(19) DANMARK

(10) **DK/EP 3322295 T3**

(12) Oversættelse af europæisk patentskrift

Patent- og Varemærkestyrelsen

(51) Int.Cl.: ***A 01 N 63/60 (2020.01)*** ***A 01 N 25/08 (2006.01)*** ***A 01 N 25/10 (2006.01)*** ***A 01 N 25/22 (2006.01)*** ***A 01 P 7/00 (2006.01)*** ***C 12 N 15/11 (2006.01)*** ***C 12 N 15/113 (2010.01)***

(45) Oversættelsen bekendtgjort den: **2022-12-19**

(80) Dato for Den Europæiske Patentmyndigheds bekendtgørelse om meddelelse af patentet: **2022-09-28**

(86) Europæisk ansøgning nr.: **16810635.9**

(86) Europæisk indleveringsdag: **2016-06-17**

(87) Den europæiske ansøgnings publiceringsdag: **2018-05-23**

(86) International ansøgning nr.: **AU2016050517**

(87) Internationalt publikationsnr.: **WO2016201523**

(30) Prioritet: **2015-06-19 AU 2015902373**

(84) Designerede stater: **AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR**

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(56) Fremdragne publikationer:
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US-A1- 2014 150 134
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DESCRIPTION

TECHNICAL FIELD

[0001] The present invention relates to, *inter alia,* compositions comprising double stranded RNA adsorbed onto a clay complex and to methods of preparing such compositions. The present invention also relates to methods of using such compositions, to deliver double stranded RNA to an insect or to protect a crop against an insect, wherein the double stranded RNA is for use with insects.

BACKGROUND ART

[0002] The discussion below especially relates to the protection of plants against insect populations, and especially the control of insect populations that eat plants. However, for the avoidance of doubt, the present invention is not limited to protection of plants or the control of such insect populations. For example, many of the issues with chemical agents for controlling insects discussed below are also of concern for non-agricultural insect control.

[0003] Two of the most common strategies for the control of insect pests on plants include breeding for disease resistance and chemical control. Chemical control in particular is frequently used to control insect populations, but chemical agents are often expensive. Furthermore, there are safety concerns relating to the potential impact of chemical agents on the environment and the use of chemical agents on, for example, fruits and vegetables to be consumed by people. These last issues are factors that have contributed to the growth of the market for organic fruit and vegetables.

[0004] A further critical factor is that insects can and have developed resistance to traditional chemical agent insecticides. For example, in Australia field populations of insecticide-resistant *Helicoverpa armigera* (Cotton bollworm) have developed, and these resistant sub-types represent a significant pest in Australia's northern grain region (which encompasses all of Queensland's production areas). Crops affected by *H. armigera* include canola, chickpea, cotton, lucerne, maize, millets and panicums, mungbeans, peanuts, sorghum, soybeans, sunflowers and winter cereals including wheat, barley, oats, canary and triticale. *H. armigera* is also a major agricultural pest endemic to Europe, Asia and Africa. It is highly polyphagous and has been shown to feed on greater than 180 species of plant in 47 families. *H. armigera* has been estimated to cause US$2 billion in crop damage annually, despite the expenditure of US$500 million worth of pesticides for its control. However, *H. armigera* is just one species in the order of Lepidoptera, which is the second largest insect order comprised of moths and butterflies. The larval stage of moths cause major damage to many economically valuable crops including cotton, tobacco, tomato, corn, sorghum, lucerne, sunflower, pulses and wheat.

[0005] One effort to reduce the use of chemical insecticides on plants has involved the development of transgenic plants. For example, over the last two decades there has been an increase in the adoption of transgenic plants expressing insecticidal proteins encoded by genes from the bacterium *Bacillus thuringiensis* (Bt). However, the availability of suitable plants is limited, and sub-types of *H. armigera,* for example, have developed resistance to both single and dual-toxin forms of Bt cotton. Furthermore, large-scale application of transgenic plants has encountered resistance from the public and from regulatory agencies, and the cost, laboriousness and time needed to develop transgenic plants makes this an unattractive option.

[0006] RNAi, or RNA interference (also known as RNA silencing), has been used for genetic research in insects and it has promise in entomology as it provides target-specific silencing of gene expression. However, there are many difficulties in using RNAi to control the impact of insect pests on plants.

[0007] The RNAi mechanism involves first introducing double stranded RNA (dsRNA) into the insect haemocoel (for example via ingestion/injection/soaking), after which the dsRNA proceeds to enter the insect cells via endocytosis or via transmembrane proteins. Next, dsRNA is digested within the insect cells by a ribonuclease III (RNaseIII) enzyme known as Dicer into short interfering RNAs (siRNAs) of 20-25 nucleotides in length. The siRNAs are then unwound and one strand (the guide strand) is loaded into the RNA induced silencing complex (RISC). Lastly, the RISC locates and binds to messenger RNAs (mRNAs) containing sequences complementary to the guide strand, resulting in degradation of mRNA of the target gene, finally leading to the death of the cell.

[0008] A major difficulty in using the RNAi mechanism is how to effectively deliver dsRNA to insects. In particular, most laboratory studies of RNAi in insects have utilised microinjection of *in vitro* synthesised dsRNAs into embryos or the hemocoel. Clearly, however, microinjection as a delivery mechanism is not feasible for large-scale plant protection. Another strategy is for insects to ingest the dsRNA by providing it mixed in the diet, but this means that the dsRNA enters the insect alimentary canal, and especially the midgut. The environment of the midgut is hostile to dsRNA, due to the pH of the midgut and the presence of gut nucleases. Nucleases capable of degrading dsRNA may also be present in the saliva of insects (as recently reported for the tarnished plant bug *Lygus lineolaris* by Allen and Walker (2012) Saliva of Lygus lineolaris digests double stranded ribonucleic acids. Journal of Insect Physiology, 58, 391-396). Furthermore, research indicates that systemic RNAi responses may be more robust in less derived insect species, but some more derived Dipteran and Lepidopteran species may be refractory to systemic RNAi.

[0009] Another difficulty is how to provide the dsRNA to insects for ingestion. dsRNA is vulnerable to nucleases in the environment, and also to ultraviolet light, specifically if it has to be provided as a topical application on the plant surface. For example, in one study dsRNA topically applied to a plant for protection against viruses could not protect the plants 7 days post application. Furthermore, when dsRNA was applied 24 hours after viral infection, the dsRNA was not able to protect the plants against the virus (Tenllado, F. & J.R. Diaz-Ruiz,

(2001) Double-stranded RNA-mediated interference with plant virus infection. Journal Virology 75: 12288-12297). In another study, when dsRNA was added to insect foods, the dsRNA levels in different diets dropped by 14% in the solid foods for beetles and moths and by 32% in the aphid fluid diet after 3 days, and by 31% and 56% after 6 days, respectively (Whyard et al (2009) Ingested double stranded RNAs can act as species-specific insecticides. Insect Biochemistry and Molecular Biology, 39, 824-832).

[0010] Major challenges for application of RNAi to insects (after effective gene targets have been identified) are the stability of dsRNA prior to administration to the insect, and the delivery of dsRNA to the insect.

[0011] WO2014/122648 describes a complex comprising RNA and a positively charged modified polysaccharide selected from starch, amylose, amylopectin, galactan, chitosan or dextrin, a pharmaceutical composition comprising it, and its use in RNA transfection. This document also describes methods for RNA transfection, gene therapy and treatment of a disease, disorder or condition comprising using the complex. In addition, this document describes the use of positively charged modified polysaccharide selected from starch, amylose, amylopectin, galactan, chitosan, or dextrin, in RNA transfection into cells.

