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Biological Activity of 2,4-D Esters on Rubber Vine (*Cryptostegia grandiflora*): Dependence on Vapour Pressure and Molecular Weight and Isomerism of the Alcohol Substituent

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Abstract

The relationship between molecular structure and biological efficacy was investigated for 16 esters of 2,4-D [(2,4-dichlorophenoxy)acetic acid] on rubber vine (*Cryptostegia grandiflora*). These included the normal (n) or straight-chain esters from C-1 (methyl) to C-8 (octyl), the n-decyl, n-dodecyl, iso-butyl, amyl (iso-pentyl), 2-ethylhexyl (iso-octyl), and the methoxy-, ethoxy-, and butoxyethyl esters. For the normal series esters, biological efficacy was found to be a function of both the molecular weight and the vapour pressure of the esters. This relationship was linear for the higher molecular weight, low volatile esters, biological efficacy decreasing with increasing molecular weight and the accompanying decrease in vapour pressure of the esters. The low molecular weight, volatile esters were more active than the higher molecular weight, low volatile esters, and increases in the vapour pressure of these low molecular weight, volatile esters were sufficient to account for the deviation from linearity of those esters. When all esters are considered, the same relationships hold but the branched-chain (iso) and chemically substituted (alkoxy alcohol) esters are less effective than the corresponding normal esters. Possible reasons for these results are discussed.

Introduction

Rubber vine (*Cryptostegia grandiflora*), a native of Madagascar, is a serious weed of the pastoral industry in north Queensland. A rampant climber, it infests more than 10 000 km of river and stream banks (Caltabiano, unpubl.) and also grows in the open as an untidy shrub. The plant is toxic (McGavin 1969; Everist 1974), but its main nuisance value lies in the economic losses that it causes by hindering mustering of stock.

Rubber vine is readily controlled by aerial application of 2,4-D [(2,4-dichlorophenoxy) acetic acid] at 2–4 kg/ha. 2,4-D esters are more effective than emulsifiable acid or amine salt formulations (Harvey 1981) as foliar sprays.

Despite a voluminous literature on the subject of 2,4-D, published systematic studies of the esters are few. Hyder *et al.* (1958) studied ethyl, butyl and iso-propyl ester formulations of 2,4-D, 2,4,5-T and MCPA for control of big sagebrush (*Artemisia tridentata*). They concluded, apparently somewhat reluctantly, that the iso-propyl esters of 2,4-D and 2,4,5-T were less effective on big sagebrush than the ethyl and butyl esters. Campbell (1959) studied the effects of the methyl, ethyl, butyl, butoxyethyl, and propylene glycol butyl ether (PGBE) esters of 2,4-D on the morphological development of strawberry (*Fragaria x ananassa*) plants. He noted 'a direct relationship between toxicity and length of the carbon chain of the alcohol', the short chain esters being more phytotoxic than the esters of higher molecular weight.

Other studies have been less detailed, and the conclusions reached somewhat contradictory. The low molecular weight alkyl esters are known to be highly volatile and highly injurious to susceptible plants (Crafts 1956*a*, 1956*b*; Leasure 1958). This has led to their replacement in the north American market by more expensive, higher molecular weight, low-volatile esters (Klingman *et al.* 1975). However, these high molecular weight esters are

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often regarded as equal to or more effective than the low molecular weight esters (Robbins *et al.* 1952; Alley 1956; Crafts 1956*a*, 1956*b*; Leonard 1958). Formulation quality affects herbicide performance, so that foresters have concluded that, for their purposes, the low-volatile esters are all essentially equal, and the differences occasionally observed have been ascribed to formulation rather than the esters themselves (Kirch 1961; Schieferstein 1961; Gratkowski 1961), with the exception that the iso-propyl ester again performs poorly on chamise (*Adenostoma fasciculatum*) (Leonard 1956, 1961).

