Published November, 1977

Effects on Cottonseed of Immersion in Acetone or Methylene Chloride[1]

John M. Halloin[2]

ABSTRACT

Acetone and methylene chloride have been advocated as carrier solvents for incorporation of growth regulators and seed protectants into seeds. The penetration and effects of these solvents on cottonseed (*Gossypium hirsutum* L.) were studied. Seeds immersed in the solvents had normal germinability, whereas excised embryos and gin-damaged seeds were killed by immersion in the solvents, apparently due to disruption of lipid systems. Seeds were soaked in solvents containing the dye oil red O; the dye penetrated the seed coat, but none entered the embryos. When excised embryos were immersed in dye solutions, the dye permeated throughout the embryos. Thus, seed coats are permeable to the solvents and the dye, but the nucellar layer surrounding embryos is impermeable, at least to the dye. Acetone and methylene chloride may be suitable for surface application of chemicals to sound cottonseed, but not for introduction of chemicals into embryos.

Additional index words: ***Gossypium hirsutum*****, Solvent infusion.**

ACETONE and methylene chloride have been used successfully to apply growth regulators and pesticides to seeds (e.g. 4, 5, 6, 8, 9). Organic solvents apparently deposit solutes within or in close proximity to embryo tissues and may increase the effectiveness of growth regulators and pesticides, especially systemic pesticides. Disagreement exists as to whether solutes are deposited within embryo tissues by organic solvents. Tao and Khan (12) reported deposition of ^{14}C-IAA within squash (*Cucurbita* sp.) and cucumber (*Cucumis sativus* L.) embryos. Conversely, Anderson (2), Brewer and Wilson (3), and Triplett and Haber (13) found that the solvents did not penetrate into lettuce (*Lactuca* sp.), oat (*Avena sativa* L.), and pigweed (*Amaranthus* sp.) embryo tissues.

Much of the work to date on mechanisms of organic solvent infusion has been done on lettuce. Cottonseed differ anatomically from lettuce achenes in several aspects which might affect penetration, distribution, and effects of organic solvents. The cotton seed coat is thick and pervious to water primarily through the chalaza (10). The embryo is attached to the seed coat only at the chalaza and is often spatially separated from the seed coat over much of its surface. Endosperm surrounds lettuce embryos and has been shown to act as a limiting barrier to penetration of organic solvents (2, 13). No prominent endosperm tissue remains in a mature cottonseed.

I studied the penetration and effects of acetone and methylene chloride on cottonseed and evaluated the potential usefulness of organic solvent infusion for application of chemicals to cottonseed.

[1] Contribution from USDA, ARS, National Cotton Pathology Research Laboratory, P. O. Drawer JF, College Station, TX 77840. Received 31 Mar. 1977.

[2] Research plant physiologist, USDA, ARS, National Cotton Pathology Research Laboratory, P. O. Drawer JF, College Station, TX 77840.

MATERIALS AND METHODS

Cottonseed (*Gossypium hirsutum* L.) used were 'Deltapine 16' (glanded) and 'Rogers GL6' (glandless). Seeds were delinted with H_2SO_4. Except where otherwise noted, any seeds that were visibly damaged or immature were discarded. Seed coats were removed from the embryos with a razor blade; visibly damaged embryos were discarded.

Sufficient solvent was applied to cover the seeds when acetone was used or to float them when methylene chloride was used. Seeds were soaked in the solvents for 0.25 to 72 hours. In one experiment, solvents were removed by vacuum drying as described by Meyer and Mayer (7). In all other experiments excess solvent was decanted and seeds were air-dried for at least 24 hours. The dye oil red 0 was applied to seeds and embryos as a 0.5% solution. Stained embryos and seeds were bisected longitudinally with a razor blade and examined for dye penetration at 6 to 50 × magnification under a stereoscopic microscope.

Seeds and embryos were germinated for 7 days in paper rolls (25 seeds per roll) under a temperature cycle of 20 C for 10 hours and 30 C for 14 hours. Treatments contained 250 seeds or embryos and germination tests were repeated three times.

Conductances of aqueous embryo leachates were measured with a Yellow Springs conductivity bridge (Model 31) with conductivity cell 3403 (K=1.0)[3]. Measurements were made after embroys were soaked in distilled water for 15 min.

[3] Mention of specific equipment or commercial varieties does not constitute endorsement by the USDA over similar equipment or other varieties not mentioned.

Solvents decanted from seeds and embryos were evaporated under reduced pressure and solutes extracted were determined by weighing. Oil was then extracted from embryos with petroleum ether by use of A.O.C.S. Official Method Aa 4-38 (1). Solutes extracted by acetone and methylene chloride are expressed as percentage changes in both embryo dry weight and extractable oil.

