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Use of isotopic tracers in studies of herbicide performance on grasses and sedges

Report of a final Research Co-ordination Meeting organized by the Joint FAO/IAEA Division of Nuclear Techniques in Food and Agriculture and held in Los Baños, Philippines, 17–21 February 1997

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FOREWORD

Herbicide products as sold to the user are a mixture or formulation of active ingredient, surfactants and other adjuvants, plus a carrier, which will be a liquid if the formulation is to be applied as a spray. The adjuvants affect the characteristics of the spray, including the retention of the droplets by plant surfaces and the penetration of active ingredient into the plant. Thus they play a critical part in determining the phytotoxicity and selectivity of the product.

Commercial formulations are usually designed for satisfactory performance in a wide range of situations from which it follows that they may not be optimum for any specific weed. In particular there may be scope to improve the rate of penetration into the plant of the active ingredient. The use of ^{14}C labelled compounds is almost essential for the necessary studies of this behaviour.

The IAEA organized a co-ordinated research programme in 1992 to explore the possibility of improving the performance of the herbicide glyphosate on *Cyperus rotundus* (purple nutsedge), commonly regarded as the "world's worst weed", by modifying the commercial formulation using penetration studies with ^{14}C labelled glyphosate as the initial screening procedure. This TECDOC summarizes the outcome of the programme and includes the papers presented at the research co-ordination meeting held in Los Baños, Philippines, 17–21 February 1997. The co-operation of the Monsanto Company, the manufacturer of the glyphosate herbicide, is gratefully acknowledged.

EDITORIAL NOTE

CONTENTS

SUMMARY OF THE CO-ORDINATED RESEARCH PROGRAMME

1. INTRODUCTION

Herbicides, like all classes of pesticide, are never used as the active ingredient (a.i.) alone. The basic reason is that they are applied at rates of a few kg down to a few g ha^{-1}, so some sort of carrier is necessary to ensure even distribution of an application. The mixture of a.i. and other components is known as a formulation.

For some purposes the carrier is a solid, the formulation is supplied as granules and is applied as such. More usually the formulation is intended to be dissolved, suspended or emulsified in water so that it can be applied as a spray. The way in which an a.i. is formulated for this purpose depends to some extent on its solubility in, or miscibility with, water. The most important types of sprayable formulations are: soluble concentrates (SLs), wettable powders (WPs), emulsifiable concentrates (ECs), suspension concentrates (SCs) and water dispersible granules (WGs). With the exception of the SL type of formulation the names indicate the presence of wetting agents, emulsifiers and other materials with surface active properties in order to maintain their physical stability and miscibility with water [1].

In addition to the compounds added to produce the required physical properties, many formulations, including SLs, contain additional adjuvants to improve the biological effect. For herbicides this means increasing toxicity to the target weed and/or improving selectivity between crop and weed. Common adjuvants include surfactants, oils and sticking agents. They may improve spray retention and rain fastness, increase spreading of the spray drops on leaf surfaces, and improve penetration of the a.i. through the cuticle. Sometimes adjuvants may affect the storage properties of the formulation or are useful only in special circumstances. In such cases they are supplied separately and are added to the spray tank by the operator.

Although there is an extensive literature on the effect of adjuvants on the processes mentioned in the two previous paragraphs, structure–activity relationships are elusive and it is generally held that in practice commercial formulations are empirically based. The major pesticide companies screen thousands of compounds for pesticide activity each year so the primary testing stage usually uses something crude like a solution in acetone. At the second and tertiary stages a standard formulation mixture that gives a satisfactory general performance is chosen. By this stage the battery of toxicological tests and environmental studies needed for registration begins so it is then too late to refine the system. From this it follows that it is often possible to improve the performance of a commercial herbicide for a specific weed.

2. PROBLEM

The most pernicious weeds include sedges and grasses such as *Cyperus* spp. (nutsedges), *Cynodon dactylon* (Bermuda grass), *Echinochloa* spp. (barnyard grass, jungle rice), *Eleusine indica* (goose grass), *Sorghum halepense* (Johnson grass), *Imperata cylindrica* (alang alang, cogon grass), and *Avena* spp. (wild oats). They are difficult to control mechanically because they either have underground perennating organs or produce seeds which are viable in the soil for several years. Herbicides are not always effective, at least partly because spray droplets are not well retained and/or the penetration and translocation of the a.i. is limited.

Cynodon dactylon (purple nutsedge) was chosen for study in this programme because it has been classified as the "world's worst weed"; it occurs in 92 countries and is a serious weed in over 50 crops [2]. It spreads by rhizomes which also produce tubers that continue to proliferate forming chains that can extend to a considerable depth in the soil. In a chain, the upper tuber exerts apical dominance over the others so suppressing their germination. If the chain is broken then the apical dominance of the upper tuber is lost and each fragment of the chain (and isolated tubers) is able to germinate. Therefore mechanical methods of control are not effective. It is also difficult to control with herbicides not only because of poor translocation and penetration in the aerial parts but also because translocation of herbicides essentially stops at the first tuber. It is not regarded as a suitable target for biological control because it can be a useful component of lawns and pastures and, of course, biological agents cannot distinguish between situations where a plant is desirable and where it is a weed [3].

3. BACKGROUND

The herbicide glyphosate, *N*-(phosphonomethyl)glycine, is a post-emergence, non-selective herbicide first reported in 1971 [4]. Because it is almost always formulated as one of its salts, concentrations are normally reported in terms of acid equivalent (a.e.) The most widely used commercial formulation is "Roundup" produced by Monsanto. The components of this formulation are known to have changed since its introduction but, of course, the exact composition is a trade secret. It has been possible to improve the activity of "Roundup" on many species with a number of additives [5]. These include surfactants, oils, and ammonium sulfate, alone and in various combinations. In some cases the activity was effectively doubled so that the application rate could be halved although in others some concentrations of ammonium sulfate were antagonistic.

Glyphosate as "Roundup" has been used to control *C. rotundus* effectively at rates as low as 2 kg ha^{-1} [6]. However, at the current (1996 Austrian) price this costs nearly US $40, so that ways to reduce the required application rate are still of the greatest interest. Given the number of examples reviewed in Ref. [5] the prospect of substantially improving the performance of "Roundup" on *C. rotundus* seemed reasonably good.

4. OBJECTIVES

The main objective was to identify ways in which the phytotoxicity to *C. rotundus* of the "Roundup" formulation of glyphosate could be increased by the addition of locally available surfactants, oils and ammonium sulfate, alone or in combination so as to reduce the cost of its control. A secondary objective was to improve the abilities of participants to undertake research on the penetration and translocation in plants using a ^{14}C labelled herbicide in the laboratory and to test the results on the field scale by observation of the phytotoxicity of non-labelled preparations.

5. APPROACH

The approach was based on the assumption that no improvement in herbicide performance could be expected unless penetration of the compound into the leaf could be increased. Therefore the screening process was to observe the effects of various additives to the "Roundup" formulation on the penetration of glyphosate using radiolabelled material as a tracer. Autoradiographs were taken or plants segmented and ^{14}C contents measured to obtain information about translocation in the plant as well. The second stage was to take the most promising components and vary their concentrations to identify one or two mixtures for field testing again on the basis of measurement of penetration of radiolabelled glyphosate.

6. THE PROGRAMME

The programme involved 7 Contract holders initially (one left after the third year because she changed her job) and 2 Agreement holders. It lasted for five years and research co-ordination meetings were held in 1992 (Vienna), 1995 (Bangkok) and 1997 (Los Baños).

As mentioned above in Section 5 the programme was divided into two phases:

(a) Laboratory studies of penetration and translocation of ^{14}C labelled glyphosate into *C. rotundus* leaves from mixtures of "Roundup" with surfactants, oils and ammonium sulfate, singly and in combinations;
(b) field testing to evaluate the toxicity to *C. rotundus* of those mixtures which produced the greatest penetration.

Details of the procedures used are set out in the Appendix.

7. RESULTS

7.1. First phase

The formulation additives examined were based on those which had proved effective in improving the efficacy of "Roundup" on the temperate weed *Elymus repens* (couch grass) [7]. They were three surfactants with and without oil, glycerol and ammonium sulfate. The surfactants, which were kindly donated by Imperial Chemical Industries plc (UK), were three polyoxyethylene aliphatic alcohols of different polarity, in the "Synperonic" series, numbers 2, 7 and 20. The numbers refer to mean molar ethylene oxide contents.

Generally the surfactants alone increased penetration but not always and there was no clear trend for penetration to increase with ethylene oxide content which would have been expected [5]. There were large site-to-site variations in uptake from the unamended formulation ranging from 20–80% after 24 h.

Most participants reported that ammonium sulfate, diesel oil and glycerol also increased penetration, at least at laboratories where penetration from the unamended formulation was low. Translocation of absorbed ^{14}C within the plant was extensive in all cases and not apparently affected by formulation. Therefore it was concluded that future work could use penetration of the leaf alone as the criterion in evaluating formulation additives.

7.2. Second phase

The polyoxyethylene aliphatic alcohols are quite cheap but may not be readily available in developing countries. As they were no more effective than diesel oil or ammonium sulfate in increasing ^{14}C penetration it was decided to concentrate on the latter additives although some participants included them. However, diesel oil is quite expensive in some countries so, where relevant, cheaper local vegetable oils were included. Additional surfactant is necessary to emulsify the added oil and Triton X-100 (t-octylphenoxypolyethoxyethanol) was used but in some cases cheap local household detergents, such as washing-up liquids, were included among the treatments.

Various combinations and rates of these additives were evaluated in the laboratory for their effect on the penetration of labelled glyphosate into the leaf. Because glyphosate is easily washed from the leaf surface by rain, speed of penetration is important so this stage of evaluation included measurements at 1 and 2 h in addition to the 24 h measurement made in the earlier work. Each participant then selected one or two mixtures to test in the field for their ability to control *C. rotundus* both grown alone and in the presence of crop competition from maize.

7.2.1. Laboratory studies

All but one participants reported one or more mixtures that increased glyphosate penetration over 24 h, in some cases by 2 or 3 fold, compared with unamended "Roundup". The exception was in Malaysia where glyphosate penetration with all treatments was of the order of 99% so there was no scope for improvement. Differences after 1 and 2 h were much smaller and in two cases did not occur. Mixtures containing both oil and ammonium sulfate (plus emulsifier) were the most effective. Diesel oil was used most commonly but in India, coconut oil, Neem oil and peanut oil were also tested and gave similar results so that coconut oil was chosen for the field study.

7.2.2. Field studies

In Pakistan the amended formulation at 1.5 kg a.e. ha^{-1} was significantly better than unamended "Roundup" at the same rate as measured by weed control rating, fresh and dry weights of *C. rotundus* and maize yield. Lower application rates did not give satisfactory weed control. In Malaysia, although amending "Roundup" did not affect penetration in the laboratory nor phytotoxicity in pot experiments, in the field a mixture containing palm oil, ammonium sulfate and surfactant caused significantly more chlorosis to *C. rotundus* than the unamended formulation. Conversely, in Thailand an oil–ammonium sulfate–detergent mixture increased phytotoxicity in pot experiments but not in the field. In all other countries the amended formulation was not statistically significantly better than unamended "Roundup".

This outcome is rather disappointing but does not mean that there is no hope of improving glyphosate performance on this weed. An important consideration is that most participants reported large variability in the field data so that apparently large differences were not significant at the 5% level of probability. This indicates that the variability in *C. rotundus* populations is high so that the 5 m × 5 m plots used in this work were too small. The management of larger plots does, however, pose problems. A related issue is that assessing *Cyperus* in more than 3 quadrates per plot would have reduced variability.

There are other possible factors that could contribute to the outcome that were missed because not enough variables could be included in the field programme. In particular herbicide was applied at only one growth stage, about 3 weeks after seed bed irrigation; earlier or later dates of application may have been more effective. Similarly an application volume rate of 200 L ha^{-1} was suggested in the protocol but a different volume may have given different results. This is supported by the report from Malaysia that 100 L ha^{-1} was more effective than 400 L.

These possibilities are, in principle, easy to investigate and some participants plan to do this. As far as this programme was concerned this was not done systematically simply because the workload on participants would have been overwhelming.

8. CONCLUSIONS

1. A large number of combinations of oil, surfactant and ammonium sulfate added to the commercial "Roundup' formulation substantially increased the penetration of ^{14}C labelled glyphosate into the leaf of *C. rotundus*.

2. Except perhaps in Pakistan, it is not possible to recommend adjuvants that will reliably improve the performance of the "Roundup" formulation of glyphosate against *C. rotundus* under field conditions.

3. A number of experimental variables remain to be explored that could lead to the identification of effective additive combinations which were not included in this programme for logistic reasons.

9. OUTPUTS

1. Participants learned procedures for studying in the laboratory the uptake and translocation by plants of radiolabelled xenobiotic chemicals.

2. Procedures for the field evaluation of herbicides were introduced or refined in the participating institutions.

3. In many countries the idea of amending commercial pesticide formulations had not previously been considered. Further work on these lines is planned by all participants. In Malaysia, the Philippines, Thailand and possibly India, there will be commercial support.

4. Several participants reported interdisciplinary interactions were stimulated.

5. The programme was used as the basis for a PhD thesis in India.

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THE USE OF ISOTOPES FOR THE OPTIMISATION OF AGROCHEMICAL FORMULATIONS

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Abstract

This introductory paper outlines the general principles of the approach to designing pesticide spray formulations with particular reference to glyphosate and ethylene oxide surfactants.

1. INTRODUCTION

Agrochemicals that have been labelled with a radio isotope provide an invaluable tool for investigating the fate of the compound when it has reached the plant, either by diffusion to the roots or by impaction of the spray solution on the leaf surface. The two most important plant–chemical interactions studied with isotopes are:

1. Uptake and translocation of the chemical into the plant.
2. Metabolism of the chemical within the plant, on the leaf surface, or within the soil.

This report is concerned with uptake and translocation, but metabolic studies are an essential aspect of translocation studies if only to confirm that the isotope, that is observed translocating in the plant, is still part of the unchanged compound.

2. THE ROLE OF SURFACTANTS IN FORMULATIONS

At the first Research Co-ordination Meeting of this Co-ordinated Research Programme it was decided to use the herbicide active ingredient glyphosate and the three closely related surfactants Synperonic A2, Synperonic A7 and Synperonic A20. All three surfactants are condensation products of synperonyl alcohol with ethylene oxide (E0). The labels 2, 7 and 20 refer to the *mean* number of moles of ethylene oxide to each synperonyl alcohol. The range of ethylene oxides is relatively wide, for example Synperonic A2 contains free synperonyl alcohol, and Synperonic A7 may contain molecular species containing anything from 4 to 12 EO, but most will be in the 6 to 8 range. The exact range of surfactant species also depends on the manufacturing process, and interestingly some of the reputedly more effective brands of surfactant are manufactured by methods that give especially wide ranges of ethylene oxide numbers.

Research using Synperonic A2, A7 and A20, carried out prior to the first meeting of this group was concerned their effect on the uptake of the fungicide ethirimol and the insecticide permethrin. Table 1 compares the uptake of ethirimol and permethrin into orange leaves.

The important points demonstrated in Table 1 are first that the two compounds show different levels of uptake, with permethrin always penetrating better than ethirimol, and the second point is that different surfactants give maximum uptake for the two compounds, with Synperonic A2 best for permethrin and A20 best for ethirimol. It is tempting to conclude that uptake of lipophilic compounds (permethrin) are optimised by lipophilic surfactants (Synperonic A2), but such general conclusions are not justified by the small database.

TABLE 1. THE UPTAKE OF PERMETHRIN AND ETHIRIMOL INTO ORANGE LEAVES

Surfactant	Percentage uptake	
	Permethrin	Ethirimol
Arquad 2C	66	6
Synperonic A2	80	18
Synperonic A7	68	22
Synperonic A20	46	28
Aerosol OT	27	3
Synperonic NP8	35	4

Uptake was for 24 hours.
Arquad 2C is a cationic surfactant, Aerosol OT is an anionic surfactant and Synperonic NP8 is a nonylphenol-ethylene oxide surfactant.

When the same experiments were carried out on the weed morning glory (*Ipomoea* spp.) the mean uptake of ethirimol was increased two fold compared with orange, while uptake of permethrin was halved (Table 2).

TABLE 2. THE UPTAKE OF PERMETHRIN AND ETHIRIMOL INTO *IPOMOEA spp.*

Surfactant	Percentage uptake	
	Permethrin	Ethirimol
Arquad 2C	49	32
Synperonic A2	34	48
Synperonic A7	41	51
Synperonic A20	24	53
Aerosol OT	24	44
Synperonic NP8	11	31

Uptake was for 24 hours.

The optimum Synperonic A surfactant for permethrin in this case was A7 and for ethirimol it was A20 but all three surfactants gave better uptake of ethirimol compared with permethrin, with A20 giving more than a two fold difference. Aerosol OT and Synperonic NP8 also gave much better uptake of ethirimol than permethrin but Arquad gave better uptake of permethrin than ethirimol, and gave marginally better uptake of permethrin than Synperonic A7.

Changing the concentration of the surfactant also has an impact both on the rate of uptake and on the type of surfactant giving optimum uptake (Table 3).

Surfactant concentrations in agrochemical formulations are usually given in mass terms e.g. 0.1% (w/v or w/w), but when trying to understand the processes influencing uptake (and translocation) it may be more realistic the think in molar terms. The mean molecular weights of different surfactants differ widely, for instance the molecular weight of Synperonic A7 is more than double that of A2, and A20 is more than double A7, which means that if the surfactant is interacting in a specific manner with the active ingredient or with a plant component, small molecules, such as Synperonic A2 have an advantage.

TABLE 3. THE UPTAKE OF ETHIRIMOL INTO ORANGE LEAVES AT TWO CONCENTRATIONS OF SURFACTANT

Surfactant	Percentage uptake	
	0.1%	1.0%
Arquad 2C	6	16
Synperonic A2	18	34
Synperonic A7	22	61
Synperonic A20	28	32
Aerosol OT	3	2
Synperonic NP8	4	14

Uptake was for 24 hours.

The diffusion of solutes into the plant tissue is inversely proportional to the molecular radius, so small surfactants penetrate more rapidly than large ones. This means that surfactants with a small molecular weight/size, such as Synperonic A2 are more likely to have an effect within the plant, for instance causing phytotoxicity by damaging membranes. The importance of penetration of surfactants into the plant tissues is not well understood and while penetration is undoubtedly *assisted by* surfactants that have penetrated the cuticle, there is evidence that damage to membranes by surfactants may inhibit the phloem translocation of some systemic herbicides. Penetration of surfactants into the plant will also reduce the beneficial effects of surfactants on the leaf surface, in particular their ability to retain moisture within the spray deposit. The hydrophilic part of a surfactant may retain sufficient water to enable uptake to continue for several hours or even days, resulting in much increased total uptake. The ideal situation may be to have a surfactant mixture that contains components that will penetrate into the cuticle to improve its permeability characteristics without damaging membranes, while other components remain on the surface to give prolonged uptake.

Where the hydrophilic part is very large, as in A20, then larger volumes of water may be retained by the surfactant in the spray deposit, which will reduce the drying process, leading to delays in uptake of the active ingredient. Water is held more strongly by ionic surfactants but the *quantity* of water may be greater with high ethylene oxide surfactants, especially under humid conditions.

The solubilities of ionic surfactants increase at higher temperatures but the solubilities of non-ionic ethylene oxide surfactants are reduced by increasing temperatures, and they may even come out of dilute solution at higher temperatures, a characteristic measured as the cloud point.

Pesticides that have very low water solubility may have to formulated as suspensions of solid particles or as emulsified solutions in an organic solvent. Solid particles cannot penetrate the cuticle so the uptake of such active ingredients requires that the particles dissolve in the medium. Much of this occurs when the formulation is diluted, especially where the particles in the formulation are very small in size, but when the deposits land on the leaf and start drying the dissolved active ingredient becomes more concentrated and may precipitate. Surfactants play an important role in maintaining higher concentrations in spray deposits, because many compounds are much more soluble in surfactant–water solutions than they are in water, for instance, ethirimol has a solubility in water of 162 mg/L but this can increase to about 1000 mg/L in a 5% surfactant solution. Transient concentrations of surfactant of 5% or more occur when spray drops dry, with final equilibrium concentrations as high as 50%.

The ability of surfactants to retain water within the spray deposit is especially important in the case of particulate formulations because the presence of the mobile (liquid water) phase enables the particles to continue to dissolve and uptake to continue for a longer period of time. This process is often complicated by the presence of other formulation components, such as dispersing agents, that are included to assist the concentrated formulation to remain in an easily dispensable form.

The roles of surfactants in oil in water emulsions are quite different. As a general rule surfactants are only used in formulations of active ingredients dissolved in oil to enable the concentrate to be diluted with water. ULV formulations, usually of insecticides are applied without water and do not require surfactants. Where larger volumes need to be applied, as in the case of herbicides, the oil solution needs to be bulked up (diluted) with water, and the only way to ensure that the oil is evenly distributed throughout the aqueous phase is to emulsify it. Emulsification of oil in water involves covering small drops of the oil with a surfactant layer that enables the drop to bind to water. Usually two surfactants will be used, an ionic, usually anionic, surfactant, to give good water binding that is still effective at high temperatures, and a non-ionic surfactant to help the oil to associate with the ionic surfactant. The choice of surfactants will depend on the active ingredient and more especially on the solvent.