[0012] Consequently, there is a need to provide an effective alternative approach which allows for targeting of insect populations or an approach which at least partially overcomes at least one of the abovementioned disadvantages or which provides the consumer with a useful or commercial choice.

SUMMARY OF INVENTION

[0013] In a first aspect, the present invention provides a composition comprising double stranded RNA adsorbed onto a clay complex, wherein the clay complex includes: a cationic clay; and a molecule including a plurality of positively charged groups, said positively charged groups being positively charged in a solution having a pH from 5.0 to 7.0; and (i) said positively charged groups having a pKa of greater than 7.5; or (ii) said molecule is a polymer which is or includes a polydialkylaminoalkylmethacrylate; wherein the composition includes from 0.5 to 30 wt% double stranded RNA, and wherein the mass ratio of clay : molecule is from 1:1 to 10:1.

[0014] Advantageously, it has been found that when dsRNA is adsorbed onto the clay complex it is stable and the composition protects the dsRNA from UV light and from nucleases. The composition advantageously may be sprayed on crops, for example, to protect the crop against the insects. Furthermore, the dsRNA remains adsorbed on the clay complex until the composition encounters a basic pH, such as within the insects' alimentary canal. Without wishing to be bound by theory, it is expected that once in the insects' alimentary canal the dsRNA will be gradually released from the clay complex, which means that the composition provides a protective function for the dsRNA within the insect gut. When insecticidal dsRNA is used in a clay composition sprayed on plant leaves, it has been shown that a significant

proportion of insects eating these leaves are adversely affected by the dsRNA or die.

[0015] The composition accordingly provides a system for delivery of dsRNA to insects having a basic pH within its alimentary canal. The dsRNA accordingly may be any suitable dsRNA for use with insects and need not be limited to insecticidal dsRNA. However, in one embodiment, the dsRNA adversely affects the insect, and is especially insecticidal dsRNA. In this embodiment, the composition may be an insecticidal composition. The composition may be for protecting plants. Insects may also be carriers for various diseases (such as malaria and dengue fever). The composition therefore may be for use in protecting humans and animals against disease.

[0016] As used herein, dsRNA that adversely affects an insect may include dsRNA that causes insect mortality or affects the normal function or behaviour of that insect or affects the physiological function of the insect, including sterilisation, viral infection and decreased mobility.

[0017] The composition may be suitable for any type of insect, as long as the insect has a basic pH within its alimentary canal. In one embodiment, the insect is a chewing or a biting insect. A chewing or biting insect will ingest the composition upon consuming a food coated with the composition. In another embodiment, the insect is of the order *Diptera, Lepidoptera* or *Coleoptera.* Exemplary insects include one or more of the following:

- Insects of the order *Diptera* (especially flies, mosquitos, gnats or midges); especially insects of the family *Tephritidae* or *Drosophilidae;* more especially of the family *Tephritidae;* even more especially of the genus *Bactrocera* or *Vidalia;* most especially fruit flies;
- Insects of the order *Lepidoptera* (especially moths or butterflies (including caterpillars)); especially:

  ◦ Insects of the family *Tortricidae, Noctuidae, Geometridae, Sesiidae, Sphingidae, Plutellidae* or *Pyralidae;*

  ◦ Insects of the family *Noctuidae;* especially of the genus *Helicoverpa;* most especially *Helicoverpa armigera* (Cotton bollworm); or

  ◦ Insects of the family *Plutellidae;* especially of the genus *Plutella;* most especially *Plutella xylostella* (Diamondback moth);

- Insects of the order *Coleoptera* (especially example weevils or other beetles); especially insects of the superfamily *Curculionoidea,* more especially of the family *Curculionidae,* most especially weevils.

[0018] The insect may be an insect larvae (for example, for the order Lepidoptera the insect larvae may be a caterpillar). The insect may be a pest to plants (such plants may be as further defined below). In one embodiment, the insect is of the order *Lepidoptera,* especially of the

family *Noctuidae;* especially of the genus *Helicoverpa;* most especially *Helicoverpa armigera.*

[0019] The insect has a basic pH within its alimentary canal. For the avoidance of doubt, reference herein to a pH within the alimentary canal of an insect does not mean that the entire alimentary canal has that pH. The insect may have a basic pH within one or more of its foregut (stomodeam), midgut (mesenteron) or hindgut (proctodeam); especially within the midgut. The basic pH may be a pH greater than 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 or 11.0. The basic pH may be a basic pH less than 11.0, 10.5, 10.0, 9.5, or 9.0. The basic pH may be a pH from 8.0 to 11.0, 8.5 to 11.0, 8.0 to 10.5, 9.0 to 10.0, 9.0 to 9.5, 9.0 to 10.5 or 9.0 to 11.0.

[0020] The composition may be a plant-protecting composition. The composition may be for protecting any suitable type of plant (or crop including a plurality of plants). The plant may be an embryophyte, especially a spermatophyte, more especially an angiosperm (such as a monocotyledon (or monocot), dicotyledon or eudicotyledon (eudicot)) or a gymnosperm.

[0021] Exemplary monocots include plants of the order: *Asparagales* (including *Amaryllidaceae* (such as leek, onion, garlic, shallots and chives) and *Asparagaceae* (such as asparagus)); *Arecales* (including *Arecaceae* (such as palms, for example coconut palm)); *Dioscoreales* (including *Dioscoreaceae* (such as yam)); *Poales* (including *Bromeliaceae* (such as pineapple) and *Poaceae* (including corn (maize), wheat, rice, barley, millet, sorghum, oats and bamboo)); and *Zingiberales* (including *Musaceae* (including banana) and *Zingiberaceae* (including ginger and galangal)).

[0022] Exemplary eudicots include plants of the order:

- *Apiales* (including *Apiaceae* (such as parsnip, carrot and celery));
- *Asterales* (including *Asteraceae* (such as lettuce, artichoke and sunflower));
- *Brassicales* (including *Brassicaceae* (such as broccoli, cabbage, kale, cauliflower, brussel sprouts, bok choy, choi sum, kohlrabi, radish, turnip and rapeseed) and *Capparaceae* (such as capers));
- *Caryophyllales* (including *Amaranthaceae* (such as spinach, chard and beet) and *Polygonaceae* (such as rhubarb));
- *Cucurbitales* (including *Cucurbitaceae* (such as cucumber, squash, pumpkin, rockmelon, honeydew melon, zucchini and watermelon));
- *Ericales* (including *Actinidiaceae* (such as kiwifruit) and *Ericaceae* (such as blueberry));
- *Fabales* (including *Fabaceae* (such as various beans, pea, soy bean, mung bean, lentil, peanut and alfalfa (lucerne)));
- *Lamiales* (including *Oleaceae* (such as olive));
- *Malpighiales* (including *Linaceae* (such as flax));
- *Malvales* (including *Malvaceae* (such as cotton));
- *Myrtales* (including *Myrtaceae* (such as guava));
- *Rosales* (including *Cannabaceae* (such as hemp), *Rosaceae* (such as strawberry, apple, pear, apricot, plum, cherry, peach, raspberry, almond, and nectarine) and *Moraceae* (such as fig));

- *Sapindales* (including *Rutaceae* (such as citrus, for example orange, lemon, grapefruit, lime and mandarin) and *Sapindaceae* (such as lychee));
- *Solanales* (including *Convolvulaceae* (such as sweet potato) and *Solanaceae* (such as potato, tomato, eggplant, peppers (such as capsicum) and tobacco)); and
- *Vitales* (including *Vitaceae* (such as grape)).