RESULTS AND DISCUSSION

Glanded and glandless cottonseed were immersed for 24 or 72 hours in either acetone or methylene chloride. They were then air-dried and germinated. Germination was not significantly reduced (5% level, by the Kolmogorov-Smirnov test) by either of the solvents or by glandular gossypol. Similarly, gossypol applied as 1% solutions in the solvents had no effect on germination of glandless seeds. Thus, neither the solvents, nor any effect which they might have on gossypol localization had any adverse influence on germination of nondamaged cottonseed.

Less than 70% of the seeds immersed in acetone for 24 or 72 hours and vacuum-dried for 24 hours germinated in loosely-covered 1 liter containers, and emerging radicles were necrotic. Acetone vapors were present in the containers. When non-treated seeds were placed in containers and 0.25 ml of acetone was added before covering, similar results were obtained. Acetone was apparently trapped inside of the cottonseed coats during soaking and was removed completely only by repeated repressurization and decompression over a 1-week period. This difficulty was not observed with methylene chloride. No inhibition of germination or seedling necrosis was observed when

Table 1. Penetration of the dye oil red O into glandless cottonseed and embryos.

Treatment	Staining of embryos	
	Superficial	Internal
Seeds		
Acetone + Dye (1 hour)	+	–
Acetone + Dye (24 hours)	+	–
Acetone + Dye (72 hours)	+	–
Acetone (1 hour), Acetone + Dye (23 hours)	+	–
Methylene chloride + Dye (1 hour)	+	–
Methylene chloride + Dye (24 hours)	+	–
Methylene chloride + Dye (72 hours)	+	–
Methylene chloride (1 hour), Methylene chloride + Dye (23 hours)	+	–
Methylene chloride + Dye (24 hours), seed coat nicked	+	–
Excised embryos		
Acetone + Dye (24 hours)	+	+
Methylene chloride + Dye (24 hours)	+	+

seeds were air-dried for 24 hours following solvent treatments. Therefore, seeds were air-dried for at least 24 hours for all other experiments.

The absence of adverse effects from solvents, and observations that solvents failed to penetrate embryo tissues of lettuce and oats (2, 3, 13), suggested that the solvents failed to penetrate cotton embryos. Cotton is unique among the species in which the pattern of organic solvent infusion has been studied. The embryo and its thin, surrounding nucellar membrane are easily separated from the seed coat. This allows for determination of the localization of any barrier to solvent penetration. The pattern of staining of embryos with the dye oil red 0 (Table 1) was observed. When intact seeds were immersed in oil red 0 dye solutions, superficial, but never internal staining of embryos was observed. When excised embryos covered with their nucellar membranes were immersed, however, staining occurred throughout the embryo tissues. These results suggested that seed coats allowed only a small amount of solvent to enter the seeds and that this amount of solvent was sufficient only to coat the surface of the embryo.

The time course of methylene chloride uptake was determined on glandless cottonseed. Seeds were immersed in the solvent for 15 min to 72 hours. Solvents were poured off and the seeds were placed immediately in sealed vials and weighed. This revealed that a small amount of solvent was taken up by seeds within the first 15 min (65 ml/kg seed), and that no further uptake occurred through 72 hours of immersion. When seeds were immersed in solvents for 1 hour and then transferred to dye solutions for 23 hours (Table 1) staining was found on most embryos. Thus, cessation of solvent uptake was not due to development of seed coat impermeability.

Seeds were nicked to produce openings in the seed coats without damaging the nucellus or breaking the attachment of the embryo to the seed coat. This allowed for free movement of solvent and solute into the seed. When these seeds were immersed in the dye solution, no internal staining of the embryos occurred (Table 1). The nucellus acted as a barrier to penetration by the dye. This barrier was continuous, even at the point of embryo attachment to the seed coat at the chalaza. When excised embryos were immersed in dye

Table 2. Effects of immersing glandless cottonseed or embryos in acetone or methylene chloride on extractable oil, aqueous embryo leachate conductance, and embryo germination.

