

Bis Esters of Carboxylic Acid Herbicides Are Effectual in Weed Control

Ethylene glycol bis esters of carboxylic acid herbicides produce lipophilic compounds that can have low volatility, good cuticular penetration, and a high percentage content of the biologically active acid moiety. Studies with the ethylene glycol bis ester of (2,4-dichlorophenoxy)acetic acid indicate that these compounds also exhibit good herbicidal activity. Bis ester chemistry can be applied to many carboxylic acid pesticides to produce ester derivatives with a high carboxylic acid content.

Weeds cause an estimated annual loss of \$10 billion in U.S. agricultural production, and the cost of weed control amounts to more than \$6.2 billion each year (U.S., *Dep. Agric., Agric. Handb.*, 1980). Perennial weeds, which account for a significant portion of these losses, are often controlled by spraying with carboxylic acid herbicides. Carboxylic acid herbicides include phloem-mobile compounds such as 2,4-D [(2,4-dichlorophenoxy)acetic acid], dalapon (2,2-dichloropropionic acid), dicamba (3,6-dichloro-*o*-anisic acid), and glyphosate [*N*-(phosphonomethyl)glycine]. The hydrophilic acid group appears necessary for transport of the chemical to other plant parts for effectual control (Crafts, 1953).

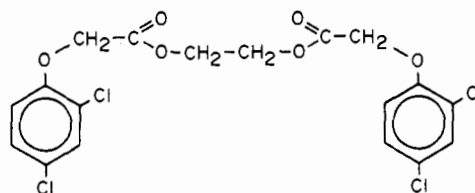
Herbicides applied to foliage must first penetrate the plant cuticle before death of the plant can occur. The impermeability of this protective barrier is determined primarily by plant species but will increase with increasing temperature, sunlight, and drought stress (Martin and Juniper, 1970). The negatively charged, lipophilic cuticle is a formidable barrier to penetration by salt formulations of carboxylic acid herbicides. An impermeable cuticle may well be responsible for the erratic control of weeds treated with these compounds in the warm, dry, and less humid climates of the world. Under these conditions, the wax content on the leaf surface increases and the hydration state of the cuticle is reduced (Martin and Juniper, 1970). The enhanced lipophilic character of the cuticular waxes predominates and causes the cuticle to become increasingly resistant to penetration by the hydrophilic salts (Richardson, 1977).

Lipophilic ester derivatives of carboxylic acid herbicides enhance penetration through plant cuticles (Norris and Freed, 1966). However, high molecular weight esters often must be used because many low molecular weight esters are extremely volatile and can damage susceptible plants growing nearby (Mullison and Hummer, 1949). After penetrating the cuticle, the ester is hydrolyzed to form the active carboxylic acid and an alcohol (Crafts, 1960). This alcohol is herbicidally inactive and can comprise a considerable portion of the ester molecule. For example, 36% of the butoxyethanol ester of 2,4-D is the alcohol.

The purpose of this study was to determine if bis esters of carboxylic acid herbicides are herbicidally active. Most bis esters would exhibit low volatility and good cuticular penetration and have the wasteful alcohol content reduced to a minimum. The ethylene glycol bis ester of 2,4-D has been chosen as a model to test the herbicidal activity of bis ester compounds.

MATERIALS AND METHODS

Chemicals. Ethylene glycol was refluxed with the acid chloride of 2,4-D (Freed, 1946) in methylene chloride to form ethylene glycol bis(2,4-dichlorophenoxyacetate). The organic phase was extracted with 10% aqueous Na₂CO₃ and evaporated to dryness in vacuo to yield crude product, which was purified by recrystallization from diisopropyl ether. Synthesis of the compound was verified by NMR,



infrared, and mass spectral analysis.

Technical-grade butoxyethanol ester of 2,4-D and XRR-0268-24 emulsifier were supplied by Dow Chemical Corp., Midland, MI.

