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(54) Title: ADJUVANTS CONTAINING UNSATURATED LIPID

(57) Abstract

The invention is directed to the use of unsaturated lipid(s) having 4 or more sites of unsaturation in the preparation of an adjuvant. The invention provides an adjuvant containing a lipid component, at least 5 % by weight of which is such unsaturated lipid(s), active compositions containing the adjuvant and methods of use of the adjuvant to enhance the uptake of active ingredients through a hydrophobic biological surface.

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ADJUVANTS CONTAINING UNSATURATED LIPID

TECHNICAL FIELD

This invention relates to an adjuvant and the use thereof. In particular, it relates to the use of unsaturated lipid as an additive to sprays to enhance uptake of active ingredients contained in the spray.

BACKGROUND

Hydrophobic biological surfaces constitute barriers which are of considerable significance in the agricultural, horticultural and animal health industries. For example, the hydrophobic waxy cuticle of the plant leaf acts as a barrier to absorption of both systemic nutrients and agrochemicals into the interior of the leaf. Nutrients and agrochemicals not immediately absorbed are therefore exposed to the effects of weathering and are often blown or washed off the leaf before absorption can occur. This results in considerable wastage of these ingredients which in turn increases the expense to the user.

Further examples of hydrophobic surfaces of significance are the waxy insect cuticle and animal skin. Both surfaces similarly provide barriers to the absorption of active ingredients of compositions such as insecticides and animal medicines. Again, this results in considerable wastage of the active ingredient which both reduces the efficacy of the composition applied and increases the cost to the user.

The inclusion of adjuvants that enhance the surface active properties of sprays containing active ingredients is now gaining wide acceptance as a means of ensuring the active ingredient reaches the target. For example agricultural sprays containing surfactants, wetters, humectants, stickers, osmoprotectants and penetrants are commonly used to enhance the uptake of the active ingredient by the plant through the leaf surface.

It will therefore be appreciated that a number of adjuvants which enhance the uptake of active ingredients through leaf surfaces are known. Many of these known adjuvants can be classified chemically as members of the non-ionic surfactant group. Examples of such adjuvants commonly in use are those compositions sold under the trade marks Contact (ICI), Citowet (BASF) and Pulse (Monsanto).

A further example of an adjuvant which comprises a component of a foliage fertilising system is that described in Australian Patent Specification No. AU-B-14270/83, Nattermann & Cie GMBH). This specification teaches the use of a polar phospholipid in the preparation of a foliar fertiliser system. The phospholipid is claimed to reduce phytotoxicity and prevent the fertilising nutrients from being washed or blown off sprayed foliage, thus achieving speedy penetration into the plant.

An alternative adjuvant available commercially in New Zealand under the trade name C-Daxoil contains vegetable oil as the surface active ingredient. Accordingly, while this adjuvant does contain a lipid component, the lipid component is either saturated lipid or predominantly unsaturated lipid with one or two sites of unsaturation. The percentage content of highly unsaturated lipid (unsaturated lipids having four or more sites of unsaturation) in the lipid component is very low, being less than 0.5% by weight.

While the products exemplified above to possess advantageous properties and do to some extent enhance transport of other ingredients across the leaf barrier, it has surprisingly been found by the applicant that superior results can be achieved through the use of highly polyunsaturated lipid. It is this finding upon which the applicant's invention is broadly based.

The applicant has also determined that these superior results are only achieved when a minimum amount of highly polyunsaturated lipid is incorporated into the adjuvant. In particular, the applicant has determined that it is essential for the adjuvant to comprise a lipid component comprising at least 5% by weight of unsaturated lipid having four or more sites of unsaturation.

It is therefore an object of the invention to promote or enhance the uptake of substances through hydrophobic biological surfaces by the provision of an adjuvant comprising a lipid component containing highly unsaturated lipids, or at least to provide the public with a useful choice.

SUMMARY OF THE INVENTION

Accordingly, in one aspect the present invention consists in the use of unsaturated lipid(s) having 4 or more sites of unsaturation in the preparation of a lipid-containing adjuvant for enhancing the uptake of an active ingredient through a hydrophobic biological surface.

In a further aspect, the invention consists in an adjuvant for combination with an active ingredient to enhance the uptake of said active ingredient through a hydrophobic biological surface, said adjuvant including an effective amount of an antioxidant and a lipid component, at least 5% by weight of the lipid component being unsaturated lipid(s) having 4 or more sites of unsaturation.

