*TORDON - 2,4-D - DIMETHYLSULFOXIDE
COMBINATION HERBICIDES FOR
USE IN ROADSIDE DEVELOPMENT*

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Progress Report

TORDON - 2,4-D - DIMETHYLSULFOXIDE COMBINATION HERBICIDES
FOR USE IN ROADSIDE DEVELOPMENT

To: G. A. Leonards, Director
Joint Highway Research Project

November 28, 1967

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From: H. L. Michael, Associate Director
Joint Highway Research Project

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The attached Progress Report is submitted on the HPR-1(5) Part II research project "Research in Roadside Development." This report on the Chemical Weed Control portion of the research is titled "Tordon - 2,4-D - Dimethylsulfoxide Combination Herbicides for Use in Roadside Development." The report has been authored by Messrs. D. J. Morre', W. R. Eisinger and H. W. Russell, members of staff of the Department of Botany and Plant Pathology of the School of Agriculture.

The report documents the effects of certain herbicides on plant growth with particular attention to roadside applications.

The report is submitted for review and comment as well as approval. It will also be submitted to the ISEC and the EPR for similar action.

Respectfully submitted,

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FOR USE IN ROADSIDE DEVELOPMENT

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I. SCOPE AND OBJECTIVES

Herbicides used in roadside development, particularly for right-of-way maintenance, must fulfill certain requirements. They must control undesirable plant species at rates that do not injure desirable plants. This requirement necessitates an exceptionally broad selectivity spectrum because of the large numbers of species common to roadside situations. The many abundant roadside species, such as Milkweed, Wild Garlic, Wild Parsnip, Canada Thistle, Wild Carrot, Curled Dock, Field Bindweed, Plantain, Johnson Grass and Dandelion are not economically controlled by any one chemical presently available. These species are either unsightly or potential invaders of adjacent crop land. Unsightly vegetation contributing to obstruction of vision, especially woody and brush species, must also be controlled. The herbicides must be economical and easy to apply and present no hazards to either the operator or to crop and noncrop plants in areas immediately adjacent to the treated areas. The tolerance margin must be sufficiently high so that reasonable deviations from the recommended rates of application (due to operator error, equipment malfunction, uneven terrain, adverse weather conditions, etc.) will neither eliminate the effectiveness of the treatment nor endanger desirable plants within or adjacent to the treated area. A single chemical which adequately meets these requirements is difficult to find.

A possible solution is offered by combining new and promising herbicides with less costly older materials (13). These combinations can kill a broader spectrum of plants at lower rates of application and thereby reduce costs. By employing combinations, one herbicide can overcome weaknesses of other herbicides in the combination. Combinations offer the possibility of broadening the spectrum of herbicidal selectivity by synergistic action. Often species notoriously resistant to one or more of the chemicals making up a combination are killed by the mixture. Similarly, an antagonistic interaction can be exploited to provide protection to desirable species. Properly chosen combinations insure against unfavorable weather conditions (13) and offer a new method of extending the duration of control over longer periods.

A popular notion is that combining herbicides is like formulating prescription drugs (17). And as in the formulation of prescription drugs, it is often desirable to know something of the mode of action or expression of the components of the herbicidal mixtures before exploring the behavior of the combinations.

In the present study, a potent (but somewhat expensive) new herbicide providing control of a broad spectrum of plant species was selected for initial use in combinations with the standard roadside herbicides 2,4-D and 2,4,5-T. The material selected was picloram (4-amino-3,5,6-trichloropicolinic acid) which is sold commercially as Tordon herbicide (Trademark of the Dow Chemical Company, Midland, Michigan).

In the first part of this report, the mode of action of Tordon herbicide is described. Extensive laboratory studies have shown its mode of action to be similar to that of the auxin herbicides 2,4-D and 2,4,5-T. The greater effectiveness of Tordon is attributed to its resistance to breakdown and greater mobility within the plant.

Interactions between Tordon and 2,4-D were also investigated and shown to be synergistic. This finding offers the opportunity of combining lesser amounts of a costly but potent herbicide (Tordon) with larger amounts of a less costly herbicide (2,4-D) into a mixture that is more effective pound for pound than either herbicide alone.

In the second part of the report, the potential of combining Tordon + 2,4-D with dimethylsulfoxide (DMSO), a potent herbicide solvent, is explored. By acting as an adjuvant, DMSO affords a second opportunity for synergistic interaction which may prove helpful against spot infestations of especially difficult to control species such as Wild Garlic.

II. STUDIES ON THE MODE OF ACTION OF TORDON (PICLORAM)

Introduction

Tordon herbicide, 4-amino-3,5,6-trichloropicolinic acid (picloram) causes stem twisting and other formative effects in dictyledonous plants (32). Studies under field conditions indicate similarities among the herbicidal effects of Tordon and the auxin herbicides 2,4-D (2,4-dichlorophenoxyacetic acid) and 2,4,5-T (2,4,5-trichlorophenoxyacetic acid). If the herbicidal properties of Tordon were similar to those of 2,4-D and 2,4,5-T, this similarity could be used as a basis of prediction with regard to application rates and methods. Differences between Tordon and 2,4-D or 2,4,5-T would be important in formulating combination herbicides.

Auxin herbicides, including 2,4-D and 2,4,5-T, are defined as compounds which when supplied in low concentrations stimulate plant shoot cell elongation (54). Thus Tordon could be classified as an auxin herbicide if it were shown to fulfill the requirements of the definition. In addition, auxin herbicides are also active in stimulation and/or inhibition of other plant responses besides those directly concerned with elongation. These effects include regulation of abscission (defoliation), induction of formative effects, and stimulation of growth of excised plant tissue in culture. However, the ability to promote cell expansion in plant shoot tissue is considered essential for a compound to be an auxin herbicide (54).

In this study, the growth regulating properties of Tordon were investigated in controlled laboratory tests and compared with those of 2,4-D and 2,4,5-T. Additional information was obtained concerning the mobility and stability of Tordon within the plant and use of Tordon - 2,4-D - 2,4,5-T mixtures for control of Milkweed and Wild Garlic along roadsides.

Promotion and Inhibition of Cell Expansion

Tordon¹ was shown to stimulate cell expansion of excised stem and coleoptile sections of etiolated plant materials grown as described by Morré and Key (54). The concentration range for optimum stimulation of elongation of etiolated Avena coleoptile, pea epicotyl, soybean

hypocotyl, and light grown mung bean epicotyl sections was between 10^{-6} and 10^{-4} M (Figures 1 to 5). Elongation of Avena coleoptile sections was inhibited by 10^{-3} M Tordon. This concentration induced little or no elongation of pea epicotyl and soybean hypocotyl sections. Comparison of fresh weight and length increases of soybean hypocotyl sections (Figures 3 and 4) suggest that growth over the concentration range of 10^{-8} to 10^{-6} M Tordon was due primarily to an increase in cell length rather than through nonpolar swelling (73% increase in fresh weight vs. 72% increase in section length at 10^{-6} M Tordon). However, at high Tordon concentrations growth was less polar (33% increase in section length vs. 52% increase in fresh weight at 10^{-3} M Tordon).

Maximal stimulation of elongation was recorded for mung bean first internode sections treated with 10^{-4} and 10^{-5} M Tordon (Figure 5). Tordon at 10^{-3} M was supraoptimal for elongation. The dose-response characteristics of growth in the presence of varying concentrations of IAA and 2,4-D were similar to those induced by equivalent concentrations of Tordon. Elongation stimulated by all three compounds at 10^{-4} M (optimal for IAA and 2,4-D) was nearly equal. Only 2,4-D was inhibitory at 10^{-3} M.

Tordon stimulated elongation of etiolated tissue sections is similar to that reported by other workers for IAA and 2,4-D (49,50,54). The growth regulator-response curve observed for Tordon treated tissues is characteristic of growth regulators of the auxin type. The response curves for Tordon have optima with descending limbs in both the supra-optimal and suboptimal dose ranges. Other workers (54; see also 49 and 50, 12 hr incubations) using 6 hour incubations have reported IAA and

1/ Tordon (Dow analytical standard (99%)), 2,4-D and IAA were dissolved in distilled water without the aid of cosolvent except in whole plant translocation and rooting studies where ethanol (100%) was used as the solvent. For control treatments the growth regulator under study was omitted from the test medium. Unless stated otherwise, treatments were not replicated within an experiment but each experiment was repeated at least 3 times.

Etiolated plant materials were grown as described by Morris and Key (54) in a constant temperature ($26 \pm 1^\circ$ C), high humidity (85 - 95%), dark growth chamber. Plant material grown in the greenhouse was maintained under a 16 hour photoperiod and transferred to constant fluorescent lighting conditions in the laboratory (400 ft-c at plant level; 40 watt Gro-Lux, Sylvania) at approximately 25° C for treatment and observation.

Figure 1. Stimulation of Elongation in Etiolated Avena Coleoptile Sections by Varying Concentrations of Tordon (Picloram). Sections (1 cm initial length) measured after 6 hours.

Figure 2. Stimulation of Elongation in Etiolated Pea Epicotyl Sections by Varying Concentrations of Tordon (Picloram). Sections (1 cm initial length) measured after 6 hours.

Figure 3. Stimulation of Elongation in Etiolated Soybean Hypocotyl Sections by Varying Concentrations of Tordon (Picloram). Sections (1 cm initial length) measured after 6 hours.

Figure 4. Stimulation of Fresh Weight Increase in Etiolated Soybean Hypocotyl Sections by Varying Concentrations of Tordon (Picloram). Sections (1 gram initial weight) were weighed after 6 hours.

2,4-D to stimulate near optimal growth of Avena coleoptile sections at concentrations (maximum ca. 10^{-5} M) lower than those reported here for Tordon (maximum ca. 10^{-4} M) using a 6 hour incubation. However, Tordon is more active than IAA at lower concentrations in stimulating growth of tissues where destruction of growth regulator might be an important factor in their effectiveness. Lower concentrations of Tordon (10^{-5} M reported here) are required to stimulate near optimal growth of soybean hypocotyl sections than IAA (5×10^{-4} M; see 54).

Tordon - 2,4-D Interactions

Equimolar mixtures of Tordon and 2,4-D were more effective in promotion of growth of Avena Coleoptile sections than either compound alone at equivalent concentrations (Table 1). The synergistic action was more pronounced at higher concentrations. Analysis of variants using randomized complete blocks and Duncan's Multiple Range Test (24) revealed that the increased stimulation measured for mixtures were significant to the 5% level.

Field trials were initiated to test 2,4-D, 2,4,5-T and Tordon herbicide alone and in combination for control of spot infestations of Common Milkweed (Asclepias syrica) along roadsides at 2 locations in Tippecanoe County, Indiana, during July and August, 1967. A combination treatment of 1 lb/A 2,4,5-T + 1 lb/A Tordon was superior to 2 lb/A of either material alone and gave complete control of top growth of milkweed in 2 to 3 weeks. The kill was sufficiently slow to permit translocation of herbicide into the root system. Samples of roots were collected for evaluation of these treatments for milkweed eradication.

The increased activity observed for mixtures of Tordon and 2,4-D in promoting cell expansion has also been observed in other field studies and such mixtures are now being used commercially. Greater effectiveness at lower application rates of herbicide per acre were reported for mixtures of Tordon and 2,4-D or 2,4,5-T in control of Macartney rose (Rosa bracteata Wendl.) (16) and blackberry (25), for use in small grains (27), and in general brush control (30). The basis of the increased activity of Tordon-2,4-D mixtures is not known although altered uptake patterns might contribute. Measurements of isotopically labeled 2,4-D uptake in the presence of Tordon would be of interest for future investigations.