Emulsifiable concentrate (EC) formulations are recognised as giving good uptake of the active ingredient, for instance, 2,4-D ester EC was used for foliar application while solutions of the 2,4-D salt were used for soil application, but in recent years, the difference in effectiveness between EC and solution formulations has decreased because better surfactant selections have resulted in improved uptake from solution formulations. There is also evidence that the good uptake from EC formulations may be largely caused by the interactions between the plant tissue, the solvent, and the emulsifiers.

Table 4 shows the effect of adding a solvent, cyclohexanone, to the Synperonic A surfactants. In all these experiments, no attempt was made to produce stable emulsions, even the Synperonic A2 alone separates from water when left to stand, so all the formulations were shaken and applied rapidly to the leaf so that all the components of the mixtures should be present in the correct proportions.

Even a stable emulsion changes its physical characteristics when left as a residue on the leaf surface because the loss of the water brings the emulsified particles or micelles into close contact. It is interesting to note that the highest uptake came from the combination of lipophilic surfactant (A2) and solvent, a mixture that can only form emulsions of water-in-solvent (invert emulsions) but which should be an effective solvent mixture for cuticle waxes, and may create aqueous layers within the cuticle.

There is no reason why surfactant mixtures selected to disperse oil in water should not disperse cutin components in water, or at least make the cuticle more permeable, even in the absence of the EC solvent. If this is the case, then surfactant mixtures that emulsify solvents may also emulsify cutin waxes. There may even be a case for selecting surfactant mixtures primarily for their ability to increase the permeability of the cuticle. It is interesting to note that the solvent mixture in Table 4 that gave the lowest uptake of ethirimol (1% A20 + 1% solvent) was physically the most stable, perhaps the solvent and surfactant cancelled each other. A number of emulsions have been tested, and found to give improved uptake but there is no way to confirm that this is the result of emulsification of the cuticle waxes or some other aspect of the uptake process, such as emulsification of the pesticide, reducing precipitation, or maintaining a supersaturated solution of the active ingredient.

TABLE 4. THE UPTAKE OF ETHIRIMOL INTO ORANGE LEAVES AT TWO CONCENTRATIONS OF SURFACTANT, WITH AND WITHOUT SOLVENT

Surfactant		Percentage	uptake	
	0.1%	Surfactant	concentration	1.0%
		+ Solvent		+ Solvent
Arquad 2C	6		16	
Synperonic A2	18	19	34	60
Synperonic A7	22	33	61	51
Synperonic A20	28	21	32	15
Aerosol OT	3		2	
Synperonic NP 8	4		14	

Uptake was for 24 hours.
Solvent 1% cyclohexanone.

3. GLYPHOSATE

From the formulation point of view, glyphosate has some physicochemical peculiarities that must be considered, in particular it is slow to penetrate and because it is phloem systemic, the formulation must be non phytotoxic. In addition, glyphosate is easily washed off leaves by rain, and uptake is highly variable between species or between growing conditions.

Glyphosate is formulated as a water soluble salt because the native compound is relatively insoluble in water despite its low molecular weight, and possessing phosphonate, carboxyl and amine groups. The reason for this is probably because it forms stable glyphosate-glyphosate complexes, possibly based on salt interactions between the amine group and the phosphonate or carboxyl groups. Many amine and alkali cation salts of glyphosate are soluble in water because the cations compete for the glyphosate–glyphosate binding sites. In addition it is possible that amines, especially long chain and branched amines also sterically hinder competition for the binding sites by other glyphosate molecules. This process my be assisted by alkyl-amine-ethoxylate surfactants which have proven to be so effective for glyphosate formulation.

Two types of surfactant have been spectacularly successful as glyphosate adjuvants, the silicon surfactants and the amine ethoxylates. The silicon surfactants were initially introduced for use with weeds, such as *Ulex* spp that were difficult to control with glyphosate. The idea behind these surfactants was that the very low surface tension they produced in the spray solution would allow the spray drops to flow directly into the open stomata, but there is some evidence that the actual process is more complex than this.

The alkyl-amine-ethoxylates combine the advantages of strong water retention of an ionic surfactant with the good adhesion of a cationic compound, with low phytotoxicity of the non-ionic surfactant, despite the amine residue. This type of herbicide is used in the Roundup formulation. Table 5 shows the effect of changing the alkyl chain length and ethylene oxide number of the surfactant.

TABLE 5. THE UPTAKE OF GLYPHOSATE, FORMULATED WITH ETHOMEEN SURFACTANTS INTO *IMPERATA CYLINDRICA*

Ethomeen	0.25%	0.1%
C2	71.5	57.7
C5	69.7	58.4
C15	55.5	35.5
T2	77.2	71.8
T5	73.6	62.4
T15	55.8	36.3
S2	74.2	70.6
S5	69.6	59.4
S15	53.8	41.0

Uptake was for 24 hours. C = coco, T = tallow, S = oleyl.
2, 5, 15 = mean ethylene oxide numbers.

The type of alkyl residue had relatively little effect on uptake, the ethylene oxide number being far more important. It is interesting to note that the C2, T2 and S2 surfactants would normally be used for water in oil emulsions, while the corresponding 15 ethylene oxide surfactants would be used for water in oil emulsions. Perhaps the 2 EO surfactants are creating or enlarging an hydrophilic pathway in the cuticle that facilitates glyphosate uptake.

4. CONCLUSIONS

Surfactants have a wide range of effects on the activity of herbicides and other pesticides. While their role as bulk, surface active chemicals, in reducing the surface tension of the spray solution is well established, their role in modifying the permeability of the cuticle, retaining fluid within the spray deposit on the leaf surface and interacting with solvents and the active ingredient within the leaf are not fully understood.

The use of isotope labelling, both of the active ingredient and with the surfactant, has helped our understanding of the overall uptake process, but the detailed analysis of the interactions within the cuticle are still poorly understood. The impact of species difference, developmental stage, growing conditions, and climate at time of application, have not been fully addressed. Far more also needs to be done on the interactions of formulation adjuvants with water relations in the tissue, and the ways in which mass flow in the walls and xylem, sink accumulation on walls and within cells, and phloem translocation, influence the diffusion of actives across the cuticle and their translocation throughout the plant.

STUDIES OF HERBICIDE PERFORMANCE IN GRASSES AND SEDGES

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Abstract

The effect of additives to a commercial formulation of glyphosate on the uptake of added ^{14}C labelled glyphosate by *Cyperus rotundus* (purple nutsedge) was studied in the laboratory. Both 1% ammonium sulfate and 1% diesel oil plus the surfactant Triton X-45 improved penetration 24 h after treatment but not after 1 or 2 h. The mixture with ammonium sulfate and Triton X-45 was tested for the control of *C. rotundus* in the field. It was not significantly better than the unamended formulation at 1.5 kg a.e. ha^{-1} but lower application rates (0.75 and 0.5 kg a.e. ha^{-1}) were at least as effective as mechanical control.

1. INTRODUCTION

The objective of this phase of the co-ordinated research programme was to evaluate the effects of adding oil and ammonium sulfate to a commercial formulation on the foliar penetration of glyphosate in *Cyperus rotundus* (purple nutsedge) 1, 2 and 24 hours after treatment and to select the most effective mixture for a field test of toxicity to this weed.

2. MATERIALS AND METHODS

2.1. Laboratory experiments

Three experiments were carried out from August to December 1995 based on the protocols developed at the second Research Co-ordination Meeting.

Tubers of *Cyperus rotundus* were collected from the CORPOICA (Corporación de Investigaciones Agropecuarias) Research Station "Nataima", located on the Tolima Valley, Colombia. Plants (one plant/pot) were grown in the glasshouse of the Agronomy Faculty, National University of Colombia, Bogota. The temperatures recorded in the glasshouse were 14°C (min.) and 38°C (max.). We selected uniformly developed plants at the 5–6 leaves stage, and the second youngest completely expanded leaf was treated with 8 µL of ^{14}C-glyphosate solutions (experiments 1 and 2) or 4 µL (experiment 3). In all cases, each plant was treated with 1.48 kBq (0.04 µCi, 88 000 dpm) of ^{14}C-glyphosate. Twenty four, 1 or 2 hours after treatment, the treated leaf was washed with 3 mL of the following two solutions:

1. Distilled water + surfactant Agrotin (0.5%).
2. Distilled water (9%) + Acetone (1%) + surfactant Agrotin (0.5%).

Radioactivity in the washes and ^{14}C-glyphosate solutions (to check the application rate) were counted in a Beckman Scintillation Counter. Ten (10) mL samples of a scintillation cocktail for aqueous solutions were used. Also, *C. rotundus* injury was recorded at 15 and 30 or 36 DAT using a visual score (Table 1). Ten (10) untreated *C. rotundus* plants per repetition in each experiment were used as controls.

TABLE 1. PHYTOTOXICITY VISUAL SCALE (J.C. Caseley, 1995, *pers. comm.*)

7 =	Untreated control.
6 =	Slight inhibition of growth, chlorosis or epinasty.
5 =	Clear inhibition of growth, marked chlorosis or epinasty
4 =	Stunted, extreme chlorosis and necrosis, and twisting steams.
3 =	Extensive stunting, necrosis, few leaves green and twisting steams.
2 =	Extensive stunting, some collapse, necrosis, very little green tissues (desiccated plants).
1 =	Moribund plants, but some green tissues. Buds may be alive.
0 =	Dead. No green tissues.

The amount of Triton X-45 needed to emulsify 1% oil in "Roundup" in the presence of ammonium sulfate (1%) was estimated. A concentration of 0.2% of Triton X-45 held this mixture in a stable emulsion for 5 minutes; 6% was needed to maintain an emulsion overnight.

2.2. The field experiment

"Roundup" + ammonium sulphate (0.5% w/v) + Triton X-45 (0.2% v/v) was the most economical and practical option to increase glyphosate penetration in *C. rotundus* in the greenhouse studies. Therefore, this formulation was tested for efficacy of control of *C. rotundus* under field conditions in 1996–1997. Three glyphosate rates were evaluated and compared with the unamended commercial glyphosate formulation "Roundup".

The field experiment was conducted at the International Center for Tropical Agriculture (CIAT) located in Palmira, Cauca Valley, Colombia. Average temperature is 24 °C and average annual rainfall is 1000 mm.

The following treatments were evaluated in cropped (rice) and uncropped plots :

1. Untreated.
2. Hand weeded control (three times: 15, 31 and 61 days after rice sown).
3. "Roundup" at 1.5 kg a.e. ha^{-1} unamended.
4. "Roundup" at 1.5 kg a.e. ha^{-1} amended with 0.5% w/v ammonium sulfate and 0.2% v/v Triton X-45.
5. "Roundup" at 0.75 kg a.e. ha^{-1} amended.
6. "Roundup" at 0.5 kg a.e. ha^{-1} amended.

The glyphosate treatments were applied at 9 a.m. to one set of plots and at 9 p.m. to another set.

The seed bed was prepared by ploughing and harrowing on October 2 1996 and immediately flood irrigated. Cropped plots were 3.5 × 4 m and uncropped plots were 3.5 × 2.5 m. One day before spraying glyphosate, numbers of *C. rotundus* shoots were counted on four 0.25 × 0.50 m quadrates per plot. Glyphosate treatments were applied on November 6 1996 using an AZ sprayer with Tee-jet-8002 nozzles at a volume rate of 300 L ha^{-1}. Three days after glyphosate application, rice (c.v. *Orizical*) was sown in cropped plots. Irrigation was maintained according to local practice. Nitrogen (urea 46%) was applied broadcast at a total rate of 110 kg N ha^{-1}. This dose was divided in three applications (November 22, December 19 1996 and January 12 1997).

C. rotundus control was assessed in cropped plots 30, 60 and 90 days after treatment (DAT) using a 0–100 visual scale (0 = no control; 100 = total control). Also, density (tillers m^2) was recorded 0, 30, 60 and 90 DAT and dry weight at 30, 60 and 90 DAT in cropped plots, in four 0.25 × 0.50 quadrats per plot. Samples (quadrats) in each plot were selected randomly at each date of evaluation. In uncropped plots, *C. rotundus* was harvested in four 0.25 × 0.5 m quadrats from one half plot 30 DAT and from the other half 60 DAT. Two weeks after harvest number of shoots of the *C. rotundus* regrowth was recorded. Rice harvest yield was not taken because an iron deficiency severely affected the crop.

3. RESULTS AND DISCUSSION

3.1. Laboratory experiments

3.1.1. Twenty-four hour ^{14}C-glyphosate penetration

In the first experiment, absorption of ^{14}C-glyphosate from mixtures of "Roundup" with additives at the concentrations set out in Table 2 ranged from 58% to 45% 24 hours after application, and 28% without additives. The mixture of diesel oil + ammonium sulfate did not increase glyphosate uptake compared with ammonium sulfate or diesel oil alone, so there is no advantage for this mixture. Diesel oil and ammonium sulfate improved glyphosate penetration equally. In general, there were slight differences among treatments in phytotoxicity to *C. rotundus*. Phytotoxicity scores ranged from 4.5 to 5.0 15 days after treatment (DAT) and phytotoxicity increased to scores of between 3.0 to 4.0 at 36 DAT. The greatest phytotoxicity was obtained with ammonium sulfate + Triton X-45.

In the second experiment, ammonium sulfate and diesel oil both in mixture with Triton X-45 were tested at different rates (Table 3). Glyphosate uptake with ammonium sulphate treatments ranged 37% to 42% and with diesel oil treatments 36% to 38.5%. Thus, at least a 10% increase in glyphosate penetration was obtained with ammonium sulphate or diesel oil as additives compared with glyphosate + Triton X-45. *C. rotundus* phytotoxicity scores 15 DAT ranged from 4.0 to 4.5. At 30 DAT scores were from 3.0 to 4.0. Considering both glyphosate uptake and *C. rotundus* phytotoxicity, there were no differences among ammonium sulfate rates but with diesel oil treatments the best result was reached at 2.0%. Thus, for the third experiment ammonium sulfate at 0.5% and diesel oil at 2.0% were selected.

3.1.2. One and two hour ^{14}C-glyphosate penetration

There were no differences in either ^{14}C-glyphosate uptake (20% to 22.5%) nor purple nutsedge phytotoxicity (4.0 to 5.5, 15 DAT) among ammonium sulfate and diesel oil treatments, and among 1 and 2 HAT. Apparently, both additives do not improve glyphosate uptake during the two first hours after treatment, as compared with glyphosate alone (Table 4).

As a general conclusion, both ammonium sulfate or diesel oil in mixture with Triton X-45 improve glyphosate uptake and *C. rotundus* injury. Ammonium sulfate could be used at 0.5% and diesel oil at 1.0% or 2.0%. The use of ammonium sulphate under field conditions has the advantage that the mixture is easier to prepare.

TABLE 2. ^{14}C-GLYPHOSATE UPTAKE IN *CYPERUS ROTUNDUS* L. FROM MIXTURES OF "ROUNDUP" WITH ADDITIVES 24 HOURS AFTER TREATMENT (HAT), AND PHYTOTOXICITY 15 AND 36 DAYS AFTER TREATMENT (DAT)

No. Treatment solutions	Uptake (%) 24 HAT x ± s.d.	Phytotoxicity 15 DAT	Phytotoxicity 36 DAT
A. Glyphosate (^{14}C-G + Roundup)	27.8 ± 8.6	4.8	4.2
B. Glyphosate +Ammonium Sulfate (1.0% w/v)	53.5 ± 7.7	5.1	4.1
C. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v)	45.2 ± 7.2	4.9	3.6
D. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Amm. Sulf. (1% w/v)	58.3 ± 9.9	4.4	3.3
E. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Diesel oil (1.0% v/v)	57.3 ± 9.4	5.0	4.0
F. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Amm. Sulf. (1% w/v) + Diesel oil (1.0% v/v)	44.9 ± 3.4	4.9	3.7

Data are means of four repetitions and 5 plants per repetition.
Phytotoxicity was evaluated using a visual scale (Table 1).
Uptake (%) = dpm in ^{14}C-solutions – dpm in washes /dpm ^{14}C-solutions * 100.

TABLE 3. ^{14}C-GLYPHOSATE UPTAKE IN *CYPERUS ROTUNDUS* L. IN MIXTURE WITH AMMONIUM SULFATE AND DIESEL OIL AT DIFFERENTS RATES, 24 HOURS AFTER TREATMENT (HAT), AND PHYTOTOXICITY 15 AND 30 DAYS AFTER TREATMENT (DAT)

No. Treatments solutions	Uptake (%) 24 HAT x ± s.d.	Phytotoxicity 15 DAT	Phytotoxicity 30 DAT
A. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v)	27.9 ± 3.3	4.4	3.9
B. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Amm. Sulf. (0.5% w/v)	38.7 ± 12.5	4.2	3.3
C. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Amm. Sulf. (1.0% w/v)	41.7 ± 8.7	4.4	3.0
D. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Amm. Sulf. (2.0% w/v)	36.9 ± 13.2	4.5	3.3
E. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Diesel oil (0.5% v/v)	35.7 ± 12.1	4.1	4.0
F. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Diesel oil (1.0% v/v)	36.5 ± 11.4	4.5	3.8
G. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Diesel oil (2.0% v/v)	38.5 ± 11.8	4.3	3.3

Data are means of four repetitions and 5 plants per repetition.
Phytotoxicity was evaluated using a visual scale (Table 1).
Uptake (%) = dpm in ^{14}C-solutions – dpm in washes / dpm ^{14}C-solutions * 100.

TABLE 4. EXPERIMENT NO. 3. ^{14}C-GLYPHOSATE UPTAKE IN *CYPERUS ROTUNDUS* L. IN MIXTURE WITH AMMONIUM SULFATE AND DIESEL OIL 1 AND 2 HOURS AFTER TREATMENT (HAT), AND PHYTOTOXICITY 15 DAYS AFTER TREATMENT (DAT), 1995

No. Treatment solutions	Uptake (%) 1 HAT x ± s.d.	2 HAT x ± s.d.	Phytotoxicity 15 DAT 1 HAT	2 HAT
A. Glyphosate (^{14}C-G + Roundup)	12.2 ± 6.3	21.7 ± 6.2	5.0	5.0
B. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Amm. Sulf. (0.5% w/v)	20.1 ± 7.1	22.5 ± 5.1	5.0	5.5
C. Glyphosate (^{14}C-G + Roundup) + Triton X-45 (0.2% v/v) + Diesel oil (2.0% v/v)	21.9 ± 5.8	22.0 ± 3.2	4.0	5.0

Data are means of four repetitions and 5 plants per repetition.
Phytotoxicity was evaluated using a visual scale (Table 1).
Uptake (%) = DPM in ^{14}C-solutions – DPM in washes /^{14}C-solutions * 100.

3.2. Field experiment

3.2.1. Tiller density

The density of *C. rotundus* was homogeneous in the experimental area before the glyphosate was applied. This was verified when significant differences on the density were not detected among the plots (Table 5).

TABLE 5. *CYPERUS ROTUNDUS* DENSITY (SHOOTS m^{-2}) IN CROPPED PLOTS

Treatment	Days after treatment				% of increasing (+) or reduction(–) of the density[2]
	0	30	60	90	
1. Untreated	143.4 a[1]	313.5 a	275.2 a	294.6 a	+105.0
2. Mechanical control	122.6 a	147.0 bcd	172.5 b	180.5 b	+47.0
Glyphosate application at 9 a.m.					
3. Glyphosate 1.5 kg a.e. ha^{-1} unamended	148.0 a	131.5 bcd	91.5 b	116.0 cd	-22.0
4. Glyphosate 1.5 kg a.e. ha^{-1} amended	128.0 a	108.5 cd	97.0 b	129.0 bcd	+0.8
5. Glyphosate 0.75 kg a.e. ha^{-1} amended	117.4 a	233.0 bc	75.5 b	108.0 cd	-8.0
6. Glyphosate 0.5 kg a.e. ha^{-1} amended	132.0 a	189.5 b	118.0 b	143.0 bc	+8.0
Glyphosate application at 4 p.m.					
7. Glyphosate 1.5 kg a.e. ha^{-1} unamended	136.0 a	132.0 bcd	97.0 b	81.0 d	–40.0
8. Glyphosate 1.5 kg a.e. ha^{-1} amended	140.0 a	92.0 d	95.5 b	97.0 cd	-31.0
9. Glyphosate 0.75 kg a.e. ha^{-1} amended	150.6 a	154.5 bc	119.0 b	146.0 bc	-3.0
10. Glyphosate 0.5 kg a.e. ha^{-1} amended	131.4 a	135.5 bcd	156.0 b	152.5 bc	+16.0

[1]Means in the columns followed by different letters are significantly different ($p < 0.05$).
[2]Percent of increasing (+) or reduction (–) of the density 90 DAT as comparing with the density 0 DAT.

Thirty and sixty days after the glyphosate application, the density of *C. rotundus* was significantly higher in the untreated plots than those treated with glyphosate or hand weeded. The statistical differences between the different glyphosate treatments were not significant except at 30 DAT when 1.5 kg ha^{-1} in either amended or unamended formulations was superior to lower rates.

At 90 DAT, the results were similar to those at earlier dates. A significantly greater *C. rotundus* shoot density was recorded in the untreated plots, compared to those treated. Also all 1.5 kg ha^{-1} glyphosate treatments, regardless of formulation, produced significantly lower densities than the hand weeded control. The density of *C. rotundus* increased two fold in the untreated plots during the time the experiment lasted; it increased from 143 to 295 shoots m^{-2} on the course of 90 days. In the hand weeded control plots the purple nutsedge shoot density also increased 47% in this time. Meanwhile the general tendency with the use of glyphosate was to reduce the purple nutsedge population or at least keep them stable during the period of the experiment. The glyphosate treatments at the high rate (1.5 kg ha^{-1}) produced the greatest decrease of the *C. rotundus* population, e.g. 30% to 40%, when the herbicide was applied at 4 p.m.

