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(71) Applicant: **LIPOTEC LABORATORIES LLC** [US/US]; 1003 Wilshire, Champaign, IL 61821 (US).

(72) Inventors: **WATKIN, Kenneth, L.**; 1003 Wilshire, Champaign, IL 61821 (US). **FOULY, Hanafy, M.**; 1708 E Marc Trail, Urbana, Illinois 61802-9546 (US).

(74) Agent: **HILLMAN, Lisa, M., W.**; McDonnell Boehnen Hulbert & Berghoff LLP, 300 South Wacker Drive, Suite 3200, Chicago, IL 60606 (US).

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(54) Title: LIPOSOME FORMULATIONS

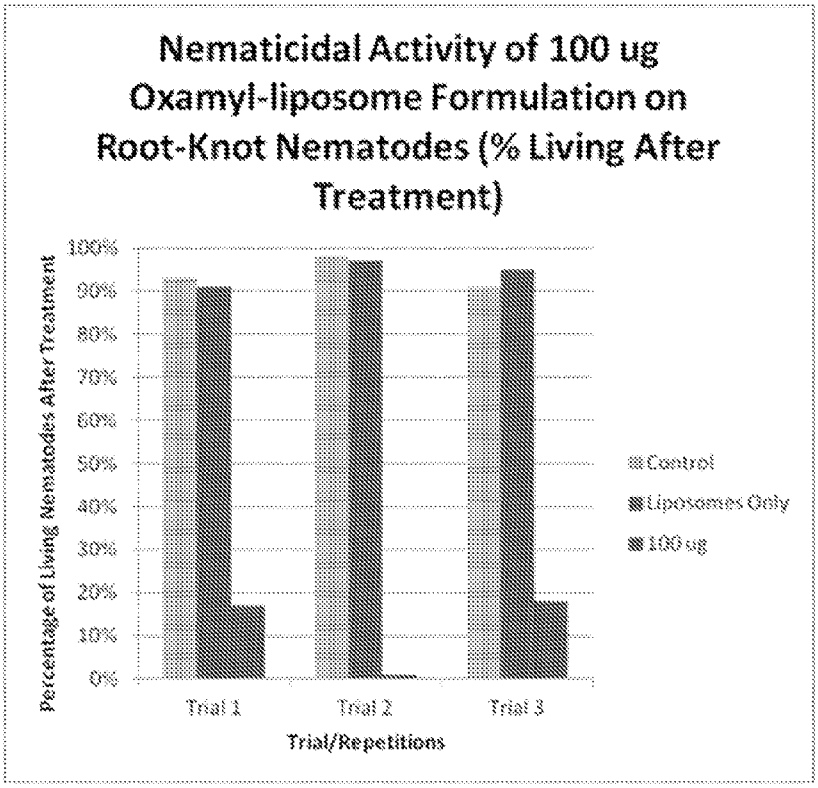

Figure 1.

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(57) Abstract: The invention provides liposomal formulations comprising pesticides, nematicides, or herbicides for control of pests and weeds. The formulations can be applied to pre- or post-emergent crops and to soil, plant media, plants, plant tissues and seeds. The liposomal formulations are also useful to treat or control pest or nematode infections of humans and animals.

TITLE: LIPOSOME FORMULATIONS

PRIORITY:

This application claims the benefit of U.S. Provisional Patent applications 61/661,210, filed June 18, 2012 and 61/794,101, filed March 15, 2013, which are incorporated by reference herein in their entirety.

BACKGROUND OF THE INVENTION

The use of nematicides, herbicides, and pesticides has been increasingly restricted over the past 30 years due to increased federal regulation and as concerns for human health and environmental safety has increased. The Food Quality Protection Action (1996) is resulting in further restrictions on the use of nematicides and pesticides. For example, the systemic nematicide fenamiphos was withdrawn from all uses in the United States in 2007. The use of aldicarb will be removed from markets by 2014. There is an urgent need to develop a low cost and quality enhancing technology, target oriented, environmentally compatible chemicals as well as suitable biological control methods for the control of pests, including insects, nematodes, and weeds. There is also a need to develop, modify, or enhance existing technologies to control pests such as insects, nematodes and weeds.

SUMMARY OF THE INVENTION

In one embodiment, the invention provides a liposome formulation comprising one or more pesticides, nematicides, or herbicides loaded in the aqueous core of liposomes, wherein the liposomes are lyophilized. One or more nematicides can be loaded into the aqueous core of the liposomes. The one or more nematicides can be 2-methyl-2-(methylthio)propionaldehyde O-methylcarbamoyloxime, 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, 2-methyl-2-(methylsulfonyl)propanal-O-(methylaminocarbonyl oximel, O,O-diethyl O-[p-(methylsulfinyl)phenyl] ester, Ethyl 4-methylthio-m-tolyl isopropylphosphoramidate, O-ethyl S,S-dipropyl phosphorodithioate, Methyl N'N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate, S-[[(1,1-dimethylethyl) thio] methyl]O,O-diethyl phosphorodithioate, thionazin, isazofos, ebufos, cleothocarb or combinations thereof. The lyophilized liposome can be loaded with about 1, 5, 10, 50, 100, 200, or 500 µg/ml of the one or more nematicides.

A liposomal composition of the invention can be a dustable powder (DP), soluble powder (SP), water soluble granules (SG), water dispersible granules (WG), wettable powders (WP), granules (GR) (slow or fast release), soluble concentrates

(SL), oil miscible liquids (OL), ultra-low volume liquids (UL), emulsifiable concentrates (EC), dispersible concentrates (DC), emulsions (both oil in water (EW) and water in oil (EO)), micro-emulsions (ME), suspension concentrates (SC), aerosols, fogging/smoke formulations, capsule suspensions (CS), powder for dry seed treatment (DS), a water soluble powder (SS), a water dispersible powder for slurry treatment (WS), a flowable concentrate (FS), a liquid solution (LS), a capsule suspension (CS), or combinations thereof.

A liposomal formulation of the invention can further comprise a fertilizer.

Another embodiment of the invention provides a method for reducing the number of live nematodes on or in plant media, soil, plants, plant tissues, or seeds, comprising administering to the plant media, soil, plants, plant tissues, or seeds an effective amount of a lyophilized liposome formulation of the invention. The lyophilized liposomes can be rehydrated before they are administered to the plant media, soil, plants, plant tissues, or seeds. The lyophilized liposomes can be rehydrated in water, liquid fertilizer or other suitable liquid. About 5-fold, 10-fold, 50-fold, or 100-fold less nematicide via the liposome formulation than is recommended for conventional, non-liposomal application of the same nematicide can be administered by the liposomal formulations of the invention. The plants or plants grown in the soil or plant media can have increased root lengths, increased stalk diameter, increased stalk length, increased leaf number, or increased leaf size as compared to plants or soil or plant media treated with non-liposomal formulations of one or more nematicides.

A liposomal formulation can be administered in an amount from about 5 g/ha to about 2000 g/ha. The nematodes can be root-knot nematodes. The liposomal composition can be applied to seeds in an amount from 0.001 g to 10 kg per 100 kg of seeds.

Yet another embodiment of the invention provides a method of increasing root lengths, increasing stalk diameter, increasing stalk length, increasing leaf number, increasing leaf size of a plant, increasing yield, increasing plant vigor or a combination thereof comprising administering a liposomal composition of the invention. The method comprises administering one or more liposome-based nematicides, herbicides or pesticides to the plant or to soil or plant media in which the plant is growing. The increases are observed when the plants are compared to

plants that were administered the same conventional, non-liposomal pesticides, herbicides or nematicides.

Still another embodiment of the invention provides a method of increasing root lengths, increasing stalk diameter, increasing stalk length, increasing leaf number, increasing leaf size of a plant, increasing yield, increasing plant vigor or a combination thereof of a nematicide treated plant or a plant grown in nematicide-treated soil or plant media. The method comprises administering one or more nematicides to the plant or the soil or plant media, wherein the one or more nematicides are present in an aqueous core of a liposome. The nematicides present in the aqueous core of a liposome can be administered at a same amount or concentration or a lower amount or concentration than the recommended administration amount or concentration of the nematicide when administered in a non-liposomal formulation.

Yet another embodiment of the invention provides a method of decreasing the amount of nematicide-induced damage to nematicide treated plants or plants grown in nematicide-treated soil or plant media. The method comprises administering one or more nematicides to the plant or the soil or plant media, wherein the one or more nematicides are present in an aqueous core of a liposome. The nematicides present in the aqueous core of a liposome are administered at a same amount or concentration or a lower amount or concentration than the recommended administration amount or concentration of the nematicide when administered in a non-liposomal formulation.

Another embodiment of the invention comprises method for reducing the number of pests, insects, or nematodes on or in an animal, comprising administering to the animal an effective amount of the liposome formulation of claim 2.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 shows nematicidal activity of 100 µg oxamyl-liposome formulation on root-knot nematodes (% living after treatment).

Figure 2 shows the effect of pre-emergent application of liposomal formulations of Avid .15 on tomato stalk height.

Figure 3 shows the effect of 5µg and 1 µg liposomal abamectin formulations ("Aba-lipo") 5µg and 1 µg non-liposomal abamectin formulations ("Aba only") on gall formation.

Figure 4 shows the effect of 5µg and 1 µg liposomal abamectin formulations ("Aba-lipo") 5µg and 1 µg non-liposomal abamectin formulations ("Aba only") on root necrosis.

Figure 5 shows the effect of 5µg and 1 µg liposomal abamectin formulations ("Aba-lipo") 5µg and 1 µg non-liposomal abamectin formulations ("Aba only") on root length.

DETAILED DESCRIPTION OF THE INVENTION

As used herein, the singular forms "a," "an", and "the" include plural referents unless the context clearly dictates otherwise.

Liposomes have received widespread attention as a carrier system for therapeutically active compounds, due to their unique characteristics such as capability to incorporate hydrophilic and hydrophobic drugs, good biocompatibility, low toxicity, lack of immune system activation, and targeted delivery of bioactive compounds to the site of action (Voinea *et al.*, J. Cell Mol. Med. 6:465 (2002)). Additionally, some achievements since the discovery of liposomes are controlled size from microscale to nanoscale and surface-engineered polymer conjugates functionalized with peptide, protein, and antibody. Progress in liposome drug delivery has led to the commercialization of liposomal anticancer drug formulations (e.g., Doxil, DaunoXome).

Liposomal formulations have now been developed that are suitable for protecting plants and plant organs (including fruits and seeds), for increasing the harvest yields, for improving the quality of the harvested material, for controlling weeds, and for controlling animal pests, in particular insects, arachnids, helminths, nematodes and molluscs, which are encountered in agriculture, in horticulture, in animal husbandry, in forests, in gardens and leisure facilities, in the protection of stored products and of materials, and in the hygiene sector. The liposome formulations can be lyophilized to produce a long lasting and storable composition which can then be further processed to meet the needs of a given application.

Advantages of Compositions of the Invention

Administration of the liposomal compositions of the invention can provide one or more advantageous properties to soil, plant medium, seeds, plants or plant tissues. Examples of such advantageous properties include a broadening of the spectrum of pesticidal activity to other pests; a reduction in the rate of application of the active ingredients; adequate control of the pests with the aid of combinations of

active ingredients, even at a rate of application at which the individual active ingredients are totally ineffective; advantageous behavior during formulating and/or upon application, for example upon grinding, sieving, emulsifying, dissolving or dispersing; increased storage stability; improved stability to light; increased advantageous degradability; improved toxicological and/or ecotoxicological behavior; improved crop characteristics including: emergence, crop yields, more developed root system (including longer roots), tillering increase, increase in plant height, increase in stalk circumference, bigger leafs, more leaves, less dead basal leaves, stronger tillers, greener leaf color, less fertilizers needed, less seeds needed, more productive tillers, earlier flowering, early grain, seed or fruit maturity, less plant verse (lodging), increased shoot growth, improved plant vigor, and early germination; or any other advantages familiar to a person skilled in the art.

An improvement in the growing (or growth) characteristics of a plant can be measured in many ways, but ultimately results in a better production of the plant, for example, an improved yield, improved vigor of the plant or quality of the harvested product from the plant. An improved yield of a plant relates to an increase in the yield of a product (e.g., as measured by plant biomass, grain, seed or fruit yield, protein content, carbohydrate or oil content or leaf area) of the plant by a measurable amount over the yield of the same product of the plant produced under the same conditions, but without the application of compositions of the invention or compared with application of conventional non-liposomal pesticides, nematicides, or herbicides. Yield can be increased by at least about 0.5, 1, 2, 3, 4, 5, 10, 15% or more. Yield can be expressed in terms of an amount by weight or volume of the plant or a product of the plant on some basis. The basis can be expressed in terms of time, growing area, weight of plants produced, or amount of a raw material used.