[0023] In one embodiment, the composition is for protecting commercial agricultural crops. Exemplary crops include cereals, vegetables (including roots and tubers), fruits, pulses, oilcrops and fibre crops. Cereals may include corn (maize), rice, wheat, barley, sorghum, millet and oats. Vegetables may include broccoli, cauliflower, cabbage, artichokes, capers, kale, spinach, lettuce, bok choy, chard, choi sum, leeks, brussel sprouts, kohlrabi, galangal, ginger, celery, rhubarb, asparagus, bamboo shoots, potatoes, sweet potatoes, yams, soybeans, mung beans, alfalfa, carrots, parsnips, beets, radishes, turnips, onions, shallots and garlic. Fruits may include tomatoes, grapes, kiwifruit, berrys (including strawberrys, blueberrys and rasberrys), guava, pears, melons (including rockmelons, watermelons and honeydew melons), citrus (including oranges, mandarins, lemons, limes and grapefruits), stonefruit (including apricots, nectarines, plums, cherries and peaches), lychees, pineapples, figs, apples, bananas, cucumbers, squash, zucchinis, pumpkins, peppers, eggplants and avocados. Pulses may include beans, peas and lentils. Oilcrops may include crops from which oil may be obtained, such as palms, soybeans, rapeseeds, sunflower seeds, peanuts, cottonseeds, palm kernels, coconuts and olives. Fibre crops may include cotton, flax, hemp and bamboo. The crop may also be tobacco or a flowering plant.

[0024] The dsRNA may be plant-protecting dsRNA (or crop-protecting dsRNA). The plant-protecting double-stranded RNA (dsRNA) may be capable of protecting a plant (especially via RNA interference) against insects. Similarly, the crop-protecting dsRNA may be capable of protecting a crop against insects. The dsRNA may be insecticidal dsRNA. Insects may be as described above.

[0025] A specific dsRNA sequence may be selected based on the insect against which protection is sought, and an appropriate sequence may be readily selected by a skilled person.

[0026] Advantageously, in order for RNA interference to occur, the RNA nucleotide sequence must match the insect perfectly. Consequently, the dsRNA is likely to be highly specific to the target insect, limiting the possibility of adverse effects on predators to the insect, on the environment or on people at the time of consumption (in the case of vegetables and fruits, for example).

[0027] As used herein, the term "plant protecting", "crop protecting" and the like means that the composition/dsRNA is able to prevent or ameliorate the impact of an insect on a plant or crop. For example, dsRNA which protects a plant or crop against an insect may kill the insect, adversely affect the insect, or may deter the insect from feeding on the plant or crop.

[0028] The length of the dsRNA may vary depending on the insect(s) against which protection is sought. Advantageously, RNases in insects (Dicer-Like enzymes) will cleave long dsRNA sequences into much smaller fragments, each of which is typically 20-25 nucleotides in length. Therefore, long dsRNA sequences of, for example, 100 to 3000 base pairs may be used, and these longer sequences would be cleaved into such smaller fragments by the insect after the insect ingests the dsRNA. It is believed that these smaller nucleotide fragments (e.g. 21 nucleotides) are involved in the RNA interference mechanism.

[0029] A single dsRNA construct may be engineered by combining specific sequences from multiple insects or genetic targets which could target multiple organisms or genetic targets (including multiple genetic targets for the same insect), as each insect will cleave the dsRNA sequence into shorter fragments. For example, a single dsRNA construct could be used to target three different insects or genetic targets. The dsRNA may target at least two insects or genetic targets, more especially from 2 to 10 insects or genetic targets, even more especially from 4 to 8 insects or genetic targets.

[0030] The dsRNA may therefore be from 20 or 21 to 3000 base pairs in length; especially from 21 to 2500, or from 21 to 2000 base pairs in length; more especially from 80 to 1750, from 80 to 1500, or from 80 to 1200 base pairs in length; most especially from 100 to 1200, or from 250 to 1200 base pairs in length. Advantageously, use of such longer dsRNA sequences provides a much greater likelihood of the sequence being cleaved to a nucleotide sequence that will match with the desired insect to affect (e.g. kill) the insect. Also, it may be less expensive to produce one longer dsRNA construct that targets multiple insects or multiple genetic targets, rather than making individual constructs and formulations. Therefore, the dsRNA may be a dsRNA construct that targets multiple insects or multiple genetic targets (for example within a single insect).

[0031] Alternatively, the dsRNA may be a plurality of dsRNAs (or two or more dsRNAs). Each dsRNA sequence in said plurality of dsRNAs may have a different genetic target, target a different insect or target a combination of different genetic targets/insects. Consequently, in one embodiment the dsRNA is a plurality of dsRNAs for targeting a plurality of insects or genetic targets.

[0032] It is expected that the clay complex is able to adsorb dsRNA sequences of a variety of lengths regardless of the sequence.

[0033] In one embodiment, the dsRNA targets the electron transport chain (ETC) of an insect, especially the Rieske gene of an insect. In another embodiment, the dsRNA targets a voltage-dependent channel of an insect, especially a voltage-dependent anionic channel (VDAC) of an insect, more especially a VDAC in the mitochondrial outer membrane of an insect. In another embodiment, the dsRNA targets the muscles of an insect, especially the Arginine Kinase (AK) gene and SERCA (sarco/endoplasmic reticulum Ca^{2+}-ATPase) gene of an insect. The AK gene is mainly involved in energy metabolism whereas the SERCA gene is involved in calcium

uptake in the endoplasmic reticulum. In another embodiment, the dsRNA targets a gut of an insect, especially the Glutathione Transferase (GTT) gene of an insect. The GTT gene is important in the detoxification of compounds. In another embodiment, the dsRNA targets the body wall and appendages of an insect, especially the Acetylcholinesterase (AchE) gene of an insect. The AchE gene is important in growth and development. In another embodiment, the dsRNA targets an intracellular receptor of an insect, especially the methoprene-tolerant (Met) receptor of an insect. In another embodiment, the dsRNA targets a GTPase hydrolase enzyme of an insect, especially the Rab4b GTPase of an insect. In another embodiment, the dsRNA targets a juvenile hormone esterase of an insect. In another embodiment, the dsRNA targets the mitochondria of an insect, especially the NV2 gene of an insect. The NV2 gene plays an important role in the electron transport chain (ETC). In another embodiment, the dsRNA targets the gut of an insect, especially the foregut of an insect, more especially the prophenoloxidase gene of an insect. The prophenoloxidase gene is involved in detoxification of compounds. In another embodiment, the dsRNA targets the cuticle and epidermis of an insect, especially the cathepsin L gene of an insect. The cathepsin L gene plays an important role in the moulting process.

[0034] Therefore, in one embodiment the dsRNA targets at least one of the group consisting of: the electron transport chain (ETC) of an insect, a voltage-dependent channel of an insect, a muscle of an insect, a gut of an insect, a body wall and/or appendages of an insect, an intracellular receptor of an insect, a GTPase hydrolase enzyme of an insect, a juvenile hormone esterase of an insect, the mitochondria of an insect, and the cuticle and/or epidermis of an insect.

[0035] In another embodiment, the dsRNA includes a strand (antisense or sense) which is complementary to or at least partly complementary to a sequence as set forth in any one of SEQ ID NOs. 1, 2 and 4-14; more especially SEQ ID NOs. 1 or 2. As used herein, a strand which is "at least partly complementary" means that one strand of the dsRNA has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with the sequence. It would be appreciated that this definition takes into account that RNA uses a U instead of a T, as found in DNA.