The amended formulation of glyphosate at 1.5 kg ha^{-1} controlled *C. rotundus* somewhat better than the others at the beginning of the experiment (30 DAT), particularly when applied during the afternoon. However, the effect was not statistically significant and disappeared by 90 DAT.

3.2.2. Visual assessment (percent of control)

The hand weeded treatment only produced a 40% to 50% control, while the glyphosate treatments gave over 60%. The best purple nutsedge control (80%) was recorded 30 DAT with a dosage of 1.5 kg ha^{-1} using the amended glyphosate formulation applied at 4 p.m. (Table 6).

3.2.3. Dry weight

The general tendency of the response of this variable to the treatments (Table 7) was similar to that of shoot density (Table 5). A significantly greater dry weight was obtained in the untreated plots than hand weeded and glyphosate treated plots (Table 7). This response was specially clear 30 DAT but not so after 60 or 90 DAT).

3.2.4. Density of C. rotundus *regrowth*

Purple nutsedge regrowth 15 d after cutting 31 DAT, was lowest in glyphosate treatments at rates of 1.5 and 0.75 kg ha^{-1} applied at 4 p.m. (treatments 7, 8 and 9) and with the 1.5 kg ha^{-1} rate of the glyphosate amended formulation applied at 9 a.m. (Table 8).

In general, the density of regrowth after cutting 61 DAT was no different in the untreated plots than those treated with the herbicide (ranged from 44 to 64 shoots m^{-2}). But the number of shoots in hand weeded plots was about double this (114 shoots m^{-2}).

TABLE 6. *CYPERUS ROTUNDUS* VISUAL ASSESSMENT (% OF CONTROL) IN CROPPED PLOTS (MEAN OF 4 BLOCKS)

Treatment	Days after treatment		
	30	90	90
1. Untreated	0	0	0
2. Mechanical control	52.5	40.0	40.0
Glyphosate application at 9 a.m.			
3. Glyphosate 1.5 kg a.e. ha^{-1} unamended	76.2	72.5	67.5
4. Glyphosate 1.5 kg a.e. ha^{-1} amended	56.2	70.0	67.5
5. Glyphosate 0.75 kg a.e. ha^{-1} amended	76.2	66.2	73.7
6. Glyphosate 0.5 kg a.e. ha^{-1} amended	65.0	62.5	65.0
Glyphosate application at 4 p.m.			
7. Glyphosate 1.5 kg a.e. ha^{-1} unamended	77.5	76.2	76.2
8. Glyphosate 1.5 kg a.e. ha^{-1} amended	81.2	73.7	71.2
9. Glyphosate 0.75 kg a.e. ha^{-1} amended	75.0	78.7	83.7
10. Glyphosate 0.5 kg a.e. ha^{-1} amended	78.7	65.0	62.5

TABLE 7. *CYPERUS ROTUNDUS* DRY WEIGHT (gm^{-2}) IN CROPPED PLOTS

Treatment	Days after treatment		
	30	90	90
1. Untreated	0.96 a	1.5 ab	0.9 ab
2. Mechanical control	0.32 b	1.9 a	1.3 a
Glyphosate application at 9 a.m.			
3. Glyphosate 1.5 kg a.e. ha^{-1} unamended	0.40 b	1.5 ab	1.2 ab
4. Glyphosate 1.5 kg a.e. ha^{-1} amended	0.32 b	1.6 ab	1.0 ab
5. Glyphosate 0.75 kg a.e, ha^{-1} amended	0.48 b	1.5 ab	0.9 ab
6. Glyphosate 0.5 kg a.e. ha^{-1} amended	0.48 b	1.0 b	1.0 ab
Glyphosate application at 4 p.m.			
7. Glyphosate 1.5 kg a.e. ha^{-1} unamended	0.40 b	1.5 ab	1.0 ab
8. Glyphosate 1.5 kg a.e. ha^{-1} amended	0.32 b	1.4 ab	0.8 b
9. Glyphosate 0.75 kg a.e. ha^{-1} amended	0.48 b	1.4 ab	1.4 a
10. Glyphosate 0.5 kg a.e. ha^{-1} amended	0.32 b	1.4 ab	1.1 ab

Means in the columns followed by different letters are significantly different ($p < 0.05$).

TABLE 8. *CYPERUS ROTUNDUS* DENSITY (SHOOTS m^{-2}) AS A PERCENTAGE OF THE REGROWTH IN UNCROPPED PLOTS

Treatment	Cutting: 31 DAT[1] Assessment: 15 DAC[2]	Cutting: 61 DAT[1] Assessment: 15 DAC[2]
1. Untreated	17.6	51.0
2. Mechanical control	14.0	114.0
Glyphosate application at 9 a.m.		
3. Glyphosate 1.5 kg a.e. ha^{-1} unamended	18.6	64.0
4. Glyphosate 1.5 kg a.e. ha^{-1} amended	8.0	53.0
5. Glyphosate 0.75 kg a.e. ha^{-1} amended	12.0	54.0
6. Glyphosate 0.5 kg a.e. ha^{-1} amended	14.0	45.0
Glyphosate application at 4 p.m.		
7. Glyphosate 1.5 kg a.e. ha^{-1} unamended	10.6	50.0
8. Glyphosate 1.5 kg a.e. ha^{-1} amended	10.0	51.0
9. Glyphosate 0.75 kg a.e. ha^{-1} amended	8.0	50.0
10. Glyphosate 0.5 kg a.e. ha^{-1} amended	14.6	44.0

[1]DAT: days after treatment.
[2]DAC: days after cutting.

As a general conclusion, amended formulations did not control *C. rotundus* significantly better than glyphosate at the rate of 1.5 kg a.e. ha^{-1} in the unamended formulation applied at the afternoon.

Hand weeding was not effective in controlling purple nutsedge. It lead to an increase in shoot and percent control was poor (40% to 50%). Mechanical control caused breakage of the tuber chains with a consequent loss of apical dominance so that large numbers of tubers germinate.

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INFLUENCE OF PALM OIL ON THE EFFICACY OF GLYPHOSATE IN THE CONTROL OF *CYPERUS ROTUNDUS* L.

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Abstract

The influence of the addition of palm oil to the formulation on the efficacy of glyphosate for the control of *Cyperus rotundus* was evaluated in the laboratory, glass-house and field. Triton X-100 failed to maintain a stable emulsion of palm oil in the formulation 10 minutes after mixing. In glass-house experiments adding mineral oil and palm oil to the glyphosate spray mixture did not increase the herbicidal efficacy. In general, glyphosate was more effective when sprayed at the volume application rate of 100 L/ha than at 400 L/ha. In contrast to the glass-house studies, in the field trial the addition of palm oil increased the efficacy of glyphosate.

1. INTRODUCTION

Cyperus rotundus (nutgrass) has been classified as one of the most noxious weeds in the world. Control of nutgrass by hand weeding or hoeing is not economical for large scale eradication while contact herbicide only provides temporary control. The systemic herbicide, glyphosate, has been proved to give effective control of *C. rotundus*. However, a high rate (4 kg a.i./ha) was needed for significant reduction of tuber numbers. This could because the foliar absorption of glyphosate was only 25–50% of the amount applied. Being water soluble, glyphosate does not readily penetrate the leaf Adding surfactant could enhance penetration of systemic herbicide and increases efficacy [1]. Non-ionic surfactants are the most commonly used with herbicides. The rates and total amount of herbicide uptake depends on the surfactant ethylene oxide (E0) content, concentration and hydrophobic-lipophilic balance, herbicide concentration and plant species [2]. Sometimes oil is added to the spray formulation to reduce evaporation and increase spreading and also to enhance penetration of herbicide on leaf surface [3]. This study evaluates the influence of adding palm oil on the effectiveness of glyphosate for the control of C. *rotundus.*

2. MATERIALS AND METHODS

2.1. Laboratory experiments

2.1.1. Miscibility of palm oil in the glyphosate spray formulation

An experiment was conducted in the laboratory by adding an emulsifier (Triton X-100) to a mixture of the commercial formulation "Roundup" (46% glyphosate), palm oil and ammonium sulfate. For each treatment, a formulation of 25 mL "Roundup" (diluted 1: 12 with water), 1 mL palm oil and 20 ml 50 g/L ammonium sulfate, and 54 mL water were mixed in a 100 mL graduated measuring cylinder. Triton X-100 at rates of 0.01, 0.05, 0.1, 0.2 and 0.5 mL were added accordingly into each measuring cylinder with the formulation. The mixtures were mixed thoroughly using a Polytron mixer for 1 minute. They were then let to stand, and the separation line of oil and water was recorded after 5, 10, 15 and 60 minutes with reference to the graduation on the cylinder.

2.1.2. Penetration studies of glyphosate into C. rotundus

The underground tubers of *C. rotundus* L. collected from Ladang 10B, Universiti Pertanian Malaysia, were sorted and trimmed of roots, rhizomes and shoots. The undamaged tubers within ±20% of the mean weight (37.5 g) were then pre-germinated for 3 days in layers of wet paper. Four tubers were planted 1-2 cm deep in each plastic pot (diameter 16 cm & height 13 cm) containing soil of 55% clay, 34% silt and 11% sand. Watering was done daily and compound fertilizer (15:15:15) was applied 4 weeks after planting. Six plants were used per treatment and each experiment was repeated on 2 different days. The treatments were as in Table 1.

TABLE 1. FORMULATIONS TESTED

Code	Description
T1	2.5 µL ^{14}C-glyphosate + 25 µL Roundup + 72.5 µL water
T2	2.5 µL ^{14}C-glyphosate + 25 µL Roundup + 25 µL $(NH_4)_2SO_4$ + 47.5 µL water
T3	2.5 µL ^{14}C-glyphosate + 25 µL Roundup + 5 µL Triton X-100 + 67.5 µL water
T4	2.5 µL ^{14}C-glyphosate + 25 µL Roundup + 25 µL $(NH_4)_2SO_4$ + 5 µL Triton X-100 + 42.5 µL water
T5	2.5 µL ^{14}C-glyphosate + 25 µL Roundup + 1 µL palm oil + 5 µL Triton X-100 + 66.5 µL water
T6	2.5 µL ^{14}C-glyphosate + 25 µLRoundup + 25 µL $(NH_4)_2SO_4$ + 5 µL Triton X-100 + µL palm oil + 41.5 µL water

Eight 0.5 drops were applied on either side of the midrib in a 2 cm area starting 10 cm from the tip. Four µL aliquots were transferred to 2 scintillation vials for checking the application rate. After 24 h, the treated area was painted with cellulose acetate solution (6%) in 9:1 acetone/water. When it had dried the acetate was removed with tweezers and dissolved in 2 mL glacial acetic acid, 8 mL scintillation fluid was added for counting. The procedure was repeated with untreated leaves to check for quenching.

2.2. Pot plant experiments

2.2.1. Effect of adding palm oil and mineral oil on the efficacy of glyphosate for the control of C. rotundus

The plants were prepared as described in the previous section and were sprayed 2 weeks later, when the test plants were at 5–6 leaves stage. The chemicals were "Roundup" and technical glyphosate MON 20058 (glyphosate isopropylamine 54% or an equivalent of 40% a.e.) applied at 1.5 kg a.e./ha. The adjuvants were palm oil ("Buruh") and mineral oil ("Orchex"). The sprayer used was a conventional manual knapsack sprayer ("CP Prima") operating at 100 kPa and 200 L/ha using a fan nozzle (05-F110, Lurmark Ltd., U.K.) and spraying 50 cm above the target. The Volume Median Diameter/Number Median Diameter (VMD/NMD) ratio was 3.0, with 216 µm and 72 µm for VMD and NMD respectively. The summary of the treatments is given in Table 2.

Effect was assessed visually and by measurement of chlorophyll content. The visual assessment of chlorosis used a scale of 0 to 100 (0 = no chlorosis and 100 = complete chlorosis). The chlorophyll content of the leaf was determined by using the Nose Method [4]. A piece of leaf with known surface area, was soaked in a vial containing 10 mL of 95% ethanol. The vial was then kept in the dark for three days until the colour of the leaf faded. Optical density was determined at 649 nm and 665 nm.

TABLE 2. SPRAY FORMULATIONS

Treatment	Formulation
T1	Control
T2	Glyphosate 1.5 kg a.e./ha
T3	Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 40g/L
T4	Glyphosate 1.5 kg a.e./ha + Triton X-100 0.5mL/L
T5	Roundup 1.5 kg a.e./ha
T6	Glyphosate + $(NH_4)_2SO_4$ 40g/L + Triton X-100 0.5mL/L
T7	Glyphosate + Triton X-100 0.5mL/L + Palm oil 5g/L
T8	Glyphosate + Triton X-100 0.5mL/L + Palm oil 2.5g/L
T9	Glyphosate + $(NH_4)_2SO_4$ 40g/L + Triton X-100 0.5mL/L + Palm oil 5g/L
T10	Glyphosate + $(NH_4)_2SO_4$ 40g/L + Triton X-100 0.5mL/L + Palm oil 2.5g/L
T11	Glyphosate + Triton X-100 0.5mL/L + Mineral oil 5mL/L
T12	Glyphosate + Triton X-100 0.5mL/L + Mineral oil 2.5mL/L
T13	Glyphosate + $(NH_4)_2SO_4$ 40g/L + Triton X-100 0.5mL/L + Mineral oil 5mL/L
T14	Glyphosate + $(NH_4)_2SO_4$ 40g/L + Triton X-100 0.5mL/L + Mineral oil 2.5mL/L

The experimental design was a Completely Randomised Design (CRD) with 10 replications, where 5 of them were for visual assessment and regrowth observation, and the remainder were for the test on chlorophyll content. Three pots were used per replicate. The data collected were subjected to the analysis of variance (ANOVA) and Duncan's New Multiple Range Test (DMRT).

2.2.2 . Effect adding Synperonic A 7 on the efficacy of spray mixture of palm oil and glyphosate

A similar procedure as described above was used in this study. The Synperonic A7 (ICI plc., UK) was supplied by IAEA, Vienna, Austria. The summary of the treatments is given in Table 3. Data collected were subjected to the ANOVA and DMRT.

2.2.3. Effect of spray volume application rates on the efficacy of a mixture of palm oil and glyphosate

A similar procedure as described above was used in this study. The nozzles used were LP 03-F110, LP 05-F110 and LP 08-F110 (Lurmark) to give spray volume application rates of 100 L/ha, 200 L/ha and 400 L/ha respectively, The summary of the treatments is given in Table 4. The experimental design was CRD with 6 replications. Six pots were used per replicate. Data collected were subjected to the ANOVA and DMRT.

2.3. Field experiment

A field plot experiment was conducted using a randomized complete block design with 4 replicates. An open field of C. *rotundus* was cut, and the field was divided into plots of 1 × 2. metres. The sedge was allowed to re-grow for a period of 3 weeks (8 to 10 leaf stage) before treatments were applied. The treatments were as in Table 5. Visual assessment of leaf chlorosis, chlorophyll content and viability of tubers, wet weight and dry weight of the plant were taken accordingly.

TABLE 3. TREATMENT FORMULATIONS

Treatment	Formulation
T1	Control
T2	Roundup
T3	Glyphosate
T4	Glyphosate + Triton X-100 0.5g/L + Synperonic A7
T5	Glyphosate + Triton X-100 0.5g/L + Palm oil 2.5g/L
T6	Glyphosate + Triton X-100 0.5g/L + Synperonic A7 + Palm oil 2.5g/L
T7	Glyphosate + Triton X-100 0.5g/L + $(NH_4)_2SO_4$ 40g/L + Synperonic A7
T8	Glyphosate + Triton X-100 0.5g/L + $(NH_4)_2SO_4$ 40g/L + Palm oil 2.5g/L
T9	Glyphosate + Triton X-1000.5g/L + $(NH_4)_2SO_4$ 40g/L + Synperonic A7 + Palm oil 2.5g/L

TABLE 4. SPRAY VOLUMES AND FORMULATIONS

Treatment	Spray volume (L/ha)	Spray formulation
T1	100	Glyphosate 1.5 kg a.e./ha + Palm oil 2.5g/L + Triton X-100 0.5g/L
T2	100	Glyphosate 1.5 kg a.e./ha
T3	100	Roundup 1.5 kg a.e./ha
T4	100	Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 40g/L
T5	200	Glyphosate 1.5 kg a.e./ha + Palm oil 2.5g/L + Triton X-100 0.5g/L
T6	200	Glyphosate 1.5 kg a.e/.ha
T7	200	Roundup 1.5 kg a.e./ha
T8	200	Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 40g/L
T9	400	Glyphosate 1.5 kg a.e./ha + Palm oil 2.5g/L + Triton X-100 0.5g/L
T10	400	Glyphosate 1.5 kg a.e./ha
T11	400	Roundup 1.5 kg a.e./ha
T12	400	Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 40g/L
T13	400	Control - no spray

TABLE 5. FIELD TREATMENTS

Treatment	Formulation
T1	1.5 kg/ha a.e. glyphosate
T2	1.5 kg/ha a.e. glyphosate + ammonium sulfate
T3	1.5 kg/ha a.e. glyphosate + palm oil
T4	1.5 kg/ha a.e. glyphosate + ammonium sulfate + Triton X-100
T5	1.5 kg/ha a.e. glyphosate + palm oil + Triton X-100
T6	1.5 kg/ha a.e. glyphosate + ammonium sulfate + palm oil + Triton X-100
T7	Control

Tubers taken from the treated plants at 2 weeks after the treatments were subjected to standard tetrazolium chloride test to determine the percentage of living cells. Each tuber was cut into two and submerged in 10g/L tetrazolium chloride solution. They were placed in the oven at 35°C for one hour. Part of the tissue stained by tetrazolium (red in colour) was used to estimate percentage of living cell.

RESULTS AND DISCUSSION

3.1. Laboratory experiments

3.1.1. Miscibility of palm oil in the glyphosate. spray formulation

The results (Table 6) showed that Triton X-100 was not able to maintain the stability of the emulsion. The separation between oil and water began to show clearly after 10 minutes. After 15 minutes total separation was obtained at all rates of Triton X-100 added. Oils must be formulated with a surfactant if they are to give a stable sprayable emulsion with water [5] and Triton X-100 could not maintain the stability.

TABLE 6. EFFECT OF ADDING EMULSIFIER (TRITON X-100) TO GLYPHOSATE FORMULATION

Triton X-100	Degree of separation (volume of oil in mL)				
(mL)	5 min	10 min	15 min	30 min	60 min
0.01	0.6	1.0	1.0	1.0	1.0
0.05	0.4	0.8	0.8	0.9	0.9
0.10	0.5	0.8	1.0	1.0	1.0
0.20	0.4	1.0	1.0	1.0	1.0
0.50	0.3	0.7	1.0	1.0	1.0
Control	1.0	1.0	1.0	1.0	1.0

3.1.2. Penetration studies of glyphosate into C. rotundus

The percentage of radio activity recovered from the cellulose acetate was less than 1% for all treatments so there was no significant difference between the treatments ($p > 0.05$). Further trials are required to verify this result.

3.2. Pot plant experiments

3.2.1. Effect adding palm oil and mineral oil on the efficacy of glyphosate for the control of C. rotundus

The chlorophyll contents at 5 and 7 days for all treatments were significantly different from the control (Table 7). Treatment T3 (Glyphosate + $(NH4)_2SO_4$ + Triton X-100), T8 (Glyphosate + Triton X-100 + Palm oil 2.5 mL/L) and T1O (Glyphosate + $(NH4)_2SO_4$ + Triton X-100 + Palm oil 2.5 in L/L) reduced the chlorophyll content more than the other treatments but the difference was not statistically significant. No significant difference was observed between the palm oil concentrations of 5 and 2.5 mL/L on the performance of glyphosate. Treatments containing mineral oil (T11, T12, T13 and T14) tended to show the lowest percentage leaf chlorosis, significantly in some cases. Thus adding mineral or palm oil to glyphosate did not increase efficacy.

TABLE 7. EFFECT OF ADDING PALM OIL AND MINERAL OIL TO THE EFFICACY OF GLYPHOSATE ON *C. ROTUNDUS*

Treatment	Chlorophyll content* (mg/cm^2)		Percentage of leaf chlorosis by visual assessment*		Wet weight* (g)
	Day 5	Day 7	Day 7	Day 15	Day 15
T1	4.1 ab	4.2 a	1 f	5 f	70.5a
T2	3.5 abc	2.2 b	55 a	77 abc	56.9abc
T3	3.1 bc	0.6 c	49 abc	78 ab	49.2 bc
T4	2.8 bc	0.6 c	52 ab	73 abcd	42.5 c
T5	3.2 bc	0.7 bc	51 abc	81 a	51.6 bc
T6	2.9 bc	0.4 c	46 abcd	72 abcd	45.8 bc
T7	3.8 abc	1.1 bc	41 cde	68 cde	57.7 abc
T8	2.5 bc	0.6 bc	46 abcd	72 abcd	41.6 c
T9	3.3 abc	1.1 bc	42 bcde	72 abcd	55.8 abc
T10	2.8 bc	0.8 bc	47 abcd	70 bcde	59.8 ab
T11	3.2 bc	1.9 bc	43 bcde	66 de	48.1 bc
T12	4.9 a	1.3 bc	34 e	66 de	50.5 bc
T13	2.2 c	1.4 bc	34 e	67 de	47.0 bc
T14	3.2 bc	0.6 bc	38 de	62 e	52.2 bc

*Mean values with the same letter within the column do not differ at the 5% level of probability (DMRT)

TABLE 8. EFFECT OF ADDING PALM OIL AND SYNPERONIC A7 ON THE EFFICACY OF GLYPHOSATE

Treatment	Chlorophyll content* (mg/cm^2)	Percentage leaf chlorosis by visual assessment*		Wet weight* (g)
	Day 7	Day 7	Day 15	Day 15
T1	11.9 a	0 c	5 f	50.8 a
T2	3.1 c	25 b	77 b	32.9 bc
T3	4.9 b	18 b	68 bcd	33.5 b
T4	5.1 b	20 b	69 bcd	31.4 bc
T5	4.5 b	21 b	66 cd	27.5 bcd
T6	4.6 b	29 b	76 bc	33.5 b
T7	3.4 c	25 b	59 d	27.1 cd
T8	3.2 c	50 a	87 a	23.1 d
T9	1.9 d	25 b	61 d	32.9 bc

*Mean values with the same letter within the column are not significantly different at $p \leq 0\ 05$ (DMRT)

3.2.2. Effect of adding Synperonic A7 on the efficacy of a spray mixture of palm oil and glyphosate

Adding Synperonic A7 to the spray mixture of glyphosate + Triton X-100 + $(NH4)_2SO_4$ + Palm oil 2.5 mL/L (T9) significantly reduced the chlorophyll content at 7 days following treatment (Table 8). However, the treatment did not give the highest percentage leaf chlorosis at 15 days following application. The treatment T8 (glyphosate + Triton X-100 -+ $(NH4)_2SO_4$ + Palm oil 2.5 mL/L) gave the highest percentage of leaf chlorosis. Synperonic A7 might have increased the uptake and translocation of glyphosate but did not produce the best result. Treatment T8 also gave the lowest wet weight of C. *rotundus*.