Conveniently, the adjuvant will further include an emulsifying agent, a stabiliser, and a preservative.

In yet a further aspect, the invention consists in an adjuvant for enhancing the uptake of an active ingredient through a hydrophobic biological surface, said adjuvant comprising a lipid component being at least 5% by weight unsaturated lipid(s) having 4 or more sites of unsaturation and with at least a major proportion of said unsaturated lipid(s) being in the form of free fatty acids.

In still a further aspect, the invention consists in an active composition for application to a hydrophobic biological surface which comprises an effective amount of an active ingredient, and an adjuvant as defined above in an amount sufficient to enhance the uptake of said active ingredient through said surface.

In yet a further aspect, the invention provides a method for producing an active composition for application to a hydrophobic biological surface comprising the step of combining an effective amount of an active ingredient, an effective amount of an antioxidant and an amount of a lipid component sufficient to enhance the uptake of said active ingredient through said surface, said lipid component being at least 5% by weight of unsaturated lipid(s) having 4 or more sites of unsaturation.

In still a further aspect, the present invention provides a method of enhancing the uptake of an active ingredient through a hydrophobic biological surface which comprises the step of applying said active ingredient to said surface together with an adjuvant as defined above.

In a preferred embodiment, the hydrophobic biological surface is

the waxy plant cuticle, typically the leaf cuticle. In other embodiments, the hydrophobic biological surface is the insect cuticle or animal skin.

In a final aspect, the invention consists in a method for preparing an adjuvant for enhancing the uptake of an active ingredient through a hydrophobic biological surface, said adjuvant comprising a lipid component being at least 5% by weight unsaturated lipid(s) having 4 or more sites of unsaturation and with at least a major proportion of said unsaturated lipid(s) being in the form of free fatty acids, comprising the steps of:

(a) forming a solution which comprises disintegrated fish containing a lipid component in acid;

(b) holding said solution to hydrolyse said lipid component into free fatty acids, said hydrolysis step being of a duration such that at least a major proportion of the lipid component following hydrolysis is in the form of free fatty acids; and

(c) recovering the lipid component from the solution.

Although the present invention is broadly as defined above, it will be appreciated by those persons skilled in the art that it is not limited thereto and that it further includes the embodiments of which the following description provides examples.

DESCRIPTION OF THE INVENTION

The present invention is broadly directed to the problem of promoting or enhancing the absorption of substances into plants, insects or animals through their hydrophobic external surfaces. This is a problem common to all active compositions which are applied in the form of a spray and arises due to the nature of the barrier to absorption presented by the hydrophobic surfaces. More particularly, the hydrophobic surface blocks or impedes the passage of many substances from the surface into the interior of the plant, insect or animal, causing the substances to be retained on the external surface and to therefore be exposed to the external environment. In this way, such substances are either blown or washed off the external surface through the actions of wind, rain or movement before they can be absorbed.

The hydrophobic biological surfaces which are the particular focus of the present invention are those provided on the aerial parts of a

plant such as the waxy leaf cuticle; the insect cuticle; and animal skin. All of these surfaces have a high lipid content and resist the passage of solid or polar substances.

The present applicant has now surprisingly determined that the absorption of such substances into the plant, insect or animal through their external hydrophobic surfaces can be enhanced through the inclusion in the active composition to be sprayed onto the plant, insect or animal of an adjuvant including a lipid component, at least 5% by weight of the lipid component being unsaturated lipid(s) having 4 or more sites of unsaturation. It is this determination by the applicant upon which the present invention is generally based.

Accordingly, in one aspect, the present invention relates to the use of unsaturated lipid(s) having 4 or more sites of unsaturation in the preparation of an adjuvant. Such an adjuvant is to be combined with an active ingredient to form an active composition for application to hydrophobic biological surfaces with the adjuvant enhancing the uptake of the active ingredient through the hydrophobic surface to which the composition is applied.

It will be appreciated that the active ingredient to be combined with the lipid adjuvant in the formation of the active composition can be of any type conventionally applied in the agricultural, horticultural and animal health industries. The term "active ingredient" therefore includes but is not limited to organic, inorganic or organomineral fertilisers containing nutrients for plant growth; agrochemicals such as plant protection agents, herbicides, growth regulators and defoliants; insecticides; and animal medicines such as pour-ons applied to the animal skin. The term also covers those additives conventionally provided as part of such fertiliser, agrochemical and insecticidal compositions, and as part of an animal medicine.