Table 1. Growth Stimulatory Effects of Mixtures of Tordon and 2,4-D on Growth of *Avena* Coleoptile Sections. 1/

Treatment	Increased length (mm) after 4 hr. incubation <u>2/</u>
Control	0.4 ± .1 a
10 ⁻⁵ M Tordon	0.8 ± .1 b,c
10 ⁻⁵ M 2,4-D	1.1 ± .1 d
5 x 10 ⁻⁶ M Tordon + 5 x 10 ⁻⁶ M 2,4-D	1.5 ± .2 e
10 ⁻⁶ M Tordon	0.7 ± .1 b
10 ⁻⁶ M 2,4-D	0.6 ± .1 b
5 x 10 ⁻⁷ M Tordon + 5 x 10 ⁻⁷ M 2,4-D	0.9 ± .1 c,d

1/ Increase in length was determined after a 4 hr. incubation.

2/ Results presented as averages of three experiments of 5 sections each with standard deviations. Means followed by the same letter are not significantly different at the 5% level from analysis of variants using randomized complete blocks and Duncan's Multiple Range Test (24).

Promotion and Inhibition of Epicotyl Elongation of Shoot Cuttings

Mung bean (*Phaseolus aureus* 141bg.) seeds were soaked in running tap water (28° C) for 6 hours in the laboratory. Seedlings were grown in the greenhouse for 7 days after which plants with shoots 7 to 10 cm in length were selected and their roots removed 3 cm below the cotyledonary node. The bases of the shoot cuttings were placed in 19 X 65 mm shell vials, one cutting per vial. Ten cuttings were used for each of 3 experiments. Vials contained 4 ml of distilled water with or without growth regulator solutions. Epicotyl growth of excised mung bean cuttings was determined by measuring the length of the region of the epicotyl between the cotyledonary node and the primary leaf bases (first internode). Addition of mineral nutrients or daily replacement of the solutions did not significantly alter the results.

Histological observations were made on stem segments from the rapidly growing region of the mung bean epicotyls. Sections were excised immediately below the second node, fixed in Carnoy's fixative, embedded in paraffin, and sectioned with a rotary microtome. Longitudinal and transverse sections from Tordon treated and control epicotyls were compared with respect to cell size and number after staining with safranin and fast green.

Of the concentrations tested, 10^{-7} M Tordon elicited the greatest total growth of first internodes during the 7 day period (Figure 6). First internode elongation induced by 10^{-7} M Tordon was about twice that observed for intact plants during the same 7 days. Concentrations of Tordon greater than and including 10^{-7} M were toxic to the cuttings. However, even at herbicidal concentrations, death of cuttings was preceded by a period of rapid first internode elongation (Figure 6). Thus, 10^{-5} and 10^{-6} M Tordon produced a greater growth response during the first 24 to 48 hours than did 10^{-7} M Tordon, but resulted in death of the epicotyl tissue within 3 to 5 days.

With 10^{-7} M Tordon, the phase of rapid growth promotion continued for 3 days and loss of turgor (a measure of herbicidal response) was delayed until between 6 and 7 days. At higher concentrations of Tordon total growth (data of Figure 6) followed an inverse log-linear relationship to concentration as did the onset of severe herbicidal symptoms which was followed in turn by turgor loss and eventual death of the cuttings. The growth rate for 10^{-8} M Tordon was constant after 1 day and was about twice the rate observed for distilled water controls. In contrast to epicotyls subjected to higher Tordon concentrations, cuttings treated with 10^{-8} M Tordon remained viable during the 7 day period. IAA and 2,4-D at concentrations of 10^{-3} M, 10^{-5} M, and 10^{-7} M induced no significant stimulation of first internode elongation (Figure 7 provides a comparison of first internode growth induced by 10^{-7} M IAA, 2,4-D, and Tordon).

Histological examination of the rapidly elongating region of mung bean cuttings treated with 10^{-7} M Tordon revealed that after 5 days of growth the cortical cells of this region averaged 250 μ in length and 100 μ in diameter in longitudinal section compared with 125 μ in length and 100 μ in diameter for similar cells of control plants. Thus, Tordon treated cells were twice as long as control cells, but showed no increase in diameter.

Figure 5. Stimulation of Elongation in Light-Grown Mung Bean Epicotyl Sections by Varying Concentrations of Tordon (Picloram), IAA and 2,4-D. Sections (1 cm initial length) were measured after 6 hours.

Figure 6. Stimulation of Elongation in the First Internode of Light-Grown Mung Bean Shoot Cuttings by Varying Concentrations of Tordon (Picloram) With Time.

Figure 7. Stimulation of Elongation in the First Internode of Light-Grown Mung Bean Shoot Cuttings by 10^{-7} M Tordon (Picloram), IAA and 2,4-D With Time.

	Initial	Control	Picloram	Change due to Picloram	IAA	Change due to IAA
Increase in length (cm) per section 2/	---	0.3 ± .1	0.7 ± .1	0.4 ± .1	0.2 ± .1	-0.2 ± .1
Total Fresh Weight (mg) 2/	147.8 ± 5.6	233.9 ± 10.9	337.1 ± 19.2	103.2 ± 20.3	---	---
Weight of xylem fraction (mg) 2/	2.31 ± .3	5.4 ± .3	6.2 ± .8	0.3 ± 1.0	---	---
Extensibility (cm/g x 10 ⁻²) 2/	1.02 ± .02	0.68 ± .16	1.08 ± .02	0.10 ± .06	0.76 ± .08	0.06 ± .10

1/ The epiphylls of shoot cuttings prepared from seven day old mung bean seedlings were marked with India ink 1 cm below the base of the primary leaves. The bases of the cuttings were placed in fresh solution (distilled water, 10^{-7} M picloram, or 10^{-7} M IAA) and the cuttings were maintained for 24 hours under continuous fluorescent lighting. Determinations of length, fresh and xylem weights, and extensibility were made for this region of the epicotyl between the paired primary leaf bases and the ink mark.

2/ Results presented as averages of three experiments with standard deviations.

Table 2. Comparison of Effects of Tordon (Picloram) and IAA on Growth, Cell Wall Synthesis and Extensibility of One Centimeter Segments of the First Internode of Mung Bean Shoot Cuttings. 1/

The activity of Tordon in stimulating growth of mung bean epicotyl sections compared favorably with that of IAA and 2,4-D. The two known auxins (IAA and 2,4-D) were included in this experiment because their effect on the growth of light grown mung epicotyl sections had not been previously investigated. A significant amount of enzymatic destruction of growth regulators probably does not occur with light grown mung bean epicotyl tissue. Evidence for this is the growth stimulation by low levels (10^{-7} M) of IAA, the least stable of the three growth regulators tested. If enzymatic decomposition of auxins were to occur, IAA would be predicted to be the most affected. The latter is the situation with soybean hypocotyls when comparing IAA and 2,4-D (54).

The effectiveness of Tordon in stimulating epicotyl elongation of mung bean shoot cuttings is probably a reflection of its greater mobility than IAA and 2,4-D within the plant. Apparently Tordon is selectively moved by the plant into the shoot cells which results in their rapid elongation. These cells must not be accessible to IAA and 2,4-D when the compounds are introduced by the shoot cutting method. Since mung bean epicotyl sections floated in petri plates gave nearly equivalent responses to IAA, 2,4-D, and Tordon, intrinsic differences among the modes of action of the three compounds could not be responsible for the specificity of the mung bean epicotyl growth system for Tordon. Selective enzymatic destruction of IAA and 2,4-D over Tordon is not likely since 2,4-D is relatively stable in biological systems (54). Epicotyl tissue itself probably does not rapidly metabolize IAA since Tordon was active in stimulating growth of epicotyl sections at low concentrations.

Light grown mung bean seedlings were selected for these experiments because they were the most responsive to Tordon of the light grown tissues tested (shoot cuttings of mung bean, Red Kidney bean, Alaska pea, soybean, sunflower, and tomato). In addition mung bean seedlings grow uniformly, do not require addition of nutrients, and respond consistently to Tordon. Despite the complex nature of the cutting (i.e. leaves, apical meristem, and cotyledons), the effects of Tordon for the first 24 hours appear to be largely confined to elongation within the epicotyl.

Thus, the Tordon mung bean epicotyl growth system is distinct from other growth systems for investigation of auxin-stimulated cell expansion. The growth rate of etiolated tissue sections floated in auxin solutions

does not approach that of the intact seedling (72) and auxin stimulations are measured only under conditions where the natural auxin source (usually the apical meristem or coleoptile tip) is removed. The growth rates measured for mung bean cuttings treated with Tordon exceed those of intact mung bean seedlings under conditions where the presumed natural auxin source [the shoot tip (47)] is present. The mung bean epicotyl growth system is specific for Tordon and can be used as a bioassay for Tordon in soil extracts in the presence or absence of other auxin herbicides.

Effects on Cell Wall Synthesis and on Cell Wall Loosening

Cell wall synthesis - Cell walls were prepared from sections cut from the first internode from 25 cuttings by exhaustive disruption with a VirTis homogenizer followed by separation of the wall fraction by Miracloth filtration. Wall preparations were washed on the filter until free of starch and then washed with organic solvents to remove lipids. Wall fractions were then dried to constant weight and weighed.

After 24 hours no significant increase in weight of total cell wall isolated from epicotyl sections from the first internode of Tordon treated (10^{-5} M) mung bean shoot cuttings was observed when compared with distilled water controls (Table 2). Under these conditions increase in fresh weight for Tordon treated internode sections was 130%. Increase in weight of wall fraction for both control and Tordon treated epicotyls was approximately 100% when compared with initial values.

Cell wall loosening - Tissue extensibility was measured by bending methods (48) for pea tissue using the apparatus depicted in Figure 8 or by force extension analysis for mung bean and pea tissue following the procedure of Olson et al. (62). The bending apparatus consisted of a rotatable horizontal shaft into which sections were inserted. Following orthogonal application of load (0.750 ± 0.001 g), the course of deformation was determined from the position of the indicator after restoration of the weight to the initial position (See Figure 9). After 5 minutes the weight was removed and recoverable deformation was measured after 1 minute. With this device it was possible to measure small deformations under constant imposed load.

For force extension analysis sections were boiled in methanol for 5 minutes prior to measurement. Extensibility of rehydrated sections was determined using an Instron Universal Testing Instrument (Instron Engineering Corporation, Canton, Massachusetts).

Figure 8. Pea Epicotyl Deformability in the Presence and Absence of 10^{-5} M Tordon (Pictoram). Measuring Device Shown in Insert. After a 6 hour incubation.

Figure 9. Extensibility of Pea Epicotyl Sections Determined by Force Extension Analysis as a Function of Tordon (Pictoram) Concentration. After a 6 hour incubation.

Figure 10. Inhibition of Maize Root Growth as a Function of Tordon (Pictoram) Concentration.

Figure 11. Percent Inhibition of Maize Root Growth Expressed as Probits as a Function of Tordon (Pictoram) and 2,4-D Concentration. Intact seedlings were incubated for 12 hours.

Cell wall loosening of both pea and mung bean epicotyl tissues was stimulated by Tordon. With pea epicotyl tissue where deformability under constant imposed external load was measured (Figure 8), wall loosening increased for up to 6 hours after the beginning of treatment (10^{-5} M Tordon). Wall loosening was proportional to log Tordon concentration over the concentration range of 10^{-8} to 10^{-3} M (Figure 9) as determined by linear stress-strain analysis. As shown in Figure 2 maximum growth stimulation by Tordon of pea epicotyl sections occurred at concentrations between 10^{-6} and 10^{-4} M, and 10^{-3} M was not effective in stimulation of elongation. Wall loosening, nonetheless, continued to increase up to 10^{-3} M.