[0036] In a further embodiment, at least a portion of a strand of the dsRNA (antisense or sense) is complementary to or at least partly complementary to a sequence as set forth in any one of SEQ ID NOs. 1, 2 and 4-14; more especially SEQ ID NOs. 1 or 2. As used herein, at least a portion of a strand which is "at least partly complementary" means that at least a portion of one strand of the dsRNA has at least 50%, at least 55%, at least 60%, at least 65%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, or at least 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity with the sequence. It would be appreciated that this definition takes into account that RNA uses a U instead of a T, as found in DNA.

[0037] In a further embodiment, the dsRNA includes or includes at least a portion of a strand

(antisense or sense) which is complementary to or at least partly complementary to a fragment of a sequence as set forth in any one of SEQ ID NOs. 1, 2 and 4-14; more especially SEQ ID NOs. 1 or 2. By way of example only, a fragment may include at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90% or 95% of a sequence which is complementary to or at least partly complementary to a sequence as set forth in any one of SEQ ID NOs. 1, 2 and 4-14; more especially SEQ ID NOs. 1 or 2.

[0038] The dsRNA may be produced in any suitable way. For example, the dsRNA may be produced in vitro via a kit, in vitro or in vivo via a bacteriophage (such as via a Pseudomonas syringae dsRNA bacteriophage), or in vivo using a specialised strain of organism, especially using a bacteria (such as a strain of E. coli). Typically, in vitro methods are suitable for smaller scale dsRNA production. For large scale production, in vivo production methods are preferred. The dsRNA used may be a crude bacterial extract.

[0039] The dsRNA may be in any suitable form and be of any suitable sequence. The dsRNA may include any suitable modifications. For example, the dsRNA may include one or more modified phosphate groups, modified nucleic acids/nucleotides, modified sugars and/or modified 5 or 3 prime ends. Exemplary modified groups which may be present in the dsRNA include, for example, inosine, methylinosine, pseudouridine, morpholine, locked nucleic acids, peptides (such as peptide nucleic acids (PNA)), biotin, cholesterol, fluorophores, radionuclides and metals. The dsRNA may also be in the form of a dsRNA construct. Such modifications may enhance the stability and/or longevity of the dsRNA.

[0040] The clay complex includes a cationic clay as defined in claim 1. The clay may be a silica-based clay. The clay may be a swelling clay. The clay may be bentonite. The clay may include, consist essentially of, or consist of montmorillonite.

[0041] The clay may have a charge density of from 10 to 400 millimolar equivalent charge per 100 g of clay (meq/100 g), especially a charge density of from 20 to 300 meq/100 g, more especially a charge density of from 30 to 250, from 40 to 200, from 50 to 200, from 70 to 150, from 80 to 120, from 90 to 110, or about 100 meq/100 g.

[0042] The clay may include a plurality of clay particles. The clay particles may have a z-average value of the particle size of up to 5 μm, more especially up to 1 μm, most especially up to 750 nm or up to 500 nm. In one embodiment, the clay particles may have a z-average value of the particle size of within the range 20-800 nm, more especially 100-800 nm or 250-650 nm even more especially about 450 nm.

[0043] When the clay includes a plurality of negatively charged layers or sheets a further positively charged component is needed to enable the formation of a composition including both the clay and the dsRNA (as dsRNA carries a negative charge (due to the phosphate groups)).

[0044] The clay complex includes a molecule having a plurality of positively charged groups,

said positively charged groups being positively charged in a solution having a pH from 5.0 to 7.0. The molecule may be for complexing with a cationic clay and with dsRNA. The molecule may be adsorbed onto the clay. The molecule may be a macromolecule, especially a polymer. The molecule may be a dendrimer. The polymer may be a co-polymer or a block co-polymer.

[0045] The positive charge in the positively charged groups may be located (or at least partially located) on a nitrogen atom and/or a transition metal; especially a nitrogen atom.

[0046] The positively charged group may be a positively charged amine (including primary, secondary, tertiary, or quarternary amines), an imine (including primary and secondary imines), a guanidine group, or an aromatic amino group. Exemplary amines include alkylamine, alkylaminoalkyl or alkylamino(alkyl)alkyl (in which each alkyl group may be independently selected from C_{1-12} alkyl, especially C_{1-6} alkyl, more especially methyl, ethyl, propyl, butyl, pentyl or hexyl); and a nitrogen-containing saturated heterocyclic ring (examples include a nitrogen-containing saturated heterocyclic ring having from 1 to 10 carbon atoms; especially pyrrolidinyl, pyrazolidinyl, piperidinyl, morpholinyl and quinuclidinyl). Exemplary imines include an alkylimine and an alkyliminoalkyl (in which each alkyl group may be independently selected from C_{1-12} alkyl, especially C_{1-6} alkyl, more especially methyl, ethyl, propyl, butyl, pentyl or hexyl). An exemplary guanidine group is an alkylguanidino group ((in which the alkyl may be independently selected from C_{1-12} alkyl, especially C_{1-6} alkyl, more especially methyl, ethyl, propyl, butyl, pentyl or hexyl). Exemplary aromatic amino groups may include aromatic nitrogen containing heterocycles; especially aromatic nitrogen containing heterocycles having from 1 to 10 carbon atoms; more especially imidazolyl, triazolyl or purinyl. The molecule or positively charged groups may be optionally substituted with at least one (and especially a plurality) of pKa modifying groups, as described below. The pKa modifying groups may be geminal or vicinal to the positively charged groups (such as groups including nitrogen atoms) in the molecule.

[0047] As used herein, the term "alkyl" or "alkylene" refers to a straight chain or branched saturated hydrocarbon group. The alkyl group may have a specified number of carbon atoms, for example, C_{1-12} alkyl refers to alkyl groups having 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 carbon atoms in a linear or branched arrangement. Unless otherwise defined, the term "alkyl" may be C_{1-12}alkyl or C_{1-6}alkyl. Examples of suitable alkyl groups may include, but are not limited to, methyl, ethyl, propyl (*n*-propyl and *i*-propyl), butyl (n-butyl, *i*-butyl and *t*-butyl), n-pentyl, 2-methylbutyl, 3-methylbutyl, 4-methylbutyl, 2-ethylpropyl, n-hexyl, 2-methylpentyl, 3-methylpentyl, 4-methylpentyl, 2-ethylbutyl, 3-ethylbutyl, heptyl, octyl, nonyl, decyl, undecyl, dodecyl, cyclopentyl, cyclohexyl and cycloheptyl.

[0048] The molecule may be a molecule having a plurality of positively charged groups (or two or more positively charged groups), wherein the groups are selected from one or more of amine, imine, guanido, and aromatic amino groups. As used herein, the terms "positively charged groups", "positively charged molecule" or the like refer to a group or molecule with a positive charge at pH 5.0 to 7.0.

[0049] Without wishing to be bound by theory, it is believed that the composition may form through electrostatic interactions between, for example, a negatively charged clay, a positively charged molecule, and a negatively charged dsRNA. Consequently, if the positive charge of the positively charged groups in the molecule are neutralised (for example due to the basic pH within the insects' alimentary canal), the composition dissociates, releasing the dsRNA. Therefore, specific molecules having a plurality of positively charged groups may be selected for use with specific insects, and the pKa of the positively charged groups in the molecule can be manipulated or selected to control the release of dsRNA in the insect. By way of example, if the pH within an insects' alimentary canal is about 10.5, then a composition including a molecule with positively charged groups having a pKa of 9.0 would be expected to release dsRNA in the insects' alimentary canal more quickly and completely than a composition including a molecule with positively charged groups having a pKa of 10.5.