An improved vigor of a plant is an increase or improvement of the vigor rating, the stand (the number of plants per unit of area), plant height, stalk circumference, plant canopy, visual appearance (such as greener leaf color), root rating, emergence, protein content, increased tillering, bigger leafs, more leaves, less dead basal leaves, stronger tillers, less fertilizer needed, less seeds needed, more productive tillers, earlier flowering, early grain or seed maturity, less plant verse (lodging), increased shoot growth, earlier germination, or any combination of these factors, by a measurable or noticeable amount over the same factor of the plant produced under the same conditions, but without the administration of the instant

compositions or with application of conventional non-liposomal pesticides or herbicides.

Pests

The compositions of the invention can be used to prevent infection by or reduce the numbers of plant pests in or on soil or other plant medium and to prevent infection or reduce the numbers of plant pests on plants or plant material such as roots, fruits and seeds. In another embodiment of the invention, the compositions of the invention reduce the damaging effect of plant pests on the plant by, for example, killing, injuring or slowing the activity of the pest. Plant pests include, for example, insects, arachnids, helminths, nematodes, molluscs, bacteria, fungi, mites, oomycytes and protozoa. Compositions of the invention can be used to control, kill, injure, paralyze, or reduce the activity of one or more of any of these pests in their egg, larvae, adult, juvenile, or desiccated forms.

Nematodes that damage plants include, for example, *Meloidogyne* spp. (root-knot), *Heterodera* spp., *Globodera* spp., *Pratylenchus* spp., *Helicotylenchus* spp., *Radopholus similis*, *Ditylenchus dipsaci*, *Rotylenchulus reniformis*, *Xiphinema* spp., *Aphelenchoides* spp. and *Belonolaimus longicaudatus.*

Plant parasitic nematodes are small, aquatic, microscopic roundworms that live in films of water surrounding soil particles and plant roots. The presence of a water film is essential to the nematode for locomotion and maintenance of body fluids. The body of the nematode, when inflated with fluids, acts like a skeleton, preventing internal collapse. In dry soils body fluids are lost, the body wall collapses, and many nematodes die as a result of dehydration. However, some can survive desiccation in a suspended state for long periods, and come back to life when soil water conditions are restored. In the dried state, nematodes are more resistant to high soil temperature and nematicides. Nematodes feed on the roots or foliar tissues of plants. In many parts of the world nematodes are a major limiting factor for agricultural production, causing serious reduction in crop quantity, quality, or harvest uniformity. All fruit and vegetable crops are susceptible to nematodes. Total crop failures frequently occur when crops are planted into areas with high nematode population levels. Plant symptoms that develop in response to nematode parasitism are generally those associated with root dysfunction. Development of small, stunted, and chlorotic plants generally reflects reduced water and nutrient uptake caused by injury to the root system. Correspondingly, root damage generally increases with

nematode infestation level, particularly where plants are grown on fine to coarse textured, sandy soils with low water holding capacity. Plant-parasitic nematodes cause yield suppression in many crops species. Estimates of nematode damage to specific crops ranged from 3.3% to 20.6%, with a mean of 12.3%. Annual production losses at the farm gate were $121 billion globally and $9.1 billion in the United States (Sasser, J.N. & Freckman, 1987 *In:* Veech & Dickson, eds. Vistas on Nematology p. 7-14, Hyattsville MD, US, Society of Nematologists).

The root-knot group *Meloidogyne* spp of nematodes are particularly important to control (Sasser, Plant Disease, 104:36 (1980)). Their worldwide distribution, extensive host ranges and involvement with fungi, bacteria, and viruses in disease complexes rank them among the top major plant pathogens affecting the global food supply. Collectively, the various species of root-knot attack nearly every crop grown. The most common species are *M. incognita, M. arenaria, M. hapla* and *M. javanica* (Sasser, Phytopathology, 42:216 (1952); Sasser, Bull. Md. Agric. Exp. Stn. A-77 (Techn) p. 31 (1954)). Not only are yields greatly affected, but production quality is also reduced. Infections by root-knot nematode cause decline in the host, and under some conditions, may kill the plant (Sasser, 1980). Infected plants may be stunted and chlorotic, may usually wilt easily, and become unproductive. However, the extent of damage caused by root-knot nematode infections varies with the host, the timing of infection, and the cultural conditions present. Root-knot nematode infection is easy to identify because of the swellings in roots that look like "knots." The swellings become large and easy to see on some hosts such as squash and tomato, but may be smaller and less conspicuous on others such as the 'Chile' pepper. Multiple infections on one root result in a swollen, rough appearance. Root-knot nematodes are very small and can only be observed using a microscope.

Unlike free-living nematodes that are numerous in all soils, plant parasitic nematodes must feed on a plant host in order to complete their life cycle. Root-knot nematodes are soil borne and feed on roots (Taylor & Sasser, 1978, Biology, Identification, and Control of Root-Knot Nematodes (Meloidogyne *species*) Raleigh, NC, USA, NC State University Graphics, 111 pp.). Their life cycle includes egg, juvenile and adult stages. Eggs hatch into juveniles that infect plant roots and take nutrients from the plant as they mature, causing the characteristic knots or swellings to form. Root-knot nematodes feed by means of a stylet, a retractable mouthpart used for piercing and feeding. Those that enter the root and develop into females are

sedentary, become much enlarged, and lay hundreds of eggs in a sac on the root surface. In moist soils above 80° F, root-knot nematodes can go from egg to adult in about 25 days. In adverse conditions, the eggs can persist in the soil for long periods of time ranging from months to years.

Nematodes are most active in warm weather in moist, but well aerated, sandy soils in the presence of host plants. They are most abundant in the upper foot of soils, but will follow roots several feet deep. Three options exist for the management of root-knot nematodes: crop rotation, host plant resistance, and nematicides. For example, rotating corn with a non-host crop such as alfalfa or oats may be effective in reducing root-knot nematode populations. Because different species have different host ranges, it is always good practice to identify the particular species in the field before deciding on crop rotation as a management strategy.

Plants that are non-hosts of *M. incognita* can serve as good hosts for *M. arenaria* or *M. javanica.* Fields with successive seasons of corn will suppress populations of northern root-knot nematode, *M. hapla*, but at the same time this scheme may enhance populations of other rootknot, stubby-root, lesion, sting, lance, and ring nematodes. Resistant corn cultivars are currently unavailable for southern root-knot nematode, *M. incognita*; however, there are a few commercial cultivars that are resistant to *M. arenaria* and *M. javanica.* Lack of good cultural management alternatives leaves nematicides as the primary nematode management tool for most corn, soybean, vegetable, cotton, and fruit tree growers.

Among the crops with the greatest estimated losses due to nematode parasitism are corn, cotton, cucurbits, leguminous vegetables, peanut, solanaceous vegetables, soybean, sugarcane, and tobacco.

Insects cause two types of damage to plants. The first type of damage is direct injury done to the plant by the insect, which eats leaves or burrows into plant tissues. There are a multitude of insect species of this type, both larvae and adults, among orthopterans, homopterans, heteropterans, coleopterans, lepidopterans, and dipterans. The second type of damage is indirect damage where the insect itself does little or no harm but transmits a bacterial, viral, or fungal infection to a plant. Insects that cause these two types of damage to plants include, for example, Coleoptera (beetles, weevils), Cerambycidae (long-horned beetles), Chrysomelidae (leaf beetles), Coccinellidae (lady beetles), Curculionidae (snout beetles, weevils, billbugs), Elateridae (click beetles), Meloidae (blister beetles), Scarabaeidae (scarab

beetles), Tenebrionidae (darkling beetles), Diptera (flies), Anthomyiidae (root maggot flies), Cecidomyiidae (midges), Hemiptera suborder heteroptera (true bugs), Lygaeidae (seed bugs, chinch bugs), Miridae (plant bugs, lygus bugs), Pentatomidae (stink bugs), Hemiptera suborder homoptera (aphids, whiteflies, leafhoppers, scales), Aleyrodidae (whiteflies), Aphididae (aphids), Cercopidae (spittlebugs), Cicadellidae (leafhoppers), Membracidae (treehoppers), Lepidoptera (moths, butterflies), Noctuidae (cutworm moths), Pyralidae (snout and grass moths), Sphingidae (sphinx moths), Orthoptera (grasshoppers and crickets), Acrididae (short-horned grasshoppers), Gryllidae (crickets), Gryllotalpidae (mole crickets), Thysanoptera (thrips), Thripidae (common thrips), Acarina (mites), Tetranychidae (spider mites).

Arachnids such as earth mites (Penthaleidae), thread-footed mites (Tarsonemidae) and gall and rust mites (Eriophyoidea) can also cause damage to plants.

Molluscs, including those in the gastropod class and those in the subclass pulmonata, can cause damage to plants. Molluscs also include, for example, snails and slugs, such as *Ampullariidae* spp.; *Arion* spp. (*A. ater, A. circumscriptus, A. hortensis, A. rufus*); *Bradybaenidae* spp. (*Bradybaena fruticum*); *Cepaea* spp. (*C. hortensis, C. nemoralis*); *Ochlodina*; *Deroceras* spp. (*D. agrestis, D. empiricorum, D. laeve, D. reticulatum*); *Discus* spp. (*D. rotundatus*); *Euomphalia* spp.; *Galba* spp. (*G. trunculata*); *Helicelia* spp. (*H. itala, H. obvia*); *Helicidae* spp. (*Helicigona arbustorum*); *Helicodiscus* spp.; *Helix* spp. (*H. aperta*); *Limax* spp. (*L. cinereoniger, L. flavus, L. marginatus, L. maximus, L. tenellus*); *Lymnaea* spp.; *Milax* spp. (*M. gagates, M. marginatus, M. sowerbyi*); *Opeas* spp.; *Pomacea* spp. (*P. canaticulata*); *Vallonia* spp. and *Zanitoides*.

Any type of plant, plant tissue, seed or plant media, or soil can be treated with the compositions of the invention. Plants include algae, bryophytes, tracheophytes, and angiosperms. Angiosperms include, for example, flowering plants, cycads, Ginkgo biloba, and conifers. Plants include seedlings, mature plants, trees and turf. Plant tissues can include, for example, roots, leaves, stems, flowers, seeds, and fruits.