The 2,4-D esters registered for sale in Queensland are the ethyl, iso-butyl, butoxyethyl and iso-octyl. As noted above, the ethyl ester is characterized by having high activity coupled with high volatility which poses serious potential risks to non-target species (Gilbey *et al.* 1984), particularly under high temperature conditions such as prevail in north Queensland. This has led to legally imposed restrictions on its use in some parts of Australia, including defined hazardous areas in Queensland. Nevertheless, the ethyl ester remains the most widely used 2,4-D ester in Australian agriculture, and, partly for this reason, it is currently the most economic to use. However, because its use, particularly in mixed cropping areas, appears to be increasingly questioned, it was considered prudent to investigate the possibility of using other 2,4-D esters for rubber vine control. The economics of these may still be more attractive than other herbicide systems. A study was made of the effectiveness of 16 esters of 2,4-D for the control of rubber vine.

Materials and Methods

All the straight-chain esters of 2,4-D from C-1 (methyl) to C-8 (octyl), the n-decyl, n-dodecyl, iso-butyl, amyl (iso-pentyl), iso-octyl (2-ethylhexyl), and the methoxy-, ethoxy-, and butoxyethyl esters were synthesized in the laboratory and formulated as per normal commercial formulations to give 400 g/l a.e. concentrates. The solvent consisted of a 50/50 (v/v) mixture of toluene and diesel distillate containing 300 g/l calcium dodecylbenzene sulfonate (I.C.I. Alkanate CS) plus 300 g/l nonionic (I.C.I. Teric 200; alkylphenol alkalene oxide derivative) emulsifying surfactants. Details of the preparation, purity data, and HPLC retention times of the esters are given elsewhere (Harvey 1986).

Rubber vine seed was collected from the field near Rockhampton, Qld. Over 9 000 seedlings were raised in an evaporatively cooled glasshouse under natural light (approximately 60% of full sun) until they were approximately 45 cm in height at about 8 months of age. Maximum day/minimum night temperatures in the glasshouse were 28/17°C; humidity was not controlled but usually exceeded 70% R.H. The required number of plants were then selected for uniformity and removed from the glasshouse 24 h prior to spraying with 0·3% a.e. emulsions of the esters in water as carrier using a knapsack sprayer with an adjustable cone nozzle. Temperatures outside averaged 25/14°C before and after spraying, while conditions at the time of spraying in the early morning were clear with a very slight breeze and the temperature still below 20°C. Equal volumes (600 ml) of spray (water plus 2,4-D concentrate) were used in all treatments.

The experimental design was a randomized complete block with eight replications of 50 plants/plot. Death (= 100% dieback) or per cent dieback to ground level was recorded for all plants approximately 7 months after treatment. Data were analysed by Analysis of Variance using SYSTAT^{R} (Wilkinson 1986), except that plants from the control (untreated) plots did not die, and so were excluded from the analysis.

Results

Results of this experiment are given in Table 1. Only a very few of the more and less effective esters differed significantly from each other in the Analysis of Variance.

However, a plot of biological efficacy (dieback) against molecular weight or carbon number (Fig. 1) indicated a systematic but nonlinear relationship, so the treatment means were then subjected to nonlinear regression, again using SYSTAT^{R} (Wilkinson 1986). Variables used in the nonlinear estimation included molecular weight (MW), carbon number (CNO), (octanol/water) partition coefficients (log P) and a factor to describe isomerism (I), all of the alcohol moiety, plus HPLC retention times and estimates of evaporation rate of the 2,4-D esters. These variables were selected because they are, or are a measure of,

Table 1. Treatments, treatment means (% dieback), molecular weight (MW), carbon number (CNO) isomer factor (*I*) and log *P* values of the alcohol moiety used in preparation of the 2,4-D esters
Values followed by the same letter do not differ significantly ($P=0.05$) using Tukey's LSR procedure