Extensibility of first internode Tordon treated mung bean shoot cuttings were measured after 24 hours of treatment. Sections from Tordon (10^{-5} M) treated cuttings were approximately 60% more extensible than equivalent sections cut from distilled water control cuttings (Table 2). IAA (10^{-5} M), which did not stimulate epicotyl elongation under these conditions, did not significantly alter the extensibility of the tissue.

Cell wall loosening or relaxing of wall bonding is associated with cell elongation stimulated by auxins (36). Wall extensibility induced by Tordon in pea epicotyl sections is similar in magnitude to that reported for IAA (53). Cleland (11) and Morré and Coartney (53) have shown that cell wall loosening can proceed independently of auxin-induced cell elongation. With pea epicotyl tissue uncoupling of elongation from wall loosening is observed at high (10^{-3} M) Tordon concentrations.

Inhibition of Root Growth

Growth regulatory compounds which stimulate shoot cell elongation also inhibit root growth (47), a property used to investigate the mode of action of Tordon. Corn seeds (WF-9 X M-14) were germinated in the dark and after 48 hours seedlings with primary roots 2 to 4 cm in length were transferred to petri plates containing 10 ml of test solution. Growth inhibition values expressed as percent of control growth were converted to probit units using the method of Bliss (10). The probit conversion is a statistical method providing symmetrical probability factors for the conversion of sigmoidal dose response functions into linear segments amenable to analysis. Curves shown in Figure 11 were fitted by a step-wise linear regression using an IBM 7094 computer.

Tordon, like NAA, 2,4-D, and other auxins, inhibited root growth in a striking fashion. Growth of the primary root of etiolated maize seedlings was inhibited by concentrations of Tordon as low as 10^{-6} M after 12 hours (Figure 10). Fifty percent inhibition was achieved at approximately 5×10^{-6} M Tordon (compared with ca. 2×10^{-6} M for 2,4-D) and near maximal inhibition was achieved with 10^{-4} M Tordon. Roots treated with 10^{-3} M Tordon were flaccid following the 12 hour incubation.

The relationship between root growth inhibition, expressed as probits, and concentration of Tordon or 2,4-D was linear at intermediate (5×10^{-7} M to ca. 5×10^{-5} M) and high (ca. 5×10^{-5} M to 10^{-3} M) concentrations (Figure 11). The dose response curves for the two compounds were parallel at intermediate concentrations. Equivalent responses were observed for high concentrations of Tordon and 2,4-D.

Kinetic analysis of dose toxicity response relationships have been used to compare the modes of action of toxic agents (55, 65). If two compounds produce a response by affecting the same regulatory site within the organism, the activity kinetics of the two compounds are similar. The parallel inhibition kinetics of Tordon and 2,4-D indicate that the two compounds may be affecting the same control system within the root. The greater effectiveness of 2,4-D for root inhibition at intermediate concentrations is probably a reflection of its greater ability to reach the regulation site. This difference is not observed at high growth regulator concentrations where rate of entry might be a less important consideration than at intermediate concentrations.

Correlative Effects

In addition to stimulation of polarized cell expansion, auxins stimulate and/or inhibit other plant responses not directly related to elongation. Tordon was shown to influence several of these responses including promotion of rooting stimulation of growth of tissue in culture, induction of formative and curvature effects, and regulation of leaf abscission. Crane (18) has reported that Tordon induces parthenocarpic fruit set in *Calimyrna* fig.

Rooting--To demonstrate root initiation activity, the basal 1 cm of *Coleus* cuttings was dipped for 15 seconds in the solution prepared in 50% ethanol and rooting was continued in moist sand in the greenhouse.

Rooting was evaluated after 8 to 10 days.

Like 2,4-D, IAA and NAA, Tordon stimulated adventitious rooting. With *Coleus* cuttings, a significant increase in the number of roots was obtained with 10^{-5} M Tordon applied as an alcohol dip. Lower concentrations were less effective and concentrations of 10^{-3} M or greater were inhibitory (Table 3, Figure 12).

Growth of tissues in culture--Tobacco (*Nicotiana tabacum* L.) pith explant disks (0.5 cm in diameter and 0.5 cm in length) were aseptically cut from mature plants. Tissue disks were aseptically transferred to an autoclaved 1% agar nutrient medium (58) plus or minus growth regulator (Tordon, NAA, IAA, or 2,4-D). Eight disks were used per treatment. Growth was measured as increased fresh weight over initial values after a 10 day incubation in the dark at 28° C.

Tordon (10^{-5} M) promoted an increase in fresh weight of tobacco pith tissue in culture (Table 4) comparable to that resulting from equivalent concentrations of IAA and 2,4-D. In the presence of Tordon, 2,4-D or IAA cell proliferation was restricted to a few peripheral cell layers. In the presence of NAA a white friable callus was formed. In the absence of exogenous growth regulators the pith explants were swollen but proliferation was not observed. That IAA and Tordon produced equivalent results in this experiment does not reflect directly upon their activities because IAA is unstable during autoclaving (D. K. Dougall, personal communication). Tordon with a decomposition temperature of 215° C (23) would be less affected by autoclaving.

Regulation of leaf abscission--Abscission responses were determined by the explant method of Rubenstein and Leopold (69). Red Kidney bean plants were grown in soil in the greenhouse for 10 or 15 days. For explant tests, the upper (distal) abscission zone of primary leaves was used. Explants consisted of 5 mm of petiole, the abscission zone, the pulvinus, and a triangular portion of leaf tissue (approximately 10 mm long and 5 mm wide at the base). The petiolar portion of the explant was inserted into 1% agar contained in 5 cm petri plates. Twenty explants were used per treatment for each experiment. After 0, 24, 48, 72, or 96 hours explants were transferred to fresh agar or to agar containing 5×10^{-5} M, 5×10^{-7} M, or 5×10^{-8} M Tordon. After 24, 48, 72, 96, 120, and 144 hours approximately 10 grams of force was applied downward on the upper surface of the leaf portion of the explant. Explants which separated at

Table 3. Effects of Tordon on Rooting of Coleus

<u>Tordon conc. (M)</u>	<u>Number of roots per cutting</u>	<u>Percent of the cuttings dead</u>
0	15 $\pm$ 2	0
10 ⁻⁸	21 $\pm$ 2	0
10 ⁻⁷	23 $\pm$ 7	0
10 ⁻⁶	25 $\pm$ 9	0
10 ⁻⁵	34 $\pm$ 9	0
10 ⁻⁴	21 $\pm$ 8	0
10 ⁻³	1 $\pm$ 1	30
10 ⁻²	0 $\pm$ 0	100

Figure 12. Stimulatory Effect of Tordon
Applied as a 50% Ethanol Dip on the Rooting
of Coleus Cuttings. A = 50% ethanol control.
B = 10⁻⁸ M Tordon. C = 10⁻⁷ M Tordon. D =
10⁻⁶ M Tordon. E = 10⁻⁵ M Tordon. F = 10⁻⁴
M Tordon. After 10 days.

Addition to basal medium	Percent increase in fresh weight in ten days. <u>2/</u>
None	48
Picloram, 10^{-5} M	111
2,4-D, 10^{-5} M	92
IAA, 10^{-5} M	114
NAA, 10^{-5} M	158

None Picloram IAA NAA 2,4-D

1/ Pith explants grown on Murashige's (45) medium with indicated auxin modifications.
2/ Results presented as percent increase in fresh weight of eight pith explants from each treatment.

Table 4. Comparison of Tordon (Picloram), 2,4-D, IAA and NAA in Stimulating Increase in Fresh Weight of Tobacco Pith Tissue in Culture. 1/

Treatment	Average curvature in degrees <u>1/</u>
Control	$0.6 \pm .5$ a
IAA 1.0×10^{-6} M <u>2/</u>	15.4 ± 2.1 b
Picloram 1.0×10^{-6} M	$0.7 \pm .8$ a
2.1×10^{-6} M	$0.7 \pm .6$ a
4.2×10^{-6} M	$0.8 \pm .4$ a
6.2×10^{-6} M	$0.2 \pm .5$ a

1/ Results presented as averages from grouped data with standard deviations. Means followed by the same letter are not significantly different at the 5% level from analysis of variance using randomized complete blocks and Duncan's Multiple Range Test (16).
2/ Concentration of growth regulator in agar block.

Table 6. Effect of Tordon (Picloram) on Avena Coleoptile Curvature.

		Percent Abscission 2/															
		Concentration of picloram in agar															
		5 X 10 ⁻⁵ M				5 X 10 ⁻⁷ M				5 X 10 ⁻⁸ M							
		Time (hrs) from excision to transfer to agar plus picloram															
Time after excision (hrs)	Control	0	2h	4h	72	96	0	2h	4h	72	96	0	2h	4h	72	96	
2h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
4h	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
72	92 ± 4	0	0	12	-	3	15	25	-	0	10	13	-	-	-	-	
96	29 ± 16	0	0	15	33	-	16	30	48	37	-	87	29	60	-	-	
120	60 ± 17	0	0	17	33	50	27	43	70	66	70	50	73	62	90	87	
144	92 ± 8	3	0	20	43	67	63	50	78	77	83	95	100	77	100	100	

Retarded Abscission
 Accelerated Abscission

□ - Retarded Abscission
 [] - Accelerated Abscission

1/ The petiolar portions of petiole-pulvinus explants prepared from 10 or 15 day old Red Kidney bean plants were inserted into 1% agar. At 2h intervals groups of explants (20 per treatment) were transferred to fresh agar or to agar containing picloram. Abscission of explants was determined by manual application of pressure to the leaf portion of explants.
2/ Results presented as averages of three experiments. Standard deviations presented for control explants.

Table 5. Percent Abscission of Bean Petiole-Pulvinus Explants as a Function of Time of Tordon (Picloram) Treatment. 1/

Figure 13. Stem Curvature Response of Red Kidney Bean Seedlings to Applications of 15 µg Tordon (Picloram) or 2,4-D to One Primary Leaf.

the abscission zone after application of force were considered to have abscised.

Tordon was active in regulating abscission of bean leaf explants. As with IAA and 2,4-D, whether Tordon promoted, inhibited, or did not influence abscission depended upon both the concentration of Tordon and the time interval between excision and treatment (Table 5). Abscission of explants was retarded throughout the time of the experiment by 5×10^{-5} M Tordon when the transfer to agar containing the Tordon was accomplished within 24 hours after excision. Abscission measured 144 hours after the beginning of the experiment was found to be retarded by both high (5×10^{-5} M) and intermediate (5×10^{-7} M) concentrations of Tordon in those explants transferred after 72 hours or less. Slight acceleration of abscission rates were observed at both low (5×10^{-8} M) and intermediate (5×10^{-7} M) concentrations of Tordon in explants transferred 24 to 96 hours after excision. Standard deviations presented for control explants provide an index of reproducibility.

Tordon was active in defoliating intact Red Kidney bean plants. Ten micrograms of Tordon were as effective as 100 micrograms of the lithium salt of 2,4-D in stimulating leaf abscission when the compounds were applied in a droplet of water to one primary leaf of 10 day old bean plants.

Auxin-induced abscission responses have been attributed to stimulation of ethylene production by the treated tissue (1). Thus, the regulation of abscission by Tordon could be mediated through ethylene production. Measurements of ethylene production by Tordon treated bean explants would be of interest in future investigations.

Curvature and Formative Effects--Formative effects were estimated visually from 15 day old bean plants (*Phaseolus vulgaris* L. var. Red Kidney, Grand Rapids Growers, Grand Rapids, Michigan) grown in the greenhouse in soil and treated with a solution of Tordon or the lithium salt of 2,4-D (5 or 50 μ g in 100 μ l of water) applied as a droplet to one primary leaf. The Avena curvature test was conducted as described by Leopold (46).