Nematicides, Pesticides, and Herbicides

Pesticides are active agents that kill or inhibit the growth of pests such as insects, arachnids, helminths, nematodes, molluscs, bacteria, fungi, mites,

oomycytes and protozoa. Herbicides are active agents that kill or inhibit the growth of unwanted plants. Liposomes of the invention can comprise pesticides, including nematicides, and herbicides. Examples of pesticides and herbicides that can be used in the liposomal formulations of the invention include, for example, 1-bromo-3-chloro-5,5-dimethylhydantoin, 2,4-D Amine, 2,4-D low volatile ester, 2,4-DB, 2,4-D + fenoxaprop-p-ethyl + MCPA + thifensulfuron methyl, abamectin, acephate, acetamiprid, acetic acid, Agrobacterium radiobacter, aluminum phosphide, amitraz, amitrole, ancymidol, anilazine, atrazine, atrazine & bentazon, atrazine & etolachlor, azinphos-methyl, azoxystrobin, Bacillus thuringiensis (Bt), bendiocarb, bensulide, bentazon, boscalid, brodifacoum, bromadiolone, bromethalin, bromoxynil, bromoxynil + MCPA, bromoxynil + 2,4-D ester, captan, captan + diazinon + thiophanate-methyl, captan + thiophanate methyl, carbaryl, carbathiin, carbathiin + captan, carbathiin + clothianidin + thiram + metalaxyl, carbathiin + imidacloprid + thiram, carbathiin + oxycarboxin + thiram, carbathiin + thiabendazole, carbathiin + thiram, carbofuran, chloroneb, chlorophacinone, chlorothalonil, chlorothalonil + propamocarb HCl, chlorpropham, chlorpyrifos, chlormequat chloride, clethodim, clodinafop-propargyl, clodinafop-propargyl + thifensulfuron-methyl + tribenuron-methyl, clofentezine, clopyralid, clopyralid + glyphosate, clopyralid + MCPA ester, clothianidin, clothianidin + carbathiin + thiram + metalaxyl, copper 8-quinolinolate, copper hydroxide, copper oxychloride, copper sulphate, cyfluthrin, cyhalothrin-lambda, cymoxanil, cymoxanil + famoxadone, cypermethrin, cyprodinil, cyromazine, daminozide, dazomet, deltamethrin, desmedipham + phenmedipham, diazinon, diazinon + captan, diazinon + captan + thiophanate-methyl, diazinon + cypermethrin, dicamba, dicamba + atrazine, dicamba + glyphosate, dicamba + MCPA, dicamba + mecoprop + 2, 4-D dicamba + mecoprop, dicamba + mecoprop + MCPA, dicamba + 2, 4-D, dichlobenil, diclofop-methyl, diclofop-methyl + bromoxynil, dicloran, dichloropropene, dichloropropene + chlorpicrin, dichlorprop + 2,4-D dichlorvos, dichlorvos + pyrethrins + piperonyl butoxide, dichlorvos + pyrethrins + piperonyl butoxide + di-n-propylisocinchomeronate Dicofol, didecyl dimethyl ammonium chloride, didecyl dimethyl ammonium chloride + dimethyl benzyl ammonium chloride, difenoconazole, difenocanazole + metalaxyl-M, difenoconazole + metalaxyl-M + fludioxonil, difenoconazole + thiamethoxam + metalaxyl-M + fludioxonil, difenzoquat, diflubenzuron, dimethoate, dimethomorph, dimethomorph + mancozeb, dinocap + mancozeb, diphacinone, diquat, diuron, dodemorph-acetate, dodine, endosulfan,

EPTC, ethalfluralin, ethametsulfuron-methyl, ethephon, etridiazole, famoxadone + cymoxanil, fatty acids, fenbuconazole, fenbutatin-oxide, fenhexamid, fenoxaprop-p-ethyl, fenoxaprop-p-ethyl + bromoxynil + MCPA, fenoxaprop-p-ethyl + MCPA + thifensulfuron methyl, fenoxaprop-p-ethyl + MCPA + 2,4-D + thifensulfuron methyl, fenaoxprop-p-ethyl + thfensulfuron methyl + tribenuron methyl, ferbam, florasulam + glyphosate, florasulam + MCPA ester, fluazifop-p-butyl, fludioxonil + difenoconazole + metalaxyl-M, fludioxonil + difenoconazole + thiamethoxam + metalaxyl-M, fluroxypyr, fluroxypyr + 2,4-D ester, fluroxypyr + MCPA ester, fluroxypyr + clopyralid + MCPA ester, flusilazole, folpet, formaldehyde, formetanate hydrochloride, fosetyl-aluminum, gibberellic acid, gibberellins + benazladenine, glufosinate ammonium, glyphosate, glyphosate + 2,4-D glyphosate + dicamba, glyphosate + florasulam, Heterorhabditis megidis, hexazinone, imazamethabenz, imazamox + imazethapyr, imazethapyr, imazethapyr + pendimenthalin, imidacloprid, imidacloprid + carbathiin + thiram, iprodione, isoxaben, kinoprene, kresoxim-methyl, lime sulphur, linuron, malathion, maleic hydrazide, mancozeb, mancozeb + dimethomorph, mancozeb + dinocap, mancozeb + metalaxyl-M, mancozeb + zoxamide, maneb, MCPA + MCPB, MCPA dimethylamine, MCPA dimethyl amine + dicamba + mecoprop, MCPA ester, MCPA ester + bromoxynil, MCPA ester + clopyralid, MCPA ester + fenoxaprop-p-ethyl + thifensulfuron methyl, MCPA ester + fenoxaprop-p-ethyl + 2,4-D + thifensulfuron methyl, MCPA ester + fenoxaprop-p-ethyl + bromoxynil, MCPA ester + florasulam, MCPA ester + fluroxypyr, MCPA potassium salt, MCPA potassium salt + dicamba, MCPA sodium salt, MCPB, MCPB + MCPA, mecoprop, mecoprop + MCPA dimethyl amine + dicamba, mefenoxam (s-isomer) + etalaxy-M, metalaxyl, metalaxyl-M + chlorothalonil, metalaxyl-M + difenoconazole, metalaxyl-M + mancozeb, metaldehyde, metam sodium, methamidophos, methomyl, methomyl + Z-9 tricosene, methoxyfenozide, methoprene, methyl bromide, methyl bromide & chloropicrin, metiram, metolachlor/ s-metolachlor, metolachlor + atrazine, metribuzin, metribuzin + tribenuron methyl, metsulfuron methyl, mineral & vegetable oil, myclobutanil, NAA, naled, napropamide, naptalam, napthalene acetamide, nicosulfuron, nicotine, oxadiazon, oxamyl, oxine benzoate, oxycarboxin, oxycarboxin + carbathiin + thiram, oxyfluorfen, paclobutrazol, paraquat, pendimethalin, pendimenthalin + imazethapyr, permethrin, permethrin + pryethrins + piperonyl butoxide, piperonyl butoxide + dichlorvos + pyrethrins, phenmediphan + desmedipham, phosalone, phosmet, pirimicarb, prohexadione ca, prometryne, propamocarb hydrochloride, propamocarb

HCl + chlorothalonil, propanil, propiconazole, propiconazole + azoxystrobin, propyzamide, putrescent whole egg solids, pyraclostrobin, pyrethrins, pyrethrins + piperonyl butoxide, pyrethrins + piperonyl butoxide + dichlorvos, pyrethrins + piperonyl butoxide + malathion, pyridaben, quinclorac, quinclorac +thifensulfuron methyl + tribenuron methyl, quintozene (PCNB), rimsulfuron, sethoxydim, simazine, soaps, spinosad, Steinernema feltiae, stoddard solvent, streptomycin sulfate, strychnine, sulphur, tebuconazole + thiram, tebufenozide, tefluthrin, terbacil, terbufos, tetrachlorvinphos, thiabendazole, thiabendazole + carbathiin, thiamethoxam + difenoconazole + metalaxyl-M + fludioxonil, thifensulfuron methyl, thifensulfuron methyl + tribenuron methyl, thifensulfuron methyl + tribenuron methyl + quinclorac, thifensulfuron methyl + MCPA ester + fenoxaprop-p-ethyl, thifensulfuron methyl + tribenuron methyl + fenaoxprop-p-ethyl, thifensulfuron-methyl + tribenuron-methyl + clodinafop-propargyl, thiophanate methyl, thiophanate methyl + captan, thiophanate-methyl + diazinon + captan, thiophanate methyl + imidacloprid + mancozeb, thiram, thiram + carbathiin, thiram + carbathiin + oxycarboxin, thiram + carbathiin + imidacloprid, thiram + carbathiin + clothianidin + metalaxyl, thiram + tebuconazole, thiram + triticonazole, tralkoxydim, tralkoxydim + bromoxynil + MCPA, tralkoxydim + clopyralid + MCPA, triadimenol, triallate, triallate + trifluralin, tribasic copper sulphate, tribenuron methyl, tribenuron methyl + 2,4-D, tribenuron methyl + metribuzin, tribenuron-methyl + thifensulfuron-methyl + clodinafop-propargyl, tribenuron methyl + thifensulfuron methyl, tribenuron methyl + thfensulfuron methyl + fenaoxprop-p-ethyl, tribenuron methyl + thifensulfuron methyl + quinclorac, trichlorfon, trifluralin, trifluralin + triallate, triforine, trinexapac-ethyl, triticonazole + thiram, uniconazole, vinclozolin, warfarin, warfarin + sulfaquinoxaline, zinc phosphide, zineb, ziram, zoxamide + mancozeb or combinations thereof.

Nematicides are, by definition, chemicals that kill nematodes (-cides). Two broad categories of nematicides are currently registered and available for use (Whitehead, 1998, Plant Nematode Control. CAB International, Walling Ford, UK). The classification system is based upon the way these chemicals move in soil. Fumigant nematicides, including methyl bromide, methyl iodide, chloropicrin, ethylene dibromide, 1,3-dichloropene, dimethyl dibromide and metam sodium and potassium, dazomet, methyl isothiocyanate, are formulated as liquids which rapidly vaporize and move through open air spaces in soil as a gas. Non-fumigant nematicides, including 2-methyl-2-(methylthio)propionaldehyde O-

methylcarbamoyloxime (Temik®, Bayer CropScience), 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate (Furadan), 2-methyl-2 (methylsulfonyl)propanal-O-(methylaminocarbonyl oximel (StandakTM, BASF), O,O-diethyl O-[p-(methylsulfinyl)phenyl] ester (Dasanit), Ethyl 4-methylthio-m-tolyl isopropylphosphoramidate (Nemacur, Makhteshim Agan Group), O-ethyl S,S-dipropyl phosphorodithioate (MOCAP®, Bayer CropScience), Methyl N'N'-dimethyl-N- [(methylcarbamoyl)oxy]-1-thiooxamimidate (Vydate®, Dupont), and S-[[(1,1-dimethylethyl) thio] methyl]O,O-diethyl phosphorodithioate (Counter), thionazin (Nemafos), Isazofos (Miral), Ebufos (Rugby), Cleothocarb (Lance) are organophosphates and/or carbamates. The non-fumigant nematicides are often further classified as contact or systemic nematicides, depending on whether they kill nematodes in soil by contact, or are taken up by the plant first and affect nematodes when they feed from cellular fluids within the plant.

Any pesticide, nematicide, or herbicide can be loaded into a liposome of the invention.

Liposomes

Liposomes of the invention include, for example, small unilamellar vesicles (SUVs) formed by a single lipid bilayer, large unilamellar vesicles (LUVs), which are vesicles with relatively large particles formed by a single lipid bilayer, and multi-lamellar vesicles (MLVs), which are formed by multiple membrane layers. Liposomes can be of any particle size, for example the mean particle diameter can be about 10 to about 2000 nm. In one embodiment of the invention, the mean particle diameter is about 10, 20, 25, 30, 40, 50, 100, 200, 300, 400, 500, 600, 700, 800, 900, 1,000, 1,250, 1,500, 1,750, 2,000 nm (or any range between about 10 and about 2,000 nm) or more. In one embodiment of the invention, the mean particle diameter is about 2,000, 1,750, 1,500, 1,250, 1,000, 900, 800, 700, 600, 500, 400, 300, 200, 100, 50, 40, 30, 25, 20, 10 nm (or any range between about 2,000 and 10 nm) or less. The mean particle diameter may be about 20 to about 1,000 nm, about 100 to about 1,500 nm, about 100 to about 1,000 nm, about 100 to about 700 nm, about 200 to about 2,000 nm, about 1,000 to about 2,000 nm, or about 750 to about 1,500 nm. Particle diameter refers to the diameter of a particle measured by dynamic light scattering.

Liposome manufacture comprises, for example, drying down of the lipids from organic solvents, dispersion of the lipids in aqueous media, purification of the

resultant liposomes, and analysis of the final product. Some methods of liposome manufacture include, for example, extrusion methods, the Mozafari method, the polyol dilution method, the bubble method, and the heating method. Pesticides and herbicides can be entrapped in lipid vesicles by any method including, for example, reverse-phase evaporation technique, ether injection/vaporization technique and the freeze-thaw method.

Examples of lipids that can be used to make liposomes of the invention include soybean lecithin, hydrogenated soybean lecithin, egg yolk lecithin, phosphatidylcholines, phosphatidylserines phosphatidylethanolamines, phosphatidyl inositols, sphingomyelins, phosphatidic acids, long-chain alkyl phosphates, gangliosides, glycolipids, phosphatidyl glycerols, and cholesterols. Phosphatidylcholines include, for example, dimyristoylphosphatidylcholine, dipalmitoylphosphatidylcholine, and distearoyl phosphatidylcholine. Phosphatidylserines include, for example, dipalmitoyl phosphatidylserine, dipalmitoyl phosphatidylserine (sodium salt), and phosphatidylserine (sodium salt) derived from bovine brain. Phosphatidylethanolamines include, for example, dimyristoyl phosphatidylethanolamine, dipalmitoyl phosphatidylethanolamine, and distearoyl phosphatidylethanolamine. Phosphatidyl inositols include, for example, phosphatidylinositol (sodium salt) derived from wheat. Sphingomyelins include for example, sphingomyelin derived from bovine brain. Phosphatidic acids and long-chain alkyl phosphates include, for example, dimyristoyl phosphatidic acid, dipalmitoyl phosphatidic acid, distearoyl phosphatidic acid, and dicetyl phosphate. Gangliosides include, for example, ganglioside GM1, ganglioside GD1a, and ganglioside GT1b. Glycolipids include, for example, galactosyl ceramide, glucosyl ceramide, lactosyl ceramide, phosphatide, and globoside. Phosphatidyl glycerols include, for example, dimyristoyl phosphatidylglycerol, dipalmitoyl phosphatidylglycerol, and distearoyl phosphatidylglycerol.