[0050] Accordingly, the pKa of the positively charged groups may be greater than 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 or 11.0. The pKa of the positively charged groups may be less than 11.0, 10.5, 10.0, 9.5, or 9.0. The pKa of the positively charged groups may be from 8.0 to 11.0, 8.5 to 11.0, 8.0 to 10.5, 9.0 to 10.0, 9.0 to 9.5, 9.0 to 10.5 or 9.0 to 11.0.

[0051] Furthermore, the dsRNA may be released from the composition at a pH of greater than 7.5, 8.0, 8.5, 9.0, 9.5, 10.0, 10.5 or 11.0. The dsRNA may be released from the composition at a pH of less than 11.0, 10.5, 10.0, 9.5, or 9.0. The dsRNA may be released from the composition at a pH of from 8.0 to 11.0, 8.5 to 11.0, 8.0 to 10.5, 9.0 to 10.0, 9.0 to 9.5, 9.0 to 10.5 or 9.0 to 11.0. If the pKa of the positively charged groups is, for example, 9.0, then it would be expected that dsRNA would be released from a composition including a molecule with those positively charged groups at a pH of 9.0.

[0052] The molecule having a plurality of positively charged groups may be of any suitable type. In one embodiment, the molecule is a polymer. The polymer may have a molecular weight of greater than 200, especially greater than 400, more especially greater than 600. The polymer may have a molecular weight of from 400 to 100,000, especially from 600 to 80,000. The polymer may have a molecular weight of from 10,000 to 50,000; especially from 10,000 to 40,000; most especially about 15,000 or about 33,000.

[0053] The polymer may be a co-polymer or a block co-polymer formed with at least one monomer providing at least one positively charged group. The polymer may also be a homopolymer, formed by only one monomer providing at least one positively charged group.

[0054] The polymer may have a plurality of positively charged nitrogen atoms (the positively charged groups may be positively charged nitrogen atoms). The pKa of the nitrogen atoms may be as described above for the positively charged groups. The polymer may be a branched or linear polymer. The polymer may be or include an optionally substituted polyalkyleneamine (may be known as polyalkyleneimine). An exemplary polyalkyleneamine may be polyhexyleneamine (may be known as polyhexyleneimine), polypentyleneamine (may be known as polypentyleneimine), polybutyleneamine (may be known as polybutyleneimine),

polypropyleneamine (may be known as polypropyleneimine) or polyethyleneamine (may be known as polyethyleneimine); especially polyethyleneamine. Polyethyleneamine includes amino groups having pKa values from 10.5 to 11.0 and thus would be expected to release dsRNA at a pH of about or greater than 10.5.

[0055] The optional substituents for the polyalkyleneamine may be pKa modifying groups or a plurality of pKa modifying groups. Accordingly, the polymer may be a polyalkyleneamine substituted by a plurality of pKa modifying groups. The pKa modifying groups may be electron withdrawing groups or electron donating groups. The pKa modifying groups may be geminal or vicinal to the amine groups in the polymer. Exemplary electron withdrawing groups may include carbonyl-containing groups (such as esters (including -CO-O-alkyl groups) and carboxylic acids (including a -CO-OH group)), nitro groups, cyano groups, protonated amino groups (such as a tetra-alkyl substituted nitrogen) and sulfate groups. The pKa modifying groups may also include -alkyl, -OH, ethers (including -O-alkyl groups), and amines (including -NH-alkyl and -N(alkyl)-alkyl groups), in which the alkyl groups may be optionally substituted with one or more of -OH, -O-alkyl, -halo (including -F, -Cl, -Br of -I), -CO-alkyl, -CO-O-alkyl, -O-CO-alkyl, -NH-alkyl, $-N(alkyl)_2$, -CO-NH-alkyl and -NH-CO-alkyl.

[0056] In one embodiment, the polymer is a polyalkyleneamine substituted with pKa modifying groups geminal or vicinal to the amine groups in the polymer. The polymer may be a polyalkyleneamine substituted with hydroxyl groups or -O-alkyl groups vicinal to the amine groups in the polymer. The polymer may be ethoxylated polyethyleneamine. Ethoxylated polyethyleneamine includes amino groups having pKa values of about 9.0 - 9.5. For the avoidance of doubt, a polyalkyleneamine may include either or both of amine and imine groups. For example, ethoxylated polyethyleneamine may include one or more of the following units: - $NH(CH_2CH_2OH)$, $-N(CH_2CH_2OH)_2$, and $-N(CH_2CH_2OH)-$.

[0057] The polymer may be polyalkylene-based; especially polyhexylene-based, polypentylene-based, polybutylene-based, polypropylene-based or polyethylene-based; most especially polyethylene-based. The polyalkylene-based polymer may include a positively charged group geminal or vicinal to a pKa modifying group (especially an electron withdrawing group). In one embodiment, the alkylene group (especially an ethylene group) in the polyalkylene-based polymer may be substituted by an amino group, and optionally vicinal to the amino group is an electron withdrawing group. The polymer may be polyethylene-based, wherein the ethylene group in the polyethylene-based polymer is substituted by an amino group and optionally substituted by an electron withdrawing group vicinal to the amino group. The amino group may be a dialkylamino group; more especially a diethylamino or dimethylamino group; most especially a dimethylamino group. The electron withdrawing group may be an alkyleneester (-R-COO-), alkyleneether (-R-O-) or alkylene-carbonyl (-R-CO-); more especially an ethylene-CO-O- group). The electron withdrawing group may be derived from a methacrylate. In an exemplary embodiment, the polymer may be or include a polydialkylaminoalkylmethacrylate. In a further exemplary embodiment, the polymer may be poly(2-dimethylaminoethyl methacrylate) (or pDMAEMA). pDMAEMA includes alkylated amino groups having pKa values of about 7.0-7.5. In another exemplary embodiment, the polymer

may be poly(2-(diethylamino)ethyl methacrylate) (or pDEAEMA). pDEAEMA has similar pKa values to pDMAEMA.

[0058] The polymer may be a homopolymer, co-polymer or block co-polymer including a dialkylaminoalkylmethacrylate (such as a 2-(dimethylamino)ethyl methacrylate or DMAEMA). The co-polymer or block co-polymer including a dialkylaminoalkylmethacrylate may also include a second dialkylaminoalkylmethacrylate, a polyalkyleneoxide (such as polyethyleneoxide or PEO), or a polyalkylacrylamide (such as poly(N-isopropylacrylamide) or PNIPAM). Exemplary co-polymers or block co-polymers include pDMAEMA-pDEAEMA, pDMAEMA-PEO, and pDMAEMA-PNIPAM. In such polymers the pKa of the amino group may vary, allowing the dsRNA to be released at different pHs.

[0059] The composition includes up to 30 wt% dsRNA, more especially up to 25 wt% or 20 wt% dsRNA. The composition includes more than 0.5 wt% dsRNA; especially more than 1 wt% dsRNA, 2 wt% dsRNA, 3 wt% dsRNA, 4 wt% dsRNA or 7 wt% dsRNA; more especially more than 5 wt% dsRNA. Specifically, the composition includes from 0.5 to 30 wt% dsRNA, e.g. from 1 to 30 wt% dsRNA, from 5 to 30 wt% dsRNA or from 5 to 20 wt% dsRNA; more especially from 5 to 25 wt% dsRNA or from 7 to 25 wt% dsRNA; most especially about 8 to 25 wt% dsRNA.

[0060] When a molecule having a plurality of positively charged nitrogen groups is present in the composition, the N:P ratio in the composition (i.e. the nitrogens in the molecule (N):the phosphorous in the dsRNA(P)) may be at least 1, 2, or 3, especially at least 4, more especially at least 5. The N:P ratio in the composition may be less than 30, less than 20, less than 10, or less than 7; especially less than 6. The N:P ratio in the composition may be from 3-7, especially from 4-6, more especially about 5.