3.2.3. Affect of spray volume application rates on the efficacy of a spray mixture of palm oil and glyphosate

All treatments significantly reduced chlorophyll content compared to control (T1 - no spray) (Table 9). In general, adding palm oil {T1, T5 and T9 (glyphosate + Palm oil + Triton X-100) at 100 L/ha, 200 L/ha and 400 L/ha respectively } reduced the chlorophyll content faster and to a greater extent than the treatment with glyphosate alone. However, treatment T9 failed to give similar leaf chlorosis as T1 and T5. The volume application rate affected the efficacy of glyphosate (Table 9). In general lower volume application rates performed better than the high volume (400 L/ha). Treatment T1 (glyphosate + Palm oil + Triton X-100 at 100 L/ha) gave significantly lower chlorophyll content and higher chlorosis compare to T9 (glyphosate + Palm oil + Triton X-100 at 400 L/ha). A similar result was obtained with "Round-up" alone. Jordon [6] showed that the presence of surfactant at a volume rate of less than 187 L/ha enhanced phytotoxicity, but not at a volume rate of 374 L/ha.

TABLE 9. EFFECT OF SPRAY VOLUMES AND FORMULATIONS ON THE EFFICACY OF GLYPHOSATE

Treatment	Chlorophyll content* (mg/cm^2)	Percentage leaf chlorosis by visual assessment*		Wet weight (g)
	Day 7	Day 7	Day 15	Day 15
T1	9.9 d	59 a	97 a	9.4 ed
T2	7.2 d	43 c	76 c	7.7 ed
T3	26.0 bcd	52 ab	89 b	10.0 cde
T4	25.1 bcd	43 c	70 cd	11.3 bcd
T5	11.4 cd	54 a	75 c	8.3 de
T6	32.4 b	43 c	60 e	11.0 bcde
T7	31.5 bc	46 bc	64 de	9.4 cde
T8	19.1 bcd	40 dc	57 e	10.5 bcde
T9	18.8 bcd	39 dc	61 e	7 6 e
T10	22.8 bcd	40 dc	58 e	9.6 cde
T11	13.9 bcd	39 dc	61 e	13.7 b
T12	25.4 bcd	35 d	55 e	12.7 bc
T13	60.4 a	1 e	5 f	21.9 a

*Mean values with the same letter within the column are not significantly different at $p \leq 0.05$ (DMRT).

3.3. Field experiments

By visual assessment (Table 10) chlorosis increased from day 3 to day 10 for all treatments. Although there was variation in chlorosis in the early assessment period, by the 10th day leaf chlorosis was consistently high, attaining more than 82% in all the treatments.

The addition of Triton X-100 to various glyphosate formulations seems to influence the effectiveness. Significant increase in leaf chlorosis was obtained (95%) compared to those without Triton X-100 with only 82 to 85% chlorosis.

The addition of palm oil seems to increase the effectiveness of glyphosate, significantly causing chlorosis to the leaves of the C. *rotundus*. However, the addition of Triton X-100 to these formulations caused no further increase in the chlorosis.

Chlorophyll content of the treated plants also showed variations on the 3rd day. However, the results seem to stabilize on day 6 and further on to day 10 (Table 10). Evaluation of the chlorophyll content showed that there was no significant difference between all treatments regardless of whether it was just glyphosate or glyphosate plus surfactant and/or emulsifier. Therefore, the high percentage of chlorosis through visual assessment did not always correspond with the result of the chlorophyll analysis. All treatments produce significant results when compared to the untreated control.

TABLE 10. VISUAL ASSESSMENT AND CHLOROPHYLL CONTENT OF THE EFFECT OF GLYPHOSATE FORMULATIONS ON *C. ROTUNDUS*

Treatment	Percentage leaf chlorosis*			Chlorophyll content* (mg/g)		
	Day 3	Day 6	Day 10	Day 3	Day 6	Day 10
T1	17.5 cd	40.0 c	85.0 c	60.7 b	10.5 b	12.3 b
T2	17.5 cd	42.5 cd	82.5 c	43.4 bc	13.2 b	11.4 b
T3	25.0 bc	55.0 abc	97.5 a	37.2 bcd	10.2 b	11.2 b
T4	32.5 ab	62.5 ab	95.0 ab	28.4 cd	12.1 b	11.6 b
T5	37.5 a	70.0 a	95.0 ab	14.2 d	13.9 b	11.5 b
T6	30.0 ab	62.5 ab	95.0 ab	22.9 cd	7.8 b	11.9 b
Control	10.0 d	10.0 d	10.0 d	84.8 a	60.4 a	21.3 a

*Mean values with the same letter within the column are not significantly different at $p \leq 0.05$ (DMRT).

The percentage of living cells in treated samples from all treatments were low, ranging between 15 to 48%, giving a significant difference when compared to the untreated control (Table 11). This indicated the translocation of the glyphosate within the plants reduced the ability of the treated tubers to regenerate.

The dry and wet weights of *C. rotundus* from the treatments were significantly different from the control. However, no significant difference was observed between treatments.

TABLE 11. PERCENTAGE OF LIVING CELL IN *C. ROTUNDUS* TUBER AFTER TREATMENT WITH GLYPHOSATE

Treatment	Percentage of living cells[1]	Wet Weight (g)[1,2]	Dry weight (g)[1,2]
T1	35.0 c	1.60 b	0.80 b
T2	27.5 cd	1.37 b	0.79 b
T3	20.0 cd	1.55 b	0.89 b
T4	15.0 e	1.45 b	0.84 b
T5	47.5 b	1.26 b	0.74 b
T6	37.5 c	1.38 b	0.75 b
Control	100.0 a	4.09 a	1.33 a

[1]Mean values with the same letter within the column are not significantly different at $p \leq 0.05$ (DMRT).
[2]Dry weight and wet weight of 10 plants collected at random.

4. CONCLUSIONS

Triton X-100 could not emulsify the palm oil and glyphosate spray mixture for more than 10 minutes. Adding the mineral oil and palm oil to the glyphosate spray mixture did not increase the efficacy of glyphosate in the potted plants studies. However, the field trial showed adding palm oil increased the efficacy of glyphosate.

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STUDIES ON THE PERFORMANCE OF GLYPHOSATE IN THE CONTROL OF GRAMINACEOUS WEEDS IN RICE FIELDS

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Abstract

Absorption and translocation of ^{14}C-labelled glyphosate was studied from the commercial formulation 'Roundup' to which three different surfactants, singly or in combination, four different oil adjuvants and ammonium sulfate were added. Increased penetration of the herbicide was observed after 1, 2 and 24 h by the addition of Triton X-100, ammonium sulfate and coconut oil. Addition of Neem oil instead of coconut oil also showed increased penetration after 24 h. Addition of Tween 80 and diesel oil increased the penetration with or without ammonium sulfate; whereas, Tween 20 with the added peanut oil or coconut oil and Tween 80 with Neem oil increased penetration only in the presence of ammonium sulfate. Therefore, the absorption of glyphosate seems to be dependent on the nature of surfactant and the oil (adjuvant) used. Field tests were carried out to study the effect of hand weeding, one dose of unamended and three different doses of amended (0.5, 0.75 and 1.5 kg a.i. ha^{-1}) 'Roundup' on weed control. The herbicide was applied at 9 am and 4 pm in cropped and uncropped plots. Visual rating after 15 days suggested better control in hand weeded plots. However, this may be due to the fact that the hand weeding was performed later than the herbicide application. Herbicide treatment with unamended Roundup resulted in significantly better weed control than the amended Roundup plots.

1. INTRODUCTION

Glyphosate is an important herbicide that can be used for the control of most perennial weeds. It is relatively non-selective and is essentially non phytotoxic as a soil application because it is tightly bound to most soils and is characteristically unavailable to plants. Studies on the absorption and translocation of foliar applied glyphosate in perennial weeds strongly suggest that it is readily absorbed by leaves and is quite mobile via the symplastic system [1–6]. It appears that the translocation of glyphosate follows the typical source–sink relationship with accumulation of active ingredient in roots (storage organs) and young growing leaves. The usefulness of surfactants for aiding in wetting, spreading and sticking of spray solutions to leaf surfaces has been noted since the turn of the century [7].

Absorption and translocation of herbicides may be increased by the presence of adjuvants, which include surfactants, wetting agents, penetrants and oils, in the spray solution [8–10]. Adjuvants enhance biological activity of herbicides by improving deposition and retention of the spray and penetration of the active ingredient [11].

Plant species differ in response to adjuvants with glyphosate [12] and the interaction of the leaf surface with surfactants is important for the absorption of the herbicide [13]. Spray carrier salts have been shown to affect herbicide toxicity [8, 14]. Recently, it has been shown that two adjuvants, Atplus 201 and Ethomeen T/25, increased ^{14}C glyphosate uptake by protoplasts isolated from quack grass (Elytrigia repense) [15]. The addition of the surfactant, monoxynol (*o*-(*p*-nonylphenyl)-ω -hydroxypolyoxyethelene] 9 to 10 POE to the treatment solution frequently increased the translocation of radiolabelled ^{14}C-glyphosate applied to the leaves of cotton, *Gossypium hirsutum* [8].

It is known from several studies that surfactants enhance phytotoxicity [16–21]. Herbicide retention varies with species, herbicide formulation, and surfactant or oil adjuvant in the spray carrier [22]. Environmental factors like light, temperature and Relative Humidity also play a major role in the retention and subsequent translocation of herbicides. [5, 23, 24] or soil moisture [25, 26]. The most common symptoms of glyphosate injury reported for a number of plant species are foliar chlorosis followed by necrosis [4, 27, 28]. These foliar symptoms are often seen on regrowth following glyphosate treatment rather than on the sprayed foliage. In perennial grasses, inhibition of rhizome or stolon growth has been reported following glyphosate application [2, 25, 28].

However, our knowledge on the performance of oil adjuvants with different surfactants in the presence and absence of $(NH_4)_2$ SO_4 on the penetration of glyphosate in purple nutsedge (*Cyperus rotundus* L.) is scanty. Therefore the objectives of the investigation were to 1) optimise the mixtures of oils, surfactants and $(NH_4)_2$ SO_4 in the formulation in terms of penetration into purple nutsedge plants in short term laboratory experiments and 2) to identify and test the best formulation for herbicide performance in the field.

2. MATERIALS AND METHODS

2.1. Laboratory experiments

Laboratory experiments were carried out from July 1995 to 1996 July where additional surfactants like Triton X-100, Tween-80 and Tween-20 with oil adjuvants such as coconut oil, peanut oil, neem oil and diesel oil with ammonium sulphate were added with ^{14}C glyphosate (specific activity of 7.4 kBq/0.02 mL) to "Roundup". The mixtures contained the following:

2.5 mL of ^{14}C glyphosate
25 mL of "Roundup" (diluted in the ratio of 1:12 to give 0.03 mg a.e./mL)
20 mL of ammonium sulfate (5%)
x mL of surfactants (Triton X-100, Tween 80 and Tween-20)
y mL of oil (Coconut oil, Peanut oil, Neem oil and diesel oil)
z mL of water to give 100 ml of mixture.

The values of x and y were determined based on the amount of surfactant needed to emulsify 1% of oil. The following are the amounts of surfactants needed for emulsification of different oils:

Triton X-100:	coconut oil – 1.0 mL; peanut oil – 0.4 ml; Neem oil – 0.2 mL; diesel oil – 0.1 mL
Tween–80:	coconut oil – 0.01 mL; peanut oil – 0.02 mL; Neem oil – 0.01 mL; diesel oil – 0.05 mL
Tween-20:	coconut oil – 0.2 mL; peanut oil – 0.2 mL; Neem oil – 0.05 ml; diesel oil – 0.01 mL.

The tubers were germinated and the plants were grown in plastic pots as in the protocol. Three plants of uniform size were selected for the experiments. Six plants were used for each treatment which was repeated on four different days. The herbicide application was carried out at 9 a.m. on each day of application. The second mature leaf from the apex was used for the treatment. The different treatments consisted of:

(A) "Roundup" alone: 2.5 µL ^{14}C - glyphosate solution + 25 µL "Roundup" (diluted 1:12) + 72.5 µL water (i.e. 0.185 KBq) labelled glyphosate/ µL
(B) "Roundup" + ammonium sulfate: As in A 25 µL of water was replaced by 25 µL 5% $(NH_4)_2$ SO_4
(C) Roundup + surfactant

(D) Roundup + surfactant + $(NH_4)_2SO_4$
(E) Roundup + surfactant + oil
(F) Roundup + surfactant + oil + $(NH_4)_2SO_4$.

Eight 0.5 μL droplets were applied on either side of the midrib in a 2 cm area starting 10 cm from the tip without touching the leaf. At the same time, 4-μL aliquots were transferred to scintillation vials to check the application rate. After 24 hours, the treated area was painted with 6% cellulose acetate (prepared in 9:1 acetone/water). The dried cellulose acetate film was removed with tweezers and dissolved in 2 ml of glacial acetic acid in a scintillation vial and 10 ml of scintillation fluid (Beckman Ready solvent, TM Multipurpose) was added and counted with Beckman Liquid Scintillation System (Model 1701).

The results of the laboratory experiments indicated that "Roundup" amended with Triton X-100, coconut oil and $(NH_4)_2SO_4$, or with Tween-20, coconut oil and $(NH_4)_2SO_4$ produced greater penetration than other formulations. Therefore, short term penetration studies were carried out with the above three formulations and the penetration was measured for a period of 1 hour and 2 hours. This experiment was carried out with the objective to identify a mixture which might improve rain fastness.

2.2. Field experiments

Field experiments were carried out with the over all objectives to reduce dose rate and to optimise time of application, volume rate and drop size. The laboratory experiments indicated that glyphosate formulated with Triton X-100 surfactant, coconut oil and $(NH_4)_2SO_4$ showed the greatest absorption into the nutsedge plant. Therefore, the field experiments were designed to find out the optimum dose and optimum time of application of this formulation ("Roundup" amended as above) and the effect of unamended "Roundup" applied at the rate of 1.5 kg a.e. ha^{-1} as applied either in the morning or the evening.

2.2.1. Cultivation details

2.2.1.1. Field preparation

The experimental field was ploughed twice with a tractor-drawn cultivator and it was levelled. The plots were irrigated and kept for 15 days to allow the *Cyperus* tubers to emerge and grow.

Healthy and well developed seeds of maize variety MHH-69 having a good germination percentage were sown in 5 × 5 m plots. The spacing was 50 cm inter row and 15 cm intra-row. Sowing was delayed from 3 day to 10 days after herbicide spraying due to rains. The rate of fertilizer application was 120 kg N, 60 kg P_2O_5 and 40 k K_2O ha^{-1}. One half dose of nitrogen and an entire dose of phosphorus and potassium were applied at the time of sowing 5 cm below and 5 cm away from the seed row.

2.2.1.2 . Herbicide application

"Roundup" was diluted with distilled water in the ratio of 1:12 and 5% ammonium sulfate in distilled water was prepared. The remaining components of the formulation. (Roundup diluted, 5% Ammonium sulphate, Triton X-100 and coconut oil were diluted in water so as to make up the quantity of spray solution to 600 L ha^{-1}. At the 5–6 leaf stage of *Cyperus rotundus*, herbicide both cropped and uncropped plots. A foot sprayer was used with a flat fan noozle at a pressure of 172 kNm^{-2}.

2.2.2. Assessments

Two days before sowing, weed counts were taken in all the plots inside a 0.25 m^2 quadrat. After application of the herbicide the number of weeds that developed symptoms were recorded at five-day intervals inside three 0.25m^2 quadrats of each plot.

Fresh and dry weights of *Cyperus* shoots were taken 4 and 8 weeks after application together with the yield of maize cobs at harvest.

3. RESULTS

3.1. Laboratory experiments

3.1.1. *Effect of surfactants and oil adjuvants on the penetration of ^{14}C glyphosate.*

The quantity of each of the 3 surfactants, Triton X-100, Tween 80 and Tween-20 needed to emulsify one percent coconut oil, peanut oil, diesel oil and Neem oil was affected by the presence of ammonium sulfate.

3.1.1.1. 24 h Studies

With ^{14}C-glyphosate + "Roundup" alone after 24 hours there was 76.7% absorption of ^{14}C. The mixture containing labelled glyphosate plus Triton X-100, coconut oil and $(NH_4)_2SO_4$ enhanced ^{14}C absorption to 99.7%. When coconut oil was replaced with Neem oil in the herbicide mixture absorption was similar at 99.5 percent (Table 1).

TABLE 1. EFFECT OF TRITON X-100 AND OIL ADJUVANTS ON THE PERCENTAGE ABSORPTION OF ^{14}C GLYPHOSATE (24 HOURS AFTER TREATMENT)

Treatments		Mean	DMRT
T_1	^{14}C glyphosate + Roundup	76 7	gh*
T_2	T_1 + $(NH_4)_2SO_4$	70 6	gh
T_3	T_1 + Triton X-100	84 7	de
T_4	T_3 + $(NH_4)_2SO_4$	77 0	fg
T_5	T_1 + Triton X-100 + Coconut Oil	98 1	b
T_6	T_5 + $(NH_4)_2SO_4$	100 0	a
T_7	T_1 + Triton X-100 + Diesel Oil	87 6	d
T_8	T_7 + $(NH_4)_2SO_4$	94 5	c
T_9	T_1 + Triton X-100 + Peanut Oil	77 9	fg
T_{10}	T_9 + $(NH_4)_2SO_4$	92 9	c
T_{11}	T_1 + Triton X-100 + Neem Oil	81 3	def
T_{12}	T_{11} + $(NH_4)_2SO_4$	100 0	c

*Means followed by different letters are significantly different ($p < 0.05$)

Tween-80 with diesel oil as an adjuvant in the absence of ammonium sulfate resulted in 96.2% absorption, whereas with ammonium sulfate there was only 66.2% absorption (Table 2).

TABLE 2. EFFECT OF TWEEN-80 AND OIL ADJUVANTS ON THE PERCENTAGE ABSORPTION OF ^{14}C GLYPHOSATE (24 HOURS AFTER TREATMENT)

Treatments	Mean	DMRT
T_1 : ^{14}C glyphosate + Roundup	74.7	cd*
T_2 : T_1 + $(NH_4)_2SO_4$	70.6	defg
T_3 : T_1 + Tween 80	73.6	def
T_4 : T_3 + $(NH_4)_2SO_4$	86.2	b
T_5 : T_1 + Tween 80 + Coconut Oil	77.5	c
T_6 : T_5 + $(NH_4)_2SO_4$	95.6	a
T_7 : T_1 + Tween 80 + diesel Oil	96.2	a
T_8 : T_7 + $(NH_4)_2SO_4$	66.2	fg
T_9 : T_1 + Tween 80 + Peanut Oil	63.7	gh
T_{10} : T_9 + $(NH_4)_2SO_4$	88.5	b
T_{11} : T_1 + Tween 80 + Neem Oil	74.0	cde
T_{12} : T_{11} + $(NH_4)_2SO_4$	85.4	b

*Means followed by different letters are significantly different ($p < 0.05$).

TABLE 3. EFFECT OF TWEEN-20 AND OIL ADJUVANTS ON THE PERCENTAGE ABSORPTION OF ^{14}C GLYPHOSATE (24 HOURS AFTER TREATMENT)

Treatments	Mean	DMRT
T_1 : ^{14}C glyphosate + Roundup	74.7	de*
T_2 : T_1 + $(NH_4)_2SO_4$	70.6	ef
T_3 : T_1 + Tween-20	84.6	b
T_4 : T_3 + $(NH_4)_2SO_4$	81.0	bc
T_5 : T_1 + Tween-20 + Coconut Oil	69.1	efg
T_6 : T_5 + $(NH_4)_2SO_4$	68.3	fg
T_7 : T_1 + Tween 20 + Diesel Oil	57.1	I
T_8 : T_7 + $(NH_4)_2SO_4$	63.3	gh
T_9 : T_1 + Tween 20 + Peanut Oil	73.6	ef
T_{10} : T_9 + $(NH_4)_2SO_4$	96.9	a
T_{11} : T_1 + Tween 20 + Neem Oil	79.3	cd
T_{12} : T_{11} + $(NH_4)_2SO_4$	56.2	I

*Means followed by different letters are significantly different ($p < 0.05$).