Treatment	Dieback (%)	MW	CNO	*I*	log *P*	VPCORR (mPa)
2,4-D n-dodecyl	78·4[a]	186·34	12	1	5·055	—[A]
n-decyl	80·5[ab]	158·29	10	1	3·997	—
iso-octyl	83·7[abc]	130·23	8	2	2·809	0·440
methoxyethyl	86·6[abc]	76·09	3	3	−0·746	—
butoxyethyl	86·6[abc]	118·18	6	3	0·841	0·613
iso-butyl	87·0[abc]	74·12	4	2	0·693	9·731
ethoxyethyl	88·4[abc]	90·12	4	3	−0·217	—
n-octyl	89·8[abc]	130·23	8	1	2·939	0·196
amyl	92·0[abc]	88·15	5	2	1·222	3·81
methyl	92·5[abc]	32·04	1	1	−0·764	59·657
n-hexyl	94·2[abc]	102·18	6	1	1·881	1·293
n-heptyl	95·2[abc]	116·21	7	1	2·410	0·352
ethyl	95·2[abc]	46·07	2	1	−0·235	31·959
n-pentyl	95·5[bc]	88·15	5	1	1·352	5·053
n-butyl	97·0[bc]	74·12	4	1	0·823	7·178
n-propyl	97·1[c]	60·10	3	1	0·294	13·594

[A]Denotes missing value.

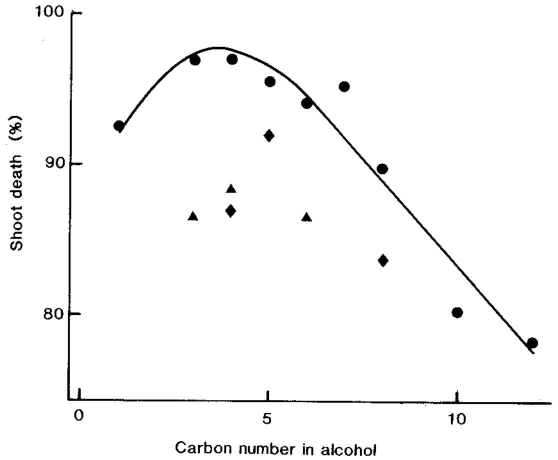

Fig. 1. Relationship between shoot death 7 months after treatment and carbon number in the alcohol moiety of 2,4-D esters for: ● n-alcohols, ◆ iso-alcohols, and ▲ alkoxy alcohols. The continuous line represents the equation (vi) in Table 3.

physical constants known to influence pesticide behaviour (Van Valkenburg 1972; Hartley and Graham-Bryce 1980).

The term describing evaporation rate used in the regression analysis was calculated as $MW^{0.5} \times VP^{CORR}$ (Hartley and Graham-Bryce 1980). This latter was derived by calculating the vapour pressures (VP) at 25°C for the C-1 to C-8 esters from the data of Jensen and Schall (1966) and comparing these with published (Hamilton 1980) and unpublished (Noble, personal communication) data from Hamilton's laboratory. Correction factors were determined for the common members of each data set, and the correction factors for the missing members estimated by interpolation using linear regression analysis. Finally, the corrected vapour pressures (VP^{CORR}; Table 1) were calculated by applying the correction factors so determined to the vapour pressures calculated from Jensen and Schall's data.

Equations used in the nonlinear regression analysis are given in Tables 2 and 3. Only those equations giving the best fit to the data (minimum value of the error sum of squares) for one, two, three or four variables are included. Table 2 is for the complete data set. Table 3 is for the normal (straight chain) series only.

Table 2. Equations giving the best fit for one, two, three or four variables for the complete data set

Equations	Error S.Sq
(i) dieback = 100·317 − 0·107MW	254·891
(ii) dieback = 112·854 − 0·200MW − 0·043$MW^{0·5}VP^{CORR}$	138·043
(iii) dieback = 114·806 − 2·391CNO − 5·305I − 0·045$MW^{0·5}VP^{CORR}$	32·737
(iv) dieback = 107·613 + 1·402CNO − 7·812I − 0·042$MW^{0·5}VP^{CORR}$ − 6·788 log P	20·246

Table 3. Corresponding equations to those in Table 2 for the normal (n) series only