Bioassays. Weed sensitivity and crop selectivity of the bis ester were compared to that of the commercially available butoxyethanol ester of 2,4-D. Emulsified formulations of the ethylene glycol bis ester and the butoxyethanol ester were sprayed at the rate of 1.1 kg of acid equiv (a.e.)/ha on 12 species of 3-week-old plants grown in the greenhouse. The 12 plant species included alfalfa (*Medicago sativa* L.), bluegrass (*Poa pratensis* L.), corn (*Zea mays* L.), cotton (*Gossypium hirsutum* L.), crabgrass [*Digitaria sanguinalis* (L.) Scop.], cucumber (*Cucumis sativus* L.), jimsonweed (*Datura stramonium* L.), morning glory [*Ipomoea hederacea* (L.) Jacq.], pigweed (*Amaranthus retroflexus* L.), soybean (*Glycine max* L.), tomato (*Lycopersicon esculentum* Mill.), and velvetleaf (*Abutilon theophrasti* Medic.). Herbicidal injury to the plants was recorded 14 days after treatment.

Volatilities of the bis ester and the butoxyethanol ester were determined with a cucumber bioassay (Leasure, 1958). Six 6-cm Petri dishes containing three cucumber seeds each and one 6-cm Petri dish containing 200 mg of pure compound were placed in a 20-cm recrystallizing dish. Two milliliters of distilled water was then added to each of the seed-containing Petri dishes. Each recrystallizing dish was covered with a glass plate and sealed with silicone grease. The recrystallizing dishes were placed in a dark growth chamber (32 °C) for 4 days, and then root and shoot lengths were measured to the nearest millimeter.

Two bioassays were used to determine the activities of both ester compounds. Herbicidal activity on whole plants was measured with a cotton leaf-width bioassay as explained by Leonard et al. (1962). One cotyledon of 12-day-old cotton plants was treated with variable amounts of emulsified herbicide. Lateral growth of the first and second true leaves was recorded to the nearest millimeter 15 days after treatment. The amount of herbicide applied was either 0, 16, 32, 64, 125, 250, 500, 1000, or 2000 ng of a.e./plant. Intrinsic herbicidal activity was measured with an oat (*Avena sativa* L.) coleoptile growth test (Mitchell and Livingston, 1968). Ten 4-mm oat coleoptile sections were placed in Petri dishes containing 20 mL of a 10 mM potassium phosphate buffer (pH 7.0) and either 0, 0.25, 0.5, 1.0, 5.0, or 10.0 μM a.e. emulsified herbicide formulation. The dishes were gently shaken in the dark for 24 h, and coleoptile length was measured to the nearest 0.5 mm.

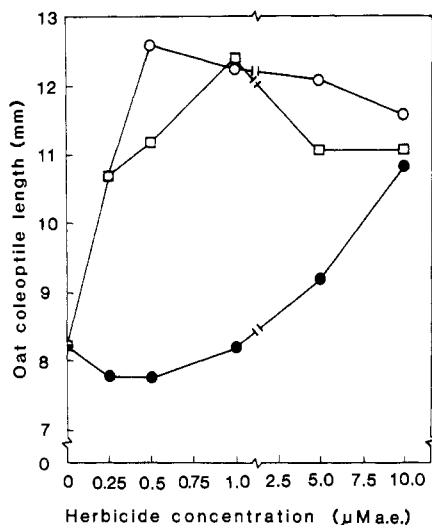


Figure 1. Emulsifier formulation affects the hormonal activity of the bis ester of 2,4-D. Symbols: (●) bis ester in XRR-0268-24 emulsifier; (○) bis ester in XRR-0268-24 emulsifier plus 1% Me₂SO; (□) butoxyethanol ester in XRR-0268-24 emulsifier. There was no statistical difference in the activity of the butoxyethanol ester formulated in either XRR-0268-24 emulsifier or XRR-0268-24 emulsifier plus 1% Me₂SO. The least significant difference between two data points is 1.45 mm at the 5% level.

RESULTS AND DISCUSSION

Species Selectivity. The susceptibility of all the species was the same for the two ester compounds. The broadleaf plants developed malformed leaves and epinastic stems, characteristic of phenoxy injury (Klingman and Ashton, 1975). The grass species were not affected by the herbicide treatments.

Volatility. An absolute measure of volatility cannot be determined with the cucumber bioassay, but the relative level of volatility of the ethylene glycol bis ester was similar to that of the butoxyethanol ester. The diester inhibited cucumber shoot growth by 60%, whereas the butoxyethanol ester inhibited shoot growth by 40%. For every molecule of bis ester volatilized, two acid moieties are released once the molecule penetrates into the plant. This may explain the somewhat higher biological activity of the bis ester in this assay. In the greenhouse, susceptible plants growing next to plants treated with either ester herbicide showed no herbicidal injury. The butoxyethanol ester of 2,4-D is considered a low volatile compound ("Herbicide Handbook of the Weed Science Society of America", 1979); therefore, volatilization of the bis ester in field applications should also be low.