The unsaturated lipid for use in this aspect of the invention may be any known or available unsaturated lipid having the necessary degree of unsaturation. Examples of suitable unsaturated lipids are the unsaturated fatty acids (both as free fatty acids and as esters of glycerol in the form of neutral mono-, di- and tri-acyl glycerols), long chain hydrocarbons and alcohol-fatty acid derivatives such as waxes. Particularly preferred unsaturated lipids are the fatty acids arachidonic acid, ecosapentaneoic acid and docosahexaenoic acid.

The unsaturated lipids for use in this aspect of the invention may also be obtained from any suitable source. For example, the preferred longer chain fatty acids ecosapentaenoic acid and docosahexaenoic acid are obtainable from fish. Their extraction from such sources can be achieved by conventional techniques.

Although the lipid component employed in preparing the adjuvant can be of a single type (for example, a solution containing only ecosapentaenoic acid), it will be appreciated that mixtures of two or more unsaturated lipids can effectively be used. Indeed, mixtures containing a number of different unsaturated fatty acids are particularly effective.

The presently preferred unsaturated lipid additive is such a mixture. This mixture is the lipid component obtained from fish offal which is stored in acid, optionally in the presence of lipases and phospholipase. When stored under these conditions (e.g. for 2 weeks), chemical and enzymatic hydrolysis of both the mono-, di- and tri-acylglycerides and the polar phosphatidyl derivatives present in the fish offal occurs, resulting in a predominance of free fatty acids. Such free fatty acids can be extracted from the hydrolysed mixture as a part of the lipid component by techniques known in the art.

If it is desired that the lipid component be solely unsaturated lipid having 4 or more sites of unsaturation, the fatty acids having the necessary degree of unsaturation can then be separated from the remainder of the lipid component, again by conventional techniques. However, such separation is not always necessary where a significant proportion of the lipid component is unsaturated lipid having 4 or more sites of unsaturation. Indeed, it has been found by the applicant that a proportion of even polar lipid can be tolerated as a contaminant provided the lipid component as a whole contains at least 5% by weight of unsaturated lipid having 4 or more sites of unsaturation.

As determined by the applicant, there are a number of properties of unsaturated lipids which give rise to their ability to enhance the uptake of active ingredients through a hydrophobic surface. These properties will now be described with particular reference to the waxy cuticle of a plant leaf.

The first property is the low melting point of unsaturated lipids in general and of the preferred highly unsaturated fatty acids in

particular. This property ensures that the active ingredient does not solidify but instead remains in semi-liquid form on the leaf surface. This is particularly important in ensuring retention of the active ingredient on the leaf surface for the period necessary for its absorption as solids are easily blown or washed off the leaf.

A further property of unsaturated lipids is their capacity to dissolve other lipids including saturated lipids. The solvent ability of the unsaturated lipids results in the partial solubilisation of the leaf cuticle which itself includes a significant lipid component. This partial solubilisation facilitates the passage of the active ingredient from the leaf surface into the interior of the leaf.

A further most important property of highly unsaturated lipids is their general hydrophobicity with pockets of lesser hydrophobicity associated with sites of increased electron density. This property has a two-fold effect in that the active ingredient interacts hydrophobically with the lipid to form hydrophobic molecular clusters, which clusters can then readily penetrate the extremely hydrophobic cuticle of the leaf. In this way, active ingredients are "packaged" into micelle-like structures for transport into the leaf. Without the hydrophobic unsaturated lipid coating, the passage of such active ingredients would be impeded if not blocked by the leaf cuticle.

It will be appreciated that the applicant believes the critical features of the lipid component and the feature which gives rise to the advantages of the invention is the general unsaturation of the lipids, and the particularly high degree of unsaturation now claimed. However, it will further be appreciated that while the above properties are believed by the applicant to result in the effectiveness of highly unsaturated lipid in enhancing uptake through hydrophobic surfaces, the applicant is no way bound by such reasoning.

As a further aspect of the invention, there is provided an adjuvant suitable for combination with an active ingredient to form an active composition. When the adjuvant is present in the composition, the uptake of the active ingredient through a hydrophobic surface is enhanced.