A liposome composition of the invention can comprise about 0.001, 0.01, 0.1, 1.0, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, (or any range between about 0.001 and 20) or more wt% of a pesticide or herbicide, for example a nematicide. A liposome composition of the invention can comprise about 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 2, 1.0, 0.1, 0.01, 0.001 (or any range between about 20 and 0.001) or less wt% of a pesticide or herbicide, for example a nematicide. For example, a liposome composition can comprise about 0.001 to about 0.01 wt%, about 0.01 to about 0.1

wt%, about 0.1 to about 1 wt%, about 1 to about 5 wt%, or about 5 to about 10 wt%, about 10 to about 20 wt% of a pesticide or nematicide. A liposome composition can comprise about 2, 3, 4, 5, 7, 10, 12, 15, 16, 17, 18 wt% (or any range between about 2 and 18 wt%) or more lipid phase and about 82, 83, 84, 85, 88, 90, 93, 95, 96, 97, 98 wt% (or any range between about 82 and 98 wt%) aqueous phase. The lipid phase may comprise about 2, 5, 10, 15, 20, 30, 40, 50, 60, 70, 75, or 80 wt% phospholipids, for example about 25 to about 44 wt % phospholipids.

A liposome of the invention can be loaded with about 1, 5, 10, 50, 100, 200, or 500, 1,000, 2,000 (or any range between about 1 and 2,000) or more µg/ml of nematicides, pesticides or herbicides. A liposome of the invention can be loaded with about 2,000, 1,000, 500, 200, 100, 50, 10, 5, 1 (or any range between about 2,000 and 1) or less µg/ml of nematicides, pesticides or herbicides.

The lipid phase may optionally comprise one or more additional agents such as thickeners, gelling agents, preservatives, stabilizers, wetting agents, pH buffering agents, emulsifiers, stearylamine, phosphatidic acid, dicetyl phosphate, sterols, cholesterol, cholesterol stearate, lanolin extracts, hydroxypropylmethycellulose, carboxymethycellulose, sodium acetate, sorbitan monolaurate, triethanolamine oleate, and sorbitol. An additional agent may be present at about 0.01, 0.1, 1, 2, 5, 7, 10, 12, or 15 wt% of the lipid phase.

A liposome composition can also include one or more additives to improve the biological performance of the composition (for example by improving wetting, retention or distribution on surfaces; resistance to rain on treated surfaces; or uptake or mobility of a liposome formulation). Such additives include surface active agents, spray additives based on oils, for example certain mineral oils or natural plant oils (such as soy bean and rape seed oil), and blends of these with other bio-enhancing adjuvants (ingredients which may aid or modify the action of a liposome formulation).

After formation and loading of liposomes with one or more pesticides or herbicides, including, for example one or more nematicides, the liposomes can be freeze-dried or lyophilized. See U.S. Pat. No. 4,311,712. The liposomes can be reconstituted on contact with water or another liquid. Other components can be added to the lyophilized or reconstituted liposomes, for example, water, fertilizer, pesticides, or herbicides.

In one embodiment of the invention the pest, for example, a nematode, ingests the liposomes of the invention. In another embodiment of the invention, the

liposome delivers the pesticide or herbicide within the liposome to soil, plant media, plant, plant tissue, seed or fruit via slow release from the liposome, where the pest, weed, or nematode then comes in contact with the pesticide or herbicide.

Due to their structure, chemical composition and colloidal size, all of which can be well controlled during preparation protocols, liposomes exhibit several properties that can be useful in various applications. The most important properties are colloidal and special membrane and surface characteristics. The colloidal stable liposomes make them work well as a carrier of different molecules, *i.e.,* drug molecules. They also include bilayer phase behavior, its mechanical properties and permeability, charge density, presence of surface bound or grafted polymers, or attachment of special ligands, respectively. Additionally, due to their amphiphilic character, liposomes are a powerful solubilizing system for a wide range of compounds. Liposomes have a non-equilibrium structure and are less sensitive to external changes than equilibrium structures, such as micelles. In addition to these physico-chemical properties, liposomes exhibit many special biological characteristics, including (specific) interactions with biological membranes and various cells.

The liposome formulations can be chosen from a number of formulation types, including dustable powders (DP), soluble powders (SP), water soluble granules (SG), water dispersible granules (WG), wettable powders (WP), granules (GR) (slow or fast release), soluble concentrates (SL), oil miscible liquids (OL), ultra-low volume liquids (UL), emulsifiable concentrates (EC), dispersible concentrates (DC), emulsions (both oil in water (EW) and water in oil (EO)), micro-emulsions (ME), suspension concentrates (SC), aerosols, fogging/smoke formulations, capsule suspensions (CS) and seed treatment formulations. The formulation type chosen in any instance will depend upon the particular purpose envisaged and the physical, chemical and biological properties of the liposome formulation.

Dustable powders (DP) may be prepared by mixing a liposome formulation with one or more solid diluents (for example natural clays, kaolin, pyrophyllite, bentonite, alumina, montmorillonite, kieselguhr, chalk, diatomaceous earths, calcium phosphates, calcium and magnesium carbonates, sulfur, lime, flours, talc and other organic and inorganic solid carriers) and mechanically grinding the mixture to a fine powder.

Soluble powders (SP) may be prepared by mixing a liposome formulation with one or more water-soluble inorganic salts (such as sodium bicarbonate, sodium carbonate or magnesium sulfate) or one or more water-soluble organic solids (such as a polysaccharide) and, optionally, one or more wetting agents, one or more dispersing agents or a mixture of said agents to improve water dispersibility/solubility. The mixture is then ground to a fine powder. Similar compositions may also be granulated to form water soluble granules (SG).

Wettable powders (WP) may be prepared by mixing a liposome formulation with one or more solid diluents or carriers, one or more wetting agents and, preferably, one or more dispersing agents and, optionally, one or more suspending agents to facilitate the dispersion in liquids. The mixture is then ground to a fine powder. Similar compositions may also be granulated to form water dispersible granules (WG).

Granules (GR) may be formed either by granulating a mixture of a liposome formulation and one or more powdered solid diluents or carriers, or from pre-formed blank granules by absorbing a liposome formulation (or a solution thereof, in a suitable agent) in a porous granular material (such as pumice, attapulgite clays, fuller's earth, kieselguhr, diatomaceous earths or ground corn cobs) or by adsorbing a liposome formulation (or a solution thereof, in a suitable agent) on to a hard core material (such as sands, silicates, mineral carbonates, sulfates or phosphates) and drying if necessary. Agents which are commonly used to aid absorption or adsorption include solvents (such as aliphatic and aromatic petroleum solvents, alcohols, ethers, ketones and esters) and sticking agents (such as polyvinyl acetates, polyvinyl alcohols, dextrins, sugars and vegetable oils). One or more other additives may also be included in granules (for example an emulsifying agent, wetting agent or dispersing agent).

Dispersible Concentrates (DC) may be prepared by dissolving a liposome formulation in water or an organic solvent, such as a ketone, alcohol or glycol ether. These solutions may contain a surface active agent (for example to improve water dilution or prevent crystallization in a spray tank).

Emulsifiable concentrates (EC) or oil-in-water emulsions (EW) may be prepared by dissolving a liposome formulation in an organic solvent (optionally containing one or more wetting agents, one or more emulsifying agents or a mixture of said agents). Suitable organic solvents for use in ECs include aromatic

hydrocarbons (such as alkylbenzenes or alkylnaphthalenes, exemplified by SOLVESSO® 100, SOLVESSO® 150 and SOLVESSO® 200; SOLVESSO®), ketones (such as cyclohexanone or methylcyclohexanone) and alcohols (such as benzyl alcohol, furfuryl alcohol or butanol), N-alkylpyrrolidones (such as N-methylpyrrolidone or N-octylpyrrolidone), dimethyl amides of fatty acids (such as C_8-C_{10} fatty acid dimethylamide) and chlorinated hydrocarbons. An EC product may spontaneously emulsify on addition to water, to produce an emulsion with sufficient stability to allow spray application through appropriate equipment. Preparation of an EW involves obtaining a liposome formulation either as a liquid (if it is not a liquid at ambient temperature, it may be melted at a reasonable temperature, typically below 70°C) or in solution (by dissolving it in an appropriate solvent) and then emulsifying the resultant liquid or solution into water containing one or more SFAs, under high shear, to produce an emulsion. Suitable solvents for use in EWs include vegetable oils, chlorinated hydrocarbons (such as chlorobenzenes), aromatic solvents (such as alkylbenzenes or alkylnaphthalenes) and other appropriate organic solvents which have a low solubility in water.

Microemulsions (ME) may be prepared by mixing water with a blend of one or more solvents with one or more SFAs, to produce spontaneously a thermodynamically stable isotropic liquid formulation. A liposome formulation is present initially in either the water or the solvent/SFA blend. Suitable solvents for use in MEs include those hereinbefore described for use in ECs or in EWs. An ME may be either an oil-in-water or a water-in-oil system (which system is present may be determined by conductivity measurements) and may be suitable for mixing water-soluble and oil-soluble pesticides in the same formulation. An ME is suitable for dilution into water, either remaining as a microemulsion or forming a conventional oil-in-water emulsion.

Suspension concentrates (SC) may comprise aqueous or non-aqueous suspensions of finely divided insoluble solid particles of a liposome formulation. SCs may be prepared by ball or bead milling the solid liposome formulation in a suitable medium, optionally with one or more dispersing agents, to produce a fine particle suspension of the compound. One or more wetting agents may be included in the composition and a suspending agent may be included to reduce the rate at which the particles settle. Alternatively, a liposome formulation may be dry milled and added to

water, containing agents hereinbefore described, to produce the desired end product.

Aerosol formulations comprise a liposome formulation and a suitable propellant (for example n-butane). A liposome formulation may also be dissolved or dispersed in a suitable medium (for example water or a water miscible liquid, such as n-propanol) to provide compositions for use in non-pressurized, hand-actuated spray pumps.

A liposome formulation may be mixed in the dry state with a pyrotechnic mixture to form a composition suitable for generating, in an enclosed space, a smoke containing the compound.

Capsule suspensions (CS) may be prepared in a manner similar to the preparation of EW formulations but with an additional polymerization stage such that an aqueous dispersion of oil droplets is obtained, in which each oil droplet is encapsulated by a polymeric shell and contains a liposome formulation and, optionally, a carrier or diluent therefor. The polymeric shell may be produced by either an interfacial polycondensation reaction or by a coacervation procedure. The compositions may provide for controlled release of the liposome formulation and they may be used for seed treatment. A liposome formulation may also be formulated in a biodegradable polymeric matrix to provide a slow, controlled release of the compound.

A liposome formulation may also be formulated for use as a seed treatment, for example as a powder composition, including a powder for dry seed treatment (DS), a water soluble powder (SS) or a water dispersible powder for slurry treatment (WS), or as a liquid composition, including a flowable concentrate (FS), a solution (LS) or a capsule suspension (CS). The preparations of DS, SS, WS, FS and LS compositions are very similar to those of, respectively, DP, SP, WP, SC and DC compositions described above. Compositions for treating seed may include an agent for assisting the adhesion of the composition to the seed (for example a mineral oil or a film-forming barrier). In a seed treatment a liposomal composition can be applied in an amount of about 0.0001, 0.001, 0.01, 0.1, 1.0, 5, 10, 100, 1,000, 5,000, 10,000 g per 100kg of seeds. For example from about 0.001 g to about 10 kg per 100 kg of seeds.