[0061] In the claimed composition, the clay complex includes a clay and a molecule having a plurality of positively charged groups. The mass ratio of clay : dsRNA may be from 1:1 to 10:1, especially from 3:1 to 8:1, more especially from 4:1 to 7:1 or 5:1 to 7:1, most especially about 6:1 or about 5:1. The mass ratio of clay : molecule is from 1:1 to 10:1, e.g. from 3:1 to 8:1, more especially from 4:1 to 7:1 or 5:1 to 7:1, most especially about 6:1 or about 5:1. The mass ratio of clay : molecule : dsRNA may be about 5:1:2.

[0062] Without wishing to be bound by theory, the loading ratio of different components in the composition may affect the release of dsRNA. For example, at the same basic pH a composition including a higher proportion of positively charged groups would be expected to release dsRNA more slowly than a composition including a lower proportion of positively charged groups (as the base has a greater amount of positive charges to neutralise).

[0063] In one embodiment, the composition has a z-average value of particle size of up to 5000 nm, especially up to 3000 nm, more especially of up to 2500 nm. In another embodiment, the composition has a z-average value of particle size of at least 50 nm, 100 nm, 250 nm or 500 nm, especially at least 1000 nm, more especially at least 1500 nm, most especially at least 2000 nm. In one embodiment, the composition has a z-average value of particle size of about

2100 nm. The composition may have a z-average value of particle size of from 50 nm to 5 µm, especially from 100 nm to 1 µm. The ideal particle size of the composition may vary depending on the food to which the composition is to be applied.

[0064] In another embodiment, the composition has a positive zeta potential, especially a zeta potential of greater than 0.5 mV. In another embodiment, the composition has a zeta potential of less than 25 mV, especially less than 15 mV, more especially less than 10 mV, even more especially less than 5 mV. In a further embodiment, the composition has a zeta potential of about 1.3 mV.

[0065] As used herein, the term "dsRNA adsorbed onto a clay complex" and the like includes circumstances in which dsRNA is adsorbed onto the surface of the clay complex, circumstances in which dsRNA is intercalated between clay complex layers, and circumstances in which dsRNA is adsorbed onto the molecule having a plurality of positively charged groups where the dsRNA/molecule is adsorbed on the surface of a clay or between clay layers. Therefore, the term "adsorbed" includes both adsorption onto the surface of the clay or clay complex, as well as intercalation between clay or clay complex layers.

[0066] Without wishing to be bound by theory, it is believed that when dsRNA is adsorbed onto the clay complex, the dsRNA is afforded some protection against RNases and U.V. light, and thus the dsRNA is significantly more stable. Thus, it is believed that the clay complex acts as a protective coating for the adsorbed dsRNA.

[0067] Advantageously, it is expected that when dsRNA is adsorbed onto the clay complex, the dsRNA substantially does not degrade, even when stored for more than 60 days. When the dsRNA is adsorbed onto the clay complex, the clay complex advantageously protects the dsRNA against RNase and UV light.

[0068] In a second aspect, the present invention relates to a preparation including the composition of the first aspect of the present invention. Context permitting, features of the second aspect of the present invention may be as described for the first aspect of the present invention.

[0069] The preparation may include one composition of the first aspect of the present invention, or a plurality of compositions of the first aspect of the present invention. For example, a user may be provided multiple compositions (for example in solid or powder form) which each target one insect pest. If the user wishes to target three insect pests when spraying a crop, for example, the user may mix three compositions and a fluid to prepare a single preparation for spraying.

[0070] The preparation may be in any suitable form. For example, the preparation may be in the form of a solid, ointment, gel, cream, powder, paste, suspension, colloid, foam or aerosol; especially a suspension or a colloid. Solid forms of the preparation may include dusts, powders, granules, pellets, pills, pastilles, tablets, filled films (including seed coatings) and the

like, which may be water-dispersible ("wettable"). In one embodiment, the preparation is in the form of a concentrate, especially a concentrate in the form of a colloid or suspension.

[0071] In one embodiment the preparation is heterogeneous, especially comprising a solid phase dispersed within a fluid phase. The solid phase may comprise the composition. The fluid phase may be, for example, a liquid, a gas, or a free flowing solid, or a combination thereof; especially a liquid; more especially an aqueous liquid; most especially water. The water may be sterile or non-sterile. The solid-phase may be dispersed within the fluid phase in any suitable way. This will depend upon the nature of the solid-phase and the fluid-phase.

[0072] Depending on the form of the preparation, the preparation may include a variety of other agents. Exemplary agents include, but are not limited to, one or more of the following types of ingredients: diluents, carriers, excipients, suspension agents, agglomeration agents, bases, buffers, bittering agents, fragrances, preservatives, propellants, thixotropic agents, surfactants, anti-freezing agents, and colouring agents. Suitable agents may be selected by a skilled person.

[0073] The preparation may also include one or more other active ingredients. An active ingredient, as defined herein, is an ingredient that provides benefit to plant protection. The active ingredient may be, for example, an insecticide, a pesticide, a fungicide, an antibiotic, an insect attractant, an anti-parasitic agent, an anti-viral agent, or a nematicide.

[0074] When the preparation is in the form of a colloid or suspension, it may include the composition at up to 30% w/w, up to 20% w/w, up to 10% w/w, up to 5% w/w, up to 2% w/w, up to 1% w/w, less than 1% w/w, less than 0.1% (less than 1 mg/ml) or less than 0.01% w/w (less than 0.1 mg/ml). The preparation may be in a concentrated form which requires dilution prior to application to an insect food, plant or crop. The preparation for application to an insect food, plant or crop may include the composition at less than 1 %w/w, less than 0.1% (less than 1 mg/ml) or less than 0.01% w/w (less than 0.1 mg/ml).

[0075] In another embodiment, when the preparation is in the form of a colloid or suspension, it may include the composition at up to 100 mg/L; especially up to 50 mg/L; more especially up to 20 mg/L or up to 10 mg/L; most especially less than 10 mg/L. In one embodiment, the concentration of the composition in a colloid or suspension is from 1-100 mg/L.

[0076] The preparation may be formulated for administration to a plant, or to any part of a plant, in any suitable way. For example, the preparation may be formulated for administration to the leaves, stem, roots, fruit, vegetables, grains and/or pulses of the plant. In one embodiment, the preparation is formulated for administration to the leaves of the plant, and is especially sprayable onto the leaves of the plant. The preparation may be sprayable onto the plant. The preparation may be administered to the plant as a metered dose. The preparation may be formulated for administration to the plant, for example, by spraying, by brush or by another applicator. The preparation may be formulated for administration to the plant by spraying (including via a fine mist), drip-feeding and/or irrigation.

[0077] The preparation may be in the form of a suspension, in which case the composition may be sprayable onto the plant. The suspension may be substantially stable. As used herein, a "substantially stable" suspension is a suspension in which, once formed, the solid phase remains sufficiently dispersed (i.e. does not significantly aggregate) in the fluid phase (especially a liquid phase, more especially water) for the suspension to be sprayed onto a plant. In one embodiment, the solid phase remains dispersed in the fluid phase for at least 24 hours after the suspension is formed, especially at least 5 days after the suspension is formed, more especially at least 10, 15, 20 or 30 days after the suspension is formed, most especially at least 60 days after the suspension is formed. If the suspension is not substantially stable, then the solid phase may aggregate leading to blockages in equipment when the suspension is sprayed onto plants, or alternatively leading to variable amounts of solid phase material being applied in a given area, resulting in incomplete protection for plants.