The adjuvant includes lipid as the principal component. This lipid component will consist of at least 5% by weight of unsaturated lipid(s) having 4 or more sites of unsaturation, preferably at least 15% by weight

unsaturated lipid having 4 or more sites of unsaturation, more preferably at least 30% by weight unsaturated lipid having 4 or more sites of unsaturation, still more preferably at least 50% by weight unsaturated lipid having 4 or more sites of unsaturation, and most preferably at least 70% by weight unsaturated lipid having 4 or more sites of unsaturation. Such unsaturated lipids may be any of those described above with a mixture predominantly consisting of unsaturated low melting point free fatty acids derived from fish offal being preferred.

The adjuvant will also include an antioxidant component. This component is included in an amount effective to protect the highly labile unsaturated lipids from oxidation and breakdown.

Examples of suitable antioxidants are propyl gallate, ascorbic acid, ascorbyl palmitate, tocopherol, butylated hydroxytoluene, butylated hydroxyanisol and ethoxyquin.

The adjuvant will commonly also include a number of further components to maintain the lipid in a stable and commercially useful form. Such additional components are conveniently an emulsifying agent, a stabiliser and a preservative.

Each of these components can be selected from amongst those known in the art. For example, the emulsifying agent can be a surfactant (such as an ammonium phosphate, a polyalkoxyether, a polyalkoxyester, a polyalkoxyamide, a fatty acid ester of polyhydric alcohol or a fatty alcohol), a hydrophilic colloid (such as acacia gum, agar, carrageenan, an alginate, guar gum, xanthan gum, carboxymethyl ether or gelatin), or a finely divided solid (such as a heavy metal hydroxide).

The stabiliser can suitably be a polysaccharide hydrocolloid such as guar gum or gelatin.

The preservative component can be any component which can be added to prevent or at least substantially inhibit bacterial decomposition. Suitable preservatives include acidulants, alkalis, mono- and di-carboxylic acids and their derivatives, phytoncides and antioxidants with antimicrobial activity. Examples of acidulants include sulphuric acid, formic acid, acetic acid and lactic acid; examples of alkalis include CaO, $Ca(OH)_2$ and KOH; examples of mono- and di-carboxylic acids include propionic acid, benzoic acid, sorbic acid and salts thereof; and examples of phytoncides include allicin, cinnamaldehyde, anethole (-propenyl-

anisole) and linalool. Mixtures of these can be used where a broad spectrum antimicrobial activity is required.

It will be understood by those persons skilled in the art that a number of the additives listed above are capable of fulfilling dual functions in the adjuvant. For example, a number of the polysaccharide hydrocolloids are capable of functioning as both emulsifying agents and stabilisers.

In addition to the above components, the adjuvant may include an odour masking agent such as a perfume and/or a deodorising agent such as ferric or magnesium sulphate. This component is included where it is viewed necessary or desirable to mask the strong odour of the adjuvant composition.

In yet a further aspect, the invention provides an active composition. The composition includes as a first component an effective amount of an active ingredient as defined above. As a second component, the composition includes an amount of lipid component sufficient to enhance the uptake of the active ingredient through a hydrophobic surface to which it is applied. Again, at least 5% by weight of the total lipid component will consist of unsaturated lipid(s) having 4 or more sites of unsaturation.

A "sufficient" amount of the lipid component will of course vary depending on both the type and quantity of the active ingredient and the composition of the lipid component itself. It can however be defined as the amount of lipid which is sufficient to at least partially coat the hydrophobic surface to which the composition is applied and to interact with the active ingredients.

The highly unsaturated lipids which make up the essential 5% by weight proportion of the lipid component can be any of those highly unsaturated lipids discussed above but preferably are highly unsaturated fatty acids. For example, the lipid component can usefully be that extracted from fish offal which has been subjected to hydrolysis in acid (pH<4) for 2 weeks and which includes approximately 85% free fatty acids.

The final component the active composition must include is an antioxidant. As discussed above, this component is included to protect the lipid component from oxidation. Without such protection, the highly labile lipids will quickly oxidise and lose their ability to enhance the

uptake of the active ingredient through the hydrophobic layer.