Methods of Use of Liposomes Formulations of the Invention

The liposomal formulations of the invention can reduce leaching of pesticides, herbicides and nematicides into soil, can prevent migration of pesticides, herbicides, and nematicides through soil (due to slow release from liposomes), can prevent the binding of biological (i.e. DOBA) or chemical (i.e. Abamectin) pesticides, herbicides and nematicides to organic materials, and can be formulated to bind to plant roots. The liposomal formulations of the invention therefore can help to efficiently deliver pesticides, herbicides or nematicides to the site of action where pests and plants interact, thereby improving control. In addition, compositions of the invention can be formulated to control the release of pesticides, herbicides and nematicides into different soil types. Compositions of the invention can also be integrated with crop rotation to control pesticides, herbicides and nematicides that infect wide range of hosts. Different formulations with effective pesticide, herbicide and nematicide doses can also be developed and integrated with soil textures maps to reduce pesticide, herbicide and nematicide use and run off in the environment. Compositions of the invention also can be effective to control nematodes, pests, and weeds in fields with varying soil textures or that need to application at different rates and different times of plant growth stages.

Formulations of the invention are effective against larvae, eggs, juveniles, and adult insects, nematodes, and other pests. Formulations of the invention can kill or paralyze insects, nematodes and pests. They can also reduce the numbers of larvae, eggs, and adult pests, insects, and nematodes on plants, plant tissues, and in or on soil or plant media.

The method of application of the compositions of the invention to soils, plant media, plants, seeds, seedlings or plant tissues is important. Compositions of the invention can be applied to soils or plant media when the plants are pre-emergent or post-emergent.

Compositions of the invention can be applied by mechanical sprayers. Sprayers convert a formulation of the invention which is mixed with a liquid carrier, such as water or fertilizer, into droplets. The droplets can be any size. Boom sprayers and air blast sprayers can also be used to apply formulations of the invention to pre-emergent or post-emergent crops. Air blast sprayers inject formulations of the invention mixed with a liquid carrier into a fast-moving air stream. Boom sprayers, aerial sprayers, ultra-low volume sprayers, drip irrigation, sprinkler irrigation, and foggers can also be used to apply formulations of the invention.

Where the formulations of the invention are in a solid, powder or granule form, they can be applied with granule or dust application equipment. Liposomal formulations of the invention can also be applied to soil, plant media, plants, plant tissues or seeds as a fumigant.

In one embodiment of the invention freeze-dried or lyophilized liposomes containing one or more pesticides (e.g., nematicides) or herbicides or a combination thereof are applied directly to non-emergent crops, emergent crops, seeds, soil, plant medium, seeds or plant tissues. In another embodiment of the invention freeze-dried or lyophilized liposomes are reconstituted or rehydrated with water, another liquid (e.g., fertilizer, pesticide, herbicide, nematicide), or any other suitable liquid or gel and then applied directly to non-emergent crops, emergent crops, seeds, soil, plant medium, seeds or plant tissues. The liquid can be fertilizer or can contain fertilizer or other components.

Pesticides are usually recommended for field application as an amount of pesticide per hectare (g/ha or kg/ha) or the amount of active ingredient or acid equivalent per hectare (kg a.i./ha or g a.i./ha).

Advantageously, a much lower amount of herbicide or pesticide, e.g., nematicide, is required to be applied to soil, plant media, seeds plant tissue, or plants to achieve the same results as where the pesticide is applied in a non-liposomal formulation. For example, the amount of herbicide, pesticide or nematicide is applied at levels about 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 20, 30, 50, or 100-fold (or any range between about 2 and about 100-fold, for example about 2- to 10-fold; about 5- to 15-fold, about 10- to 20-fold; about 10- to 50-fold) less than the same herbicide, pesticide or nematicide applied in a non-liposomal formulation, e.g., direct application of the same pesticide, herbicide or nematicide. For example, oxamyl in a non-liposomal formulation has a suggested application rate for potatoes of 4.0 to 5.5 kg a.i. /ha. When oxamyl is incorporated into the liposome formulations of the invention, the application rate would fall to about 0.4 to 0.55 kg a.i./ha (10-fold less).

Liposome formulations of the invention can be applied at about 0.0001, 0.001, 0.005, 0.01, 0.1, 1, 2, 10, 100, 1,000, 2,000, 5,000 (or any range between about 0.0001 and 5,000) kg/ha. For example, about 0.0001 to about 0.01, about 0.01 to about 10, about 10 to about 1,000, about 1,000 to about 5,000 kg/ha.

Surprisingly, where pesticides or nematicides in the liposomal formulations of the invention are applied at the same concentration as non-liposomal formulations, the liposomal formulations have unexpected advantages. Firstly, liposomal formulations of the invention when applied at the same concentrations as non-liposomal formulations are more effective at controlling pests, weeds, and nematodes and at reducing damage to plants such as gall formation and root necrosis. Secondly, the use of liposomal formulations of the invention applied at the same or lower concentrations as non-liposomal formulations result in longer root length of plants, enhanced stalk growth, enhanced leaf growth, and healthier plants having enhanced vigor.

Therefore, the invention includes methods of increasing root lengths, increasing stalk diameter, increasing stalk length, increasing leaf number, increasing leaf size of a plant, increasing plant vigor or a combination thereof of a nematicide, herbicide or pesticide treated plant or a plant grown in nematicide-, herbicide-, or pesticide-treated soil or plant media comprising administering one or more nematicides, herbicides, or pesticides to the plant or the soil or plant media, wherein the one or more nematicides, herbicides or pesticides are present in an aqueous core of a liposome. The nematicides, herbicides or pesticides present in the aqueous core of a liposome can be administered at a same amount or a lower amount or concentration than the recommended administration amount or concentration of the same nematicide, herbicides or pesticides when administered in a non-liposomal formulation.

Another embodiment of the invention provides a method of decreasing the amount of nematicide-, pesticide-, or herbicide-induced damage to nematicide, pesticide or herbicide treated plants or a plants grown in nematicide-, pesticide-, or herbicide-treated soil or plant media comprising administering one or more nematicides, pesticides, or herbicides to the plant or the soil or plant media, wherein the one or more nematicides, pesticides or herbicides are present in an aqueous core of a liposome. The nematicides, pesticides, or herbicides present in the aqueous core of a liposome can be administered at a same amount or concentration or a lower amount or concentration than the recommended administration amount or concentration of the same nematicides, pesticides or herbicides when administered in a non-liposomal formulation.

Nematicide-, pesticide-, or herbicide-induced damage to plants can include, for example, root necrosis, gall formation, decreased yields, less developed root system (including shorter roots), tillering decrease, decrease in plant height, decrease in stalk circumference, smaller leafs, less leaves, more dead basal leaves, more fertilizers needed, more seeds needed, less productive tillers, later flowering, later grain, seed or fruit maturity, more plant verse (lodging), decreased shoot growth, decreased plant vigor, or a combination thereof.

Treatment of Humans and Animals

Liposomal compositions of the invention can also be used to treat animals, including mice, rats, horses, cattle, sheep, pigs, dogs, cats, and primates. The compounds of the invention are also effective for use in humans. Administration of the liposomal compositions can reduce or alleviate the symptoms of an animal infected with or in contact with one or more pests or nematodes. Administration can also eliminate or reduce the number of pests or nematodes infecting or in contact with an animal.

The liposomes of the present invention can be administered by any suitable means including, but not limited to, for example parenterally, intraarticularly, subcutaneously, intramuscularly, intradermally, intravenously (including an intravenous drip), intraperitoneally (including bolus injection), intramedullary, intrathecally, intraventricularly, transdermally, subcutaneously, intranasally, orally, rectally, topically (including transdermal, aerosol, buccal and sublingual), vaginally, or intravesically.

Liposomes of the invention can be present in a pharmaceutical formulation. For example, in addition to the active ingredients, liposomal pharmaceutical compositions of the invention can contain suitable pharmaceutically-acceptable carriers comprising excipients and auxiliaries which facilitate processing of the active compounds into preparations which can be used pharmaceutically. Further details on techniques for formulation and administration may be found in the latest edition of Remington's Pharmaceutical Sciences (Maack Publishing Co., Easton, Pa.).

Pharmaceutical compositions for oral administration can be formulated using pharmaceutically acceptable carriers well known in the art in dosages suitable for oral administration. Such carriers enable the pharmaceutical compositions to be formulated as tablets, pills, dragees, capsules, liquids, gels, syrups, slurries, suspensions, and the like, for ingestion by the patient.

The concentration of liposomes in the pharmaceutical formulations can vary widely, *i.e.*, from less than about 0.05%, usually at or at least about 2-5% to as much as 10 to 30% by weight and will be selected primarily by fluid volumes, viscosities, etc., in accordance with the particular mode of administration selected. For example, the concentration can be increased to lower the fluid load associated with treatment. Alternatively, liposomes can be diluted to low concentrations to lessen inflammation at the site of administration. The amount of liposomes administered will depend upon the particular nematicides or pesticides used, the disease state being treated and the judgment of the clinician, but will generally, in a human, will be between about 0.001 and about 50 mg per kilogram of body weight, for example, between about 0.001 and 10 mg/kg or between about 5 and about 40 mg/kg of body weight. Higher lipid doses are suitable for other animals, for example, 50-120 mg/kg.

Dosage for the liposomal compositions will depend on the administrating physician's opinion based on age, weight, and condition of the patient, and the treatment schedule. Doses of pesticides or nematicides in humans will be effective at ranges as low as from 0.015 mg/M^2/dose and will still be tolerable at doses as high as 15 to 75 mg/M^2/dose, depending on dose scheduling. Doses may be single doses or they may be administered repeatedly every 4 h, 6 h, or 12 h or every 1 d, 2 d, 3 d, 4 d, 5 d, 6 d, 7 d, 8 d, 9 d, 10 d or combination thereof. Scheduling may employ a cycle of treatment that is repeated every week, 2 weeks, three weeks, four weeks, five weeks or six weeks or combination thereof.

In certain embodiments, the liposomal compositions of the invention can be administered as a preventative measure. Prevention or preventing refers to a reduction of the risk of acquiring a pest or nematode infection. The compositions of the invention can be administered as a preventative measure to a subject even though symptoms of pest or nematode infection are absent or minimal.

About, as used herein, means that the value varies up or down by 5%. For example, for a value of about 100, means 95 to 105 (or any value between 95 and 105).

All patents, patent applications, and other scientific or technical writings referred to anywhere herein are incorporated by reference herein in their entirety. The invention illustratively described herein suitably can be practiced in the absence of any element or elements, limitation or limitations that are not specifically disclosed

herein. Thus, for example, in each instance herein any of the terms "comprising", "consisting essentially of", and "consisting of" may be replaced with either of the other two terms, while retaining their ordinary meanings. The terms and expressions which have been employed are used as terms of description and not of limitation, and there is no intention that in the use of such terms and expressions of excluding any equivalents of the features shown and described or portions thereof, but it is recognized that various modifications are possible within the scope of the invention claimed. Thus, it should be understood that although the present invention has been specifically disclosed by embodiments, optional features, modification and variation of the concepts herein disclosed may be resorted to by those skilled in the art, and that such modifications and variations are considered to be within the scope of this invention as defined by the description and the appended claims.

In addition, where features or aspects of the invention are described in terms of Markush groups or other grouping of alternatives, those skilled in the art will recognize that the invention is also thereby described in terms of any individual member or subgroup of members of the Markush group or other group.

The following are provided for exemplification purposes only and are not intended to limit the scope of the invention described in broad terms above.

Examples

Example 1: Egg Extraction

M. incognita eggs collected from tomato cultures by NaOC1 extraction (Hussey and Barker, 1973). Briefly, six to twelve week old infected tomato roots were cut into 1-2 segment. Root segments were shacked vigorously in 200 ml of a 0.5% Na O Cl solution for 4 min. Then, the Na O Cl solution was passed quickly through a 200-mesh (75-um), nested over a 500-mesh sieve to collect freed eggs. The a 500-mesh sieve with eggs was quickly placed under a stream of cold water to remove residual Na O Cl and rinsed several times. About 50ml aqueous suspension of eggs was collected and number of eggs per unit volume will be counted. The egg suspension was allowed to sit at room temperature for 4 days. The hatching juveniles were collected and used in the subsequent experiments.

