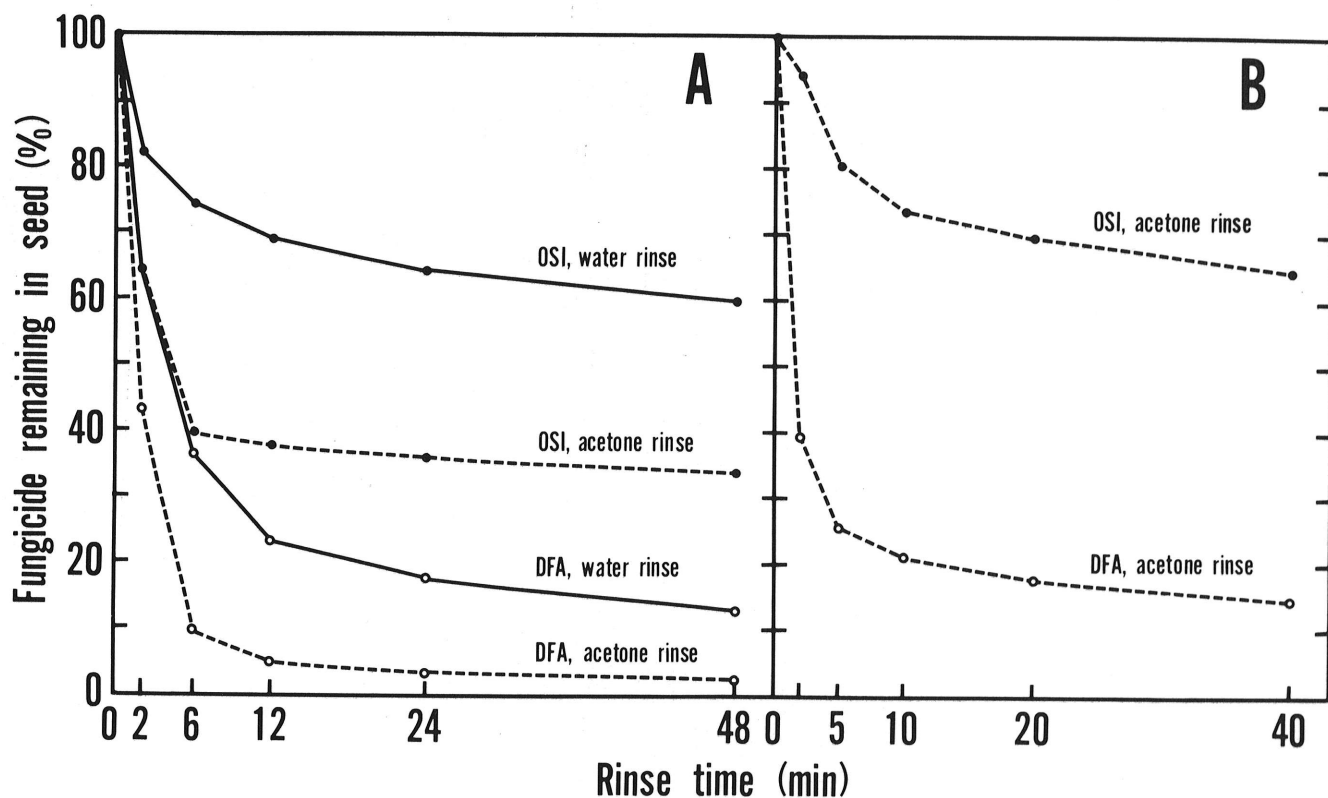


Fig. 3. Retention of fungicides in seeds treated with organic solvent (acetone) infusion (OSI) or direct fungicide application (DFA) after rinses in acetone or water for specified lengths of time. A, Cottonseed treated with ^{14}C -labeled carboxin by DFA and with OSI for 1 hr with ^{14}C -labeled carboxin in acetone, which deposited 2.3 mg a.i./g of seed, 6.0 $\mu\text{Ci/g}$ a.i. B, Soybean seed treated by DFA and OSI with ^{14}C -labeled ethazol for 1 hr, which deposited 1.0 mg a.i./g of seed, 5.0 $\mu\text{Ci/g}$ a.i.