The ^{14}C glyphosate mixture containıng the surfactants, Tween-20, peanut oil and ammonıum sulfate caused a higher percentage of penetration of herbicide (96.9%) when compared to other treatments (<85%) (Table 3).

3.1.1.2. Short term penetration studies

Short term penetratıon studies for 1 and 2 hours indicated that Triton X-100 with coconut oil and ammonium sulfate in the herbicide mixture caused 69.1% penetration in 1 hour and 98.5% in 2 hours. A higher level of absorption of ^{14}C glyphosate was also observed in the herbıcide mıxture without $(NH_4)_2SO_4$ (90.4%) (Table 4).

TABLE 4 PERCENTAGE PENETRATION OF ^{14}C-GLYPHOSATE IN THE PRESENCE OF TRITON X-100, COCONUT OIL AND $(NH_4)_2SO_4$ OVER 1 AND 2 HOURS

No	Treatments	1 hour	2 hour
I	^{14}C-glyphosate + Roundup	32 5 (± 1 23)	43 5 (± 2 38)
II	^{14}C-glyphosate + Roundup + $(NH_4)_2SO_4$	38 5 (± 1 63)	51 2 (± 1 10)
III	^{14}C-glyphosate + Roundup + Tnton X-100	66 1 (± 1 16)	85 1 (± 0 78)
IV	^{14}C-glyphosate + Roundup + Tnton X-100 + $(NH_4)_2SO_4$	60 8 (± 0 55)	84 3 (± 1 75)
V	^{14}C-glyphosate + Roundup + Tnton X-100 + Coconut oıl	67 3 (± 0 90)	90 4 (± 1 05)
VI	^{14}C-glyphosate + Roundup + Tnton X-100 + Coconut oıl + $(NH_4)_2SO_4$	69 1 (± 0 99)	98 5 (± 0 61)

Fıgures ın parenthesıs are standard errors

Tween-80 with Neem oil in the presence of ammonium sulfate caused highest percentage penetration both at 1 hour and 2 hours after application (55.7 and 66.7 respectively) (Table 5).

TABLE 5. PERCENTAGE PENETRATION OF ^{14}C-GLYPHOSATE IN THE PRESENCE OF TWEEN-80, NEEM OIL AND $(NH_4)_2SO_4$ OVER 1 AND 2 HOURS

No	Treatments	1 hour	2 hour
I	^{14}C-glyphosate + Roundup	32 5 (± 1 23)	43 5 (± 2 38)
II	^{14}C-glyphosate + Roundup + $(NH_4)_2SO_4$	38 5 (± 1 63)	51 2 (± 1 10)
III	^{14}C-glyphosate + Roundup + Tween 80	38 3 (± 1 11)	42 9 (± 3 81)
IV	^{14}C-glyphosate + Roundup + Tween 80 + $(NH_4)_2SO_4$	40 6 (± 1 18)	61 4 (± 1 52)
V	^{14}C-glyphosate + Roundup + Tween 80 + Neem oıl	44 7 (± 2 71)	55 7 (± 2 81)
VI	^{14}C-glyphosate + Roundup + Tween 80 + Neem oıl + $(NH_4)_2SO_4$	55 7 (± 2 97)	67 7 (± 7 23)

Fıgures ın parenthesıs represents standard error (±)

Tween-20 with coconut oil and ammonium sulfate resulted in highest percentage penetration of ^{14}C glyphosate both after 1 hour and 2 hours of application (Table 6).

TABLE 6. PERCENTAGE PENETRATION OF ^{14}C-GLYPHOSATE IN THE PRESENCE OF TWEEN-20, OIL AND COCONUT $(NH_4)_2SO_4$ OVER 1 AND 2 HOURS

No.	Treatments	1 hour	2 hour
I.	^{14}C-glyphosate + Roundup	32.5 (± 1.23)	43.5 (± 2.38)
II.	^{14}C-glyphosate + Roundup + $(NH_4)_2SO_4$	38.5 (± 1.63)	51.2 (± 1.10)
III.	^{14}C-glyphosate + Roundup + Tween 20	50.4 (± 1.70)	72.1 (± 1.97)
IV.	^{14}C-glyphosate + Roundup + Tween 20 + $(NH_4)_2SO_4$	48.5 (± 1.71)	79.3 (± 2.21)
V.	^{14}C-glyphosate + Roundup + Tween 20 + Coconut Oil	52.4 (± 1.36)	82.3 (± 0.94)
VI.	^{14}C-glyphosate + Roundup + Tween 20 + Coconut Oil + $(NH_4)_2SO_4$	58.7 (± 1.64)	88.9 (± 1.30)

Figures in parenthesis represents standard error (±).

3.2. Field experiments

3.2.1. Weed control

Field experiments were carried out with three different dosages of amended Roundup (0.5, 0.75 and 1.5 kg a.e. ha^{-1}) one dosage of unamended Roundup at the rate of 1.5 kg a.e. ha^{-1} and one hand weeding treatment with two times of application i.e. before 9.00 a.m. and after 4 p.m. both in cropped and uncropped plots.

The visual control rating was carried out 0–100 scale (0 is no injury, 100% complete kill) (Table 7).

TABLE 7. AVERAGE OF PERCENTAGE WEED CONTROL AS AFFECTED BY DIFFERENT TREATMENTS AT 15 DAYS AFTER APPLICATION OF HE (0 -SCALE)*

Treatments	Main plots		Times of application		Herbicidal treatments (kg a.e. ha^{-1})				Hand weed
	Crop	No crop	9 am	4 pm	Amended			Round-up	
					0.5	0.75	1.5	1.5	
Mean	59.25	60	57.75	61.55	50	43.12	53.12	68.75	83.12
SE (d)	6.32						2.62		
CD%	20.12						5.29		

*Rating (% injury)	Description of main categories
0	No weed control
10	Very poor
20	Poor
30	Poor to deficient
40	Deficient
50	Deficient / Moderate
60	Moderate
70	Some what satisfactory
80	Satisfactory
90	Very good to excellent
100	Complete.

There was no significant difference in fresh weight of weeds recorded between treatments after 4 weeks of herbicide application except in unweeded control (Table 8). The results were in accordance with other findings [29–31]. They reported that one half of the recommended dose of "Roundup" had almost as much effect as the full dose, overall, the level of control with the full recommended dose was so good that there was little opportunity for ammonium sulfate to give further improvements. However, the dry weight of weeds was least with 1.5 kg a.e. ha^{-1} amended herbicide application in the evening hours and it was followed by unamended herbicide application at the rate of 1.5 kg a.e. ha^{-1} in the morning. All the herbicide treatments were similar, except for the evening application of amended "Roundup" at the rate of 1.5 kg a.e. ha^{-1}.

Though all the weed control treatments were equivalent as measured by fresh weight of weeds at 4 weeks after spraying, they differed significantly at 8 weeks after spraying (Table 8).

In general spraying the herbicide in the evening was superior to spraying in the morning. As measured by fresh weight weed control was effective with amended "Roundup" applied at the rate of 1.5 kg a.e. ha^{-1} in the evening but it was at par will all the other treatments except unweeded control and amended "Roundup" at the rate of 0.5 kg a.e. ha^{-1} applied in the morning.

TABLE 8. FRESH AND DRY WEIGHTS (g) OF SHOOTS HARVESTED FROM 3 M^2 UNCROPPED PLOTS AT 4 AND 8 WEEKS AFTER HERBICIDE APPLICATION

No	Treatments	After 4 weeks		After 8 weeks	
		Fresh weight	Dry weight	Fresh weight	Dry weight
1	Unweeded control	216.75 b	82.13 b	132.20 ab	78.0 a
	Amended Roundup				
2.	0.5 kg a.e. ha^{-1} (M)	149.0 a	72.56 b	146.20 a	67.5 ab
3.	0.5 kg a.e. ha^{-1} (E)	131.0 a	50.35 b	102.50 abc	55.0 ac
4.	0.75 kg a.e. ha^{-1} (M)	153.0 a	57.35 b	101.0 abc	66.2 abc
5.	0.75 kg a.e. ha^{-1} (E)	138.0 a	52.08 b	120.0 abc	55.5 bcd
6.	1.5 kg a.e. ha^{-1} (M)	148.0 a	52.96 b	92.5 abc	47.75 cd
7.	1.5 kg a.e. ha^{-1} (E)	102.0 a	35.13 a	84.75 bc	39.0 cd
	Unamended Roundup				
1.	1.5 kg a.e. ha^{-1} (M)	138.0 a	51.16 b	101.25 abc	64.2 abc
2.	1.5 kg a.e. ha^{-1} (E)	162.0 a	60.56 b	99.75 b	49.75 bcd

M – Morning. E – Evening.
Means followed by different letters are significantly different ($p < 0.05$).

Weed control as measured by dry weight of weeds was effective with 1.5 kg a.e. ha^{-1} of amended "Roundup" applied in the evening and it was equivalent to all the other treatments except 0.5 kg a.e. ha^{-1} amended "Roundup" applied in the morning and unweeded control.

3.2.2. Maize yield

Fresh weight of cobs (four) recorded was highest (377.5g) with unamended Roundup applied at the rate of 1.5 kg a.e. ha^{-1} and it was at par with all the other treatments except unweeded control and amended Roundup applied at the rate of 0.5 kg a.e. ha^{-1} which were significantly lower (Table 9).

TABLE 9. FRESH WEIGHT (g) OF COBS RECORDED AT HARVEST AS INFLUENCED BY THE TREATMENTS

No.	Treatment	Means
1.	Unweeded Control	191.25 c
	Amended Roundup	
2.	0.5 kg a.e. ha^{-1} (M)	203.75 c
3.	0.5 kg a.e. ha^{-1} (E)	248.75 bc
4.	0.75 kg a.e. ha^{-1} (M)	263.75 abc
5.	0.75 kg a.e. ha^{-1} (E)	273.75 abc
6.	1.5 kg a.e. ha^{-1} (M)	340.0 ab
7.	1.5 kg a.e. ha^{-1} (E)	367.50 a
	Unamended Roundup	
8.	1.5 kg a.e. ha^{-1} (M)	362.5 ab
9.	1.5 kg a.e. ha^{-1} (E)	377.5 a
10.	Hand weeding (once)	268.75 abc

M : Morning E : Evening.
Means followed by different: letters are significantly different ($p < 0.05$).

4. CONCLUSIONS

The laboratory experiments indicated that penetration of ^{14}C glyphosate varied with the type of surfactant, oil and $(NH_4)_2 SO_4$ in the "Roundup" formulation. The surfactant Triton X-100 and coconut oil in the presence of $(NH_4)_2 SO_4$ showed faster penetration than other formulations as indicated by short term experiments.

In the field experiments there was comparable weed control with all the treatments as measured by fresh and dry weight of weeds. On the basis of fresh weight of maize cobs the conclusion is similar although all 1.5 kg/ha "Roundup" treatments, amended and unamended were significantly better than the unweeded control and 0.5 kg amended "Roundup" applied in the morning.

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EFFECT OF SURFACTANTS ON THE PENETRATION OF ^{14}C-GLYPHOSATE IN *CYPERUS ROTUNDUS* IN PAKISTANI AGROCLIMATIC CONDITIONS

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Abstract

The penetration of ^{14}C-glyphosate was studied in *Cyperus rotundus* with three nonionic surfactants. Among the three surfactants Synperonic A20 was more effective than A2 and A7 in enhancing penetration of glyphosate 24 hours after treatment both in dry and wet seasons The addition of diesel oil to Synperonic A20 further increased penetration of glyphosate in both seasons.

1. INTRODUCTION

The cotton crop with its multifarious advantages can be rightly called the life line of Pakistan's economy. It accounts for 60 percent of the export earnings and 55 percent of the domestic edible oil production in the country. It also provides raw material to 1035 ginning factories, 262 textile mills and 13,000 oil expelling units, and job opportunities to millions of people.

Cotton production is badly affected by insects and weeds. With the increase in use of insecticides, the losses due to insects have been significantly controlled. However, losses due to perennial weeds like *Cyperus rotundus; Trianthema partulacastrum; Digera alternifolius* and *Ephorbia hirta* are still significant and may range from 15–55%. These weeds are difficult to control mechanically because they either have underground perennating organs or produce seeds which are viable for several years [1]. Herbicides are not always effective at least partly because spray droplets are not well retained and/or the penetration and translocation of the active ingredient is limited. Commercial herbicide formulations together with application specifications have usually been developed for a range of weeds and are thus not optimum for any one in particular. Also they are commonly designed for temperate and not tropical weeds. Thus improved performance on particular targets is possible by manipulating formulation and applications factors and this has been achieved for some grass weeds [2]. The present investigations are therefore, aimed to study the effect of three non-ionic surfactants on the penetration of ^{14}C-glyphosate in *Cyperus rotundus* L. in Pakistani climatic conditions.

2. MATERIALS AND METHODS

The herbicide used was ^{14}C-glyphosate and the test plant selected was *Cyperus rotundus.* The additives were Synperonic A2, A7 and A20 at 0.1% in the spray solution and each was used with $\pm$1% ammonium sulfate. The optional additives used were 1% diesel and 1% glycerol.

Cyperus rotundus tubers were pre-germinated in wet blotting paper and average weight was noted. The time period noted for the pre-germination of the sprout in the blotting paper was at room temperature. The germinated sprouts were transferred to the pots and the times noted for the emergence of the plant from the soil and reaching the 5–6 leaf stage. The temperature during this period was also noted. Four treatments each containing 7.2 kBq per 4 μL of labelled glyphosate along with other constituents were applied on the 2nd leaf of *C. rotundus* when the plant was at the 5–6 leaf stage. Eight 0.5 μL drops were applied on either side of the midrib in a 2 cm area starting 10 cm from the tip without touching the leaf. Aliquots (4 μL) were added to 2 scintillation vials to check the application rates. After 24 h, the treated area was painted with cellulose acetate in 9:1 acetone/water. After drying, the cellulose acetate film was removed with tweezers and dissolved in 2 ml glacial acetic acid and 5 – 10 ml of the scintillator permaflour was added for counting. The treated leaves and plants were harvested after 4 and 24 h and 5 d.

3. RESULTS AND DISCUSSION

The emergence of the plants took two days and the 5-6 leaf stage was reached after 15-21 days during summer and 75 days in winter. In the summer (August) minimum temperatures were 24–29°C and maxima 37–42 °C; corresponding winter (January–March) temperatures were 7–21°C and 13–32°C. Relative humidities in summer were 68–71% in the morning and 42–54% in the evening. Winter values were 62–88% and 43–58% respectively. The only rainfall in both periods consisted of light showers at night.

The amount of radioactivity in the cellulose acetate film, treated zone, remaining zone, 10 cm zone and remaining part of the plant harvested after 4, 24 hours and 5 days after five treatments are recorded in Tables 1 and 2.

TABLE 1. EFFECT OF SURFACTANTS ON THE PENETRATION OF GLYPHOSATE IN *C.ROTUNDUS* AFTER APPLICATION AT THE 5 TO 6 LEAF STAGE IN SUMMER

	Distribution of C-14 (% of applied)					
Sr. No. Formulation additive	Cellulose acetate	Treated zone	Remaining zone	10 cm zone	Remaining plant	Total uptake
	(After 4 hours)					
1. Synperonic A-2	83.6	1.5	2.9	1.4	3.3	9.1
2. Synperonic A-7	88.2	1.3	2.5	2.0	2.7	8.5
3. Synperonic A-20	54.2	9.4	10.1	2.5	13.2	35.2
4. Synperonic A20 + Diesel oil	40.6	15.9	11.1	7.0	12.7	46.7
5. 'Roundup alone	90.3	3.5	1.4	1.6	1.1	7.6
	(After 24 hours)					
1. Synperonic A-2	56.3	4.6	8.7	5.3	20.2	38.8
2. Synperonic A-7	48.4	3.3	4.4	5.8	26.6	40.1
3. Synperonic A-20	27.6	5.0	14.0	5.7	42.2	66.9
4. Synperonic A20 + Diesel oil	12.4	8.8	14.8	6.3	49.6	79.5
5. 'Roundup' alone	61.2	9.1	6.2	5.8	13.4	34.5
	(After 5 days)					
1. Synperonic A-2	49.5	3.3	14.2	5.4	21.6	43.3
3. Synperonic A-20	16.6	4.8	13.8	8.3	47.4	74.3
4. Synperonic A20 + Diesel oil	8.5	7.3	11.8	8.7	54.6	82.4
5. 'Roundup alone'	54.2	5.0	8.6	7.8	19.3	40.7

There was a significant decrease in the amount of ^{14}C-radioactivity in the cellulose acetate and treated zone samples after 24 hours and five days. The amount of radiocarbon significantly increased in the remaining part of the plant after 24 hours and 5 days. However, ^{14}C-glyphosate was distributed through the treated zone, the remaining zone, the 10 cm zone and remaining part of the plant when harvested after five days (Table 1). Formulation No. 4 (Synperonic A20 plus diesel oil) produced significantly greater penetration of glyphosate than the other treatments. Synperonic A2 and A7 and 'Roundup' produced similar glyphosate penetration patterns whereas Synperonic A20 alone gave penetration intermediate between these and formulation No. 4.

TABLE 2. EFFECT OF SURFACTANTS ON THE PENETRATION OF GLYPHOSATE IN *C.ROTUNDUS* AFTER APPLICATION AT THE 5 TO 6 LEAF STAGE IN WINTER

	Distribution of C-14 (% of applied)					
Sr. Formulation No. additive	Cellulose acetate	Treated zone	Remaining zone	10 cm zone	Remaining plant	Total uptake
	(After 4 hours)					
1. Synperonic A-2	85.3	2.3	3.5	1.2	3.2	10.2
2. Synperonic A-7	80.6	1.9	2.8	1.7	2.7	9.1
3. Synperonic A-20	59.3	4.9	2.2	2.6	20.6	30.3
4. Synperonic A20 + Diesel oil	45.9	20.5	7.0	4.0	11.2	42.6
5.. 'Roundup' alone	88.4	4.3	2.6	1.4	1.3	9.6
(After 24 hours)						
1. Synperonic A-2	59.5	4.7	7.2	3.1	18.7	33.7
2. Synperonic A-7	62.7	7.2	5.4	4.8	13.4	30.8
3. Synperonic A-20	21.5	12.9	8.1	9.4	37.9	68.3
4. Synperonic A20 + Diesel	63.6	7.5	8.6	6.4	7.2	29.7
	(After 5 days)					
1. Synperonic A-2	51.3	6.2	4.2	3.4	24.1	37.9
2. Synperonic A-7	54.2	4.2	4.7	3.2	28.3	40.4
3. Synperonic A-20	7.4	5.6	12.9	8.7	46.5	83.2
5. 'Roundup' alone	62.4	4.5	9.8	7.4	10.6	32.3

Results presented in Table 2 showed the effect of three non-ionic surfactants on the penetration of ^{14}C-glyphosate in *C. rotundus* during the winter season. They are similar to those obtained in the summer It is, therefore, concluded that formulation No. 4 with Synperonic A20 plus diesel oil is the most effective combination for enhancing the penetration of glyphosate.

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USE OF ISOTOPIC TRACERS IN STUDIES ON ^{14}C-GLYPHOSATE PERFORMANCE ON *CYPERUS ROTUNDUS* IN POT AND FIELD CONDITIONS

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Abstract

The effect of surfactants and oil on bioefficacy of the herbicide, glyphosate in controlling *Cyperus rotundus* L. was evaluated using potted plants. A mixture of the commercial formulation, "Roundup" with 0.2% Triton X-100, 1% diesel oil and 1% of 4% aqueous ammonium sulfate produced the most penetration into the leaf. The results of the field experiments suggested that this mixture applied at a rate of 1.5 kg/ha glyphosate amended "Roundup" can effectively control *C. rotundus* in the field.

1. INTRODUCTION

The methods of formulating an agrochemical, particularly the addition of surfactant adjuvants, can have a profound influence on the efficiency of control of the target plant, by affecting uptake, redistribution and persistence and consequently its ultimate biological performance. Optimising the performance of most commercial pesticide formulations is still done primarily by trial and error but work on the effect of surfactants on the uptake of neutral model organic compounds with disparate physico-chemical properties offers a more rational guideline for adjuvant selection and formulation design using a predictive response surface model approach [1, 2].

Glyphosate, a non-selective, systemic, post-emergence herbicide, controls *Cyperus rotundus,* purple nutsedge, to some extent under many conditions [3–7]. The commercial formulations and application specifications are usually designed to give acceptable performance on a range of species. It is possible to improve performance on particular targets by manipulating formulation and application factors such as the addition of surfactants [8]. Surfactants of different properties may enhance the activity of glyphosate by improving penetration and translocation [9,10] or reduce it by antagonizing uptake [11].

The aim of the studies was to:

(a) standardize the type and concentration of the additives to be used with the "Roundup"commercial formulation of glyphosate for effective penetration in *C. rotundus* under pot conditions.

(b) to assess the time course of penetration over 24 hours.

(c) and finally to apply the results of the pot experiments to studies under field conditions.

2. MATERIALS AND METHODS

2.1.Laboratory experiments

2.1.1. Estimation of the amount of Triton X-100 needed to emulsify 1% oil in Roundup in the presence of ammonium sulfate

The basic mixture used consisted of 54 mL, water. 25 mL "Roundup" (diluted 1:12 to give 30 mg a.e./mL). 1 mL oil (diesel was used through out being the cheapest). 0.01, 0.02, 0.05, 0.1, 0.2 and 0.5 mL Triton X-100 was added to 100 mL of the above mixture which was then shaken and allowed to stand over night. The lowest quantity of Triton X-100, that maintained the emulsion was selected. Besides Triton X-100, two other locally available detergents were also tried.