Example 2: Liposomes preparations

Liposomes having an aqueous core and phospholipid bilayers were prepared using the thin-film dehydration-rehydration method obtaining, multilamilar vesicles (MLVs) and small unilamellar vesicles (SUVs) (Bangham *et al.*, J. Mol. Biol. 13:238-

252 (1965); Gosangari & Watkin, Pharm Dev Technol. 17:383-8 (2012)). Using thin-film hydration method, briefly, required amounts of lipids [1,2-Dipalmitoyl-sn-glycero-3-phosphocholine (DPPC) liposomes and 1, 2-distearoyl-*sn*glycero- 3-phosphocholine (DSPC)] were dissolved in chloroform, and a thin film will be formed on the inner side of the round bottom flask, by evaporating the solvent under vacuum using a rotavapor. The lipid film formed was stored overnight in vacuum desiccator to eliminate traces of chloroform. The film was then hydrated at 58°C, above the *Tc* of DSPC, using 10mL of phosphate-buffered saline (PBS, 20mM Na2HPO3–NaH2PO3, 150mM NaCl, pH 7.0) containing different concentration of Oxamyl. The hydration process of Oxamyl liposomes was done with vigorous agitation to form multilamellar vesicles (MLV). The formed liposomes were sonicated using a probe sonicator in 5 cycles of 2 min each. The MLVs were centrifuged at 10000 x g for 15 minutes to purify the liposomes from the un-encapsulated Oxamyl (Mohammed *et al.,* Int J Pharm. 285:23-342004). To form small unilamellar vesicles (SUV), the multilamellar liposomes were extruded through polycarbonate membranes of pore sizes 1.0 µm (Olson *et al.*, Biochim Biophys Acta. 557:9-23 (1979)). The unencapsulated oxamyl was then separated using Sephadex G-50 macrospin columns and the encapsulation efficiency was calculated spectrophotometrically at 280 nm. The liposomal fractions collected from the Sephadex columns were pooled and lyophilized after addition of suitable amount of sucrose as a cryoprotectant. Addition of sugars to liposome formulation prevent vesicle fusion and have been attributed to the formation of a stable glassy state as well as direct interaction between the polar head groups of phospholipids and sugars (Crowe & Crowe, Biochim Biophys Acta. 939:327-34 (1988)).

Example 3: Fluorescent Uranin-liposomes:

We have developed a micron sized liposome that has the ability to encapsulate different concentrations of oxamyl (or other insecticide/nematicide). It is an efficiency method that suppresses the root-knot nematodes. To prove this concept we used liposomes loaded with 100 mM of the hydrophilic fluorescent reagent uranin to test oral administration of water-soluble substances to the plant parasitic nematode. Liposomes prepared as mentioned above. Uranin solution (2 mg/ml) in PBS buffer was added to thin film of liposomes during rehydration at above 50 °C. Unencapsulated uranin was removed through gel chromatography. About 50

ul of uranin liposome solution was mixed with nematode suspension and incubated for 2-3 days at room temperature. The mix was then visualized by fluorescent microscope. Our data demonstrate that ingestion of liposomes loaded with fluorescent dye resulted in successful oral delivery of chemicals into the intestines of Root-knot and Spiral nematodes. Spiral nematodes fed 25 µl of liposomes containing uracin showed clear fluorescence along their esophagus digestive tracts. Root nematodes fed 25 µl of liposomes containing uracin showed clear fluorescence along several organs of their bodies.

Example 4: Effect of Oxamyl (Vydate) and Thiocarb (Larvin) liposome formulation on Root-Knot nematodes *in vitro*

Determine the efficient concentration of nematicides (Oxamyl and Thidiocarb) that kill or suppress Root-Knot Larvae.

M. incognita eggs collected from tomato cultures by NaOC1 extraction (Hussey & Barker, Plant Disease Reporter. 57:1025–1028 (1973)). Egg suspension was incubated at room temperature until larvae were hatched (4-5 days). The juveniles (J2) were counted and evaluated for activity/mobility for the duration of the study. Four different concentrations of both nematicides (Oxamyl and Thiodicarb) were used to assess their efficacy in killing the nematodes. These were untreated control, 200 ug, 1mg, 2 mg, and 10 mg. Three replicates of the each concentration were mixed with J2 suspensions and incubated at room temperature for 2 days.

Root-Knot larvae (J2)		
Treatment	**Alive**	**Dead**
Control 1	97	3
Control 2	93	7
Control 3	100	0
Larvin (Thiodicarb) 200 ug	76	23
Larvin (Thiodicarb) 200 ug	86	14
Larvin (Thiodicarb) 200 ug	89	11
Larvin (Thiodicarb) 1 mg	63	37
Larvin (Thiodicarb) 1 mg	75	25
Larvin (Thiodicarb) 1 mg	82	18
Larvin (Thiodicarb) 2 mg	70	30
Larvin (Thiodicarb) 2 mg	85	15
Larvin (Thiodicarb) 2 mg	65	35
Larvin (Thiodicarb) 10 mg	70 non-mobile	30
Larvin (Thiodicarb) 10 mg	80 non-mobile	20
Larvin (Thiodicarb) 10 mg	70 non-mobile	30
Oxamyl (Vydate) 200 ug	60 non-mobile	40
Oxamyl (Vydate) 200 ug	58 non-mobile	42
Oxamyl (Vydate) 200 ug	50 non-mobile	50
Oxamyl (Vydate) 1 mg	0	100
Oxamyl (Vydate) 1 mg	0	100
Oxamyl (Vydate) 1 mg	0	100

Oxamyl (Vydate)	2 mg	0	100
Oxamyl (Vydate)	2 mg	0	100
Oxamyl (Vydate)	2 mg	0	100
Oxamyl (Vydate)	10 mg	0	100
Oxamyl (Vydate)	10 mg	0	100
Oxamyl (Vydate)	10 mg	0	100

Based on the data mentioned above, we eliminated Thiodicarb (Larvin) because it required higher concentration to kill Root-Knot (J2). We studied the efficiency of Oxamyl; 200 ug/ml and 100 ug/ml in suppression of J2. We found that both concentrations lead to 100 % mortality of J2 larvae. We used 100ug of Oxamyl in subsequent studies with liposomes. Oxamyl-liposome formulation was created and it demonstrated its efficiency to suppress Root-knot nematodes as follows.

Treatment	**Nematicidal activity of 100 ug/ml Oxamyl-liposome formulation on root-knot nematodes**
Control 1	7 % dead
Control 2	2 % dead
Control 3	9 % dead
Liposomes only 1	9 % dead
Liposomes only 2	3 % dead
Liposomes only 3	5 % dead
100 ug	83 dead 17 non mobile
100 ug	100 dead
100 ug	82 dead 18 non mobile

Where no oxamyl was added, the larvae were free and active. Where oxamyl was added the larvae were dead or paralyzed. See also, Figure 1. Figure 1 shows the nematicide activity of 100 µg of oxamyl-liposome formulations on root-knot nematodes.

Dosage

The lethal effect of nematicides is determined by two components. The first is concentration (C) of the nematicide in soil solution, usually expressed as 5 parts per million (PPM). The second is the length of time (T) the nematode is exposed, expressed in minutes, hours or days. The level of nematode control is then related to dosage, the amount of pesticide placed in the environment of the nematode for a known length of exposure time (concentration X time). Total exposure is the sum of CT products.

For most organisms, nematodes included, there is a nematicide concentration level, below which kill is not obtained regardless of the length of exposure. If

exposure to 10 ppm for 20 days (200 CT) is the minimum dosage required to kill a nematode, then exposure to 4 ppm for 50 days (200 CT) will be totally ineffective even though the nematode has received the same cumulative dosage. In this example, a minimum concentration of 10 ppm was required to effectively contribute to the lethal or disorientating activity of the nematicide. For most nematodes, long exposures to low concentrations of fumigant nematicides above the minimum concentration appear to be more effective than short exposures to higher concentration. All nematode species are not equally susceptible to a given nematicide nor are all life stages of a given species equally sensitive given the same exposure time. For example, after a 24 hour exposure to the fumigant nematicide EDB, only 75% of a population of free living nematodes in soil was killed while the citrus nematode did not survive a 0.5 hour exposure to EDB at the same concentration. In dry soils, many nematodes which can survive in a dehydrated state can tolerate 10 times the lethal dose of methyl bromide compared to active forms in moist soil. In practice, fumigant nematicides are commonly injected through a series of uniformly spaced shanks into soil. As the liquid volatilizes, gases begin moving in mass flow, diffusing radially outward in all directions from the point of injection. Since diffusion is greater in air above the soil surface, upward mass flow and diffusion is usually greater than downward movement, and much of the gas may escape the soil and enter the atmosphere. As the nematicide front moves through soil, gaseous molecules are adsorbed to particle surfaces, redissolve into soil solution, and fill empty air spaces between soil particles. Maximum nematicide concentration decreases as do the sums of CT products with distance from the point of injection. Eventually, with time and distance, concentrations fall below an immediate killing level. The number of nematodes killed by fumigant treatment within these areas depends on the number of CT units which develop within the nematicide treated zone.

The relationship between nematicide application rate and nematode control is therefore not only a measure of pesticide toxicity but chemical dispersion as well. If dispersal is good, increases in chemical application rates will result in higher CT values and provide control to a greater soil volume. If dispersal is poor, increases in application rates will not provide control to a larger soil volume. Unlike fumigant nematicides where water may effectively block efficient dispersion in soil, nonfumigant nematicides must be carried by rainfall or irrigation water into soil to be

effective. Nematicide concentration and its persistence above a certain effective concentration is also important for nematode control with nonfumigant nematicides. The apparent failure to control nematodes with nonfumigant nematicides in many instances, is very likely the result of excessive rainfall or irrigation and poor chemical retention within the primary rooting zone of the crop. Unless otherwise explained, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which a disclosed disclosure belongs.

Example 5: Pre-Emergent Soil Treatment

The use of the liposomal formulations for the pre-emergent treatment of soil was tested. Peat chips were placed in a container with 3 rows of 5 chips each. The chips were hydrated with tap water and kept at room temperature for two days. The experimental and control treatments were administered to the wood chips. The chips were allowed to rest for one or two days. Two to three tomato seeds were planted on each chip. The plant stalk and leaf growth was monitored during plant emergence.

The normal control condition comprised no delivery of any type of Avid .15 (Syngenta) to the chips. For the experimental conditions one of three dosage levels of liposomal Avid .15 were applied to the chips:

(1) 56 µl, which is the commercially recommended dose level adjusted for the given area of the test chips;

(2) 1 µl

(3) 0.5 µl.

The doses were applied to the center of the chip using a Gilson Pipette Man.

The results are shown in Figure 2. Pre-emergent treatment of the chips with the liposomal formulations enhanced emerging plant growth compared to the untreated normal growth conditions. Lower doses (1 µl and 0.5 µl) enhanced stalk and leaf growth and lead to healthier plants as compared to the higher dose (56 µl). The plants administered the higher dose (56 µl) performed better than the normal control, but had inhibited leaf growth as compared to the normal control plants. At two weeks from planting the normal controls had large multi-leaf growth and strong stalks. The high dose (56 µl) plants had thin stalks and very small leaves. The low dose plants (1µl) had multiple large leaves. The very low dose plants (0.5 µl) had medium to large multi-leaf growth.

Example 6: Abamectin Liposomal Formulations

Abamectin at 5µg or 1µg was directly applied to soil prior to planting. Alternatively abamectin was loaded into liposomes at either 5µg or 1µg and applied to soil prior to planting. Gall formation was detected. The results are shown in Figure 3. The non-liposomal abamectin 1µg application resulted in the most gall formulation followed by the non-liposomal abamectin 5µg application. The liposomal abamectin 5µg or 1µg applications had almost non-detectable levels of gall formation. Additionally, the non-liposomal abamectin 5µg application resulted in the most root necrosis followed by the non-liposomal abamectin 1µg application. The liposomal abamectin 5µg or 1µg applications resulted in less root necrosis. *See* Figure 4. Additionally, the liposomal abamectin 5µg or 1µg applications resulted in longer root length than for the non-liposomal abamectin 1µg or 5 µg applications. See Figure 5. Therefore, liposomal abamectin formulations enhance root length as compared to non-liposomal abamectin formulations.