Equations	Error S.Sq
(v) dieback = 100·574 − 1·558CNO	138·035
(vi) dieback = 107·818 − 2·083CNO − 0·039$MW^{0·5}VP^{CORR}$	6·549
(vii) dieback = 46·344 + 23·067CNO − 0·039$MW^{0·5}VP^{CORR}$ − 47·543 log P	6·549

Discussion

The data presented in Table 1 confirm Campbell's (1959) finding that biological activity of the esters of 2,4-D decreases with increasing molecular weight of the alcohol substituent when applied as foliar sprays. Table 1 also shows that the branched-chain isomers are less effective than the straight chain, or normal isomers, a finding consistent with that of Hyder *et al.* (1958). Introduction of an ether oxygen molecule into the alcohol chain of the ester improves the water solubility of the ester and may improve the stability of ester-in-water spray emulsions, but again decreases the efficacy of the ester. The greater efficacy of the straight chain esters suggests that penetration into the leaf may be the main factor limiting effectiveness of the esters.

An examination of the regression equations (Tables 2 and 3) emphasizes the importance of the variables carbon number (CNO) or molecular weight (MW) and isomer (I), a factor which includes both isomerism and, in the case of the ether alcohols, chemical substitution. For the five esters derived from the normal or straight chain alcohols between C-6 (n-hexyl) and C-12 (n-dodecyl), the relationship between dieback, molecular weight and vapour pressure is described by the linear equation

$$\text{dieback} = 118·874 - 3·509\text{CNO} - 0·256MW^{0·5}VP^{CORR} \qquad (r^2 = 0·935).$$

This simple linear relationship between increasing effectiveness and decreasing molecular weight holds until we reach the 'volatile' esters, defined as those esters in which the carbon number of the alcohol is five or fewer (Leasure 1958; Jensen and Schall 1966). Then increasing volatility apparently results in the ester being lost from the leaf to the extent that uptake into the plant and the resultant biological activity are both reduced. Increases in the vapour pressure and hence the evaporation rate are sufficient to account for the deviation from linearity.

The partition ratio (log *P*) had little to contribute in the regression equations, and is assumed to be relatively unimportant, a finding consistent with that of Price and Anderson (1985), who found uptake of 10 compounds into 10 plant species to be largely independent of the partition coefficient.

It was noted, during the course of this experiment, that the lower molecular weight esters were faster acting than the high molecular weight esters. Crafts (1956*a*) and Leonard (1958) have noted the same. The observation allows an explanation of the above data and of the uptake of phenoxy acid and other pesticide esters into leaves.

The explanation depends on three factors: (i) esters of 2,4-D and other herbicides are hydrolysed during uptake into leaves (Crafts 1960; Morré and Rogers 1960); (ii) an esterase capable of this hydrolysis has been found to occur in the cuticle (Price 1982); and (iii) low molecular weight esters are more easily hydrolysed than the higher molecular weight esters (Smith 1972).

More rapid hydrolysis of the lower molecular weight esters than the high molecular weight esters by a cuticular esterase would lead to more rapid uptake of the hydrolysed 2,4-D acid and a more rapid expression of responses such as stem curvature. The ease of hydrolysis of the low molecular weight esters and the effect of isomerism and chemical substitution of the alcohol moiety in this experiment suggest that steric factors are important in determining the rate of hydrolysis by the cuticular esterase. The size of the ester molecule as reflected in its molecular weight would be more important in this system than its relative solubility in the aqueous and lipid phases in the cuticle and cell wall. That is, the partitioning of the ester would be determined by the rate of hydrolysis by the esterase, not by the partition ratio, log *P*. Uptake would be largely independent of log *P* as found by Price and Anderson (1985) and in this experiment.

Alternative explanations exist in that diffusion of the ester through the cuticle, and solubility of the ester in the cuticle would both be affected by the size (CNO) and isomer (I) of the alcohol moiety. Differences in the rate of hydrolysis of ester to acid could be affected by the rate at which the ester is presented to the esterase, as well as the postulated rate at which the ester can hydrolyse the ester. Since both processes would behave in a parallel manner, it is not possible to distinguish between them on the data presented here.

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