Tordon induced stem proliferation, leaf and stem bending, loss of chlorophyll from treated leaves, adventitious rooting, and other formative effects when applied to primary leaves on Red Kidney bean plants. The formative responses produced by Tordon resembled those produced by equal amounts of 2,4-D, on an acid equivalent basis but were generally more pronounced with Tordon.

Tordon was not active in producing curvature in the Avena test (Table 6). No significant curvature was measured for coleoptiles which received agar blocks containing Tordon. However, coleoptiles which received blocks containing IAA produced a highly significant curvature response.

Translocation

Translocation of Tordon within whole bean plants was investigated using a technique described by Day (20). Red Kidney bean seeds were planted in soil and grown for 10 days in the greenhouse. Forty-eight plants were selected for uniformity of growth (first trifoliate leaves not yet expanded) and randomly arranged in three groups. Either 15 μ g of Tordon or 2,4-D in 10 μ l of ethanol or 10 μ l of ethanol alone was applied as a droplet to the mid-vein of one primary leaf 1 cm from the leaf blade-pulvinus juncture. Droplets were confined with a lanolin ring 1 cm in diameter. Curvature of the stem away from the treated leaf was measured every 30 minutes for 3 1/2 hours. This experiment was repeated 5 times. Curvature was measured by placing the midpoint of a protractor over the center of curvature. Deflection of the upper portion of the stem was recorded in degrees. The elapsing time between application and response was estimated by back extrapolating the linear portion of the response curve to zero curvature.

Tordon was shown to be translocated by whole bean plants following foliar application (Figure 13). Significant stem curvature was measured for Tordon and 2,4-D treated plants one and one half hours after application. Back extrapolation of the linear portions of the Tordon and 2,4-D response curves indicated that the two compounds had the same application-response lag period.

Tordon and 2,4-D were shown to have approximately the same application to response lag time when applied to leaves of whole bean plants. The response lag is thought to represent a summation of the lag times for several processes involved in the movement of the growth regulators and response of the plant. These processes may include foliar penetration, entry into the phloem (38), translocation, movement out of the phloem, and stimulation of growth in the stem cells. Although the time required for each process might not be the same for picloram and 2,4-D, the total application to response time was nearly equal for the two compounds as determined by back extrapolation of the linear phases of the response curves.

Tordon, like 2,4-D, does not induce curvature in the Avena curvature

test. Apparently these compounds are not transported in *Avena* coleoptiles in the same way as IAA.

Structural Requirements and Mode of Action

Auxin is a generic term for compounds primarily characterized by their capacity to stimulate cell elongation in shoot cells of excised plant parts (54). Tordon is an auxin herbicide by this definition since it promotes cell elongation in plant shoot sections of etiolated *Avena* coleoptiles, pea epicotyls, soybean hypocotyls, and light grown mung bean epicotyls. In addition Tordon promotes growth for whole shoot cuttings. In addition, Kefford and Case (40) report that Tordon stimulated growth of etiolated wheat coleoptile sections.

Although Tordon is structurally different from the benzoic acid derivatives, the phenoxyacetic acid derivatives, and IAA and its functional analogues, it appears to function as a growth regulator of the auxin type with a herbicidal action similar to that of 2,4-D. The structural requirements for auxin activity proposed by Thimann and co-workers (75) can be met by Tordon. It contains a planar ring and a potential electropositive region (the amino group) and a potential electronegative group (the carboxyl group) capable of a conformation in which these regions are separated by a distance of 5.5 Å. Thus, Tordon is a new type of synthetic auxin, a substituted picolinic acid. It is structurally different from other synthetic auxin herbicides, but stimulates elongation of shoot cells of plants and meets the above proposed structural requirements for auxin activity.

Review of Tordon Applications

Tordon was developed for control of brush and deep-rooted herbaceous plant species in non-crop areas (78). The phenoxyacetic acid derivatives, 2,4-D and 2,4,5-T, have not been effective in control of such species as Canada thistle (*Cirsium arvense* L.), black berry (*Rubus* sp. L.), field bindweed (*Convolvulus arvensis* L.), leafy spurge (*Euphorbia esula* L.), Russian knapweed (*Centaurea repens* L.), and black locust (*Robinia pseudo-acacia* L.) (34, 76, 78).

Picloram was effective for long term control of these (3, 28, 44, 78) and other problem species including kudzu (*Pueraria lobata* Willd.) (14), wild buckwheat (*Eragrostis sagittata* Gillet.) (27, 30), Japanese bamboo (*Polygonum cuspidatum* L.) (63, 64), poison oak (*Rhus toxicodendron* L.) (33), hawkweed (*Hieracium* sp. L.) (9), woollyleaf franseria (*Franseria tomentosa* Gray) (22) and mesquite (*Prosopis juliflora* Sw.) (66). Other promising agronomic applications of Tordon include pasture

renovation (7, 25) and selective control of broad-leaved species in flax (52), wheat (31), corn and sorghum (6).

An important factor in the effectiveness of Tordon is its persistence in the soil which is reported to be greater than that of 2,4-D and 2,4,5-T (4, 21, 30, 44, 51). Regrowth of susceptible species and reinfestation by Canada thistle, leafy spurge, field bindweed, and common millweed (Asclepias syriaca L.) did not occur in areas treated with herbicidal rates of Tordon up to two years after application (4, 44, 79).

Another factor contributing to the effectiveness of Tordon in controlling deep-rooted and woody plants is its rapid absorption and mobility within the plant (78). From patterns of symptom development Wiltse (80) concluded that following foliar sprays and soil applications for brush control, Tordon is rapidly absorbed by both the roots and leaves and translocated throughout the plant. Watson and Mesler (77), Arend (5), and Morrow (58) reported both acropetal and basipetal translocation of Tordon when applied to hardwood species by either basal frill or tree injection methods. The greater basipetal translocation of Tordon compared with that of 2,4-D and 2,4,5-T may contribute to its effectiveness in controlling woody species (80).

Mobility of Tordon was investigated by Hurtt and Foy (38). Application of the herbicide to leaves of Black Valentine bean (Phaseolus vulgaris L.) resulted in formative effects even in adjacent untreated plants growing in the same container. Results of soil analysis and steam girdling experiments suggested that the herbicide was absorbed by the leaves, translocated in the phloem to the roots, exuded into the soil, and absorbed by the roots of adjacent plants.

Herbicidal responses induced in Tordon treated plants are similar to those induced by comparable treatments using the substituted phenoxyacetic acids. Watson and Wiltse (78) reported 2,4-D-like stem twisting and leaf curl as a part of the death pattern of Tordon treated plants. A more complete comparison of 2,4-D and Tordon induced formative effects was provided by Hamaker et al. (32). They reported that Tordon treatment resulted in herbicidal responses comparable to those induced by 2,4-D. These responses included adventitious root formation, stem proliferation, and loss of chlorophyll from treated leaves.

Tordon, like the phenoxyacetic acid derivatives, is less toxic to grasses than to broad-leaved dicotyledonous plants (25, 32, 78). For example, Herr, Stroube, and Ray (35) reported that corn (Ohio 162), oats

(Clintland), and barley (Moore) were less sensitive to Tordon soil residues than alfalfa (Vernal) and soybeans (Harosoy).

Reports based on field observations indicate that Tordon is generally more toxic to dicotyledonous species than either 2,4-D or 2,4,5-T at equivalent doses (26, 60, 61, 70, 71). However, the similarity between formative effects produced by Tordon and the substituted phenoxyacetic acids has lead to speculation that these herbicides might operate through the same mechanism (32). The susceptibility of some roadside species to Tordon is given in Table 7. This is only a partial listing and more species will be added as testing of Tordon continues.

Table 7. Susceptibility of Plant Species to Tordon

Species	Rate for 100% Control for 1 year; Acid equivalent	Reference
Aspen	1 lb/ A	78
Black Locust	1/2 lb/100 gal	78
Canada Thistle	4 lb/ A	44
Common Milkweed	1 lb/ A	44
Conifers	1 lb/ A	78
Curly Dock	1 lb/100 gal	44
Fennel	5 lb/ A	45
Field Bindweed	2 lb/ A	34
Field Horsetail	1 lb/ A	44
Goldenrod	1 lb/100 gal	44
Hardwood Trees	3 lb/100 gal	77
Hawkweed	1/2 lb/ A	9
Honeysuckle	5 lb/ A	45
Japanese Bamboo	1 lb/100 gal	64
Knotweed	5 lb/ A	63
Ludzu	5 lb/ A	14
Leafy Spurge	2 lb/ A	4
Maples	1/2 lb/ A	78
Nightshade	3 lb/ A	78
Oak Brush	1 1/2 lb/ A	34
Plantain	1 lb/ A	60
Poison Ivy	5 lb/ A	45
Poison Oak	4 lb/100 gal	33

Table 7. (Cont'd). Susceptibility of Plant Species to Tordon

Species	Rate for 100% Control for 1 year; Acid equivalent	Reference
Pokeweed	5 lb/ A	45
Ragweed	1 lb/100 gal	60
Redbud	4 lb/ A	80
Russian Knapweed	1 lb/ A	4
Sassafras	1/2 lb/ A	78
Sumac	4 lb/ A	80
Tall Larkspur	3 lb/100 gal	34
Trumpet Vine	5 lb/ A	45
Virginia Creeper	5 lb/ A	45
Wildbuckwheat	3/4 oz/ A*	27
Wild Lettuce	1 lb/ A	60
Wild Rose	4 lb/ A	80
Willow	4 lb/ A	80
Woolyleaf Franseria	2 lb/ A	22
Yellow Wood Sorrel	1 lb/100 gal	44

* In wheat

Table 7. (Cont'd). Susceptibility of Plant Species to Tordon

Species	Rate for 100% Control for 1 year; Acid equivalent	Reference
Pokeweed	5 lb/ A	45
Ragweed	1 lb/100 gal	60
Redbud	4 lb/ A	80
Russian Knapweed	1 lb/ A	4
Sassafras	1/2 lb/ A	78
Sumac	4 lb/ A	80
Tall Larkspur	3 lb/100 gal	34
Trumpet Vine	5 lb/ A	45
Virginia Creeper	5 lb/ A	45
Wildbuckwheat	3/4 oz/ A*	27
Wild Lettuce	1 lb/ A	60
Wild Rose	4 lb/ A	80
Willow	4 lb/ A	80
Woolyleaf Franseria	2 lb/ A	22
Yellow Wood Sorrel	1 lb/100 gal	44

* In wheat

III. DIMETHYLSULFOXIDE AS A HERBICIDE CARRIER AND ADJUVANT

Introduction

Dimethylsulfoxide (DMSO), a byproduct of the paper making industry, is an exceptionally powerful solvent for organic herbicides (19, 41), making a concentrated true solution with most herbicides, even with water insoluble materials such as Atrazine. It is easy and safe to use and DMSO by itself has an extremely low toxicity (42, 43). DMSO has a remarkable ability to penetrate skin and to carry solutes into tissues under conditions where solute penetration would not otherwise occur (19, 67, 68, 73). The low order of toxicity of DMSO, its ability to penetrate tissues and its solubility characteristics combine to indicate that DMSO is potentially useful as a carrier of herbicides in formulations for roadside development (41).