The active composition can be prepared by combining the active ingredient and the lipid component according to any conventional technique. By way of example, a composition comprising the active ingredient and antioxidant, and including as optional components an emulsifying agent, a stabiliser and a preservative (whether as part of the active ingredient itself or as separate additives) can have freshly extracted lipid of the appropriate degree of unsaturation added to it. Alternatively, the lipid component can be in the form of the adjuvant of the invention defined above which comprises the highly unsaturated lipid and the antioxidant, preferably in combination with an emulsifying agent, a stabiliser and a preservative. In this form, the adjuvant can simply be added to a composition which comprises the active ingredient(s) to be applied.

Where, as is preferred, the adjuvant comprises highly unsaturated low melting point fatty acids derived from fish offal, the active composition will also include an odour masking agent such as a perfume and/or deodorising agent. This agent is desirable in view of the strong and characteristic odour associated with matter derived from such sources.

It will further be appreciated that where the active ingredient of the composition is a plant nutrient such as a digested organic material, it will again be desirable to include an odour masking agent. Suitable examples of such masking agents which can be included are ferric or magnesium sulphate.

Upon combination of the desired components, the pH of the active composition thus formed can be modified if desired to optimise the conditions under which the uptake of active ingredients will occur. These conditions are those under which the active ingredients interact with the lipid component to form the hydrophobic molecular clusters.

The optimal conditions of pH for such interaction will of course vary depending on the nature of the active ingredients. For example, where the active ingredient is an organic fertiliser containing digested protein, the pH can be adjusted to a value of from 3 up to 6, preferably from 5 to 5.5. At such pHs, most amino acids and peptides are at or close to their isoelectric points which ensures optimal hydrophobic binding between the unsaturated lipid and the proteins occurs.

Once the active composition has been prepared, it can be applied immediately or formulated for storage. If the composition is to be stored for later use, it can be concentrated as either an aqueous suspension or emulsion concentrate or if appropriate dried to provide a wettable powder. In this latter form, the desired composition can be easily reconstituted by the addition of the necessary amount of water or other appropriate diluent.

Once the composition is in the form necessary or desired for application, it can be applied by any of those methods known in the art. By way of example, the composition can be applied to the foliage of plants by controlled droplet application.

Once applied, the composition coats and adheres to the hydrophobic surface. The active ingredients are dispersed throughout the coating and at least partially contained within the hydrophobic molecular clusters. The lipid component partially solubilises the external hydrophobic surface of the plant, insect or animal to which it is applied and increases the ease of transport of the molecular clusters into the interior of the plant, insect or animal. Once absorbed, the active ingredients within the clusters are released and become available for assimilation.

The following non-limiting examples are provided to illustrate the present invention and in no way limit the scope thereof.

Example 1 - Preparation of the Highly Unsaturated Lipid Adjuvant (HULA)

The adjuvant was prepared by extraction and centrifugation from fish offal as follows:

Fish offal is mechanically disintegrated into a slurry with water and pumped into a reaction tank. The pH of the slurry in the tank is adjusted to approximately 3.7 with formic acid and digested with a mixture of pepsin and Rhizopus acidic protease for 12 hours. The slurry is heated to 35°C and stirred during the digestion. Prior to digestion antioxidants (butylated hydroxytoluene and Vitamin E) are added.

After addition of diammonium phosphate to the digested slurry to 3% phosphate (w/w), papain and pancreatin are added to effect a second digestion. The digestion is carried out for 6 hrs at 35°C with continuous stirring after which urea and potassium nitrate are added to

give a product having an NPK ratio of 8:3:3.

The lipid adjuvant is extracted from the resulting product by centrifugation using a Sharples ASA-26 high speed centrifuge. High speed centrifugation results in the separation of a crude lipid preparation from the slurry which is purified to produce an amber-coloured lipid adjuvant by a second pass through the centrifuge.

A total lipid profile obtained by capillary gas-liquid chromatography of the prepared adjuvant is presented in Table 1.

TABLE 1 - ANALYSIS OF THE MAJOR SATURATED AND UNSATURATED COMPONENTS OF THE PREPARED LIPID ADJUVANT

Saturates		% weight composition
Palmitic 16:0		25.44
Stearic 18:0		6.69
Unsaturates		
Palmitoleic	16:1	7.60
Oleic	18:1	22.90
Linoleic	18:2n-6	1.17
Linolenic	18:3n-3	0.40
Arachidonic	20:4n-6	3.07
Eicosapentaenoic	20:5n-3	5.43
Docoasahexaenoic	22:6n-3	7.80

The separated protein hydrolysate and lipid adjuvant were used in the succeeding experiments, with the highly unsaturated lipid adjuvant being denoted as HULA.