[0078] In a third aspect, the present invention provides a method of preparing the composition of the first aspect of the present invention. The method includes the steps of adsorbing dsRNA onto a molecule having a plurality of positively charged groups; and adsorbing the dsRNA/molecule onto a clay.

[0079] Advantageously, it has been found that loading the dsRNA onto the molecule and then loading the dsRNA/molecule onto the clay provides significantly higher loading of dsRNA in the composition. Furthermore, this method also provides improved release parameters. In one embodiment, the molecule has a plurality of positively charged nitrogen groups. In this embodiment, in the dsRNA/molecule the N:P ratio (i.e. the nitrogens in the molecule (N):the phosphorous in the dsRNA(P)) may be at least 1, 2 or 3, especially at least 4, more especially at least 5. In this embodiment, in the dsRNA/molecule the N:P ratio may be less than 30, less than 20, less than 10, or less than 7, especially less than 6. In this embodiment, in the dsRNA/molecule the N:P ratio may be from 3-7, especially from 4-6, more especially about 5.

[0080] The step of adsorbing dsRNA onto the molecule may include contacting or incubating the dsRNA with the molecule in an aqueous solution with shaking or inversion. This step may be performed below 25 °C, especially below 15 °C, more especially below 10 °C, most especially below 5 °C. This step may be performed on ice. This step may be performed at about pH 7.

[0081] The step of adsorbing the dsRNA/molecule onto a clay may include contacting or incubating the dsRNA/molecule with the clay in an aqueous solution with shaking or inversion. This step may be performed below 25 °C, especially below 15 °C, more especially below 10 °C, most especially below 5 °C. This step may be performed on ice. This step may be performed at about pH 7. In the claimed invention, the composition formed by the method includes a clay, a molecule having a plurality of positively charged groups, and dsRNA. The mass ratio of clay : dsRNA may be from 1:1 to 10:1, especially from 3:1 to 8:1, more especially from 4:1 to 7:1 or from 5:1 to 7:1, most especially about 6:1 or about 5:1. The mass ratio of clay : molecule is from 1:1 to 10:1, such as from 3:1 to 8:1, more especially from 4:1 to 7:1 or

from 5:1 to 7:1, most especially about 6:1 or about 5:1.

[0082] Features of the third aspect of the present invention may be as described above for the first aspect.

[0083] In a fourth aspect, the present invention relates to a method of delivering dsRNA to an insect having a basic pH within its alimentary canal, the method comprising administering (or applying) the composition of the first aspect or the preparation of the second aspect of the present invention to an insect food, wherein the double stranded RNA is for use with insects.

[0084] The composition may be administered to any insect food. Such foods may include plants and plant parts (including fruits and vegetables, for example), but also may include synthetic insect food sources, insect baits or water traps. In one embodiment, the insect food is a plant (including parts of plants). Advantageously, the insect may ingest the composition while ingesting the food. The step of administering the composition to an insect food may involve spraying, dripping or pouring the composition or preparation onto the food, or brushing the composition or preparation onto the food.

[0085] In a fifth aspect the present invention relates to a method of protecting a crop against an insect having a basic pH within its alimentary canal, the method comprising the step of administering (or applying) to a crop the composition of the first aspect or the preparation of the second aspect of the present invention, wherein the double stranded RNA is for use with insects. The crop may include a plurality of plants as defined above. The step of administering the composition or preparation to the crop may involve administering the preparation or composition to the leaves, stem, roots, fruit, vegetables, grains and/or pulses of the plants within the crop. The step may involve spraying the composition or preparation onto the crop, especially onto the leaves of the plants in the crop. The step may involve brushing the composition or preparation onto the crop, especially onto the leaves of the plants within the crop. The step may involve administering the composition or preparation to the crop by spraying, drip-feeding or irrigation.

[0086] In one embodiment of the fifth aspect the dsRNA is insecticidal dsRNA. In this embodiment, the composition is an insecticidal composition.

[0087] Features of the fourth and fifth aspects of the present invention may be as described for the first and second aspects of the present invention, context permitting.

[0088] Further features of the fourth to fifth aspects of the present invention may be as described below.

[0089] In one embodiment, the composition or preparation, once applied to a plant or crop, is capable of protecting the plant or crop for at least 15 days, especially at least 20 days, more especially at least 25 days, most especially at least 30 days. In a further embodiment, the composition, once administered to a plant or crop, is capable of protecting the plant or crop for

from 2 to 8 weeks, especially from 3 to 6 weeks, most especially from 4 to 5 weeks. Once administered to a plant or crop, the composition may degrade at a rate of 10-30% per week, especially 15-25% per week, more especially at a rate of 15-20% per week. The duration of protection may be affected, for example, by the amount of rainfall on the plant or crop. Therefore, in a drier climate it is expected that the duration of protection would be increased, whereas a shorter duration of protection may be provided in a wetter climate.

[0090] The composition may also be administered to the plant or insect food at any suitable concentration, and advantageously the dsRNA in the composition may be effective at relatively low concentrations. In one embodiment, less than 100 µg of dsRNA per plant may be administered, especially less than 50 µg, more especially less than 40, 30, 20, 10 or 5 µg, most especially less than 1 µg or 0.5 µg of dsRNA per plant. When the composition is administered to the leaf of the plant, less than 100 µg of dsRNA per leaf may be administered to the plant, especially less than 50 µg, more especially less than 40, 30, 20, 10 or 5 µg, most especially less than 1 µg or 0.5 µg or 0.1 µg of dsRNA per leaf is administered to the plant.

[0091] The composition or preparation may include an effective amount of dsRNA. The term "effective amount" means that a sufficient quantity of dsRNA is administered so as to affect the targeted insect.

[0092] In a sixth aspect, the present invention provides a kit comprising:

1. (i) a clay complex as defined in claim 1; and
2. (ii) dsRNA;

wherein the dsRNA is adsorbable onto the clay complex to form the composition of the first aspect.

[0093] In one embodiment of the sixth aspect, the clay complex (or the clay and the molecule having a plurality of positively charged groups) and/or the double-stranded RNA is provided in solid or liquid (especially aqueous) form. The clay complex (or the clay and the molecule having a plurality of positively charged groups) and/or the dsRNA may, depending on their form, include a variety of other agents. Exemplary agents include, but are not limited to, one or more of the following types of ingredients: diluents, carriers, excipients, suspension agents, agglomeration agents, bases, buffers, bittering agents, fragrances, preservatives, propellants, surfactants, thixotropic agents, anti-freezing agents, and colouring agents. Suitable agents may be selected by a skilled person.

[0094] The kit may also include one or more other active ingredients. An active ingredient, as defined herein, is an ingredient that provides benefit to a plant. The active ingredient may be, for example, an insecticide, a pesticide, a fungicide, an antibiotic, an insect attractant, an anti-paracitic agent, an anti-viral agent, or a nematicide.

[0095] Features of the sixth aspect may be as described for the first aspect of the present invention.

[0096] Any of the features described herein can be combined in any combination with any one or more of the other features described herein within the scope of the invention.