Claims:

We claim

1. A liposome formulation comprising one or more pesticides, nematicides, or herbicides loaded in the aqueous core of liposomes, wherein the liposomes are lyophilized.
2. The liposomal formulation of claim 1, wherein one or more nematicides are loaded into the aqueous core of the liposomes.
3. The liposome formulation of claim 2, wherein the one or more nematicides are 2-methyl-2-(methylthio)propionaldehyde O-methylcarbamoyloxime, 2,3-Dihydro-2,2-dimethyl-7-benzofuranyl methylcarbamate, 2-methyl-2-(methylsulfonyl)propanal-O-(methylaminocarbonyl oximel, O,O-diethyl O-[p-(methylsulfinyl)phenyl] ester, Ethyl 4-methylthio-m-tolyl isopropylphosphoramidate, O-ethyl S,S-dipropyl phosphorodithioate, Methyl N'N'-dimethyl-N-[(methylcarbamoyl)oxy]-1-thiooxamimidate, S-[[(1,1-dimethylethyl) thio] methyl]O,O-diethyl phosphorodithioate, thionazin, isazofos, ebufos, cleothocarb or combinations thereof.
4. The liposome formulation of claim 2, wherein the lyophilized liposome is loaded with about 1, 5, 10, 50, 100, 200, or 500 µg/ml of the one or more nematicides.
5. The composition of claim 1, wherein the liposome formulation is a dustable powder (DP), soluble powder (SP), water soluble granules (SG), water dispersible granules (WG), wettable powders (WP), granules (GR) (slow or fast release), soluble concentrates (SL), oil miscible liquids (OL), ultra-low volume liquids (UL), emulsifiable concentrates (EC), dispersible concentrates (DC), emulsions (both oil in water (EW) and water in oil (EO)), micro-emulsions (ME), suspension concentrates (SC), aerosols, fogging/smoke formulations, capsule suspensions (CS), powder for dry seed treatment (DS), a water soluble powder (SS), a water dispersible powder for slurry treatment (WS), a flowable concentrate (FS), a liquid solution (LS), a capsule suspension (CS), or combinations thereof.
6. The liposomal formulation of claim 1, wherein the composition further comprises a fertilizer.

7. A method for reducing the number of nematodes on or in plant media, soil, plants, plant tissues, or seeds, comprising administering to the plant media, soil, plants, plant tissues, or seeds an effective amount of the liposome formulation of claim 2.
8. The method of claim 7, wherein the lyophilized liposomes are rehydrated before they are administered to the plant media, soil, plants, plant tissues, or seeds.
9. The method of claim 8, wherein the lyophilized liposomes are rehydrated with water, liquid fertilizer, or other suitable liquid.
10. The method of claim 7, comprising administering about 5-fold, 10-fold, or 50-fold less nematicide via the liposome formulation than is recommended for conventional, non-liposomal application of the same nematicide.
11. The method of claim 7, wherein the plants or plants grown in the soil or plant media have increased root lengths, increased stalk diameter, increased stalk length, increased leaf number, increased leaf size, increased yield, or increased vigor as compared to plants or soil or plant media treated with non-liposomal formulations of the same one or more nematicides of the administered liposome formulation.
12. The method of claim 7, wherein the liposome formulation is administered in an amount from about 5 g/ha to about 2000 g/ha.
13. The method of claim 7, wherein the nematodes are root-knot nematodes.
14. The method of claim 7 wherein the liposomal composition is applied to seeds in an amount from about 0.001 g to about 10 kg per 100 kg of seeds.
15. A method of increasing root lengths, increasing stalk diameter, increasing stalk length, increasing leaf number, increasing leaf size of a plant, increasing yield, increasing plant vigor, or a combination thereof comprising administering a composition of claim 2 to the plant or to soil or plant media in which the plant is growing.
16. A method of increasing root lengths, increasing stalk diameter, increasing stalk length, increasing leaf number, increasing leaf size of a plant, increasing yield, increasing plant vigor or a combination thereof of a nematicide treated plant or a plant grown in nematicide-treated soil or plant media comprising administering one or more nematicides to the plant or the

soil or plant media, wherein the one or more nematicides are present in an aqueous core of a liposome.

17. The method of claim 16, wherein the nematicides present in the aqueous core of a liposome are administered at a same amount or concentration or a lower amount or concentration than the recommended administration amount or concentration of the nematicide when administered in a non-liposomal formulation.

18. A method of decreasing the amount of nematicide-induced damage to nematicide treated plants or plants grown in nematicide-treated soil or plant media comprising administering one or more nematicides to the plant or the soil or plant media, wherein the one or more nematicides are present in an aqueous core of a liposome.

19. The method of claim 18, wherein the nematicides present in the aqueous core of a liposome are administered at a same amount or concentration or a lower amount or concentration than the recommended administration amount or concentration of the nematicide when administered in a non-liposomal formulation.

20. A method for reducing the number of nematodes on or in an animal, comprising administering to the animal an effective amount of the liposome formulation of claim 2.

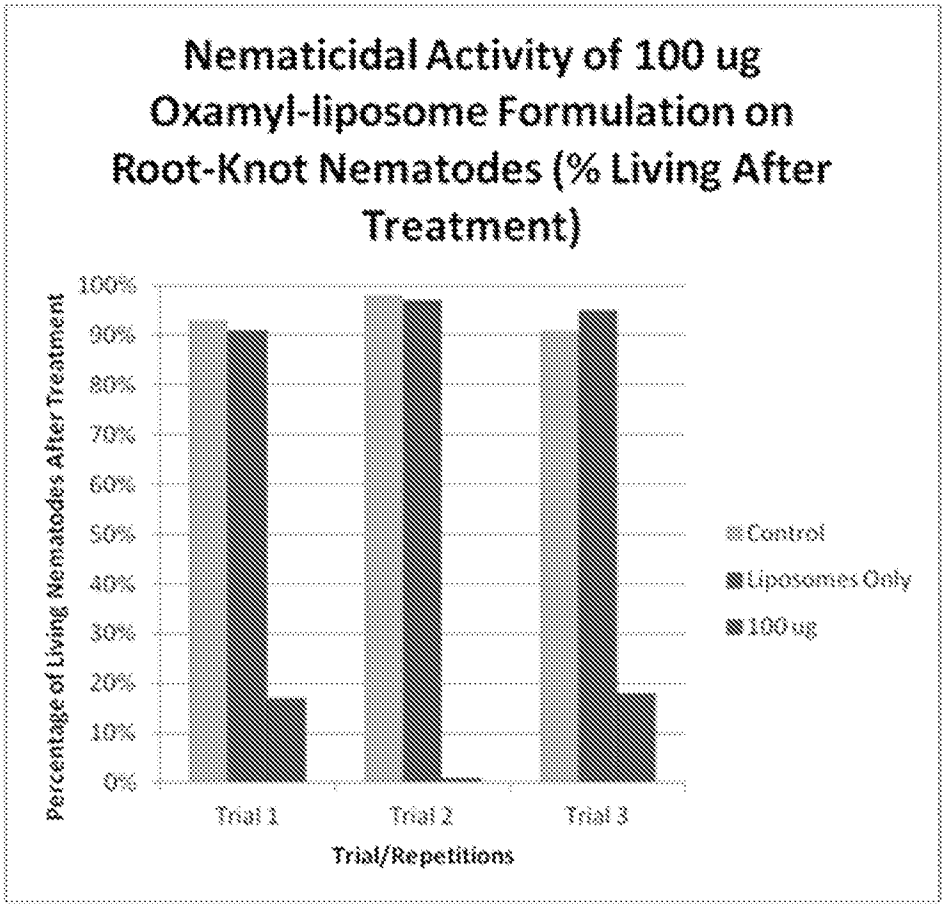
Nematicidal Activity of 100 ug Oxamyl-liposome Formulation on Root-Knot Nematodes (% Living After Treatment)
Percentage of Living Nematodes After Treatment
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Trial 1
Trial 2
Trial 3
Trial/Repetitions
Control
Liposomes Only
100 ug

Figure 1.

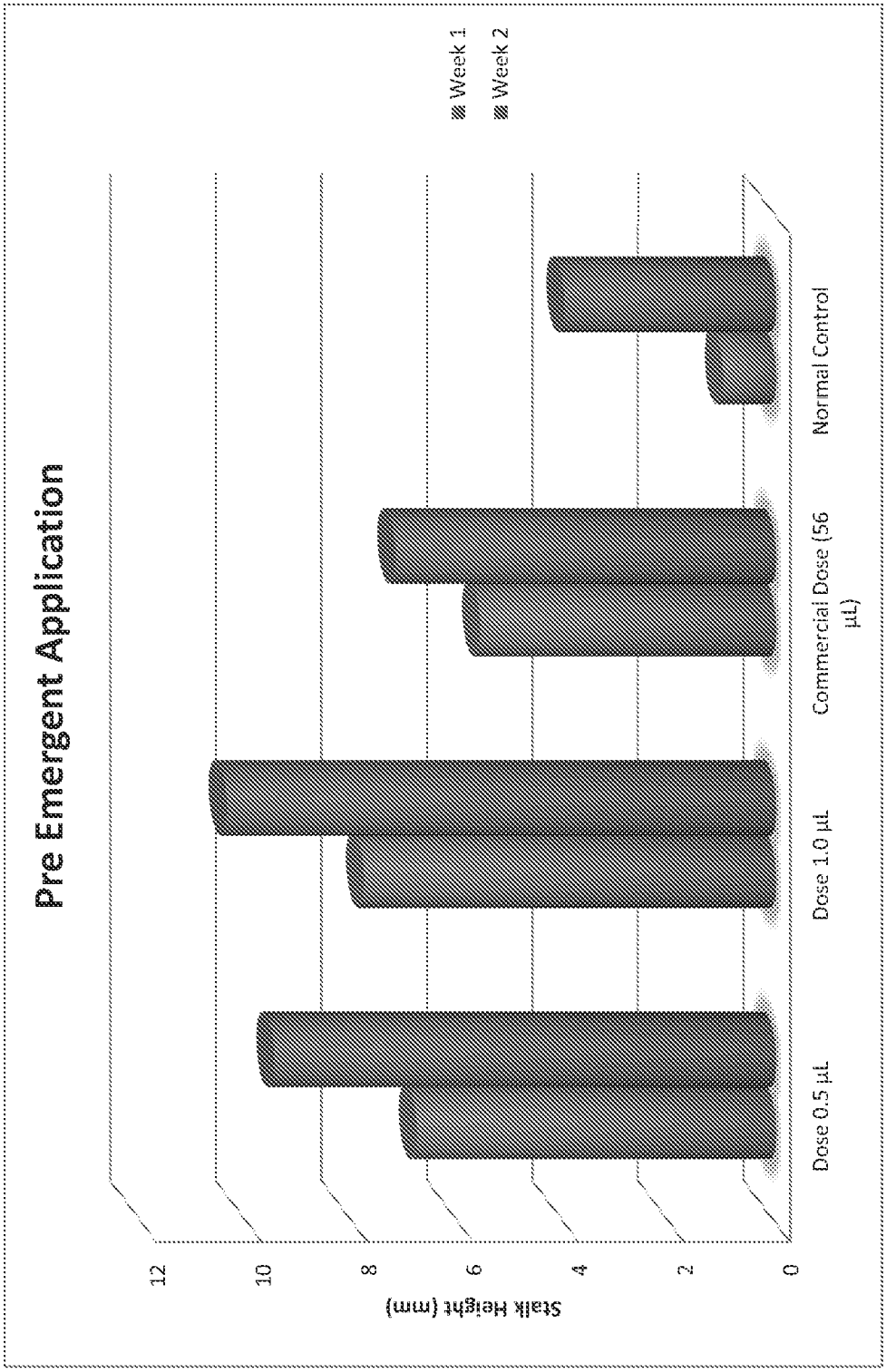
Pre Emergent Application
Week 1
Week 2
Stalk Height (mm)
0
2
4
6
8
10
12
Dose 0.5 µL
Dose 1.0 µL
Commercial Dose (56 µL)
Normal Control