The research undertaken was concerned with 1) The extent to which DMSO would increase herbicidal effectiveness through facilitated uptake; 2) the mechanisms of DMSO-facilitated herbicide uptake; 3) DMSO phytotoxicity and 4) DMSO-herbicide combinations for use in roadside development. Preliminary laboratory investigations were followed by greenhouse tests and field studies. The results are reported under the following headings:

Enhancement of herbicidal effects of 2,4-D and Tordon by application
in DMSO

DMSO effects on membrane permeability

Phytotoxicity of DMSO-containing solvent systems

Greenhouse evaluation of DMSO - 2,4-D interactions

Field studies.

Enhancement of Herbicidal Effects of 2,4-D and Tordon by Application in DMSO

In work similar to that reported by Hull (37), we have observed enhancement of 2,4-D and Tordon activity when the herbicides were applied using DMSO as the carrier. Figures 1 to 8 show the stems of red kidney beans treated with the indicated test solutions 14 days after treatment. The 2,4-D applied in DMSO elicited a bending response at concentrations as low as 10 µg per plant, while 2,4-D applied in water did not result in any response at concentrations less than 100 µg per plant (Table 8). Also the stem proliferation seen in the 100 and 200 µg treatments in DMSO was not observed in any of the 2,4-D treatments applied in water. Similar results were obtained with Tordon with greater herbicidal effectiveness observed when the herbicide was applied in DMSO.

Figure 14. Comparison of Formative Effects of 14 Day Old Bean Plants Treated With Varying Amounts of 2,4-D With DMSO or Water as the Carrier.

Figure 14 (Cont'd.). Comparison of Formative Effects of 14 Day Old Bean Plants Treated With Varying Amounts of 2,4-D With DMSO or Water as the Carrier.

Table 8. Apparent Enhancement of Foliar Penetration of 2,4-D by DMSO.

<u>Amount of 2,4-D</u>	<u>Herbicidal symptoms</u>	
	<u>H₂O</u>	<u>DMSO</u>
25 µg	-	+
50 µg	-	+
100 µg	-	+
200 µg	+	+

When assayed in the laboratory, with petiole explants, leaf abscission can be promoted or retarded by auxins such as 2,4-D depending on the concentration and the method of application (2, 8). However with intact plants, induced abscission is less readily achieved (3). In this study, the ability of 2,4-D to promote leaf abscission from intact plants was studied and used as an assay method to determine the effectiveness of DMSO in enhancing the herbicidal activity of 2,4-D.

Bean plants (Phaseolus vulgaris L. cv. Red Kidney) were grown in the greenhouse under a 16 hour photoperiod. Plants were treated when the first trifoliate leaf was fully expanded (18 to 22 days old) by application of 50 µl of the test solution to one primary (monofoliate) leaf 1 cm from the leaf blade-pulvinus junction. Absorptive leaf area for the test solutions was confined by a 1.5 cm lanolin ring (Figure 17). Where indicated, 50 µl of test solution was injected into the bean hypocotyl 8 cm below the cotyledons using a hypodermic needle. Treatment rates are all reported as µg acid equivalent of 2,4-D. Experiments were repeated a minimum of 4 times with at least 25 plants per treatment.

Abscission was determined by manual application of pressure to the treated leaf until resistance was felt or separation occurred. A mechanical device applying a uniform load of 5 g on the treated leaf gave comparable results.

Preliminary results using constant amounts of 2,4-D in varying volumes of solvent indicated that the abscission response in this system was dependent upon the quantity of 2,4-D present and independent of the solvent volume. With this in mind, all further experiments were designed using a constant volume of solvent (50 µl) varying the quantity and chemical form of the 2,4-D.

Abscission of primary leaves receiving 2,4-D in DMSO occurred between 12 and 120 hours after application with the use of 2,4-D acid resulting in more rapid abscission than the lithium salt of 2,4-D (Li-2,4-D) (Figure 15).

Figure 15. Comparison of Abscission of Leaves Treated by 50 µg Acid or the Acid Equivalent of the Lithium Salt of 2,4-D Applied in DMSO or water. ●, ○ - 2,4-D Acid; ■, □ - Li-2,4-D; closed symbols = 50 µl DMSO; open symbols = 50 µl water.

Figure 16. Effect of 2,4-D Concentration on 2,4-D Induced Abscission of Treated Leaves. 2,4-D was supplied as acid in 50 µl DMSO (closed symbols) or an acid equivalent concentration of Li-2,4-D in 50 µl water (open symbols). ▼, ▽ - 25 µg; ●, ○ - 50 µg; ■, □ - 100 µg; ▲, △ - 200 µg.

Figure 17. Defoliation of Bean Plants by 200 µg 2,4-D in DMSO (Dotted Bars) or 200 µg Acid Equivalent of Li-2,4-D in Water (Open Bars). Treatments were applied as a single 50 µl drop to one primary leaf.

Less than 20% of the treated leaves abscised when 2,4-D acid or Li-2,4-D was applied in water with abscission beginning about 24 hours later than in comparable DMSO formulations. DMSO alone did not affect plant growth but a small necrotic spot developed in the DMSO treated area 24 to 36 hours after treatment. 2,4-D induced abscission was complete in all treatments irrespective of 2,4-D concentration after approximately 120 hours, with the percent abscission in the DMSO treatments consistently four- to fivefold higher than that induced by 2,4-D applied in water (Figure 15 and 16). Addition of a wetting agent (Tween 80) to the above treatments did not affect the rates of abscission. Primary leaves on untreated plants and plants receiving DMSO alone did not abscise during these experiments.

Induced abscission was not restricted to treated leaves (Figure 17). When one primary leaf was treated as above and all leaves on the plant were tested for abscission after 120 hours, abscission induced by auxin-DMSO formulations was four- to fivefold greater than that induced by comparable auxin-water formulations regardless of leaf position. Using DMSO as the solvent, 200 µg of 2,4-D per plant were required to achieve 50% defoliation in 120 hours or less. These trifoliate leaves do not normally abscise for several months. All 2,4-D treated plants expressed sublethal herbicidal symptoms. Stem deformation and proliferation were increased by application of the 2,4-D in DMSO. The low levels of 2,4-D induced abscission achieved with the water formulations indicate that red kidney bean plants are particularly sensitive to auxin applied in this manner, although other species including cotton and soybean were also defoliated by applications of 2,4-D in DMSO (Table 9). The higher rates of abscission occurring in the DMSO treatments suggest that the DMSO may be facilitating foliar penetration. To investigate this possibility further, 2,4-D formulations in water and DMSO were injected into the hypocotyl of the bean plants. These injections resulted in identical symptom development regardless of the solvent used, suggesting that the DMSO enhancement of 2,4-D induced abscission in the foliar treatments was an effect on penetration rather than on translocation or activity.

Effects due to 2,4-D entry through necrotic tissue by a wick effect were tested by applying 2,4-D formulations to necrotic spots comparable to those which appeared as a result of treatment with DMSO alone. Localized leaf areas were killed with liquid nitrogen. The frozen tissue

Table 9. Comparison of Application Method, 2,4-D Concentration and Amount of DMSO on Defoliation of Treated Leaves of Bean, Soybean and Cotton.

<u>Species</u>	<u>Age of Plants, Days</u>	<u>Duration of Experiment, hrs</u>	<u>DMSO, μl</u>	<u>Amount/leaf 2,4-D, μg</u>	<u>Method of treatment</u>	<u>Treated leaves, No.</u>	<u>Defoliation, %</u>	<u>DMSO Toxicity</u>
Bean	28	120	6	25	Spread	60	26	Chronic
			6	25	Droplet	60	51	Local
			6	0	Spread	60	5	Chronic
			6	0	Droplet	60	0	Local
			6-10	25	Spread	270	14	Acute
			6-10	25	Droplet	270	25	Local
Soybean	40	216	10	25	Spread	80	0	Acute
			10	25	Droplet	80	0	Acute
			2	25	Spread	80	0	Acute
			2	25	Droplet	80	27	Chronic
			2	0	Spread	80	0	Acute
			2	0	Droplet	80	0	Chronic
Cotton	40	240	2	250	Droplet	80	0	Chronic
			10	25	Spread	50	34	Chronic
			10	25	Droplet	40	2	Local
			10	250	Droplet	40	0	Local

was thawed and treated as above. Pre-killing the treated area did not enhance the effectiveness of 2,4-D applied in water and reduced the effectiveness of 2,4-D applied in DMSO (Table 10).

Table 10. Effect of Pre-Killing Treated Tissue by Freezing on the Abscission Response of the Treated Leaves.

Treatment	Abscission after 120 hours (%)	
	Frozen	Not frozen
Untreated	5	0
DMSO	5	0
DMSO + 100 µg 2,4-D	70	90
Water + 100 µg a.e. Li-2,4-D	20	20

When treated areas were covered with a parafilm seal over the lanolin ring, abscission induced by 2,4-D applied in 80% ethanol was greater than that induced by 2,4-D applied in water, but was considerably less than that achieved with 2,4-D formulated in DMSO (Table 11). Thus, the DMSO enhancement of 2,4-D induced abscission is an effect of the solvent properties of DMSO. It is not, for example, a manifestation of differential uptake of the acid from over the lithium salt (Figure 15). Covering treated areas containing 2,4-D-water formulations did not enhance the effectiveness of these treatments. Thus, the DMSO effect cannot be attributed to an increase in the time the solution is retained at the leaf surface by virtue of its low vapor pressure.

When the effectiveness of water and DMSO formulations of 2,4-D acid or equivalent amounts of the *n*-octyl ester of 2,4-D (octyl-2,4-D) were compared, octyl-2,4-D (unformulated) and 2,4-D acid formulated in DMSO were equal in promoting abscission (Table 11). Abscission induced by octyl 2,4-D was not enhanced by application in DMSO. Since 2,4-D esters readily penetrate leaf surfaces (55), these results support our suggestion that the effect of DMSO on 2,4-D induced abscission is one of facilitated foliar penetration of the 2,4-D acid and its salts.

DMSO Effects on Membrane Permeability

As reported in the previous section, DMSO rapidly penetrates biological membranes (see also, 39, 67, 68) and as a result may enhance herbicide penetration. Alternatively the physiological properties of DMSO

Table 11. Comparison of Abscission of Primary Leaves Following Applications of 50 µg of 2,4-D Acid or Acid Equivalent of Octyl-2,4-D.

Treatment	Abscission after 120 hours (%)
DMSO	0
DMSO + 2,4-D acid	81
Ethanol (80%)	0
Ethanol (80%) + 2,4-D acid	19
Ethanol (80%) + 2,4-D acid; sealed ¹	34
Water	0
Water + 2,4-D acid	20
Water + 2,4-D acid; sealed ¹	23
Octyl-2,4-D	87
DMSO + octyl-2,4-D	85

¹Lanolin ring covered with parafilm to retard evaporation.

might be derived from an alteration of membrane permeability. To help distinguish between these two possibilities, the effects of DMSO on several parameters of membrane permeability were measured including hydraulic permeability, electrolyte efflux and solute uptake.

Uniformly labeled glucose was selected as a solute because it was readily available, penetrates slowly and is translocated rapidly. Autoradiographic analysis 8 hours following application of 0.5 µc C¹⁴ glucose to primary leaves of bean plants indicated little or no penetration of the compound using either water or DMSO as the carrier. As a comparison, 5 µc of C¹⁴-glucose were applied to the soil around the roots of 14 to 16 day old bean plants. Autoradiographs indicated a low level of absorption of the label when water was used as the carrier but extensive absorption of the solute when DMSO was used as the carrier (Figure 18). Similar results were obtained with plants grown in Krum (a silicate soil conditioner) watered with Hoagland's nutrient solution. These results with glucose may arise from facilitated uptake although an alteration in root permeability cannot be excluded. In control experiments, the osmotic properties of roots were not irreversibly altered by the DMSO treatments.

