SOME ASPECTS OF ABSORPTION, TRANSLOCATION AND METABOLISM OF ^{14}C -PICLORAM IN GORSE

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Summary

Growth chamber and laboratory experiments were undertaken with gorse (*Ulex europaeus*) plants grown from cuttings, to study factors relating to the absorption, translocation and metabolism of ^{14}C -picloram applied with unlabelled 2,4,5-T. The effects of two non-ionic surfactants, lowering the pH and removing cuticular wax with chloroform were to increase *in vitro* absorption by 44 to 350%. Misting after spraying increased *in vivo* absorption by 82 to 107%. Autoradiographs and stem sections suggest that picloram was readily translocated from the treated stem to the roots, although re-distribution of picloram to other untreated areas did not appear to occur. Source-sink relationships were observed. No metabolites of picloram were detected from spine and stem sections.

INTRODUCTION

Previous studies of the effect of ^{14}C -2,4,5-T on gorse (*Ulex europaeus*) have suggested that this herbicide is poorly absorbed (Leonard and Yates 1959; Schicke and Linden 1963). Additives such as picloram, have been used to improve the effectiveness of 2,4,5-T. However, there is apparently no published information on the absorption, translocation or metabolism of picloram, either applied as an additive with 2,4,5-T or applied singly to gorse. This paper reports on some aspects of the absorption, translocation and metabolism of ^{14}C -picloram (potassium salt) applied with unlabelled 2,4,5-T.

METHOD

The method has been described in detail (Rolston 1974) and is briefly outlined below. Plants were raised as clonal material propagated from shoot cuttings, and reached a mature state after six months (viz. hard spines and flowering). The radio-active herbicide used was the potassium salt of carboxyl- ^{14}C -picloram mixed with a commercial herbicide formulation (5% w/v picloram as the potassium salt plus 20% w/v 2,4,5-T as the butyl ester) diluted 1:250 with distilled water. Detection of the ^{14}C -picloram was by liquid scintillation spectrometry using the channels ratio method of quench correction (Herberg 1965).

In vitro absorption was determined by incubating the excised terminal six cm of an actively growing branch in 10 ml of the ^{14}C -picloram mixture. Each segment was placed in a separate test tube, incubated at $27 \pm 1^\circ\text{C}$ for 12 hours with light at 7 000 lux; then washed, to remove unabsorbed ^{14}C -picloram from the surface, oven dried, weighed, ground and the radio-activity determined. There were 10 replicates per treatment. The following factors affecting *in vitro* absorption are reported; the effect of pH (4.15, 5.25 and 6.65), the

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effect of two non-ionic surfactants, Triton X-100 and Dowfax 9N9, and the effect of surface wax removal by a 10 second chloroform wash.

In vivo absorption was determined using whole plants (eight months old), placed in a growth chamber at $22 \pm 2^\circ\text{C}$, $70 \pm 10\%$ relative humidity and light at 7 000 lux. The plants were initially sprayed with unlabelled picloram:2,4,5-T (1:4, diluted 1:250) mixture followed by an application to a selected area of 5 micro litres of the ^{14}C -picloram mixture. At the end of the experimental period the treated area was excised and the unabsorbed herbicide recovered by washing. Absorption was calculated as the difference between applied and unabsorbed ^{14}C -picloram. Using this method the effect of re-wetting dry herbicide deposits with a mist of distilled water (taking care to avoid run-off) was studied.

The plants used for translocation studies were treated by the method described for *in vivo* absorption. At the end of the experimental period, the treated stems and roots were excised from the rest of the plant and the roots washed to remove adhering potting mixture. Plants used for autoradiography were harvested 1,2,4,7 and 16 days after picloram application and were immediately frozen in liquid air to prevent further translocation. After thawing the sections were pressed for 14 days before mounting on light-weight cardboard and placed in light proof sachets containing X-ray film. In other plants the treated stem was sectioned into 2.5 cm lengths. The plant sections were oven dried, weighed, ground and the radio-activity in each section determined.

The possibility of metabolites of picloram causing artifacts during autoradiography was examined by incubating branch tips in a ^{14}C -picloram mixture for 24 hours. The tissue was then washed and ground with fine sand in 0.1 M NH_4HCO_3 , pH 7.3. The extracts were centrifuged, and the liquid was divided into two equal portions and in one fraction the soluble proteins were precipitated with 5% ZnSO_4 . The precipitated soluble protein solution was centrifuged and the radio-activity of the pellets and liquid determined. Descending chromatography on Whatman No. 1 paper in the upper phase of an n-butanol:acetic acid:water (40:12:50) mixture was used to separate possible metabolites and after drying the ^{14}C distribution on the paper was detected with a Packard Radiochromatogram Scanner.

RESULTS

Absorption

Of the two non-ionic surfactants, Dowfax 9N9 more effectively enhanced ^{14}C -picloram absorption than Triton X-100. The surfactants increased *in vitro* absorption by 44 to 123% (Table 1). Lowering the pH of the incubation solution increased *in vitro* absorption by 120%, while the effect of removing the cuticular wax layer was to increase absorption by 350%.