Activity. We found it imperative to properly emulsify the bis ester before herbicidal activity studies were begun. The XRR-0268-24 emulsifier, which was developed to produce optimal activity for the butoxyethanol ester of 2,4-D, did not properly emulsify the ethylene glycol bis ester derivative and produced a mixture with low intrinsic biological activity. However, the addition of 1% (v/v) dimethyl sulfoxide (Me₂SO) restored the biological activity of the bis ester to that of the butoxyethanol ester (Figure 1). Adding 1% Me₂SO to the butoxyethanol ester had no significant effect on its biological activity. The bis ester was stable in 1% Me₂SO. After 10 days, the amounts of bis ester extractable with diethyl ether from aqueous solutions with or without Me₂SO were not significantly different.

In a cotton leaf width bioassay, 2 µg of a.e. of the butoxyethanol ester formulated in XRR-0268-24 emulsifier decreased the leaf width by 42% compared to that of

untreated plants. Me₂SO concentrations varying from 0 to 1% (v/v) did not affect the amount of leaf width inhibition induced by the butoxyethanol ester. Two micrograms of a.e. of the bis ester formulated in XRR-0268-24 emulsifier inhibited lateral leaf growth by 11%. As the Me₂SO content was increased to 1% (v/v), leaf growth inhibition by the bis ester also increased until it equaled that of the butoxyethanol ester. Properly emulsified formulations of both esters induced leaf curling in cotton plants treated with as little as 64 ng of a.e. of either ester compound.

When pure, the ethylene glycol bis ester of 2,4-D is crystalline and does not exhibit the oil-like penetration properties of the liquid, butoxyethanol ester. A good emulsifier must compensate for these crystalline properties by maintaining a proper oil-like formulation on the leaf surface (Hess et al., 1981). Common procedures for initial testing of a compound for herbicidal activity usually include spraying the compound onto the plant with an acetone in water solution. This procedure can result in dissolution of the mixture on the plant cuticle surface that may allow little or no penetration of the compound through the plant cuticle. The chemical would appear to have no herbicidal activity when, in reality, the chemical may be highly active. Proper emulsification of any potential postemergent herbicide prior to application is essential before herbicidal activity can be accurately assessed.

Bis ester herbicides have advantages over mono ester herbicide derivatives. First, a greater content of the biologically active carboxylic acid can be incorporated into 1 mol of the bis ester compound than into 1 mol of the mono ester. For example, 2,4-D acid comprises 87% of the bis ester molecule and 64% of the butoxyethanol ester molecule. Therefore, the same degree of weed control can be obtained with a lesser weight of bis ester material. Second, the increased 2,4-D acid content of the bis ester may be advantageous in the warm, dry, and less humid climates of the world where the amount of penetration of the biologically active acid group is the limiting factor in successful weed control. If an equivalent number of mono and bis ester molecules are able to penetrate a well-developed cuticle, the bis ester will yield, upon hydrolysis, twice as many active acid moieties. In addition, the penetration characteristics of a properly emulsified bis ester could potentially be better than those of the mono ester. These effects of bis esters may not be seen on plants grown in the greenhouse because greenhouse plants have easily penetrable cuticles (Martin and Juniper, 1970).

The importance of this communication is not to report a new herbicide derivative of 2,4-D but to convey the idea that bis ester derivatives of carboxylic acid herbicides may be beneficial and practicable compounds. As with any new herbicide, these compounds will have to be thoroughly tested under a wide range of environmental conditions to determine their practical efficacy. We have also synthesized the ethylene glycol bis ester of dalapon and the ethylene glycol bis ester of 2,4-DB [4-(2,4-dichlorophenoxy)butyric acid] and have found both compounds to be herbicidally active. The chemistry of bis ester synthesis is applicable to many carboxylic acid herbicides (Table I). The bis ester derivative of many of these compounds should have low volatility, good cuticular penetration, and a high percentage content of the active acid moiety. In addition to effective weed control, some crop and weed species may exhibit different selectivities when treated with a bis ester as compared to a mono ester derivative. This could result when different plants enzymatically hydrolyze these esters to different degrees or when the