Figure 18. Autoradiographic Demonstration of DMSO-Enhanced C¹⁴-Glucose Uptake by Bean Shoots Comparing Root Applications in Water (A) or DMSO (B). The autoradiograph is shown on the right. The plant from which the autoradiographs were made is given on the left for comparison.

The effects of DMSO on electrolyte efflux were determined by measuring changes in conductance of deionized water containing plant tissue in a ratio of 1.25 g of tissue to 50 ml water. Chloroform was included in this study because of its reported membrane activity. A 5 second exposure to chloroform was sufficient to effect maximal electrolyte efflux (Figure 19). It is apparent from these data that neither prolonged contact with aqueous solutions of DMSO, nor brief exposure to the pure compound significantly altered electrolyte efflux.

The methods of Glinka and Reinhold (29) were used to measure the effects of DMSO on hydraulic permeability. This involves observing the weight changes of carrot xylem parenchyma exposed to equiosmotic concentrations of solutes. Figure 21 shows cryoscopic determinations of the osmotic potential of various concentrations of sucrose mannitol and DMSO. The effects of these three solutes upon the thermodynamic properties of water were essentially identical in the ranges tested. These results are of interest in view of the fact that DMSO is highly polar and is believed to form hydrogen bonds with water (15). Since DMSO, once it has entered the cell, does not readily reequilibrate with water, the harmful effects of high concentrations of DMSO reported in the next section may arise through replacement of water.

Figure 20 indicates weight changes in carrot xylem parenchyma sections in response to the indicated solutions. Alterations in membrane properties appear as changes in equilibration rate against the osmoticum, or as the inability to return to original weight when placed in water. Chloroform has been reported to alter hydraulic permeability in an irreversible manner, and is included here for comparison. As can be seen, DMSO, in a concentration identical to chloroform, apparently does not alter hydraulic permeability. In Figure 23 we see the response of carrot tissue to a variety of other membrane active agents, all of which seem to cause some degree of alteration in hydraulic permeability.

The tissue response to equimolar concentrations of DMSO and mannitol, and response to one after preincubation in the other is summarized in Figure 24. From the equilibration rate of the tissue immersed in DMSO, it can be seen that DMSO penetrates rapidly and apparently does not act as a functional osmoticum. Mannitol, on the other hand, does not penetrate readily, but functions as an osmoticum. It is also apparent from a comparison of the kinetics of water efflux due to mannitol in the presence and absence of a DMSO pretreatment, that preincubation in DMSO does not

Figure 19. Electrolyte Efflux (Conductance) from Carrot Xylem Parenchyma Disks Comparing Water-, DMSO- and Chloroform-Treated Tissues.

Figure 20. Effect of Mannitol and DMSO on Water Flux Into and Out of Carrot Disks.

Figure 21. Cryoscopic Determinations of the Osmotic Potential of Various Concentrations of Sucrose, Mannitol and DMSO.

Figure 22. Changes in Dry Weight of Carrot Disks Due to DMSO Not Readily Re-equilibrated With Water.

Figure 23. Effect of Mannitol With and Without Diethyl Ether, Amyl Alcohol or Glutaraldehyde on Water Flux Into and Out of Carrot Disks.

Figure 24. Effect of Preincubation in Mannitol or DMSO on Water Flux Into and Out of Carrot Disks.

Figure 25. Water Uptake in Pea Seeds With Intact Seed Coats After Soaking in DMSO.

Figure 26. Water Uptake in Pea Seeds With Ruptured Seed Coats After Soaking in DMSO.

affect the hydraulic permeability. Recovery is also not affected, as is demonstrated by a comparison of the rates of recovery of all tissues when returned to water. The final recovery point of the tissue treated with only DMSO indicated that there were no irreversible effects upon membrane function.

Tissue response to DMSO after preincubation in mannitol is probably a manifestation of alteration of the reflection coefficient as discussed by Glinka and Reinhold (29) resulting from interactions due to the simultaneous influx of both DMSO and water. Slight changes in recovery rate when the tissue is returned to water might be explained on the basis of internal effects of DMSO upon hydrostatic pressures upon the membrane, or an alteration in internal solute concentration.

The fact that tissue treated with mannitol never fully recovered was either a function of mannitol toxicity or a manifestation of the hysteresis effect resulting from plasmolysis of the tissue (29).

The absorption of DMSO by seeds was also investigated. Twenty pea and 20 maize seeds were placed in pure DMSO and pure water for 21 hours and the amount of liquid taken up was determined. Water uptake in seeds with both ruptured and intact seed coats was studied using seeds that had been previously soaked 90, 60 or 30 minutes in pure DMSO and compared with a control group of seeds. At 15 minute intervals the seeds were blotted dry and weighed.

The 20 maize seeds placed in water for 21 hours showed a net gain in weight of 3.647 g as opposed to 4.960 (136% of control) for those in pure DMSO for the same length of time. The 20 pea seeds soaked in water gained a total of 2.892 g and those soaked in DMSO lost 1.055 g presumably through extraction of starch. Amount of water uptake in pea seeds (25 seeds) with both ruptured and intact seed coats (Figures 25 and 26) show a response pattern complicated by both extraction and DMSO binding phenomena. However, these results confirm the observations that DMSO was taken up without markedly affecting hydraulic permeability.

The data of Figure 22, obtained by incubating tissue in the indicated DMSO concentrations for 1 hour, then in water for 1 hour prior to drying indicated that a portion of the DMSO that enters the cells does not readily re-equilibrate with water. This is either an indication of differential permeability with respect to direction of flux, or DMSO binding in the cells.

In summary, DMSO enters plant cells at a rate comparable to that of water and in so doing may enhance solute absorption. It does not seem to alter either electrolyte efflux from plant tissues or hydraulic permeability as a result. Some of the DMSO which enters tissues is not readily re-equilibrated with water and may be bound.

Phytotoxicity of DMSO-containing Solvent Systems

To determine the effects of chronic DMSO exposure upon plant growth, various species of plants were treated under various conditions and stages of development with DMSO concentrations ranging from 0.1 to 100% with water as the diluent. When 7 day old cotton plants were sprayed with DMSO concentrations ranging from 0.1 to 25% DMSO, the only visible effects appeared at concentrations of 15% or greater even when the plants were sprayed daily to the point of runoff for a period of 4 weeks. At the end of two weeks of such daily exposure to DMSO solutions, a slight tip burn appeared around the edges of the primary leaves of the group receiving 25% DMSO. The same symptoms appeared with 15% DMSO after 18 days. Single applications of pure DMSO did not cause significant injury to 7 day old cotton plants but resulted in local necrosis when applied to mature cotton plants.

The toxicity of pure DMSO applied at a rate of 30 gal/A was tested in the greenhouse to approximately 40 species of seedling and rapidly growing plant species from 18 families (Table 12). Monocots (Gramineae; Grass Family and Liliaceae; Lily Family) and chickweed (Caryophyllaceae; Pink Family) were uniformly resistant to toxic effects of DMSO. Pigweed (Amaranthaceae; Pigweed Family), Composites (Compositae; Sunflower Family), Peppermint (Labiatae; Mint Family), Legumes (Leguminosae; Legume Family), Strawberry (Rosaceae; Rose Family) and especially members of the Potato Family (Solanaceae; Tomato and Jimsonweed) were most sensitive to DMSO. Only Lespedeza (Leguminosae; Legume Family) and Jimsonweed (Solanaceae; Potato Family) were completely killed by the DMSO. Other species were intermediate and appeared to survive the treatment without permanent effects. Elodea (*Anacharis canadensis*) plants survived for several months in a 10% aqueous DMSO solution. All DMSO-induced disorders appeared to be temporary (contact injury) and none of the treated plants showed signs of systemic disorders.

The effects of DMSO upon seed germination were evaluated using seed from 10 plant species, soaked in various concentrations of DMSO and

Table 12. Susceptibility of Plant Species to DMSO.

PLANT SPECIES	Age of plants (weeks)	Leaf Necrosis Days after treatment			
		2	4	6	8
<u>Gramineae</u>					
Corn	2	1	2	1	2
Wheat	3	1	1	2	3
Oats	3	1	2	3	3
Barley	3	1	2	2	3
Crabgrass	5	2	2	1	2
<u>Amaranthaceae</u>					
Redroot Pigweed	4	4	4	4	3
<u>Caryophyllaceae</u>					
Chickweed	4	0	1	1	1
<u>Chenopodiaceae</u>					
Sugar Beet	2	2	4	3	3
Lambsquarters	5	3	3	4	3
<u>Compositae</u>					
Ragweed	5	5	6	7	9
Canada Thistle	5*	1	5	5	5
<u>Convolvulaceae</u>					
Morning Glory	4	4	3	3	3
<u>Cruciferae</u>					
Cabbage	3	3	4	4	4
Yellow Rocket	5	2	3	3	3
<u>Cucurbitaceae</u>					
Pumpkin	2	3	5	5	3
Muskmelon	2	1	3	4	2
<u>Labiatae</u>					
Peppermint	2**	5	8	5	2
<u>Liliaceae</u>					
Onion	5	0	0	2	2
Asparagus	5	0	1	2	3
<u>Leguminosae</u>					
Alfalfa	5	2	3	3	2
Birdsfoot Trefoil	5	3	3	3	3
Red Clover	5	5	5	5	4
Ladino Clover	5	3	4	3	3
Lespedeza	5	5	10	10	10

Table 12 (Cont'd). Susceptibility of Plant Species to DMSO.

PLANT SPECIES	Age of plants (weeks)	Leaf Necrosis Days after treatment			
		<u>2</u>	<u>4</u>	<u>6</u>	<u>8</u>
Snapbean	2	4	5	5	5
Soybean	2	5	4	3	3
<u>Malvaceae</u>					
Velvet Leaf	5	4	4	5	3
Prickly Side	5	3	4	4	3
<u>Plantaginaceae</u>					
Buckhorn Plantain	5	3	2	2	2
<u>Polygonaceae</u>					
Smartweed	5	2	3	3	3
Curled Dock	4	2	4	4	3
<u>Portulacaceae</u>					
Purslane	4	2	3	5	2
<u>Rosaceae</u>					
Strawberry	2**	5	8	6	5
<u>Solanaceae</u>					
Tomato	3	6	9	9	9
Jimsonweed	3	8	10	10	10
<u>Umbelliferae</u>					
Carrot	5	3	3	3	3
Wild Parsnip	4	3	3	5	3

0 = No effect

10 = Complete necrosis

* From date rhizome pieces planted

** From date of transplanting

germinated under standard greenhouse conditions. In the first experiment, tomato, cucumber and bean seeds were divided into two groups; one group receiving a 6 hour presoak in water, the other group being placed directly in DMSO solutions (10^{-4} to 10^{-1}) (Figures 27 A, E and D). Soaking time was 24 hours. In the second experiment, seeds of 7 plant species were soaked in water for 6 hours and DMSO for 18 hours prior to germination (Figures 27 C and D). Figures 27 A to E indicate that there was no

Figure 27. Effects of DMSO on Seed Germination.

In the first group (A, B and E), tomato, bean and cucumber seeds were divided into two groups; one group receiving a 6 hour presoak in water; the other group being placed directly in DMSO solutions indicated. Soaking time was 24 hours. In the second group (C and D), seeds of 7 plant species were soaked in water for 6 hours and DMSO for 18 hours prior to germination.

consistant effects of DMSO upon germination over the concentration ranges of 10^{-4} to 1 M. Concentrations greater than 2.5 M DMSO depressed germination rates somewhat. All plants that germinated showed normal morphology and development during a 2 week observation period.