Example 2

This experiment was designed to test the uptake of ^{14}Carbon labelled amino acids through the leaves of potted pepper plants in the presence of HULA. Comparisons were also made with other lipids including fatty acids of varying degrees of unsaturation to demonstrate the advantages associated with the use of an adjuvant having a high degree of unsaturation.

Method

Preparations were used containing ^{14}C protein hydrolysate obtained from Amersham UK and (1) olive oil; (2) sunflower oil; and (3) HULA.

Olive oil is monoenoic, containing oleic acid as the predominant fatty acid; whereas sunflower oil is dienoic containing linoleic acid as the predominant fatty acid. Both olive and sunflower oil contain substantially less than 1% by weight of unsaturated lipid having four or more sites of unsaturation.

The preparations were applied in 5 µl volumes to pepper leaves and left for 16 hours. The control preparation contained ^{14}C labelled protein hydrolysate only. After 16 hours the leaves were vigorously washed with 70% alcohol three times and the collected fluid evaporated in scintillation vials. After addition of scintillating fluid the ^{14}C counts were measured on an LKB scintillation counter. The results are shown in Table 2.

Results

Results in Table 2 are expressed as % uptake into the leaf of the ^{14}C protein hydrolysate compared with the control.

TABLE 2

Adjuvants	% uptake
Olive oil (monoenoic)	22.6
Sunflower oil (dienoic)	47.0
HULA	76.5

From the Table, it can be seen that HULA is more effective in increasing the foliar uptake of ^{14}C labelled amino acids than the other less unsaturated preparations. Thus HULA can be used together with fertilisers or other agricultural compositions containing protein components to increase the foliar uptake of the protein.

Example 3

This example was designed to field test the adjuvant properties of HULA on grass dry matter yields when applied with a fertiliser. A monoenoic adjuvant (olive oil) was also included in the experimental design.

Method

A fertiliser of NPK = (8:3:3 (elemental)) and from which the lipid component had been extracted was prepared as described in Example 1. The following 10 square metre plots were set:

	Plot	Application Rate
1.	Control (water)	20 L/Hectare
2.	Fertiliser (without adjuvant)	20L/Hectare
3.	Fertiliser (with monoenoic oil adjuvant)	20L/Hectare
4.	Fertiliser (with HULA)	20L/Hectare

After three weeks the plots were cut and the weight of the dry matter measured.

Results

The results are expressed as a dry matter increase % of the control non-fertilised plot.

TABLE 3

	% increase in weight of dry matter over control
Fertiliser (20L/H) rate	62%
Fertiliser (20L/H) + monoenoic oil adjuvant	67.9%
Fertiliser (20L/H) + HULA	123.0%

These results clearly show that (1) inclusion of an adjuvant enhances the dry matter yield; and (2) the HULA preparation is much more effective than the monoenoic oil in enhancing fertiliser uptake.

Example 4

This example was designed to examine the effect of HULA on herbicide weed kill. As before, olive oil and sunflower oil which contain predominantly oleic acid (one site of unsaturation) and linoleic acid (two sites of unsaturation) respectively were also included in the experimental design for comparison.

Method

Plots were sprayed with Roundup (N-phosphonomethyl glycine, Monsanto), or MCPA (2-methyl-4-chlorophenoxyacetic acid, Dow-Elanco) in combination with the HULA adjuvant or the comparison oils and assessed for weed kill.

Results

Results are expressed as the % of weeds that are killed in each plot.

TABLE 4

	Olive Oil Adjuvant	Sunflower Oil Adjuvant	HULA
Roundup	62	77	100 *1
MCPA	61	65	92 *2

Notes: *1 Combination of the HULA adjuvant with Roundup also resulted in a complete clover kill.
*2 Californian Thistle kill.

The surprising effect of increasing the degree of unsaturation of the lipid component in the adjuvant used in combination with the herbicide is evident from the data. The HULA adjuvant was extremely effective at increasing the weed kill of Roundup and MCPA.

This result, when combined with Example 3, shows the general ability of HULA to increase uptake of any active ingredient.

APPLICATION OF THE INVENTION

Thus, in accordance with the present invention there is provided a method by which the uptake of substances through hydrophobic biological surfaces can be enhanced. This is achieved through the provision of an adjuvant comprising highly unsaturated lipid which advantageously modifies both the surface and the presentation of the active ingredients to the surface for transport therethrough. In this way, the wastage of valuable active ingredients such as agrochemicals is minimised and the active ingredients are made quickly available for assimilation.