Table I. Herbicides Suitable for Bis Ester Chemistry^a

acifluorfen	3,6-dichloropicolinic acid	mecoprop
benazolin	dichlorprop	naptalam
benzadox	endothall	picloram
bifenox	fenac	silvex
chloramben	flurenol	2,4,5-T
2,4-D	glyphosate	2,3,6-TBA
dalapon	glyphosine	Cl ₃ CCO ₂ H
2,4-DB	MCPA	triclopyr
dicamba	MCPB	

^a Common name as determined by the Weed Science Society of America ("Herbicide Handbook of the Weed Science Society of America", 1979).

penetration, translocation, and detoxification characteristics of the esters vary between species. These selectivity differences, when coupled with the efficient penetration of the bis ester compound, may transform a carboxylic acid herbicide with moderate efficacy into a useful chemical for weed control.

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Effects of Repeated Application of Dichlobenil in a Commercial Apple Orchard

A commercial apple orchard of Idared on Malling 7 rootstock was treated with dichlobenil (2,6-dichlorobenzonitrile) at the maximum recommended rate (8.97 kg/ha a.i.) every other year from 1974 to 1978. Residues of the herbicide and the metabolite 2,6-dichlorobenzamide were determined in leaves, fruits, and soil. 2,6-Dichlorobenzamide was present in considerably greater amounts in the leaves and soil samples than the parent compound. Small amounts of 2,6-dichlorobenzamide was also detected in fruit. The residues levels were higher, and foliar phytotoxicity symptoms, characterized by leaf margin yellowing and leaf tip burn, were more marked in fruiting trees. However, removing the fruit to encourage growth resulted in a reduction in the leaf symptoms as well as the levels of dichlobenil and 2,6-dichlorobenzamide.

Dichlobenil (2,6-dichlorobenzonitrile) is recommended for the selective control of a wide range of annual and perennial weeds including both grasses and broadleaf species (Verloop, 1972; Beynon and Wright, 1972). Apple trees have been considered very tolerant to dichlobenil even at excessive rates (Sanford, 1962). However, Lord et al. (1973) and Lord and Damon (1974) observed that the annual applications of dichlobenil at the recommended rates caused foliar phytotoxic symptoms on young apple and peach trees. A comparison between control and dichlobenil-treated "McIntosh" apple trees showed no differences in tree growth and yield, although some relationship between phytotoxicity severity and tree growth and yield was noticed (Lord et al., 1973). In a later study, Lord and Green (1975) observed that the registered rates of dichlobenil usage in orchards, 4.47-8.97 kg/ha, had no adverse effect on tree growth even though foliar phytotoxic symptoms were present.

The occurrence of leaf margin chlorosis due to the use of dichlobenil as a soil-applied selective herbicide varies in accordance with geographical location and climatic conditions (Verloop, 1972). The leaf chlorosis varies from year to year (Verloop, 1972), probably due to soil conditions when dichlobenil is metabolized to 2,6-dichlorobenzamide, the causative agent of the leaf yellowing (Leach et al., 1971).

The herbicide dichlobenil has been used in eastern Ontario to effectively control weeds under mature apple trees. The present study was designed to determine the residue levels of dichlobenil and its metabolite, 2,6-dichlorobenzamide, in soil, leaf, and fruit after repeated applications of the herbicide in a commercial apple orchard. It was also of interest to determine whether any phytotoxicity symptoms occurred following the dichlobenil usage.

EXPERIMENTAL SECTION

Samples. Samples were collected on Aug 28, 1980, from a commercial apple orchard of Idared on Malling 7 rootstock growing on Newcastle silt loam (organic matter 5%). The trees were planted in 1974. Dichlobenil was applied (broadcast) at the maximum recommended rate of 65 g of product (8.97 kg/ha a.i.) on an area 2 × 2 m under each tree in the fall of 1974, 1976, and 1978 (Heeney et al., 1981). Excellent weed control was obtained in the area under the treated trees. The trees were allowed to fruit at an early age (1978). In 1980, fruit was removed from some of the trees to encourage growth. On Aug 28, 1980, midshoot leaf samples were collected from control and treated fruiting and defruited trees having similar dichlobenil treatments. In each case the leaves were collected from four trees and mixed thoroughly, and a subsample was retained for