Translocation

The following observations were made from the autoradiographs:

- the roots were labelled within 24 hours, although further increases in ^{14}C concentration were not observed,
- both translocation towards stem tip and roots occurred, with the latter being predominantly confined to the treated stem and roots with other branches accumulating little or no ^{14}C ,
- source-sink relationships occurred with nodules and seed pods being more heavily labelled than the surrounding roots and stem.

The analysis of branch sections showed marked differences in the pattern of translocation depending on the application site of the

Scrub Weeds

¹⁴C-picloram. When the stem tip was treated, it retained 40% of the ¹⁴C label 48 hours after application and a further 30% of the ¹⁴C label was in the adjacent 6 cm of stem. By treating the stem below the tip, considerable translocation in a basal direction occurred (Fig. 1).

TABLE 1: THE EFFECT OF pH, SURFACTANT AND A 10 SECOND CHLOROFORM DIP ON THE *in vitro* ABSORPTION OF ¹⁴C-PICLORAM (potassium salt)

Treatment	Absorption ¹ (dpm. gDW ⁻¹ x 10 ⁴)	t ²
pH		
6.25 (control)	3.26	
5.25	5.72	**
4.15	7.20	**
Surfactant		
No surfactant	2.27	
0.25% w/v Triton X-100	3.28	*
0.50% w/v Triton X-100	3.71	**
0.25% w/v Dowfax 9N9	5.07	**
0.50% w/v Dowfax 9N9	4.90	**
Chloroform dip		
Control	2.90	
Chloroform dip (10 seconds)	10.15	**

¹ dpm. gDW⁻¹ = disintegrations per minute per gram dry weight.

² * P > 0.1%.

** P > 0.01%.

Misting 24 and 48 hours after spraying compared to no mist increased *in vivo* absorption by 82 to 107% (Table 2).

TABLE 2: THE EFFECT OF MISTING WITH DISTILLED WATER ON THE *in vivo* ABSORPTION OF ¹⁴C-PICLORAM (potassium salt). PLANTS HARVESTED FIVE DAYS AFTER HERBICIDE APPLICATION

Treatment	% absorbed	t
Not misted	27	
Misted at 24 hours after application of the picloram mixture.	51	P > 0.001
Misted at 24 and 48 hours after application of the picloram mixture.	59	NS

Metabolism

After incubation for 24 hours and grinding, 98.80% of the absorbed radio-activity was recovered in the soluble fraction, with most of the remaining activity being in the insoluble (cell residue) fraction. Only a trace (0.06%) of the radio-activity was associated with the protein precipitate. The radiochromatogram scan of the supernatant (soluble fraction) had only one distinct peak (Rf = 0.76) which corresponded to the Rf value obtained for pure picloram.

Scrub Weeds

Fig. 1: The distribution of the ¹⁴C-picloram formulated as the K-salt along a 30 cm length of stem following application of the herbicide 6 cm below the stem tip.

DISCUSSION

The stability of picloram suggests that ^{14}C -picloram and not a breakdown product of picloram was measured. Loeffler and van Overbeek (1971) note that picloram is reasonably stable in many plant species and this contributes to its effectiveness as a herbicide.

The two non-ionic surfactants tested markedly enhanced the *in vitro* absorption of ^{14}C -picloram. The only published field trials on the effect of surfactants on the control of gorse (Houlker 1960) showed that the sodium salt of a secondary alkyl sulphate gave improvements in the initial control of gorse, but did not improve long term control. G. W. Mason (pers. comm.) notes that final kill of gorse in the field has never been consistently enhanced by the addition of any surfactant with brushweed herbicides. Lowering the pH of the incubating ^{14}C -picloram mixture from 6.65 to 4.15 doubled its absorption and this is in contrast with results previously reported for other species (Ashton and Crafts 1973).

The marked increase in ^{14}C -picloram absorption when wax was removed suggest that cuticular surfaces are a barrier to absorption. The increase in absorption following misting indicates that little absorption occurs once the spray droplets have dried and further absorption can occur only if the deposits are redissolved. Upritchard (1973) has already observed that the effectiveness of 2,4,5-T is improved in areas of reliable summer rains compared with areas where summer droughts regularly occur. While this may result from reduced absorption due to rapid drying of spray droplets under drought conditions an alternative explanation is that poor growth may occur under these conditions resulting in limited herbicide translocation.

The results obtained suggest that absorption is influenced by spray, plant and environmental factors and these factors must be considered both in respect to the formulation and the application of a herbicide to gorse.

The pattern of translocation obtained from the autoradiographs is similar to that of 2,4,5-T in gorse (Schicke and Linden 1963). The apparent lack of build up of ^{14}C -picloram in the roots may be related to the stage of growth as translocation in a basal direction appeared to be greater in the quantitative analysis than in the autoradiographs. These two studies were made on plants of different ages and the difference may represent stage of growth or seasonal influences. The poor translocation into other lateral branches supports field observations that complete coverage of gorse bushes is necessary for adequate control.

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Scrub Weeds

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