2.1.2. 24 h penetration studies

Cyperus rotundus tubers were collected, selected by weight to ensure uniformity, germinated and plants were grown in the pots as reported earlier. Six uniform plants were selected when they had 6 leaves and the 2nd oldest leaf were used for the operation in all studies and each treatment was repeated on four different days. The herbicide application was done at the same time of the day on each occasion.

The six treatments applied were:

A. "Roundup" alone: 2.5 µL ^{14}C-glyphosate solution + 25 µL "Roundup" diluted 1:12 + 72.5 µL water (i.e. 0.185 KBq/0.005 µCi labelled glyphosate which was one-tenth of the activity used before).

B. "Roundup" + ammonium sulfate: As for A above but replaced 25 µL of the water with 25 µL 4% $(NH_4)_2SO_4$.

C. "Roundup" + Triton X-100: As for A. with addition of the amount of Triton X-100 selected from experiment 1.

D. "Roundup" + Triton X-100 + $(NH_4)_2SO_4$: As for B with the addition of Triton X-100 as in C.

E. "Roundup" + Triton X-100 + oil: As far C but replaced 1 µL of the water with 1 µL oil.

F. Roundup + Triton X-100 + oil + $(NH_4)_2SO_4$: As far E but replaced 25 µL of water with 25 µL 4% $(NH_4)_2SO_4$.

As described previously, 8 × 0.5 µL drops were applied on either side of the midrib in a 2 cm area starting 10 cm from the tip without touching the leaf. Four µL aliquots were added in to 2 scintillation vials to check the application rates. After 24 hours, the treated area was painted with 6% cellulose acetate in 9:1 acetone/water. The dried cellulose acetate was removed with tweezers and dissolved in 2 mL glacial acetic acid and 5–10 mL "Permaflour" was added for counting. Cellulose acetate was also applied to untreated leaves.

2.1.3. Modification of treatment studies

The best treatment from experiment 2 was selected and formulation No. F was modified by replacing 1% diesel oil with 0.5% or 2% diesel oil or with 1% glycerol.

2.1.4. Short term penetration studies
Formulations D and F were selected for the above experiment and penetration was measured over 1 hour and 2 hours following the revised protocol.

2.2. Field experiments

2.2.1. Layout

The best modified formulation found in the pot experiments was tested at rates of 1.5, 0.75 and 0.5 kg/ha a.e. glyphosate and compared with 1.5 kg a.e. glyphosate in unamended "Roundup". The application rate was 200 L/ha using a spray pump made by GLORIA 172-R Germany with a nozzle flow rate 500 ml/min at a pressure of 6 bar. There were two times of application, 8 a.m. and 5 p.m. for both the cropped (with maize) and uncropped plots.

The design was split split in RCBD with 4 replications plot (cropped/uncropped); the size of the cropped plots was 5 × 5 m and that of the uncropped 5 × 3m. There were untreated control + 4 glyphosate treatments × 2 times of application × cropped/uncropped = 5 × 2 × 2 = 20 treatments with 4 replicates, to give 80 plots. In addition, the cropped plots included a hand-weeded control, to make a total of 84 plots. The experimental area was assessed to confirm a natural stand of *Cyperus*. The area was ploughed, the seed bed prepared and irrigated following local agricultural practices. Two days before glyphosate application, the number of *Cyperus* shoots were counted in three 0.25 × 0.25 m quadrats per plot. Seeds of the maize variety (China) were sown three days after glyphosate applications.

2.2.2. Assessment

Weed control was visually estimated on the *Weed Science* scale of 0, 100, where 0 = no weed reduction or injury and 100 complete weed reduction, 4 and 8 weeks after treatment. In uncropped plots *Cyperus* was harvested from half the plot after four weeks and the other half after 8 weeks. Fresh and dry weights were recorded. Yield of the maize crop was measured and all vegetation (crop + weed) was cut to 2 cm above the ground and visual observations were recorded after two weeks and the field was further assessed after eight weeks. The effect of the treatment on tuber formation of *Cyperus* was noted ten weeks after cutting the vegetation.

2.2.3. Statistical analysis

The data collected were subjected to the analysis of variance (ANOVA) and Duncan's New Multiple Range Test [12].

3. RESULTS AND DISCUSSION

During the experimental period between May and September, 1995 daily temperature minima were 22–30°C and maxima 35–48°C. Relative humidities ranged from 62–70% in the morning and 45–56% in the evening. There were 3 showers in September.

The results of the studies made to select the best emulsifier that can hold and maintain the emulsions to be applied weed indicated that 0.02 and 0.05% of Triton X-100, could emulsify 1% diesel oil so 0.02% was chosen. The two locally available surfactants tested were no better than 0.02% Triton-100.

TABLE 1. EFFECT OF FORMULATION ON PENETRATION OF GLYPHOSATE

Treatments	% Recovery of ^{14}C-Glyphosate from cellulose acetate on four different days				
	Day 1	Day 2	Day 3	Day 4	Mean
A	52.6	50.7	54.2	56.3	53.4 b
B	64.1	62.7	56.6	61.7	61.3 a
C	51.1	53.3	47.6	55.8	52.0 b
D	35.7	38.4	33.9	34.2	35.5 d
E	41.7	51.7	48.2	50.6	47.9 c
F	34.0	35.3	30.9	31.8	33.0 e

Means in the same column followed by different letters are significantly different ($p < 0.05$).

The 24 hours penetration studies are presented in Table1. Out of six treatments during four different timings, treatment F produced the best glyphosate penetration followed by D. Treatments A and C were not significantly different and D and E gave more penetration but values were lower than for F. The results of further modification in the selected formulation are presented in Table 2. There was no difference between 2% diesel oil and 1% but 0.5% produced lower penetration. Replacement of diesel oil by glycerol did not improve performance. Therefore 1% diesel oil was chosen.

TABLE 2. MODIFICATION OF TREATMENT MIXTURES

Treatments	Recovery of ^{14}C-glyphosate from cellulose-acetate						
	1	2	3	4	5	6	Average
M-1	48.5	46.2	45.6	48.5	42.9	48.0	46.6 a
M-2	36.9	39.3	38.3	46.3	40.9	41.3	40.5 b
M-3	34.5	35.1	37.6	32.1	32.7	34.5	34.4 c

Means in the same column followed by different letters are significantly different ($p < 0.05$).
Treatments:
M - 1 = Mixture with Glycerol.
M - 2 = Mixture with 0.5% diesel oil.
M - 3 = Mixture with 2% diesel oil.

The shorter term studies over 1 and 2 hours (Table 3) did not show differences between D and F so did not discriminate between formulations as effectively as the 24 h study. However, in tropical conditions, rainfastness is important for glyphosate so the shorter term assessment may have more practical value but this point was not explored further.

TABLE 3. SHORT TERM PENETRATION STUDIES

Treatments	Time (h)	% recovery of ^{14}C-glyphosate from cellulose-acetate					
		1	2	4	5	6	Average
D	1	70.2	73.8	77.9	71.0	76.2	73.4 b
	2	60.6	56.3	61.2	53.5	56.0	57.3 c
F	1	65.9	70.2	67.9	72.3	69.5	69.3 b
	2	55.8	52.7	58.1	56.3	53.1	55.1 c

Means in the same column followed by different letters are significantly different (p <0.05).

TABLE 4. BEFORE SPRAYING GLYPHOSATE, NUMBER OF *CYPERUS* SHOOTS IN THREE 0.25 m × 0.25 m QUADRATS PER PLOT (MORNING)

Treatments	Uncropped plots				cropped plots			
	plot-1	plot-2	plot-3	plot-4	plot-1	plot-2	plot-3	plot-4
Control	9 7 10	6 10 8	7 5 8	8 6 11	8 6 5	10 7 6	8 8 10	7 5 11
Two days before 1st treatment	8 8 10	12 6 7	4 6 7	5 8 12	7 8 12	9 6 4	6 8 7	5 9 9
Two days before 2nd treatment	3 7 10	8 6 7	7 6 11	8 8 10	7 6 6	8 4 3	9 8 4	6 8 5
Two days before 3rd treatment	11 7 5	9 6 5	8 6 5	9 7 8	12 7 9	10 6 9	7 5 12	8 6 9
Two days before 4th treatment	10 8 6	8 7 6	8 6 10	6 8 9	8 9 9	7 5 13	9 8 10	11 7 8

TABLE 5. BEFORE SPRAYING GLYPHOSATE, NUMBER OF *CYPERUS* SHOOTS IN THREE 0.25 m × 0.25 m QUADRATS PER PLOT (EVENING)

Treatment	Uncropped plots				Cropped plots			
	plot-1	plot-2	plot-3	plot-4	plot-1	plot-2	plot-3	plot-4
Control	8 7 10	7 4 9	7 8 10	5 9 8	10 8 10	8 8 8	9 12 7	10 8 7
Two days before 1st treatment	11 6 8	7 9 5	6 8 11	6 12 8	9 11 7	6 9 8	6 9 10	11 10 8
Two days before 2nd treatment	9 6 9	6 8 8	10 5 9	7 8 11	8 12 9	10 8 6	8 10 10	7 11 9
Two days before 3rd treatment	10 8 7	8 5 9	8 7 8	5 9 5	7 12 9	8 9 7	7 9 12	9 9 6
Two days before 4th treatment	5 8 8	7 7 11	8 10 9	10 10 8	8 6 10	8 9 9	8 5 9	4 9 7

The assessment of weed population in the field plots made two days before herbicide application showed that the population was uniform over the whole site (Tables 4 and 5).

The percent weed control ratings recorded in Table 6 show that the maximum efficacy was given by 1.5 kg/ha amended "Roundup", followed by 1.5 kg/ha unamended 'Roundup". The difference between these treatments was highly significant and both were significantly better than the other three. These treatment differences were the same for plants treated at 4 and 8 weeks, for the cropped and uncropped plots and for the morning and evening times of application.

TABLE 6. WEED CONTROL RATING OF GLYPHOSATE FORMULATIONS APPLIED ON *CYPERUS ROTUNDUS*

Treatment	Uncropped plots					Cropped plots				
	Morning		Evening		Average	Morning		Evening		Average
	4 week	8 week	4 week	8 week	Average	4 week	8 week	4 week	8 week	Average
Control	0	0	0	0	0 e	0	0	0	0	0 e
1 5 kg/ha unamended "Roundup"	60	60	60	60	60 b	60	60	60	60	60 b
1 5 kg/ha amended "Roundup"	80	90	80	90	85 a	80	90	80	90	85 a
0 75 kg/ha amended "Roundup"	30	30	30	40	32 c	30	30	30	30	30 c
0 5 kg/ha amended "Roundup"	20	20	20	20	20 d	20	20	20	20	20 d

All values are average of four replicates
Means in the same column followed by different letters are significantly different ($p < 0\ 05$)

TABLE 7 FRESH WEIGHT OF *CYPERUS* PLANTS IN PLOTS TREATED WITH DIFFERENT CONCENTRATIONS AND FORMULATIONS OF GLYPHOSATE

	Morning plots		Evening plots		Av weight
	After 4 week	after 8 week	After 4 week	after 8 week	
Control	2 2	2 4	2 1	2 5	2 3a
1 5 kg/ha unamended "Roundup"	1 8	1 6	1 7	1 6	1 7b
1 5 kg/ha amended "Roundup"	1 1	1 9	1 1	1 0	1 1c
0 75 kg/ha amended "Roundup"	1 8	1 7	1 9	1 6	1 8b
0 5 kg/ha amended "Roundup"	2 5	2.1	2 3	2 2	2 3a

* The values are average of 4 plots
Means in the same column followed by different letters are significantly different ($p < 0\ 05$)

TABLE 8. DRY WEIGHT OF *CYPERUS* PLANTS IN PLOTS TREATED WITH DIFFERENT CONCENTRATIONS AND FORMULATIONS OF GLYPHOSATE

Treatment	Morning plots		Evening plots		Average weight	Weight loss
	After 4 week	After 8 week	after 4 week	After 8 week		
Control	0.66	0.68	0.62	0.68	0.66a	71.6 a
1.5 kg/ha unamended "Roundup"	0.62	0.50	0.53	0.51	0.54c	68.6 b
1.5 kg/ha amended "Roundup"	0.33	0.32	0.30	0.29	0.31 d	71.5 a
0.75 kg/ha amended "Roundup"	0.58	0.53	0.57	0.53	0.55c	68.7 b
0.5 kg/ha amended "Roundup"	0.69	0.59	0.65	0.60	0.63b	72.0 a

The values are average of 4 plots.
Means in the same column followed by different letters are significantly different ($p < 0.05$).

TABLE 9. EFFECT OF DIFFERENT CONCENTRATIONS OF GLYPHOSATE ON MAIZE YIELDS

Treatment	Average weight of 10 maize plants (kg)			Average weight of 10 maize cobs (kg)		
	Morning (plots)	Evening (plots)	Average	Morning (plots)	Evening (plots)	Average
Control	5.54	4.610	5.07 e	2.23	2.13	2.18 e
1.5 kg/ha unamended Roundup"	9.70	9.72	9.71 b	3.52	4.20	3.91 b
1.5 kg/ha amended "Roundup"	11.57	12.59	12.08 a	5.05	4.80	4.93 a
0.75 kg/ha amended "Roundup"	9.23	6.41	7.84 c	3.22	2.65	2.94 c
0.5 kg/ha amended "Roundup"	7.5	5.86	6.68 d	2.77	2.52	2.65 d

* These values are average from four plots.
Means in the same column followed by different letters are significantly different ($p < 0.05$).

The fresh weight data of the plants in the uncropped pots (Table7) show that 1.5 kg/ha amended and unamended "Roundup" and 0.75 kg/ha amended "Roundup" significantly reduced fresh weight of *Cyperus* after 4 weeks compared with controls but the 0.5 kg/ha amended treatment did not. There were no differences between plots treated in the morning and evening. Plants in the control plots increased in weight at 8 weeks but the treated plants did not. Corresponding measurements were not made in the cropped plots because visual assessment (Table 6) indicated no differences between cropped and uncropped plots.

Dry weight data (Table 8) follow he same pattern except the 0.5 kg/ha amended treatment also gave a significantly lower value than control.

The results presented in Table 9 show that the yield of maize plants and cobs in all treatments treated was significantly higher than the control with the amended "Roundup" at 1.5 kg/ha the highest followed by the unamended "Roundup" at 1.5 kg/ha. Even the treatments which did not produce a measurable effect on *Cyperus* increased total maize yield with plots treated in the morning plot producing higher weights than those sprayed in the evening. However, there was no significant difference in the yield of cobs.

4. CONCLUSIONS

Amendments could enhance the toxicity of the "Roundup formulation of glyphosate against *C. rotundus* in both pot and field experiments. The improved efficacy of glyphosate against the weed may be attributed to faster penetration and translocation of the compound in the plant caused by the additives as indicated by the tracer studies.

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EFFECT OF DIESEL OIL AND AMMONIUM SULFATE ON EFFICACY OF GLYPHOSATE ON *CYPERUS ROTUNDUS* UNDER FIELD CONDITIONS

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Abstract

Under Gezira field conditions, excellent and lasting *Cyperus rotundus* suppression was achieved, irrespective of application time or cropping conditions. From visual assessments, suppression was achieved when glyphosate as "Roundup" at 1.5 kg a.e./ha was applied alone or with the addition of ammonium sulfate, diesel oil emulsified with Triton X-100. At the rate of 0. 75kg a.e./ha the herbicide was moderately effective on the cropped fields but on the uncropped fields it was as effective as the higher rate at 8 weeks. At the lowest rate tested (0.5 kg a.e./ha) the herbicide was less effective and was not significantly improved by the addition of adjuvants or changing the time of spray application. The fresh and dry weights in the 0.75 kg a.e./ha treatments were reduced by 85% to 98% compared with the controls confirming the visual assessments. Unrestricted competition from the natural weed population combined with *C. rotundus*, reduced the maize stand by 59%, height by 55%, straw yield by 60% and grain yield by 87%, *C. rotundus* alone was less competitive reducing maize stand, height, straw and grain yields by 21%, 2%, 61% and 68% respectively.

1. INTRODUCTION

Cyperus rotundus (Purple nutsedge) is classified [1] as one of the worst weeds of the world. The weed is a perennial and propagates mainly by tubers. Control of *C. rotundus* by hand weeding is not effective as the weed regenerates from underground tubers. Work undertaken in the Sudan showed that in arable areas the weed could be adequately controlled by deep ploughing and prolonged exposure of tubers on the soil surface during summer [2]. However, under plantation crops such, as fruit trees and on irrigation canals and water ways, where deep ploughing and cultivation are not possible, other means of control have to be considered.

Glyphosate is a systemic, non-selective herbicide, which is effective against a variety of perennial weeds including *C. rotundus.* However, good and reliable control of *C. rotundus,* across sites and seasons, is only achieved at relatively high rates. Reduction of the herbicide rate is imperative in order to reduce treatment costs and attain economically acceptable control. To achieve this an integrated approach based on improved herbicide formulation and cultural practices viz combining herbicide treatments with cropping should be adopted.

The enhancement of penetration and translocation are key factors in improving the performance of foliar applied systemic herbicides. Important conclusions of the Second RCM of this programme were that glyphosate translocates easily in C. *rotundus* and that penetration into the leaf is the critical step that limits herbicidal efficacy.

In general, penetration is governed by a multitude of variables including factors pertaining to the spray solution, the herbicide, the environment and the plant in question. Factors, such as high temperature and low humidity, which enhance spray droplet drying tend to reduce penetration and consequently reduce activity of foliar applied herbicides. Numerous reports have shown that various spray adjuvants including organic and inorganic compounds increase glyphosate toxicity to *C. rotundus.*

The present investigation was undertaken at the Gezira Research Station with the primary objective of increasing the herbicidal efficacy of glyphosate on *C. rotundus* through the use of spray adjuvants and the manipulation of spraying time. The environment was also modified in some cases by planting a crop after spraying the *C. rotundus* plants.

2. MATERIALS AND METHODS

A *C. rotundus* population density of 9 to 30 plants/m^2 was achieved by planting 20 pre-germinated tubers in plots previously infested with *C. rotundus.* Glyphosate, as "Roundup", was supplied by the International Atomic Energy Agency (IAEA). The product was used as supplied (unamended) or after addition of ammonium sulfate $(NH_4)_2 SO_4$ (20g/L), diesel oil (10 mL/L) and Triton X-100 (0.5 mL/L) (amended). Amended glyphosate was applied at 1.5, 0.75, and 0.5 kg a.e./ha. Unamended glyphosate was applied at 1.5 kg a.e./ha. The herbicide was applied as an aqueous spray with a knapsack sprayer at a volume rate of 375 L/ha using a flood jet nozzle (Lurmark) at 103–206 kPa on the 2nd of October 1996. Two equivalent sets of treatments were made, one set was sprayed in the morning (before 9 a.m.), when temperature and relative humidity were 34°C and 47%, respectively, and the second set was applied in the evening (4 to 5 p.m.), when temperature and relative humidity were 32^{0}C and 48%, respectively.

The controls included plots infested with *C. rotundus* alone, clean weeded plots, and plots infested by a combination of the natural weed flora and *C. rotundus.* Three days after herbicide application, seeds of maize, *(Zea mays* L.) c.v. Geiza 2, were planted in holes 4 cm deep and 20cm apart, on ridges 120cm apart. The emerged seedlings were thinned to one plant per hole 15 days after emergence. Nitrogen as urea (190 kg/ha) and superphosphate (95 kg/ha P_20_5) were applied at sowing. The experimental layout was a split plot, with cropped/ uncropped as the main plot, and treatments plus timing of application as the sub-plots. Plot size was 5x5 metres for all treatments and the plots were irrigated every 12 days. The weeded plots were hand-weeded 4 times at 21 day intervals, but some of the herbicide treated plots received supplementary weedings at 4 and 8 weeks after spraying. All weedings were by hand hoe. Two sprays with the insecticides methomyl as "Lannate" and carbaryl as "Sevin" were applied to control stem borers.

Percentage cover of C. *rotundus* was assessed on random areas of 0.1 m^2 at 4 and 8 weeks after spraying. The data are expressed as a percentage of the weed cover of the untreated control, and transformed to a scale of 0 to 10, where 0 means no control and 10 means complete control [3]. *C. rotundus* was harvested from half of the uncropped plots at 4 weeks and from the other half at 8 weeks. Fresh and dry weights of the weeds were determined. Maize height, and straw and grain yields were determined at harvest.

3. RESULTS

Based on visual estimates, "Roundup" at 1.5 kg a.e./ha, irrespective of adjuvants or application time, gave excellent and lasting control of *C. rotundus* both under cropped and uncropped conditions (Table 1).

At 0.75 kg a.e./ha the product gave moderate weed control under cropped conditions, but under uncropped conditions the results were good for morning applications but variable for evening applications, ranging from poor to satisfactory at 4 weeks to excellent at 8 weeks. At 0.5 kg a.e./ha poor to moderate control was achieved, under cropped conditions. However under no cropping conditions moderate to satisfactory control was attained. It is noteworthy that "Roundup" at 0.5 kg a.e./ha tended to be more effective at 8 weeks when applied in the morning than in the evening (Table 1).