Treatment	Solids extracted	Change in % extractable oil	Embryo leachate conductance	Germination
	% of dry weight		μmhos/g dry weight	%
Control	0 a**	0 a	18 a	92 a
Seeds, acetone†	–	−0.5 a	19 a	94 a
Seeds, methylene chloride†	–	+0.2 a	17 a	91 a
Embryos, acetone‡	1.8 b	+0.7 a	43 b	0 b
Embryos, methylene chloride‡	9.7 c	−5.3 b	106 c	0 b

** Values followed by different letters differ significantly at the 1% level according to Duncans multiple range test. † Measurements made on embryos excised from seeds following immersion in solvents.
‡ Embryos excised from seeds before immersion in solvents.

solutions for 5 or 10 min staining was most intense near the severed point of attachment to the seed coat where the permeability barrier was disrupted during excision. Failure of solvents to penetrate embryos of intact seeds was due to the nucellus, rather than to any impediment of permeability in the seed coats.

Although Tao and Khan (12) reported deposition of solutes by methylene chloride within embryo tissues, no explanation was given of how solvents could penetrate embryo tissues without causing severe disruption of lipid systems, as described by Swanson et al. (11). Others observed that scarification or cutting of lettuce, oat, and pigweed seeds to facilitate solvent penetration into embryo tissues killed the embryos (2, 3, 13). Extraction of material, changes in extractable oil, leachate conductance, and germination of embryos, as a result of immersion of seeds and embryos in solvents were measured on glandless cottonseed (Table 2). Solvent immersion had no effect on any of the parameters measured on embryos in intact seeds. However, excised embryos immersed directly in solvents were affected; materials were extracted from embryos, extractable oil decreased, leachate conductance increased, and embryos failed to germinate. Effects were especially pronounced on embryos immersed in methylene chloride, and these embryos were friable when handled.

Cottonseed frequently are cut or punctured during ginning. Experiments which demonstrated killing of excised embryos by organic solvents indicated that gin-damaged seed would be vulnerable to additional damage by organic solvents. To test this hypothesis, sound and saw-damaged glanded seeds were immersed for 4 hours in methylene chloride. Germination of nondamaged control and solvent-treated seeds was 99 and 92%, respectively, whereas, germination of comparable saw-damaged seeds was 70 and 47%, respectively.

This study, and those of others (2, 3, 13) demonstrated that treatments that allowed the solvents to penetrate embryos killed or injured them, probably due to disruption of lipid systems. In cottonseed the nucellar layer surrounding the embryo apparently functions as a barrier to solvent penetration. In lettuce the endosperm has been shown to perform a

similar function (2, 13). The usefulness of the organic solvent infusion method for applying chemicals to cottonseed may be limited by prexisting mechanical damage to the nucellar membrane.

ACKNOWLEDGEMENTS

I wish to thank J. A. Green and L. W. Young for their excellent technical assistance.

REFERENCES

1. American Oil Chemists Society. 1966. Official and tentative methods, 3rd Ed., Chicago, Ill.
2. Anderson, J. D. 1973. Dichloromethane and lettuce seed germination. Science 179:94-95.
3. Brewer, P. E., and R. E. Wilson. 1975. Dichloromethane: Variability in penetration and resulting effects on seed germination and CO_2 evolution. Bot. Gaz. 136:216-218.
4. Eckenrode, C. J., R. J. Kuhr, and A. A. Khan. 1974. Treatment of seeds by solvent infusion for control of the seedcorn maggot. J. Econ. Entomol. 67:284-286.
5. Ellis, M. A., S. F. Foor, and J. B. Sinclair. 1976. Dichloromethane: Nonaqueous vehicle for systemic fungicides in soybean seeds. Phytopathology 66:1249-1251.
6. Khan, A. A., K. L. Tao, and C. H. Roe. 1973. Application of chemicals in organic solvents to dry seeds. Plant Physiol. 52:79-81.
7. Meyer, H., and A. M. Mayer. 1971. Permeation of dry seeds with chemicals: Use of dichloromethane. Science 171:583-584.
8. Milborrow, B. V. 1963. Penetration of seeds by acetone solutes. Nature (London) 199:716-717.
9. Papavizas, G. C., and J. A. Lewis. 1976. Acetone infusion of pyroxychlor into soybean seed for the control of *Phytophthora megasperma* var. sojae. Plant Dis. Rep. 60:484-488.
10. Simpson, D. M., C. L. Adams, and G. M. Stone. 1940. Anatomical structure of the cottonseed coat as related to problems of germination. USDA Tech. Bull. No. 734.
11. Swanson, E. D., W. W. Thomson, and J. B. Mudd. 1973. The effect of lipid extraction on electron-microscopic images of plant membranes. Can. J. Bot. 51:1221-1229.
12. Tao, K. L., and A. A. Khan. 1974. Penetration of dry seeds with chemicals applied in acetone. Plant Physiol. 54:956-958.
13. Triplett, L. L., and A. H. Haber. 1973. Dichloromethane and lettuce seed germination. Science 179:95-96.