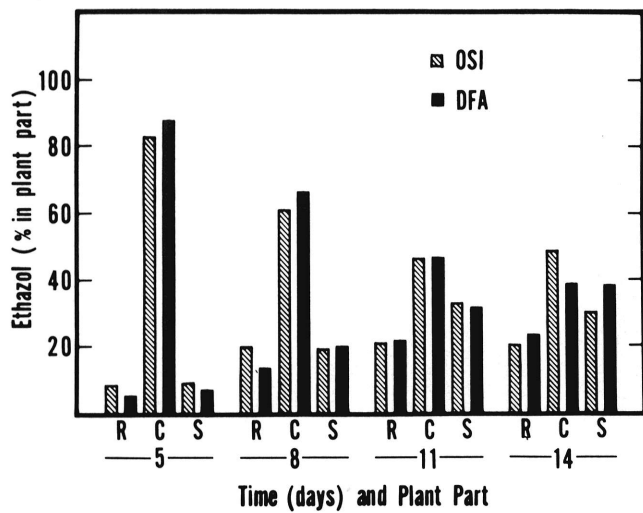


Fig. 4. Translocation and distribution of ^{14}C -ethazol in soybean plants (roots = R; cotyledons = C; shoots = S) grown from seeds that were treated with the fungicide by organic solvent (acetone) infusion (OSI) or by direct fungicide application (DFA). Soybean seeds were treated by immersion for 1 hr in ^{14}C -ethazol in acetone (OSI) or by direct application (DFA) of the radioactive fungicide. The quantity of ethazol in plant parts is expressed as a percentage of the total radioactivity averaged from six replications consisting of 10 seedlings each.

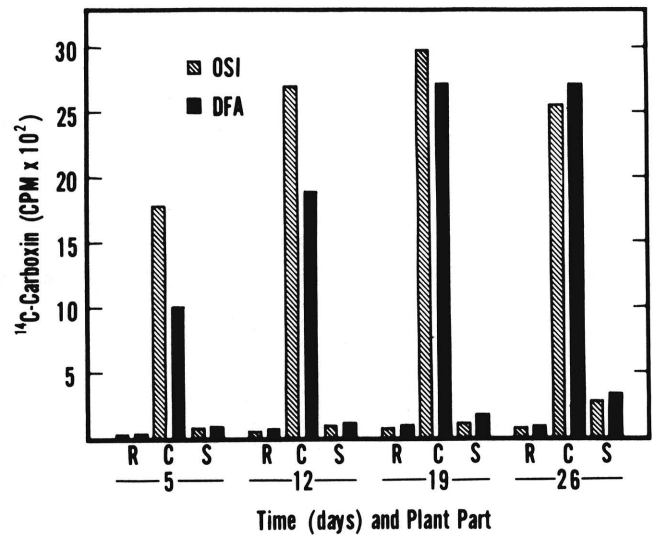


Fig. 5. Translocation and distribution of ^{14}C -carboxin in cotton plants (roots = R; cotyledons = C; shoots = S) whose seeds were treated with organic solvent (acetone) infusion (OSI) or with direct fungicide application (DFA). Cottonseed was treated by immersion for 1 hr in ^{14}C -carboxin in acetone (OSI) or by direct application (DFA) of the radioactive fungicide in quantities calculated to place equal amounts of fungicide (2.3 mg a.i./g of seed) upon or within seeds following either treatment. The quantity of carboxin is expressed directly as the radioactivity (counts per min) in a plant part from the average of six replications of 10 seedlings each.

demonstrated (5). Our experiments on translocation of ^{14}C -carboxin and ethazol make the following contributions: on an equal basis, the acetone infusion technique allows more fungicide to accumulate in cottonseed embryos during the first 12 days following exposure of seed in natural soil than does the direct fungicide application method; acetone infusion places carboxin and ethazol in a favorable, protected location in the seeds for further uptake, but the method of seed application has little effect in the long run on the developing seedlings. This may be due to subsequent uptake by seedlings of these systemic fungicides being released from gradually disintegrating seed coats remaining in soil. Measurable radioactivity increased up to the 19th day after cottonseeds treated with carboxin were planted. Uptake and distribution of fungicides, however, may be critical during the first 10–14 days of growth because of the importance of seedling diseases during that initial period of growth.

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