TABLE 1. INFLUENCE OF SPRAY ADJUVANTS AND SPRAYING TIME ON HERBICIDAL EFFICACY OF "ROUNDUP"

Treatment		*C. rotundus* control			
		Cropped		Uncropped	
		4 Wks	8 Wks	4 Wks	8 Wks
Amended "Roundup" 1.5 kg	M	10 a	9 abc	9 a	10 a
	E	10 a	9 abc	9 a	10 a
0.75 kg	M	5 def	6 de	8 abc	10 a
	E	6 bcd	6 de	4 ef	9 abc
0.5 kg	M	6 cde	5 e	3 f	8 bc
	E	5 def	3 f	4 def	6 de
Unamended "Roundup" 1.5 kg	M	9 a	9 abc	8 ab	9 ab
	E	9 a	7 cd	10 a	9 ab
Infested control*		8 ab	1 g	5 def	2 g

M = Sprayed before 9 a.m.; E = Sprayed after 4 p.m.
Scores relative to the unweeded control (= 0), in which weeds other than *C. rotundus* were removed by hand.
* = Weeds other than *C. rotundus* were not removed.
Means within rows followed by different letters are significantly different (p <0.05).

TABLE 2. INFLUENCE OF "ROUNDUP" ON FRESH AND DRY WEIGHTS OF AERIAL PARTS *C. ROTUNDUS* IN UNCROPPED PLOTS

Treatments		Fresh Wt g/m²		Dry Wt g/m	
		4 Wks	8Wks	4Wks	8 Wks
Amended "Roundup" 1.5 kg	M	3.53 c	0.55 e	1.37 d	0.19 e
	E	0.80 c	0.23 e	0.34 d	0.07 e
0.75 kg	M	6.71 c	3 00 e	2.54 cd	0.86 de
	E	6.37 c	5.61de	2.47 cd	1.85 de
0.5 kg	M	7.23 c	10.2 cd	2.76 cd	3.3 cd
	E	17.6 b	14.4 bc	5.02 bc	5.05 bc
Unamended "Roundup" 1.5kg	M	3.46 c	0.51 e	2.22 d	0.19 e
	E	0.90 c	0.20 e	0.37 d	0.06 e
Control*		43.9 a	28.7 a	13.25 a	11.15 a
Infested control**		19.80 b	12.00 b	7.24 b	6.50 b
S.E. ±		4.9	3.17	1.6	1.2

M = Sprayed before 9 a.m.; E = Sprayed after 4 p.m.
* = Weeds other than *C. rotundus* were removed by hand.
** = Weeds other than *C. rotundus* were not removed.
Means within rows followed by different letters are significantly different (p <0.05).

Data on fresh and dry weight of *C. rotundus* foliage (Table 2) showed that the weed, when grown in the absence of other weeds, had a fresh weight of 43.9 and 28.7 g/m^2 at 4 and 8 weeks, respectively. The corresponding figures for dry weight were 13.2 and 11.2 g/m^2. Competition from other weeds reduced the dry weights of *C. rotundus* to 7.24 g/m^2 (45% reduction) and 6.5 g/m^2 (42% reduction) at 4 and 8 weeks, respectively . The fresh and dry weight data confirmed the excellent and lasting activity of 0.75 kg a.e./ha, with fresh and dry weights of the weed reduced by 85 to 98%. The herbicide, when applied at 0.5 kg a.e./ha was less effective. At 4 weeks the treatments gave 60% and 84% reductions in fresh weight for evening and morning applications, respectively, the corresponding reductions in dry weight being 62% and 79%. However, at 8 weeks, the plants had recovered and showed only moderate suppression of fresh and dry weights (Table 2). Time of application had no consistently significant effects on the dry weights of *C. rotundus,* irrespective of the rate.

TABLE 3. INFLUENCE OF "ROUNDUP" RATE, AMENDMENT AND APPLICATION TIME ON MAIZE GROWTH AND YIELD

Treatments		Grain yield (t/ha)	Plant height (cm)	Maize stand (000/ha)	Straw yield (t/ha)
Amended "Roundup" 1.5 kg	M	2.04 a	160 abB	55 a	6.83 b
	E	2.00 a	145 cde	51 ab	6.80 b
0.75 kg	M	1.72 a	126 g	44 ab	5.40 b
	E	2.03 a	142 bcd	53 ab	6.85 b
0.5 kg	M	2.01 a	141 def	53 ab	6.50 b
	E	1.34 ab	131 fg	42 ab	4.68 b
Unamended "Roundup" 1.5kg	M	2.13 a	161 a	49 ab	7.03 b
	E	1.78 a	157 abc	49 ab	6.88 b
Control*		0.59 bc	131 ag	40 c	4.33 b
Infested control**		0.23 c	60 h	21 c	4.35 b
Weed free control		1.85 a	133 efg	51 ab	10.9 a
S.E.±		0.41	11.1	1.8	1.2

M = Sprayed before 9 a.m.; E = Sprayed after 4 p.m
* = Weeds other than *C. rotundus* were removed by hand.
** = Weeds other than *C. rotundus* were not removed.
Means within columns followed by different letters are significantly different ($p < 0.05$).

Table 3 shows that unrestricted competition from the natural weed population combined with *C. rotundus,* reduced the maize stand by 59%, height by 55%, straw yield by 60% and grain yield by 87%. *C. rotundus,* alone, was less competitive, the reductions in maize stand, height and straw and grain yields being 21%, 2%, 6 1% and 68%, respectively. In comparison with untreated plots infested with *C. rotundus* and the natural weed flora, "Roundup" significantly, increased maize growth and yield. The herbicide, irrespective of rate, adjuvants and spraying time, resulted in crop stands and grain yields comparable to the weed free control. However, with all herbicide treatments straw yields were lower than the weed free control. Plots infested with both the natural weed flora and *C. rotundus* yielded, significantly, shorter plants. "Roundup" at 1.5 kg/ha, irrespective of adjuvants or spraying time, yielded significantly taller plants. All other treatments yielded plants with comparable height to those grown in the weed free control.

4. CONCLUSIONS

Under the conditions specified for this experimental programme, *C. rotundus,* combined with the natural weed flora was more competitive than *C. rotundus* alone. Crop yield and yield components, as well as the growth of *C. rotundus,* were significantly reduced by the natural weeds.

The visual assessment of ground cover of *C. rotundus* treated with "Roundup" at 1.5 kg a.e./ha, irrespective of adjuvants, cropping situation or timing of application, gave excellent and lasting suppression of *C. rotundus.* At the rate of 0.75 kg a.e./ha the herbicide was moderately effective at 4 weeks, but at 8 weeks it was as effective as the high rate on the uncropped plots. At the lowest rate (0.5 kg a. e./ha) the herbicide was less effective and was not significantly improved by adjuvants or manipulation of spraying time.

Crop height was less sensitive to competition from *C. rotundus* (only 2% reduction), but crop stand, straw and grain yield were significantly reduced by competition from the weed. "Roundup", irrespective of rate, adjuvants or spraying time, resulted in a crop stand and grain yield comparable to the weed free control, but did not significantly improve straw yield or plant height.

Under the of conditions of this experimental programme, the herbicidal efficacy of "Roundup" on *C. rotundus* was more affected by herbicide rate than by spray adjuvants or spraying titne.

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EFFECTS OF ADDITIVES ON GLYPHOSATE ACTIVITY IN PURPLE NUTSEDGE

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Abstract

Effects of additives on ^{14}C-glyphosate penetration into purple nutsedge leaves were examined in the laboratory. and efficacy of glyphosate for purple nutsedge control was studied in the greenhouse and field The addition of $(NH_4)_2SO_4$ at 1.0% (v/v) + diesel oil at 1.0% (v/v) + Tendal at 1.0% (v/v) increased ^{14}C-glyphosate penetration into nutsedge leaves more than the addition of either one alone. $(NH_4)_2SO_4$ at 1 0% + diesel oil at 1.0% + Tendal at 0 12 or 0.25% increased the phytotoxicity of glyphosate at 0 5 and 0 75 kg a e./ha on nutsedge plants in the greenhouse but not in the field. Additives did not enhance glyphosate activity by reducing the number of nutsedge tubers

1. INTRODUCTION

Purple nutsedge (*Cyperus rotundus*) was ranked as one of the most serious weeds in the world [1]. It is an important weed in corn, sorghum, soybean, mungbean, peanut, cotton, upland rice, and vegetables. Mechanical control of this weed is not successful because it can sprout new shoots from tubers. Various selective pre-emergence herbicides cannot control this weed.

Glyphosate is a non-selective, translocated, foliar applied herbicide [2]. Which has been reported to control purple nutsedge [3]. However. application of glyphosate for weed control is restricted by cost. Appropriate adjuvants or additives might be used in combination with glyphosate to maintain its optimum activity but at a reduced rate.

Ammonium sulfate has been reported to increase activity of glyphosate for purple nutsedge control [3]. Furthermore, calcium antagonism of glyphosate has been overcome with $(NH_4)_2SO_4$ [4, 5], citric acid, and an organosilicone adjuvant [6].

Various additives including nonionic surfactants [7], the organosilicone Silgard 309 [8], and both petroleum and seed oils [9] were reported to increase glyphosate activity. The organosilicone Silwet 77 enhanced ^{14}C-glyphosate uptake into bean (*Vicia faba*) leaf [10]. Furthermore, oils also increased glyphosate penetration [9].

The objectives of these experiments were to determine the effect of the various additives, $(NH_4)_2SO_4$, Tendal (surfactant), and diesel oil at appropriate concentrations on ^{14}C-glyphosate penetration into purple nutsedge leaves, and to determine the effect of additives on glyphosate efficacy for purple nutsedge control.

2. MATERIALS AND METHODS

2.1. Laboratory experiments

^{14}C-glyphosate and glyphosate at 1.5 kg a.e./ha with several additives in a spray volume of 200 L/ha were applied when the purple nutsedge plants were 5-6 leaves (approximately 2 weeks after planting). Eight drops of 0.5 µl 7.7 kBq (0.02 µCi) of ^{14}C-glyphosate were applied on the same leaf of each plant. Plants were harvested at 2 and 24 hours after application. Cellulose acetate (6.0%) in 9:1 acetone/water was painted on the ^{14}C-glyphosate treated area, 2 minutes after application, dried cellulose acetate was removed and mixed with 2 ml glacial acetic acid. Two hundred µL solution of cellulose acetate in glacial acetic acid was mixed with scintillation cocktail to determine the amount of ^{14}C-glyphosate by liquid scintillation spectrometry.

The additive, Sunlite®, is a local detergent. The surfactant, Tendal®, is a blend of 60% alkyl aryl polyethoxylate and sodium salt of dialkyl sulfosuccinate plus 40% solubilizer and couplers. Triton X-100® is dioctyl sulfonosuccinate, sodium salt. The herbicide, Roundup®. contains 36% a.e. glyphosate.

Experiments were carried out in a Randomized Complete Block Design with 6 replications. The temperature and relative humidity during application of first, second, and third experiments were 32°C, 65%, 30°C, 70% and 28°C, 80% respectively.

2.2. Greenhouse experiments

Three purple nutsedge tubers were planted in polyethylene pots containing clay soil. The pots were 14 cm. in diameter and 15 cm. high . Plants were watered from the surface every day. At 2 weeks after germination, glyphosate was applied alone and in combination with additives.

Herbicide was applied by laboratory sprayer at a spray volume of 200 L/ha and pressure of 87 kg /cm^2. The nozzle was a T-jet, flat fan No 8001. During application the temperature was 28°C with 75% relative humidity.

The number of nutsedge shoots and tubers in each pot were recorded at 21 and 60 days after application. The experiment was carried out in a Randomized Complete Block Design with 4 replications.

2.3. Field experiment

The field experiment was conducted at the National Corn and Sorghum Research Institute at Pakchong, Nakornrachima. Glyphosate at 0.5,0.75 and 1.5 kg a.e./ha was applied alone and in combination with various additives. The herbicide was applied when naturally grown purple nutsedge was at the 5–6 leaves stage or approximately 2 weeks after cultivation. Herbicide was applied either before 9 a.m. or after 4.p.m. The size of each treated plot was 5 × 5 m cropped and 5 × 3 uncropped. Corn was planted at 3 days after herbicide application.

At 1, 2, 4, and 8 weeks after application, a visual weed control rating was recorded. Dry weights of nutsedge plants were recorded at 4 and 8 weeks after application in the uncropped area. The number of nutsedge plants was recorded at 6 and 10 weeks after application in the uncropped area. The yield of corn, dry weight, and number of nutsedge plants in the cropped area were recorded at harvesting.

Experiments were carried out in a Randomized Complete Block Design with 4 replications. Herbicide was applied by knapsack sprayer, with a spray volume of 200 L/ha. The nozzle was a T-jet, flat fan No 8001. The pressure was 87 kg/cm^2. The temperature before 9.0 a.m. was 27^{o}C with 70% relative humidity and after 4.0 p.m. was 32 ^{o}C with 65% relative humidity.

3. RESULTS AND DISCUSSION

3.1. Laboratory experiments

Tendal + diesel oil + $(NH_4)_2SO_4$ greatly increased penetration of ^{14}C-glyphosate at 2 hours after application. The other treatments gave similar results but the increases were lower (Table 1)

TABLE 1. EFFECTS OF VARIOUS ADDITIVES ON ^{14}C-GLYPHOSATE PENETRATION INTO PURPLE NUTSEDGE LEAVES AT 2 HOURS AFTER APPLICATION

Treatment	% absorption
Glyphosate 1.5 kg a.e./ha $(NH_4)_2SO_4$ 1.0%	6.2 d[1]
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 0.25%	24.8 bcd
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 0.5%	35.2 abc
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 1.0%	26.5 bcd
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 2.0%	26.7 bcd
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 4.0%	29.9 bcd
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 0.25% + diesel oil 0.25%	15.5 cd
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 0.5% + diesel oil 0.5%	38.6 abc
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 1.0% + diesel oil 1.0%	58.0 a
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 2.0% + diesel oil 2.0%	39.6 abc
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 4.0% + diesel oil 4.0%	46.9 ab
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Triton X-100 1.0% + diesel oil 1.0%	47.4 ab

[1]Means in the same column followed by different letters are significantly different ($p < 0.05$).

TABLE 2. EFFECTS OF VARIOUS ADDITIVES ON ^{14}C-GLYPHOSATE PENETRATION INTO PURPLE NUTSEDGE LEAVES AT 24 HOURS AFTER APPLICATION

Treatment	% absorption
Glyphosate 1.5 kg a.e./ha	63.8 bc[1]
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0%	68.2 ab
Glyphosate 1.5 kg a.e./ha + Triton X-100 2.0%	66.2 b
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Triton X-100 2.0%	78.2 a
Glyphosate 1.5 kg a.e./ha + Trion x-100 2.0%+diesel oil 1.0%	66.3 b
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Triton X-100 2.0% + diesel oil 1.0%	72.7 ab
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Sunlite 4.0%	63.7 bc
Glyphosate 1.5 kg a.e./ha + Sunlite 4.0% + diesel oil 1.0%	54.5 c
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Sunlite 4.0% + diesel oil 1.0%	65.9 b

[1]Means in the same column followed by different letters are significantly different ($p < 0.05$).

At 24 hours after application, $(NH_4)_2SO_4$ alone increased ^{14}C-glyphosate penetration into purple nutsedge leaves but not Triton X-100 or Triton X-100 + oil (Table 2).

However, $(NH_4)_2SO_4$ in combination with Triton X-100 increased ^{14}C-glyphosate penetration into nutsedge leaves more than $(NH_4)_2SO_4$ or Triton X-100 alone or Triton X-100 + oil

(Table 2). $(NH_4)_2SO_4$ + Triton X-100 + oil increased ^{14}C-glyphosate penetration into nutsedge leaves to the same degree as $(NH_4)_2SO_4$ + Triton X-100 (Table 2). Sunlite in combination with $(NH_4)_2SO_4$ or diesel oil or both did not increase ^{14}C-glyphosate penetration into nutsedge leaves (Table 1).

At 2 hours after application, with the concentration of $(NH_4)_2SO_4$ at 1.0%, Tendal at 1.0% increased ^{14}C-glyphosate penetration more than Tendal at 0.5% (Table 3).

TABLE 3. EFFECTS OF $(NH_4)_2SO_4$, TENDAL, AND DIESEL OIL ON ^{14}C-GLYPHOSATE PENETRATION INTO PURPLE NUTSEDGE LEAVES AT 2 HOURS AFTER APPLICATION

Treatment	% absorption
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 0.5%	21.6 b[1]
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 1.0%	29.8 ab
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 1.0% + diesel oil 0.25%	19.5 b
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 1.0% + diesel oil 0.5%	25.7 b
Glyphosate 1.5 kg a.e./ha + $(NH_4)_2SO_4$ 1.0% + Tendal 1.0% + diesel oil 1.0%	42.4 a

[1]Means in the same column followed by different letters are significantly different ($p < 0.05$).

Furthermore, when the concentration of $(NH_4)_2SO_4$ was at 1.0% and Tendal was at 1.0%, oil at 1.0% increased ^{14}C-glyphosate penetration more than oil at 0.25 or 0.5% (Table 3).

The increase in ^{14}C-glyphosate penetration into nutsedge leaves when using $(NH_4)_2SO_4$ might be due to a change of the glyphosate molecule to a more readily absorbed form. NMR spectroscopy showed that NH_4^+ from $(NH_4)_2SO_4$ complexed directly with the glyphosate molecule through the phosphonate and carboxylate groups and resulted in a more readily absorbed form of glyphosate [5]. It was also found that a nonionic organosilicone adjuvant increased ^{14}C-glyphosate absorption into sunflower leaves However, the organosilicone adjuvant did not directly interact with glyphosate [6]. The organosilicone adjuvants might alter the physical properties of the spray solution or the leaf cuticle to the point where ^{14}C-glyphosate could readily penetrate the leaf.

Oils have seldom been tested with water soluble herbicides, although glyphosate efficacy against wheat was increased by both petroleum and seed oils The main action of adjuvant oils was increasing herbicide penetration but, the mechanisms involved are poorly understood [9].

3.2. Greenhouse experiment

At 7 days after application, $(NH_4)_2SO_4$ at 1.0% + oil 1.0% + Tendal at 0.12% or 0.25% increased nutsedge control by glyphosate at 0.75 kg a.e./ha . However, additives did not increase the activity of glyphosate at the higher rate (Table 4).

At 14 days after application, $(NH_4)_2SO_4$ at 1.0% + oil at 1.0% + Tendal at 0.12 or 0.25 or 1.0% increased the activity of glyphosate at 0.5 and 0.75 kg a.e./ha However, at 21 days after application, the additives increased activity of glyphosate only at 0.5 kg a.e./ha (Table 4).

TABLE 4. PURPLE NUTSEDGE CONTROL WITH GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES UNDER GREENHOUSE CONDITIONS

Glyphosate (kg a.e./ha)	$(NH_4)_2SO_4$ (% v/v)	Diesel oil (% v/v)	Tendal (% v/v)	Days after application[1] 4	7	14	21
0.5	-	-	–	18 d[2]	45 e	59 c	81 b
0.75	–	–	–	23 bcd	43 e	69 bc	83 a
1.5	–	–	–	28 a–d	65 abc	98 a	100 a
0.5	1	1	0.12	20 cd	45 e	89 a	93 ab
0.75	1	1	0.12	28 a–d	63 a–d	90 a	95 ab
1.5	1	1	0.12	35 ab	75 a	100 a	100 a
0.5	1	1	0.25	25 a–d	53 cde	83 ab	90 ab
0.75	1	1	0.25	30 abc	68 abc	95 a	100 a
1.5	1	1	0.25	33 abc	73 ab	99 a	100 a
0.5	1	1	1	23 bcd	58 b–c	79 ab	93 ab
0.75	1	1	1	25 a–d	48 d–e	82 ab	90 ab
1.5	1	1	1	38 a	73 ab	91 a	91 ab
Nontreated	–	–	–	0 e	0 f	0 d	0 c

[1]% Weed control ; 0 = no control, 100 = complete control.

[2]Means in the same column followed by different letters are significantly different ($p < 0.05$).

The additives increased glyphosate phytotoxicity on nutsedge plants, but dry weight and number of tubers were not affected (Table 5).

TABLE 5. EFFECT OF GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES ON DRY WEIGHT AND NUMBER OF PURPLE NUTSEDGE TUBERS UNDER GREENHOUSE CONDITIONS

Glyphosate (kg a.e./ha)	$(NH_4)_2SO_4$ (% v/v)	Diesel oil (% v/v)	Tendal (% v/v)	Dry weight[1] (g/pot)	Number[2] of tubers/pot
0.5	–	–	–	0.43 b[3]	6 b
0.75	–	–	–	0.64 b	8 b
1.5	–	–	–	0.79 b	4 b
0.5	1	1	0.12	0.31 b	7 b
0.75	1	1	0.12	0.39 b	5 b
1.5	1	1	0.12	0.44 b	3 b
0.5	1	1	0.25	0.31 b	5 b
0.75	1	1	0.25	0.53 b	6 b
1.5	1	1	0.25	0.43 b	4 b
0.5	1	1	1	0.64 b	4 b
0.75	1	1	1	0.45 b	6 b
1.5	1	1	1	0.34 b	4 b
Nontreated	–	–	–	4.31 a	23 a

[1]At 21days after application.

[2]At 60 days after application.

[3]Means in the same column followed by different letters are significantly different ($p < 0.05$).