An approximate adherence of DMSO phytotoxicity patterns to related plants and plant families suggested that susceptibility to DMSO might be attributable to some genetic characteristic. The cuticularized layer of leaves and other plant surfaces consists of a primary cuticle, formed by the oxidation of oils on exposed cell walls plus various surface and subsurface wax deposits. It is known that DMSO and paraffinic substances such as wax and cutin are immiscible with each other and it is possible that differences in DMSO phytotoxicity arise from differences in the chemical composition and thickness of the leaf cuticle. A correlation between the presence of surface wax on mature leaves (70) and susceptibility to DMSO is given in Table 13. The correlation is reasonably good. The exceptions include cabbage which has a poorly developed cuticle although producing surface wax and several legumes (Red and Ladino Clover and Soybean) which have a very thin cuticle even though wax is present. The two other exceptions, Buckhorn Plantain and Smartweed, do not produce wax but are only moderately susceptible to DMSO may arise from differences in the cuticularized layer and do not necessarily reflect true tolerance to the solvent.

The above results and the field and greenhouse studies which follow show that although pure DMSO is toxic to many plant species, grasses are resistant and the use of DMSO as a solvent for herbicides used in roadside development should not be limited by phytotoxicity of DMSO.

Greenhouse Evaluation of DMSO - 2,4-D Interactions

DMSO - 2,4-D interactions were investigated in a series of greenhouse investigations during 1966 and 1967 comparing untreated controls with pure DMSO, 1/2 lb per acre of 2,4-D (amine salt) in 30 gal of water per acre and 1/2 lb per acre of 2,4-D (amine salt) in 30 gal of DMSO per acre, comparing approximately 40 different plant species from 18 major plant families. A synergistic interaction between application of 2,4-D in DMSO vs. application of 2,4-D in water is indicated when, in comparing herbicidal effectiveness, the combination treatment is greater than the combined effectiveness of the materials in the combination applied separately. The expected response for a combination is obtained by

Table 13. Relationship Between the Presence (+) or Absence (-) of Surface Waxes on Mature Plants and Susceptibility to DMSO (+ = Susceptible; - = Resistant; $\pm$ = Intermediate)

<u>PLANT SPECIES</u>	<u>Wax bearing</u>	<u>Susceptible to DMSO</u>
<u>Gramineae</u>		
Corn	+	-
Foxtail	+	-
Crabgrass	+	-
<u>Compositae</u>		
Thistle	-	+
Ragweed	-	+
<u>Convolvulaceae</u>		
Morning Glory	-	$\pm$
<u>Cruciferae</u>		
Cabbage	+	$\pm$
<u>Liliaceae</u>		
Onion	+	-
<u>Leguminosae</u>		
Ladino Clover	+	$\pm$
Red Clover	+	+
Soybean	+	$\pm$
Sweet Clover	+	-
<u>Malvaceae</u>		
Cotton (Primary Leaves)	+	-
Cotton	-	+
<u>Plantaginaceae</u>		
Buckhorn Plantain	-	$\pm$
<u>Polygonaceae</u>		
Smartweed	-	$\pm$
<u>Rosaceae</u>		
Strawberry	-	+
<u>Solanaceae</u>		
Tomato	-	+
Jimsonweed	-	+

taking the product of the percent of control values for the materials applied alone and dividing by $(100)^{n-1}$ where n is the number of herbicides in the combination (12). When the inhibitory effects of the combination are greater than the expected values, the materials are synergistic. When the inhibitory effects are less than the expected values, the materials are antagonistic. Examples of such calculations for effects of DMSO - 2,4-D combinations on fresh weight are given in Table 14.

Table 14. Examples of Synergistic and Antagonistic Interactions Between 2,4-D and DMSO.

PLANT SPECIES	<u>Fresh weight, % of control</u>				
	DMSO	2,4-D	Expected [(DMSO X 2,4-D)/100]	Measured DMSO + 2,4-D	Inter- action
SYNERGISTIC					
Cabbage	80	70	56	2	+54
Onion	50	80	40	4	+36
Ladino clover	75	100	75	13	+62
Alfalfa	90	90	80	10	+70
Birdsfoot Trefoil	55	95	52	22	+30
Buckhorn plantain	90	100	90	40	+50
Tomato	90	90	80	0	+80
ANTAGONISTIC					
Corn	70	100	70	100	-30
Redroot Pigweed	30	5	15	40	-25
NO INTERACTION					
Crabgrass	100	100	100	100	0
Chickweed	100	85	85	85	0
Asparagus	60	30	18	15	+3
Red clover	40	30	12	8	+4
Snap bean	5	35	2	11	+9
Velvet Leaf	60	25	15	24	-9
Prickly Sida	80	20	16	8	+8

Since the type of killing action of the solvent DMSO and the herbicide 2,4-D are quite different, some of the interactions are difficult to evaluate. As reported in the section "Phytotoxicity of DMSO", the solvent exerts a contact type of action resulting in rapid necrosis of susceptible plant parts. 2,4-D on the other hand, exhibits a systemic killing action involving aberrant growth responses including twisting and curling of plant parts, hypertrophy, hyperplasia and chlorosis. These parameters are impossible to quantitate and thus difficult to sum under present methods of evaluation. Therefore, evaluations of synergism or antagonism

based on morphogenic changes are somewhat arbitrary. Comparisons reported include in addition to formative effects (morphogenic changes) given in Figure 28, leaf necrosis after 8 days, rate of mortality between 0 to 3 weeks (Figure 29), total mortality after 3 weeks, fresh (Figure 30) and dry weights (Figure 31) after 3 weeks and vigor of plants remaining after 16 days (Figure 32). Evidence for synergistic (+), antagonistic (-) or no (o) interactions are then summarized in Table 16.

Results presented in Figure 28 are based on visual estimates of formative effects one week after treatment when most species were showing maximal 2,4-D induced changes. With most species the expression of 2,4-D induced formative effects was unaffected (eg. Red Clover, Curled Dock or Wild Parsnip) or markedly reduced (eg. Pigweed, Lambsquarters, Peppermint, Onion, Buckhorn Plantain and Carrot) by application of the 2,4-D in DMSO. Only with Ladino Clover, Snap Bean, Curled Dock and Strawberry was there evidence for enhancement of 2,4-D induced formative effects by DMSO with bean being the test plant chosen for the controlled laboratory studies reported in the previous section.

Nonetheless, the killing action of 2,4-D was enhanced in these greenhouse experiments. Data for carrot is shown in Figure 29. Even though formative effects were reduced by 2,4-D application in DMSO (Figure 28) death of the plant began to occur 5 to 6 days earlier in the DMSO treatment. Similarly, the L.D. 50 and total mortality were extended by this same 6 day interval. Thus,rapidity of killing action was one criterion by which a synergistic interaction between DMSO and 2,4-D was demonstrated in the greenhouse. Other criteria for showing synergism, included fresh and dry weight determinations (Figures 30 and 31), visual estimates of leaf necrosis after 6 days, % kill after 3 weeks and reduction in vigor of plants remaining 16 days after treatment (Figure 32). Even maize which is notably resistant to 2,4-D, responded to the DMSO-2,4-D combination by an increased tendency toward lodging (Table 15). Thus,with but few exceptions, a synergistic action between 2,4-D and DMSO as the solvent was found by one criterion or another for those plant species tested (Table 16). The more subtle differences are of academic interest but of little practical value. However, with some species such as onion and jimson-weed, the addition of DMSO resulted in the difference between partial control and eradication (see Figure 33 for results with onion).

Figure 28. Comparisons of the Effects of DMSO Alone (30 gpa); 0.5 lb/A of 2,4-D in Water (30 gpa) or 0.5 lb/A of 2,4-D in DMSO (30 gpa) = COMB on the 2,4-D - Induced Formative Effects of Various Plant Species 6 Days After Treatment.

Figure 29. Effects of DMSO Alone (30 gpa); 0.5 lb/A of 2,4-D in Water (30 gpa) or 0.5 lb/A of 2,4-D in DMSO = CORB on the 2,4-D Induced Mortality (% Kill) of Carrot (Daucus carota).

$$2 \quad 4-0 \quad \frac{1}{2} 16/11 \quad 50$$

0 10 20 30 40 50 60 70 80 90 100----0 10 20 30 40 50 60 70 80 90 100 + DMSO (full strength)

corn-----		:	:	:	:	:	:	:	:	:-----	:	:	:	:	:	:
wheat-----		:	:	:	:	:	:	:	:	:-----	:	:	:	:	:	:
oats-----		:	:	:	:	:	:	:	:	:-----	:	:	:	:	:	:
barley-----		:	:	:	:	:	:	:	:	:-----	:	:	:	:	:	:

redroot pigweed-----

chickweed-----

[illegible]

Compositae .

ragweed

morning glory-----

cabbage

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peppergrass-----, . . . . .

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shepherdspurse-----

pumpkin-----

Figure 32 (Cont'd)

$$2, 4-D \quad \frac{1}{2} \text{ lb/A}$$

2,4-D $\frac{1}{2}$ lb/A -51-
+ DMSO (full strength)

[illegible]

Table 15. Effect of DMSO Alone (30 gpa); 0.5 lb/A of 2,4-D in Water (30 gpa) or 0.5 lb/A of 2,4-D in DMSO (30 gpa) = COMB on Lodging of Corn and Small Grains 3 Days After Treatment.

<u>PLANT SPECIES</u>	<u>Number of Lodged Plants, % of Total</u>			
	<u>CONT</u>	<u>DMSO</u>	<u>2,4-D</u>	<u>COMB</u>
Corn	0	0	10	40
Wheat	0	0	14	14
Oats	0	0	20	30
Barley	0	0	7	7

Onion and wild garlic are species susceptible to 2,4-D but not easily controlled by herbicidal application and extremely difficult to eradicate. 2,4-D at a rate of 1/2 lb per acre reduced onion fresh weight by only about 20% in these experiments two weeks after treatment with the production of bulbs relatively normal in appearance. Application of the 2,4-D in DMSO resulted in a 95% inhibition in fresh weight over the same time period and complete suppression of bulbing (Figure 33). Vigor of the few remaining plants was reduced, the root systems were undeveloped and it is questionable whether these seedlings would have survived under field conditions. The results with onion prompted the initiation of the field testing program described briefly in the next section involving the use of 2,4-D - Tordon - DMSO mixtures for wild garlic eradication along roadsides.

Field Studies

General roadside and noncrop situations.-- Field trials were conducted during June, July and August, 1966 at seven locations in Tippecanoe County, Indiana, comparing 1/8, 1/2 and 2 lbs/A 2,4-D (mutoxyethanol ester) or Tordon (sodium salt) in water or pure DMSO for control of a variety of plant species in roadside and other noncrop situations. Plots were sprayed (30 gallons per acre) between 13 June and 30 June with observations taken at approximately 1 week intervals. About one-half of the species encountered showed no increased response to herbicide applied in DMSO as compared to water formulations (Table 17). The remaining species showed marginal enhancement of herbicidal activity with application in DMSO with the exception of red and white clover. Red and white clover growing in association with bluegrass or in isolated

Table 16. Summary of Criteria Showing Synergistic (+), Antagonistic (-) or No (o) Interaction Between 2,4-D and DMSO When Applied in Combination to Various Plant Species.