Figure 2

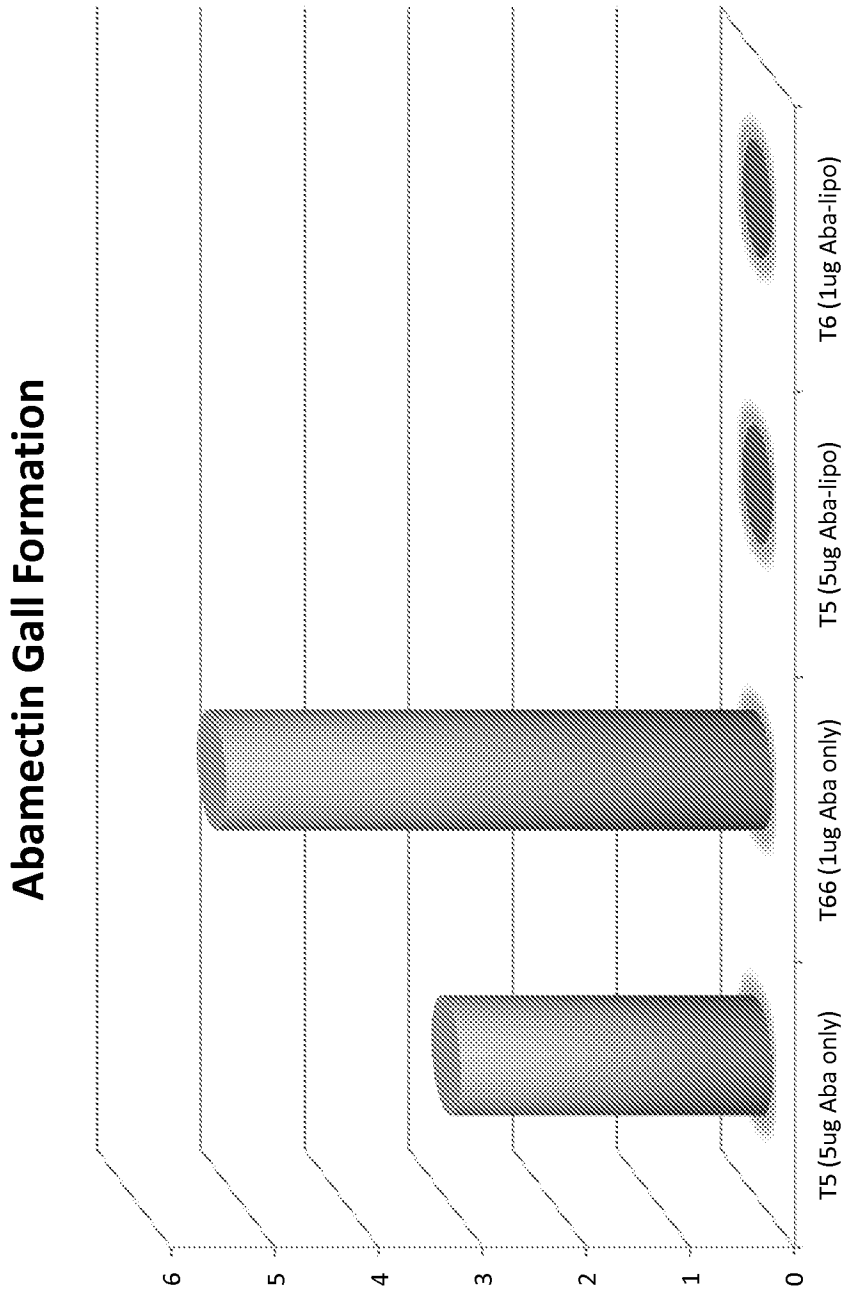
Abamectin Gall Formation
6
5
4
3
2
1
0
T5 (5ug Aba only)
T66 (1ug Aba only)
T5 (5ug Aba-lipo)
T6 (1ug Aba-lipo)

Figure 3

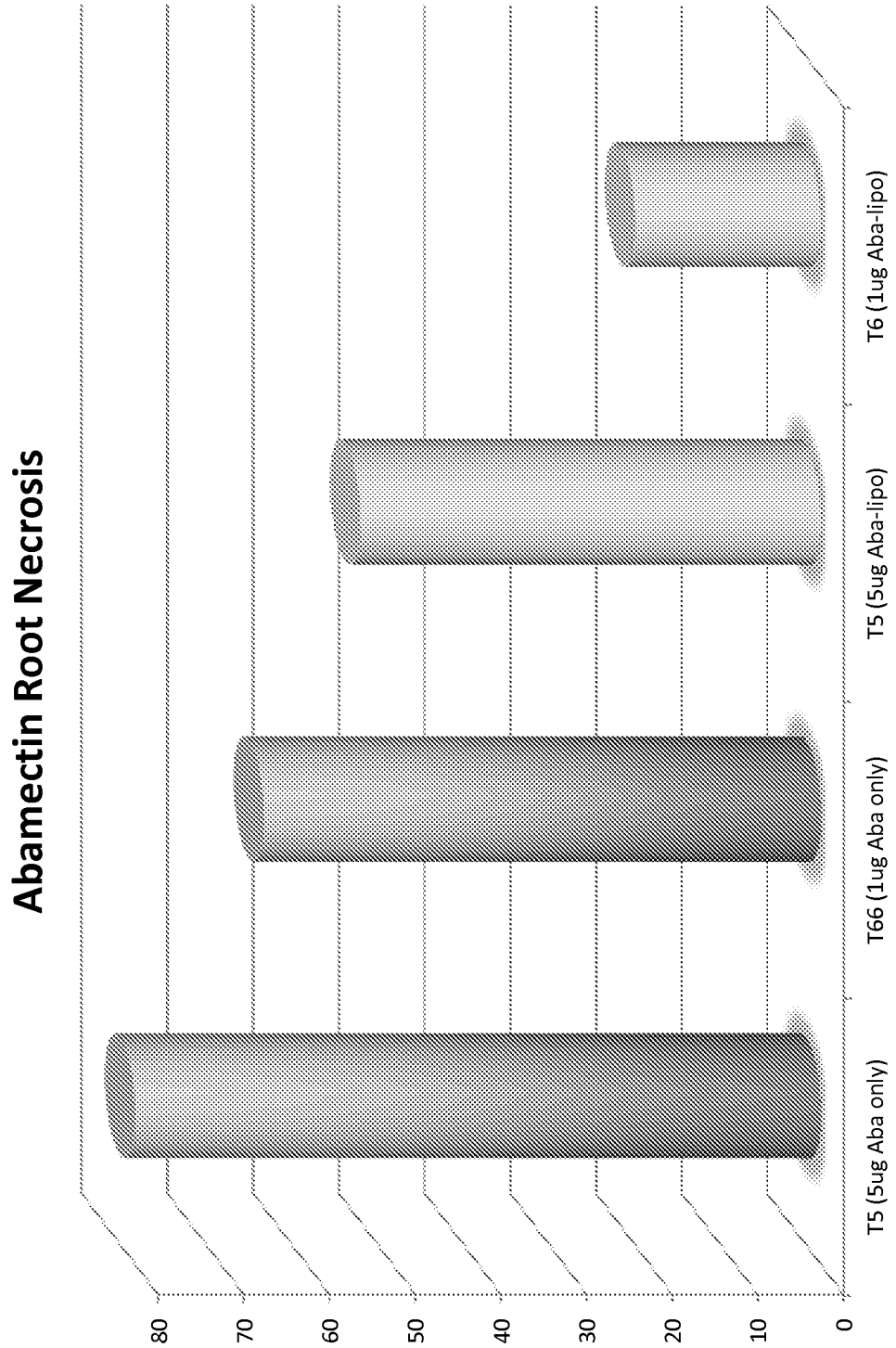
Abamectin Root Necrosis
80
70
60
50
40
30
20
10
0
T5 (5ug Aba only)
T66 (1ug Aba only)
T5 (5ug Aba-lipo)
T6 (1ug Aba-lipo)

Figure 4

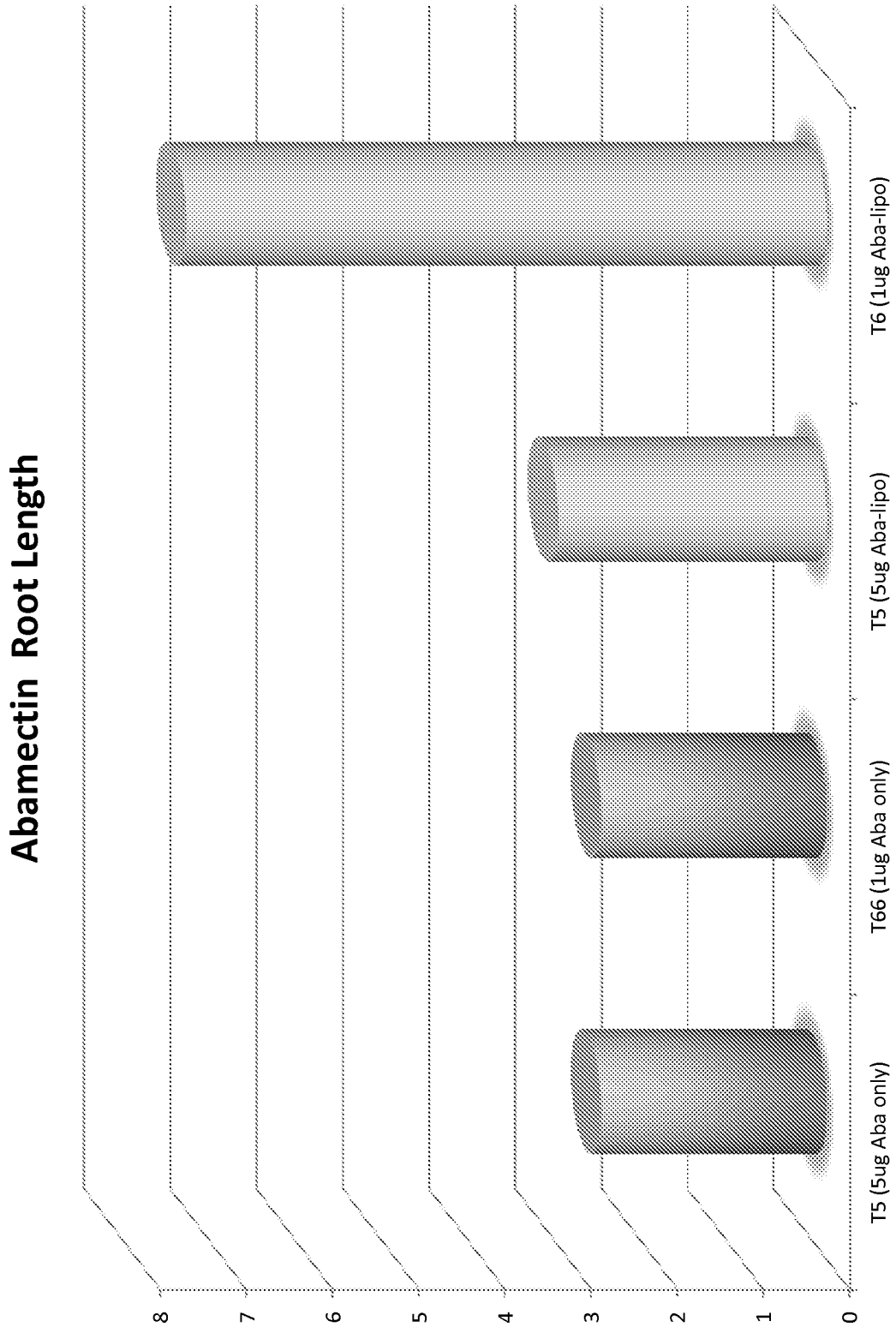
Abamectin Root Length
8
7
6
5
4
3
2
1
0
T5 (5ug Aba only)
T66 (1ug Aba only)
T5 (5ug Aba-lipo)
T6 (1ug Aba-lipo)

Figure 5

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 13/46338

A. CLASSIFICATION OF SUBJECT MATTER
IPC(8) - A61K 9/127 (2013.01)
USPC - 424/450
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
USPC 424/450
IPC A61K 9/127 (2013.01)

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
USPC 504/116.1 (Keyword limited, terms below)

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
PatBase, Google Patents, Google Scholar (NPL); Keywords: LIPOSOME PESTICIDE HERBICIDE nematicide, nematode, root knot, fertilizer, propionaldehyde, methylcarbamate, methylaminocarbonyl, isopropylphosphoramidate, phosphorodithioate, thionazin, isazofos, ebufos, deothocarb

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	US 2009/0181076 A1 (Prestidge et al.) 16 July 2009 (16.07.2009) para [0001], [0009],[0011] ? [0016], [0022],[0032], [0036], [0052]	1, 5 --- 2-4, 6-20
Y	US 2008/0146445 A1 (DeKerpel et al.) 19 June 2008 (19.06.2008) para [0180], [0181], [0194], [0195], [0223], [0230], [0239], [0244], [0293], [0294],	2-4, 6-20

☐ Further documents are listed in the continuation of Box C. ☐

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent 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 to 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 when the document is taken alone
"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

Date of the actual completion of the international search	Date of mailing of the international search report
21 October 2013 (21.10.2013)	01 NOV 2013
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-3201	Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774