A further advantage achieved by incorporation of an adjuvant according to the invention into an active composition arises out of the

retention of the active ingredient on the hydrophobic surface even when exposed to wind, rain and movement. This retention is important particularly in the case of compositions which contain compounds that are unable to gain direct entry into the plant, insect or animal because of their size. Through their retention on the hydrophobic surface, such large size compounds are made available to bacterial degradation, resulting in the eventual production of smaller size products which can be absorbed through the hydrophobic surface. This is particularly the case where the hydrophobic surface is a plant leaf.

It will be appreciated by those persons skilled in the art that the above description is provided by way of example only and that the present invention is limited only by the lawful scope of the appended claims.

CLAIMS:

1. The use of unsaturated lipid(s) having 4 or more sites of unsaturation in the preparation of a lipid-containing adjuvant for enhancing the uptake of an active ingredient through a hydrophobic biological surface.

2. The use as claimed in claim 1 wherein the lipid content of said adjuvant is entirely unsaturated lipid(s) having 4 or more sites of unsaturation.

3. The use as claimed in claim 1 wherein the lipid content of said adjuvant is at least 5% by weight unsaturated lipid(s) having 4 or more sites of unsaturation.

4. The use as claimed in claim 1 wherein the lipid content of said adjuvant is at least 15% by weight unsaturated lipid(s) having 4 or more sites of unsaturation.

5. The use as claimed in any one of claims 1 to 4 wherein the unsaturated lipid(s) are selected from the group consisting of arachidonic acid, eicosapentaenoic acid and docosahexaenoic acid.

6. An adjuvant for combination with an active ingredient to enhance the uptake of said active ingredient through a hydrophobic biological surface, said adjuvant including an effective amount of an antioxidant and a lipid component, at least 5% by weight of the total lipid component being unsaturated lipid(s) having 4 or more sites of unsaturation.

7. An adjuvant as claimed in claim 6 wherein the lipid component is entirely unsaturated lipid(s) having 4 or more sites of unsaturation.

8. An adjuvant as claimed in claim 6 wherein the lipid component is at least 15% by weight unsaturated lipid(s) having 4 or more sites of

unsaturation.

9. An adjuvant as claimed in any one of claims 6 to 8 wherein the unsaturated lipid(s) are selected from the group consisting of arachidonic acid, eicosapentaenoic acid and docasahexaenoic acid.

10. An adjuvant as claimed in any one of claims 6 to 9 further including one or more components selected from emulsifying agents, stabilisers, preservatives and odour-masking agents.

11. An adjuvant for enhancing the uptake of an active ingredient through a hydrophobic biological surface, said adjuvant comprising a lipid component being at least 5% by weight unsaturated lipid(s) having 4 or more sites of unsaturation and with at least a major proportion of said unsaturated lipid(s) being in the form of free fatty acids.

12. An active composition for application to a hydrophobic biological surface which comprises an effective amount of an active ingredient and an adjuvant as claimed in any one of claims 6 to 11 sufficient to enhance the uptake of said ingredient through said surface.

13. An active composition as claimed in claim 12 wherein the active ingredient is a plant nutrient.

14. An active composition as claimed in claim 12 wherein the active ingredient is an agrochemical selected from the group consisting of plant protection agents, herbicides, plant growth regulators and defoliants.

15. An active composition as claimed in claim 12 wherein the active ingredient is an insecticide.

16. An active composition as claimed in claim 12 wherein the active ingredient is an animal medicine.

17. A method for producing an active composition for application to a hydrophobic biological surface comprising the step of combining an effective amount of active ingredient, an effective amount of an antioxidant and an amount of a lipid component sufficient to enhance the uptake of said active ingredient through said surface, said lipid component being at least 5% by weight unsaturated lipid(s) having 4 or more sites of unsaturation.

18. A method as claimed in claim 17 wherein said antioxidant and said lipid component are added to said active ingredient in the form of an adjuvant as claimed in any one of claims 6 to 10.

19. A method of enhancing the uptake of an active ingredient through a hydrophobic biological surface which comprises the step of applying said active ingredient to said surface together with an adjuvant as claimed in any one of claims 6 to 11.