Plant Species	After 8 days		Vigor (16 days)	Rate of Mortality	After 21 days		
	Formative Effects	Leaf Necrosis			Total Mortality	Fresh Weight	Dry Weight
<u>Gramineae</u>							
Corn	o	+	o	o	o	-	-
Wheat	o	-	-	o	o	-	o
Oats	o	-	-	o	o	-	-
Barley	o	-	-	o	o	o	-
Crabgrass	o	o	o	o	o	o	o
<u>Amaranthaceae</u>							
Redroot Pigweed	-	+	-	+	-	-	-
<u>Caryophyllaceae</u>							
Chickweed	-	+	-	+	-	o	o
<u>Chenopodiaceae</u>							
Sugar Beet	o	o	o	+	+	+	+
Lambsquarters	-	++	+	++	+	+	+
<u>Compositae</u>							
Ragweed	-	o	o	++	o	o	o
<u>Convolvulaceae</u>							
Morning Glory	-	+	o	++	o	o	o
<u>Cruciferae</u>							
Cabbage	o	o	+	+	+	+	+
Yellow Rocket	o	++	-	++	+	+	+
<u>Cucurbitaceae</u>							
Pumpkin	-	++	o	++	o	o	o
Muskmelon	-	++	o	+	o	o	o
<u>Labiatae</u>							
Peppermint	-	++	o	o	+	+	+
<u>Liliaceae</u>							
Onion	-	o	+	+	+	+	+
Asparagus	-	+	+	+	-	o	o

Table 16 (Cont'd). Summary of Criteria Showing Synergistic (+), Antagonistic (-) or No (o) Interaction Between 2,4-D and DMSO When Applied in Combination to Various Plant Species.

Plant Species	After 8 days			Rate of Mortality	After 21 days		
	Formative Effects	Leaf Necrosis	Vigor (16 days)		Total Mortality	Fresh Weight	Dry Weight
<u>Leguminosae</u>							
Alfalfa	o	+	+	+	+	+	+
Birdsfoot Trefoil	o	+	+	+	-	+	+
Red Clover	o	+	+	+	o	o	o
Ladino Clover	+	+	+	+	-	+	+
Snapbean	+	o	o	+	+	o	o
Soybean	-	+	o	++	o	o	-
<u>Melvoceae</u>							
Velvet Leaf	-	+	-	+	o	o	-
Prickly Sida	-	+	+	o	o	o	-
<u>Plantaginaceae</u>							
Buckhorn Plantain	-	+	+	o	+	+	+
<u>Polygonaceae</u>							
Smartweed	-	++	+	++	+	+	+
Curled Dock	-	+	+	+	o	+	-
<u>Portulacaceae</u>							
Purslane	o	+	o	+	+	o	o
<u>Rosaceae</u>							
Strawberry	+	o	+	+	++	+	+
<u>Solanaceae</u>							
Tomato	-	o	o	++	+	+	o
Jimsonweed	o	o	o	++	o	o	o
<u>Umbelliferae</u>							
Carrot	-	++	o	+	+	o	o
Wild Parsnip	o	-	-	+	-	-	-

Figure 33. Effects of DMSO Alone (30 gpa); 0.5 lb/A of 2,4-D in Water (30 gpa) or 0.5 lb/A of 2,4-D in DMSO (30 gpa) = COMB on Growth of Onion (Allium sepa) 3 Weeks After Treatment.

Table 17. Summary of Species Tested for Enhanced Susceptibility to Herbicide (2,4-D or Tordon) Comparing Application in DMSO vs. Application in Water. Enhancement of herbicidal effectiveness refers only to the additional effect of application of the herbicide in DMSO relative to application of the herbicide in water. Based on a summation of visual ratings. Data of Wayne Rosso and D. J. Morrè.

SUSCEPTIBILITY to Herbicide (2,4-D or Tordon) NOT AFFECTED by Application in DMSO Relative to Application in Water.

SUSCEPTIBILITY to Herbicide (2,4-D or Tordon) ENHANCED (Slight to Moderate) by Application in DMSO Relative to Application in Water.

Bluegrass
Brome, Downy
Burdock
Carrot, Wild
Cinquefoil, Rough
Clover, Sweet
Crabgrass
Dock, Curled
Goatsbeard
Mullein, Common
Mustard, Tumbling
Parsnip, Wild
Peppergrass
Plantain, Buckhorn
Primrose, Evening
Trefoil, Birdsfoot
Vervain, Prostrate
Witchgrass
Wood Sorrell, Yellow

Bindweed, Field
Catnip
Clover, Red
Clover, White
Cranesbill
Dandelion
Garlic, Wild
Lettuce, Prickly
Marestail
Medic, Black
Milkweed, Common
Motherwort
Plantain, Broadleaved
Pigweed, Redroot
Ragweed
Speedwell, Thymeleaved
Speedwell, Purselane
Thistle, Field
Yellow Rocket

stands showed most effective control with herbicide applied in DMSO with no apparent injury to the grass. However, in none of the 1966 studies did we find strong synergistic interactions between the herbicides and DMSO (the effects of DMSO and herbicide were approximately additive (Table 18) for red and white clover).

Table 18. Effect of 2,4-D or Tordon Applied in Water or DMSO on Foliar of White Clover One Week After Treatment. Plants treated 30 June 1966 using 30 gallons of solvent per acre. Data of Wayne Rosso and D. J. Morre.

<u>Herbicide</u>	<u>Solvent</u>	<u>Lbs/A</u>	<u>% Necrotic Foliage</u>
2,4-D	Water	1/8	0
		1/2	10
		2	20
	DMSO	1/8	40
		1/2	20
		2	20
Tordon	Water	1/8	20
		1/2	40
		2	60
	DMSO	1/8	40
		1/2	60
		2	80
None	Water	-	0
	DMSO	-	20

Spot infestations of wild garlic.-- On the basis of greenhouse studies, field trials were initiated in 1967 to test 2,4-D - Tordon - DMSO mixtures for control of spot infestations of wild garlic along roadsides in 5 locations in Indiana and Missouri. Treatments were applied in late April and early May, 1967 when the plants were 8 to 14 inches high. 2,4-D and Tordon (1/2, 1 and 2 lbs per acre) were applied in water or DMSO alone and in equimolar (50:50) combinations. A treatment consisting of 1 lb 2,4-D + 1 lb Tordon in 30 gal of DMSO per acre was most effective and provided better control of wild garlic than 2 lbs per acre of either 2,4-D or Tordon alone in DMSO and better control of wild garlic than the combination applied in water. Further testing is scheduled and several years will be required for a complete evaluation of the

effectiveness of this treatment.

DMSO as a carrier for Atrazine.-- In preliminary field studies, the possibility of using DMSO as a carrier for Atrazine (2, chloro-4-ethylamino-6-isopropyl-amino-s-triazine) was studied. Atrazine and the related herbicide Simazine have been used for weed control in roadside plantings and are extremely insoluble in water but soluble in DMSO. In late post emergence applications, DMSO enhanced activity of Atrazine on all dicots tested but did not significantly enhance the activity of Atrazine on monocots.

IV. SUMMARY

Tordon herbicide, 4-amino-3,5,6-trichloropicolinic acid (picloram) is a potent, broad-spectrum herbicide with many potential uses in roadside development. Most turf grasses are resistant and most broad-leaved species (including 2,4-D and 2,4,5-T resistant perennials) are highly susceptible. Tordon causes stem twisting and other formative effects in susceptible broad-leaved dicotyledonous plants. The distortions of the growing stems and leaves are similar to those produced by the phenoxyacetic acids (2,4-D and 2,4,5-T) but Tordon is more toxic to plants than either 2,4-D or 2,4,5-T. Because of the many potential uses of Tordon in roadside development, its mode of action was examined in detail and compared with that of 2,4-D and 2,4,5-T.

Qualitatively, Tordon, 2,4-D and 2,4,5-T cause similar growth responses in tissue sections floated on solutions containing the growth regulator. In other studies, 2,4-D, IAA and Tordon were found to cause similar tissue responses with respect to cell elongation in a variety of tissues including soybean hypocotyl, pea epicotyl, mung bean hypocotyl and oat coleoptile sections and in the support of callus growth of tissue explants grown in culture. In addition, Tordon resulted in stem curving, cell wall loosening, root growth inhibition, formative effects and abscission responses similar to those of IAA and 2,4-D. Results of a kinetic analysis of root growth inhibition were compatible with a single mode of action for Tordon and 2,4-D and the structural requirements for auxin herbicide activity can be met by Tordon. Thus, by all criteria available, Tordon can be classified as an auxin herbicide along with 2,4-D and 2,4,5-T.

The greater herbicidal effectiveness of Tordon (compared with 2,4-D or 2,4,5-T) can be attributed to its greater mobility and resistance to breakdown within the plant. Thus, even though the type of formative effects produced by Tordon were similar to those produced by 2,4-D under field and greenhouse conditions, the effects induced by Tordon were more severe and persisted for a longer period of time.

The additional property of Tordon to markedly affect shoot growth of intact cuttings is not shared by either 2,4-D or 2,4,5-T. This property provides a convenient bioassay system for determining Tordon residues in soil.

Tordon was translocated following foliar application. In beans, the total elapse of time between application and response was approximately the same for Tordon and 2,4-D although Tordon illicited a greater response.

Kinetic analyses of Tordon-2,4-D interactions revealed that equimolar mixtures (50:50) of the two herbicides resulted in greater growth promotion than the same amounts of each auxin alone. This increased activity observed for mixtures of Tordon and 2,4-D in promoting cell expansion has also been observed in field studies and such mixtures are currently under study for control of Common Milkweed and Wild Garlic along roadsides.

In controlled laboratory investigations, an enhancement of 2,4-D and Tordon penetration and/or activity was observed when the herbicide was applied to plants using dimethylsulfoxide (DMSO) as the carrier. DMSO is a byproduct of the paper making industry and a powerful solvent for organic herbicides. At least part of the enhancement was due to facilitated foliar penetration of the herbicide due to rapid penetration of the DMSO carrier. In addition, DMSO may enhance solute absorption under some conditions but does not seem to alter drastically electrolyte permeability or hydraulic permeability of plant cells.

Rapid abscission of leaves from 18 to 22 day old bean plants was induced by 2,4-D or Tordon applied in DMSO. With 2,4-D, the time between treatment and the onset of abscission was 12 hours; 50% defoliation was achieved in less than 120 hours. The effectiveness of 2,4-D in this test system was increased fourfold by application in DMSO when compared with application in water at identical rates.

The phytotoxicity of DMSO was determined for a wide range of plants. Although pure DMSO is toxic to many plant species, grasses are resistant and the use of DMSO as a solvent or cosolvent for use in roadside development should not be limited by phytotoxicity considerations. Tolerance to concentrated DMSO solutions seems related to the cuticularized surface layers of the leaf, particularly to the existence of wax deposits.

A synergistic enhancement of 2,4-D toxicity by application in DMSO was observed in approximately 36 of 40 different species of mature or seedling plants tested from 18 plant families in preliminary greenhouse studies. 2,4-D at a rate of 1/2 lb per acre, reduced onion fresh weight by 20% during a two week period following treatment and permitted relatively normal bulb formation. Application of the 2,4-D in DMSO, however, resulted in a 95% inhibition in fresh weight over the same time period and complete suppression of bulbing. These results with onion prompted a field testing program involving 2,4-D - Tordon - DMSO mixtures for wild garlic eradication along roadsides. Seven field trials in 1966

comparing approximately 40 common roadside species of plants (Wild Garlic excluded) showed no evidence of a 2,4-D - DMSO or Tordon - DMSO interaction but the combinations proved effective for rapid control of Red and White Clover in turf. Field tests in 1967 conducted with spot infestations of Wild Garlic along roadsides indicated that a treatment consisting of 1 lb 2,4-D + 1 lb Tordon in 30 gal of DMSO per acre was potentially effective in Wild Garlic eradication. This treatment provided better initial control of Wild Garlic than 2 lbs per acre of either 2,4-D or Tordon alone in DMSO and better control of Wild Garlic than the combination in water.

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