3.3. Field experiments

At 1, 2, and 4 weeks after application, all combinations of $(NH_4)_2SO_4$ + diesel oil and Tendal did not increase the control of nutsedge by glyphosate in either cropped or uncropped plots (Tables 6–8). However, the treatment which contained all the additives increased

TABLE 6. PURPLE NUTSEDGE CONTROL WITH GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES AT 1 WEEK AFTER APPLICATION

Glyphosate (kg a.e./ha)	$(NH_4)_2SO_4$ (% v/v)	Diesel oil (%v/v)	Tendal (%v/v)	Time of application	% Weed control[1] with corn	without corn	T-test
1.5	–	–	–	9.00 a.m.	53 ab[2]	53 ab	ns[3]
1.5	–	–	–	4.00 p.m.	60 a	60 a	ns
0.5	1	1	1	9.00 a.m.	43 b	43 b	ns
0.5	1	1	1	4.00 p.m.	41 b	41 b	ns
0.75	1	1	1	9.00 a.m.	48 ab	48 ab	ns
0.75	1	1	1	4.00 p.m.	41 b	41 b	ns
1.5	1	1	1	9.00 a.m.	59 a	59 a	ns
1.5	1	1	1	4.00 p.m.	40 b	40 b	ns
Weeded	–	–	–	–	0c	0c	ns
Nonweeded	–	–	–	–	0c	0c	ns

[1]% weed control ; 0 = no control, 100 = complete control.
[2]Means in the same column followed by different letters are significantly different (p <0.05).
[3]Comparison of means within the same line; ns = not significantly different (p >0.05).

TABLE 7. PURPLE NUTSEDGE CONTROL WITH GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES AT 2 WEEKS AFTER APPLICATION

Glyphosate (kg a.e./ha)	$(NH_4)_2SO_4$ (% v/v)	Diesel oil (%v/v)	Tendal (%v/v)	Time of application	% Weed control[1] with corn	without corn	T-test
1.5	–	–	–	9.00 a.m.	63 bc[2]	63 bc	ns[3]
1.5	–	–	–	4.00 p.m.	74 b	71 b	ns
0.5	1	1	1	9.00 a.m.	43 d	41 e	ns
0.5	1	1	1	4.00 p.m.	44 d	45 de	ns
0.75	1	1	1	9.00 a.m.	50 cd	48 cde	ns
0.75	1	1	1	4.00 p.m.	46 d	43 e	ns
1.5	1	1	1	9.00 a.m.	70 b	68 b	ns
1.5	1	1	1	4.00 p.m.	61 bc	59 bcd	ns
Weeded	–	–	–	–	100 a	100 a	ns
Nonweeded	–	–	–	–	0e	0f	ns

[1]% weed control ; 0 = no control, 100 = complete control.
[2]Means in the same column followed by different letters are significantly different (p <0.05).
[3]Comparison of means within the same line; ns = not significantly different (p >0.05).

TABLE 8. PURPLE NUTSEDGE CONTROL WITH GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES AT 4 WEEKS AFTER APPLICATION

Glyphosate (kg a.e./ha)	$(NH_4)_2SO_4$ (% v/v)	Diesel oil (%v/v)	Tendal (%v/v)	Time of application	% Weed control[1] with corn	without corn	T-test
1.5	–	–	–	9.00 a.m.	62 abc[2]	59 abc	ns[3]
1.5	–	–	–	4.00 p.m.	79 a	75 a	ns
0.5	1	1	1	9.00 a.m.	35 d	38 d	ns
0.5	1	1	1	4.00 p.m.	43 cd	40 cd	ns
0.75	1	1	1	9.00 a.m.	41 d	40 cd	ns
0.75	1	1	1	4.00 p.m.	35 d	33 d	ns
1.5	1	1	1	9.00 a.m.	68 ab	63 ab	ns
1.5	1	1	1	4.00 p.m.	54 bcd	48 bcd	ns
Weeded	–	–	–	–	34 d	28 d	ns
Nonweeded	–	–	–	–	0e	0e	ns

[1]% weed control ; 0 = no control, 100 = complete control.
[2]Means in the same column followed by different letters are significantly different (p <0.05).
[3]Comparison of means within the same line; ns = not significantly different (p >0.05).

TABLE 9. EFFECT OF GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES ON DRY WEIGHT AND NUMBER OF PURPLE NUTSEDGE PLANTS IN UNCROPPED AREA

Glyphosate (kg a.e./ha)	$(NH_4)_2SO_4$ (%v/v)	Diesel oil (%v/v)	Tendal (%v/v)	Time of application	Dry weight[1] (g/0.25m^2)	% reduction of plant number[2]	Dry weight[3] (g/0.25m^2)	% reduction of plant number[4]
1.5	–	–	–	9.00 a.m.	7.6 b[5]	–32.6	20.8	–15.0
1.5	–	–	–	4.00 p.m.	8.3 b	–18.6	20.3	7.6
0.5	1	1	1	9.00 a.m.	26.7 ab	–182.4	26.3	–103.4
0.5	1	1	1	4.00 p.m.	29.4 ab	–122.0	32.3	–50.7
0.75	1	1	1	9.00 a.m.	15.8 b	–115.0	23.1	–54.2
0.75	1	1	1	4.00 p.m.	18.3 b	–202..5	35.9	–147.3
1.5	1	1	1	9.00 a.m.	23.0 ab	–42.0	19.4	–19.5
1.5	1	1	1	4.00 p.m.	13.2 b	–129.8	25.7	–91.5
Weeded	–	–	–	–	19.7 b	–204.8	37.8	–147.7
Nonweeded	–	–	–	–	44.5 a	–194.4	45.9	–79.5
					–	NS	NS	NS

[1]At 4 weeks after application.
[2]At 6 weeks after application.
[3]At 8 weeks after application.
[4]At 10 weeks after application.
[5]Means in the same column followed by different letters are significantly different ($p < 0.05$).

nutsedge control in the cropped plots more than uncropped plots, at 8 weeks after application (Table 10), presumably because of the effect of crop competition. With one exception, there was no difference between the application in the morning and the afternoon (Tables 6–9).

The additives did not increased activity of glyphosate as measured by either dry weight at 4 and 8 weeks after application or number of nutsedge shoots at 6 and 10 weeks after application (Table 9) and at harvesting (Table 11). Glyphosate alone or in combination with additives increased the weight of corn grain and corn yield (Table 12).

TABLE 10. PURPLE NUTSEDGE CONTROL WITH GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES AT 8 WEEKS AFTER APPLICATION

Glyphosate (kg a.e./ha)	$(NH_4)_2SO_4$ (% v/v)	Diesel oil (%v/v)	Tendal (%v/v)	Time of application	% Weed control[1] with corn	% Weed control[1] without corn	T-test
1.5	–	–	–	9.00 a.m.	38 bcd[2]	30 bcd	ns[3]
1.5	–	–	–	4.00 p.m.	61 a	51 a	ns
0.5	1	1	1	9.00 a.m.	15 ef	10 ef	ns
0.5	1	1	1	4.00 p.m.	21 de	13 def	ns
0.75	1	1	1	9.00 a.m.	31 de	21d–e	ns
0.75	1	1	1	4.00 p.m.	29 de	18c–f	ns
1.5	1	1	1	9.00 a.m.	50 abc	31 bc	*
1.5	1	1	1	4.00 p.m.	55 ab	36 ab	*
Weeded	–	–	–	–	24 cd	30 bcd	ns
Nonweeded	–	–	–	–	0 f	0	ns

[1]% weed control ; 0 = no control, 100 = complete control.
[2]Means in the same column followed by different letters are significantly different ($p < 0.05$).
[3]Comparison of means within the same line;* = significantly different ($p < 0.05$).
ns = not significantly different ($p > 0.05$).

TABLE 11. EFFECT OF GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES ON DRY WEIGHT AND NUMBER OF PURPLE NUTSEDGE PLANTS IN CORN AREA AT HARVESTING

Glyphosate (kg a e /ha)	$(NH_4)_2SO_4$ (%v/v)	Diesel oil (%v/v)	Tendal (%v/v)	Time of application	Dry weight ($g/0\ 25m^2$)	% reduction of plant number
1 5	–	–	–	9 00 a m	3.0	51 0
1 5	–	–	–	4 00 p.m.	1.1	72 7
0 5	1	1	1	9.00 a.m	6.3	42 6
0 5	1	1	1	4.00 p.m	3.3	57 1
0 75	1	1	1	9.00 a.m	4 0	48 1
0 75	1	1	1	4.00 p.m.	4.7	38 3
1.5	1	1	1	9.00 a.m.	2 9	57 4
1 5	1	1	1	4.00 p m	3 0	48 7
Weeded	–	–	–	–	2 0	37 6
Nonweeded	–	–	–	–	3 9	49 3

TABLE 12. EFFECT OF PURPLE NUTSEDGE CONTROL WITH PREPLANTING APPLICATION OF GLYPHOSATE IN COMBINATION WITH VARIOUS ADDITIVES ON YIELD OF CORN

Glyphosate (kg a e./ha)	$(NH_4)_2SO_4$ (%v/v)	Diesel oil (%v/v)	Tendal (%v/v)	Time of application	Weight of grain from 10 ears (g)	Yield (kg/ha)
1.5	–	–	–	9 00 a m	1,270 a[1]	4,270 a
1.5	–	–	–	4 00 p m	1,360 a	4,640 a
0.5	1	1	1	9 00 a m	1,210 a	3,850 a
0.5	1	1	1	4 00 p.m	1,310 a	4,230 a
0.75	1	1	1	9 00 a m	1,080 a	3,810 a
0.75	1	1	1	4.00 p m.	1,210 a	4,460 a
1.5	1	1	1	9 00 a.m	1,230 a	4,250 a
1 5	1	1	1	4 00 p m	1,310 a	4,710 a
Weeded	–	–	–	–	1,130 a	3,760 a
Nonweeded	–	–	–	–	799 b	2,250 b

[1]Means in the same column followed by different letters are significantly different ($p < 0\ 05$)

In the field experiment the additives did not affect glyphosate activity. This might be due to the quality of the water. Well water from the field was used for mixing the spray solution. That water might have contained cations such as Ca^{+2} and Mg^{+2}. Even though, $(NH_4)_2SO_4$ was added to spray solution, the molar ratio necessary to overcome Ca^{+2} must be 3:1 ($(NH_4)_2SO_4$:$CaCl_2$) [5]. Furthermore, glyphosate does not have soil activity, and nutsedge tubers in the field did not germinate at the same time, so late germinating tubers did not come into contact with spray droplets.

4. CONCLUSIONS

The addition of $(NH_4)_2SO_4$ at 1.0% + oil at 1.0% + Tendal at 1.0% increased ^{14}C-glyphosate penetration into nutsedge leaves more than the addition of either one alone.

The addition of $(NH_4)_2SO_4$ at 1.0% + oil at 1.0% + Tendal at 0.12 or 0.25 or 1.0% increased phytotoxicity of glyphosate at 0.5 and 0.75 kg a.e./ha at 7 and 14 days after application in the greenhouse, but not in the field. Additives in combination with glyphosate did not reduce the number of nutsedge tubers compared with glyphosate alone.

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Appendix

EXPERIMENTAL PROTOCOLS

1. FIRST PHASE

1.1. Leaf penetration study

The herbicide used will be glyphosate as "Roundup" and the test plant will be *Cyperus rotundus.*

The additives (of low, medium and high HLB) will be Synperonic A2, A7 and A20 used at 0.1% in the spray solution. Each will be used ± 1% $(NH_4)_2SO_4$, this also applies to unamended "Roundup".

Optional additional additives are 1% glycerol and 1% diesel oil.

The constituents should be mixed in the following sequence:

"Roundup" + surfactant + (oil/glycerol) + water + $((NH_4)_2SO_4$

Each treatment should be replicated four times, so that there will be 8 × 4 treatment replicates + 4 untreated controls.

"Roundup" and Synperonic surfactants will be supplied.

The experiment should be done in both wet and dry seasons for 2 years.

Tubers should be pregerminated in wet blotting paper or equivalent. Mean tuber weight should be recorded first and the range of tuber weights should be restricted to not more than ±10% of the mean. After germination, sow tubers 1–2 cm deep in a medium containing 50% sand in 7–10 cm pots. Plant more pots than will be needed, so that plants can be selected for uniformity before treatment. If possible, keep records of max.–min. temperature, rainfall, humidity, day-length and whether shaded or not. Watering should be from above.

The 2nd leaf should be treated when the plant is at the 5–6 leaf stage. The "Roundup" solution should be equivalent to 1.5 kg a.e./200 L (of formulation) and contain 1.85 kBq/0.05 mCi/mL = 0.169 mg/mL of labelled material (assuming we have a sp.act. of 50 – 60 mCi/mmole). Thus, an application of 4 mL will contain 30 mg a.e. glyphosate + 0.67 mg (7.2 kBq/0.2 mCi) labelled material. These quantities are calculated on the basis that samples will be oxidized before counting. If this is not possible, and for autoradiography experiments, the activity should be increased to 18.5 kBq/0.5 mCi per treatment (1.675 mg or 0.4188 mg/mL).

The volume to make up should be as small as possible to avoid wasting radioactive material. Glyphosate supplied by Amersham contains 1.85 MBq/50mCi in 250 mL per vial. The following dilutions are suggested:

(a) For a standard treatment of 7.2 kBq/0.2 mCi per 4 mL:
25 ml labelled glyphosate solution (° 185 kBq/5 mCi)
25 ml Roundup solution (1 mL 48% diluted to 16 mL to give 30 mg/mL; 25 mL contains 750 mg)
25 mL 0.4% surfactant
25 mL water or 4% $(NH_4)_2SO_4$.

Therefore, 1 mL of this mixture will contain 1.85 kBq/0.05 mCi labelled glyphosate, 7.5 mg cold glyphosate, 0.1% surfactant and 1% $(NH_4)_2SO_4$.

(b) For treatments including glycerol and diesel oil, this mixture could be modified to contain:
25 mL labelled glyphosate
25 mL Roundup (diluted 1:16)
20 mL 0.5% surfactant
10 mL 10% glycerol or 1 ml diesel oil + 9 mL water
20 mL 5% $(NH_4)_2SO_4$.

(c) For the higher activity treatment of 18.5 kBq/0.5 mCi per 4 mL:
25 mL labelled glyphosate
5 mL Roundup (1 mL diluted to 8 mL)
5 mL 0.8% detergent
5 mL 8% $(NH_4)_2SO_4$ or 5 mL water.

Apply the treatment in 8 × 0.5 mL drops to the upper surface on either side of the in midrib in a 2 cm area starting 10 cm from the top. ***Do not touch the leaf***. (A Hamilton 25 mL syringe with dispenser and a blunt needle will be supplied.)

Put 2 × 4 mL into scintillation vials at the same time in order to check application rate.

Record time of day, temperature and humidity. After treatment, pots should stand in a saucer or dish.

Plants should be harvested for assessment at 24 h (record exact time) and for shorter and longer periods if possible.

All procedures should be practised first to avoid waste of ^{14}C-glyphosate and so that the time interval between treating plants corresponds with the time needed for harvest.

At harvest, paint over treated area with cellulose acetate dissolved in 9:1 acetone/water. About 6% is the concentration required but exact concentration depends on the conditions. It must dry in 2 min. with a thick film. Strip off with tweezers, dissolve in 2 mL glacial acetic acid and add 5–10 mL scintillation fluid for counting.

As soon as the cellulose acetate has been removed, cut the leaf into part beyond treated zone, treated zone and area between stem and treated zone. Remove soil from plant roots, flatten the plant between blotting paper and put into deep freezer.

Subsequently, plants can be cut systematically for tissue analysis.

It is recommended that some additional plants are treated for autoradiography.

1.2. Additional experiment to assess the effect of overall treatment

Spray Roundup on plants at 1.5 kg/ha in 200 L/ha after covering area to be treated with e.g. plastic (non-adhesive) tape. Treat as soon as possible with Roundup containing ^{14}C-glyphosate as before. Include 4 plants (not treated with ^{14}C) to grow on to assess phytotoxicity.

2. SECOND PHASE

2.1. Introduction

A conclusion at the 2nd RCM in Bangkok was that glyphosate was easily translocated once inside the plant, so that penetration of the leaf was the critical step. Therefore, measurement of penetration in 24 h could be used as a primary screening procedure for formulation additives and a measurement after 2 or even 1 h would give a secondary screen.

The experiments carried out so far showed that oil and ammonium sulfate, alone or together, gave useful increases in penetration, so the second phase of the programme would concentrate on the effects of these as they should be readily available in most countries, although the cheapest oil will not be the same everywhere.

Additional surfactant will be needed to emulsify the oil. The first experiments would use Triton X-100 for this purpose but later work will try to identify a suitable locally available surfactant, such as washing-up liquid.

2.2. Laboratory experiments

2.2.1. Estimation of the amount of Triton X-100 needed to emulsify 1% oil in "Roundup" in the presence of $(NH_4)_2SO_4$

The basic mixture will be:
54 mL water
25 ml "Roundup" (*diluted 1:12 to give 30 mg a.e./mL)
1 mL oil (diesel or vegetable whichever is cheapest)
20 mL 5% $(NH_4)_2SO_4$
[*Note, the earlier protocol was wrong: "Roundup" contains 48% of the isopropylamine salt, not the acid, so 30 mg/ml a.e. requires a dilution of 1:12 not 1:16. Thanks are due to Rungsit Suwanketnikom for drawing attention to this.]

As a first step, add 0.01, 0.05, 0.1, 0.2 and 0.5 mL of Triton X-100 to 100 mL of this mixture, shake and allow to stand overnight. If 0.01 mL holds the emulsion then repeat the experiment with smaller amounts, say 0.005 and 0.001 mL or even less until the emulsion does not hold. Use the lowest quantity of Triton X-100 that will maintain the emulsion.

2.2.2. 24 h penetration studies

Germinate tubers and grow plants as in the previous set of experiments, planting about 3 times as many tubers as will be needed, so that uniform plants can be selected for the experiments.

There should be six plants per treatment and each experiment should be repeated on 4 different days. The herbicide application should be done at the same time of day on each occasion.

Plants should be used when they have 6 leaves and the second oldest leaf should be used.

The first treatments should be:

A. "Roundup" alone: 2.5 µL ^{14}C-glyphosate solution + 25 µL "Roundup" diluted 1:12 + 72.5 µL water (i.e. 0.185 kBq/0.005 µCi labelled glyphosate/µL which is one-tenth of the activity used before).

B. "Roundup" + ammonium sulfate: As for A above but replace 25 µL of the water with 25 µL 4% $(NH_4)_2SO_4$.

C. "Roundup" + Triton X-100: As for A with addition of the amount of Triton X-100 determined in 1) above.

D. "Roundup" + Triton X-100 + $(NH_4)_2SO_4$: As for B with the addition of Triton X-100 as in C.

E. "Roundup" + Triton X-100 + oil: As for C but replace 1 µL of the water with 1 µL oil.

F. "Roundup" + Triton X-100 + oil + $(NH_4)_2SO_4$: As for E but replace 25 µL of the water with 25 µL 4% $(NH_4)_2SO_4$.

As in the first phase experiments, apply 8 x 0.5 µL drops on either side of the midrib in a 2 cm area starting 10 cm from the tip without touching the leaf. At the same time, put 4 µL aliquots into 2 scintillation vials to check the application rate. The time interval between treating successive plants should be at least as long as that needed for the cellulose acetate harvesting procedure (below).

After 24 h, paint the treated area with cellulose acetate in 9:1 acetone/water (about 6% but adjust concentration, if necessary, so that it dries in 2 min, remove with tweezers, dissolve in 2 mL glacial acetic acid and add 5 – 10 mL scintillation fluid for counting.

Also apply cellulose acetate to untreated leaves, strip off and dissolve as before but add 8µL of the glyphosate treatment solution. Do this for each treatment solution to check for quenching.

2.2.3. Modification of treatment mixtures

Select the best treatment from experiment 2 and vary the quantities of additives to see if this affects penetration. If all treatments are similar, concentrate on "Roundup" plus $(NH_4)_2SO_4$, and "Roundup" plus locally available surfactants. If the best treatment contains oil, again try local surfactants and also look at other oils if available.

2.2.4. Short-term penetration studies

Select the best 2 or 3 treatments from 3 and measure penetration over 1 h and 2 h.

2.3. Field experiments

It was agreed to test the best modified formulation at rates of 1.5, 0.75 and 0.5 kg/ha a.e. compared with 1.5 kg a.e. unamended "Roundup". Application rate should be 200 L/ha and please specify nozzle type and pressure that you use.

There will be 2 times of application, before 9 a.m. and after 4 p.m. and cropped (with maize) and uncropped plots.

Thus, there will be untreated control + 4 glyphosate treatments × 2 times of application × cropped/uncropped = 5 × 2 × 2 = 20 treatments. With 4 replicates, this gives 80 plots. In addition, the cropped plots should include a hand-weeded control, so a total of 84 plots will be necessary. The uncropped plots will be 3 × 2 m and the cropped plots 5 × 5 m. Layout should be split-plot (cropped/uncropped).

Where available, use a natural stand of *Cyperus*, otherwise sow tubers at 20/m^2 a year before the experiment is due to begin.

Plough, prepare seed bed and irrigate according to local practice. Apply glyphosate treatment 3 weeks later but 1–3 d before spraying count number of *Cyperus* shoots in three 0.25 m quadrats per plot.

Sow the crop 3 d after glyphosate application. Use insecticides as necessary.

Assessments: Assess cropped and uncropped plots on the Weed Science visual scale at 4 and 8 weeks. In uncropped plots, harvest *Cyperus* from half the plot after 4 weeks and the other half after 8 weeks; record fresh and dry wt. Measure crop yield and cut all vegetation to 1–2 cm above ground. Assess visually after 2 weeks and leave as long as possible with occasional assessment. In countries where *Cyperus* goes to seed observe if there is a treatment effect.

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