20. A method as claimed in claim 19 wherein said active ingredient and said adjuvant are applied in the form of an active composition as claimed in claim 12.

21. A method as claimed in claim 19 or claim 20 wherein the active ingredient is a plant nutrient, a plant protection agent, a herbicide, a plant growth regulator or a defoliant, and the hydrophobic biological surface is the waxy plant cuticle.

22. A method as claimed in claim 19 or claim 20 wherein the active ingredient is an insecticide and the hydrophobic biological surface is the insect cuticle.

23. A method for preparing an adjuvant as claimed in claim 11 comprising the steps of:

(a) forming a solution which comprises disintegrated fish containing a lipid component in acid;

(b) holding said solution to hydrolyse said lipid component into free fatty acids, said hydrolysis step being of a duration such that at least a major proportion of the lipid component following

hydrolysis is in the form of free fatty acids; and

(c) recovering the lipid component from the solution.

INTERNATIONAL SEARCH REPORT

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent classification (IPC) or to both National Classification and IPC

Int. Cl.[5] A61K 47/44, C05G, A01N 25/02

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC	Derwent Data Bases A61K 47/44, 47/00, 9/107, A01N 25/02, 17/08, C05G 3/00 and Keywords: Fatty Acid, Waxes, Hydrocarbon, Paraffin, Lipid, Ecosapentenoic, Docosahexenoic, Uptake Enhancer, Carrier, penetration.

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

AU: IPC as above
CHEM. ABS. using Keywords Ecospentenoic and Docosahexenoic.

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category*	Citation of Document, [11] with indication, where appropriate of the relevant passages [12]	Relevant to Claim No [13]
X	EP,A, 304603 (INDENA S.p.A.) 1 March 1989 (01.03.89).	(1-12, 16-20)
P,X	Derwent Abstract Accession No.91-153782/21, Class B05, JP,A, 03-090022 (MOCHIDA PHARM KK) 16 April 1991 (16.04.91).	(1-12, 16-20)
X	EP,A, 381823 (XU ZHENG) 16 August 1990 (16.08.90). See page 7 lines 30 to 38 and page 8 lines 8 to 10 and 21 to 29.	(1-12, 16-20)
P,X	AU,A, 78223/91 (DERMAMED) 19 December 1991 (19.12.91). See page 9 line 23 and page 10 lines 25 to 26.	(1-12, 16-20)

(continued)

* Special categories of cited documents : [10]

"A" Document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior t the international filing date but later than the priority date claimed

"T" Later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
9 April 1992 (09.04.92)	10 April 1992 (10.04.92)
International Searching Authority	**Signature of Authorized Officer**
AUSTRALIAN PATENT OFFICE	JOHN G. HANSON

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

A	GB,A, 2218334 (SANDOZ LTD) 15 November 1989 (15.11.89)	
A	AU,B, 30023/84 (563760) (TROPONWERKE G.m.b.H. and Co. KG) 3 January 1985 (03.01.85).	
A	AU,B, 64463/74 (479605) (HOECHST AG) 17 July 1975 (17.07.75).	
A	WO,A, 90/08543 (B.BRAUN MELSUNGEN AG) 9 August 1990 (09.08.90).	
A	US,A, 4165385 (DIANIS CREATIONS INC.) 21 August 1979 (21.08.79).	

V. ☐ OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim numbers ..., because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claim numbers ..., because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claim numbers ..., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4a

VI. ☐ OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this international application as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims of the international application.

2. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims of the international application for which fees were paid, specifically claims:

3. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. ☐ As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

☐ The additional search fees were accompanied by applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL APPLICATION NO. PCT/AU 91/00592

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report	Patent Family Member		
EP 304603	IT 8721453	IT 210200	JP 2048528
EP 381823	CN 1042658		
AU 78223/91	CA 2044371	EP 463454	
GB 2218334	BE 1002266	CH 679119	DE 39115617
	FR 2631235	IT 8947949	JP 2017127
AU 479605	AT 331558	BE 809838	BR 7400254
	CA 1036932	CH 588209	DD 108888
	DE 2301922	FR 2213738	GB 1434866
	IL 43996	IT 1048900	JP 49101529
WO 90/08543	DE 3903057	EP 456764	
US 4165385			
AU 563760	US 4731384	DE 3323832	DE 3483285
	EP 130516	FI 842612	JP 60025921
	NZ 208693		

END OF ANNEX