BRIEF DESCRIPTION OF DRAWINGS

[0097] Various embodiments of the invention will be described with reference to the following drawings, in which:

Figure 1A provides a gel retardation image of bentonite - polyethyleneimine - dsRNA compositions (BEN-PEI-dsRNA) at different dsRNA:BEN-PEI loading ratios, in which the polyethyleneimine (PEI) has a molecular weight of 60,000;

Figure 1B provides a gel retardation image of bentonite - polyethyleneimine - dsRNA compositions (BEN-PEI-dsRNA) at different dsRNA:BEN-PEI loading ratios, in which the polyethyleneimine (PEI) has a molecular weight of 800;

Figure 2A provides a gel retardation image of dsRNA release from bentonite - polyethyleneimine - dsRNA compositions (BEN-PEI-dsRNA) at various pH, in which the polyethyleneimine (PEI) has a molecular weight of 60,000;

Figure 2B provides a gel retardation image of dsRNA release from bentonite - polyethyleneimine - dsRNA compositions (BEN-PEI-dsRNA) at various pH, in which the polyethyleneimine (PEI) has a molecular weight of 800;

Figure 3 provides a gel electrophoresis image of dsRNA-etPEI complexes with N:P ratios of 1, 2, 3, 4 and 5 (N: amine in PEI and P: phosphate in dsRNA);

Figure 4A provides a gel electrophoresis image of dsRNA release from a dsRNA-etPEI complex, with an N/P ratio of 3 (i.e. NP3; N: amine in PEI and P: phosphate in dsRNA);

Figure 4B provides a gel electrophoresis image of dsRNA release from a dsRNA-etPEI complex, with an N/P ratio of 4 (i.e. NP4; N: amine in PEI and P: phosphate in dsRNA);

Figure 5 illustrates the change in pH of a etPEI solution as increasing volumes of NaOH is added;

Figure 6A provides a gel electrophoresis image in which the presence of free dsRNA is investigated in a Bentonite - etPEI - dsRNA complex with a N:P ratio of 3 (N: amine in PEI and P: phosphate in dsRNA; denoted BReP3) at pH 8.0 and 8.5;

Figure 6B provides a gel electrophoresis image in which the presence of free dsRNA is investigated in a Bentonite - etPEI - dsRNA complex with N:P ratios of 4 and 5 (N: amine in PEI and P: phosphate in dsRNA; denoted BReP4 and BReP5) at pH 8.0 and 8.5;

Figure 7 provides a gel electrophoresis image showing the pH dependent release profile of

dsRNA from BReP4;

Figure 8 provides a gel electrophoresis image showing the pH dependent release profile of dsRNA from BReP5;

Figure 9 provides a gel electrophoresis image showing the pH dependent release profile of dsRNA from BReP6;

Figure 10A is a scanning electron microscope image of bentonite;

Figure 10B is a scanning electron microscope image of BEN-etPEI;

Figure 11 illustrates the particle size distribution of bentonite, etPEI, etPEI-dsRNA complex, and BEN-etPEI-dsRNA;

Figure 12 is a gel electrophoresis image illustrating the nuclease protection of dsRNA in BReP5 in a high salt Nuclease A bioassay;

Figure 13 is a photograph illustrating the mortality of *H. armigera* fed with DEPC water, BEN-etPEI, naked Rieske dsRNA and BReP5. Dead larvae was indicated by red arrows;

Figure 14 provides statistical data for the effect at day 12 of ingestion by larvae of *Nicotiana tabacum* leaves coated with DEPC water, BEN-etPEI, naked Rieske dsRNA and BReP5;

Figure 15 provides a gel electrophoresis image of dsRNA - pDMAEMA complexes with N:P ratios of 1/2, 1, 2, and 3 (N: amine in pDMAEMA and P: phosphate in dsRNA), in which pDMAEMA has molecular weight of 33, 000;

Figure 16 provides a gel retardation image of dsRNA release from bentonite - pDMAEMA - dsRNA (BPR) complex with an N/P ratio of 1 (i.e. NP1, N: amine in pDMAEMA and P: phosphate in dsRNA) and a mass ratio of bentonite:pDMAEMA of 5:1 (i.e. BPR5), in which pDMAEMA has molecular weight of 33, 000;

Figure 17 is a gel electrophoresis image illustrating the nuclease protection of dsRNA in BPR5 in a low salt Nuclease A bioassay;

Figure 18 is a graph of relative transcript levels of VDAC in *Helicoverpa armigera* larvae continuously fed for 3 days (once a day) on GFP dsRNA (as control), bentonite-pDMAEMA (BP), VDAC dsRNA, and bentonite-pDMAEMA-VDAC (BP VDAC). The data are presented as means ± SD; and

Figure 19 is a graph of relative transcript levels of Rieske in *Helicoverpa armigera* larvae continuously fed for 3 days (once a day) on GFP dsRNA (as control), bentonite-pDMAEMA (BP), Rieske dsRNA and bentonite-pDMAEMA-Rieske (BP Rie). The data are presented as means ± SD.

[0098] Preferred features, embodiments and variations of the invention may be discerned from

the following Examples which provides sufficient information for those skilled in the art to perform the invention. The following Examples are not to be regarded as limiting the scope of the invention in any way. The scope of the invention is defined by the claims.

EXAMPLES

Targets for topical application of dsRNA

[0099] Targets for the composition of the present invention or dsRNA gene sequences for use in the composition of the present invention include (unless otherwise stated, the sequences below are intended to target *Helicoverpa armigera*):

- Rieske gene - The Rieske gene expresses an iron sulphur cluster involved in the electron transport chain (ETC) which when targeted by RNAi causes mortality in insects. The cDNA sequence used for the Rieske gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 1 below (300bp):
  CGCATACACCAGCTGAAAAGGTGTTGGTGCACCCCTTGCCAAAAACCTCGACTG
  TGGAGTCACTGCATGGATCCCTGCCTATCCAGGGCTTGAAGGCCAGAGTAAACG
  GTCGCGTTTTGTTAAATATTTCTGGGGATTTACGACGAAAAATCGTGTTACATAA
  CACCTTGTCACTTCTAGGGCCAAGCCATGTACGTTTCGCGCATACCGACATCAG
  CTACCCCGACTTCTCGGCGTACCGTCGCAAGGAGACGCAGGATCCCACCTCAAG
  GGCTAACGACAACGTCGATGGACGTCAGT
- Voltage-Dependent Anionic Channels (VDAC) - VDACs are integral membrane proteins forming pores in the mitochondrial outer membrane. VDACs act as general diffusion pores for small molecules such as ATP, phosphocreatine, and small ions. They have also shown to be involved in apoptotic pathways. The cDNA sequence used for the VDAC gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 2 below (232bp):
  GCGCTAGACGCCGACGCGTCTCTGCATGCCAAGGTCAACAACAAATCTCTCATT
  GGTCTTGGATACCAACAGAAGTTGCGCCCAGGCGTGACTTTGACAATTTCTGCT
  GCTATCGATGGCCAGAACTTCAACGCTGGTGGACACAAAGTGGGTGTCGCCCTG
  GAGCTCGAGCCCTAAGTACACAGAGGCGCTTCGGCTTTTAGTCCTGTAGATAAT
  ACATAATGCCACACTG
- Green Fluorescent Protein (GFP) - GFP was used as a negative control in some experiments. The cDNA sequence used for GFP (which corresponds to the RNA sequence) is provided in SEQ ID NO. 3 below (339bp):
  AGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCGACACC
  CTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACAT
  CCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCATGGC
  CGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATCG
  AGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGC

GACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTG
AGCAAAGACCCCAACGAGA

- Control - MEGAscript® RNAi kit control (500 bp).
- Arginine Kinase (AK) gene - targeting the AK gene may affect the muscles of an insect. The AK gene is mainly involved in energy metabolism. The cDNA sequence for the AK gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 4 below (200bp):
GATGACAGGCTTGGTTTCCTGACTTTCTGCCCCACCAACTTGGGAACCACCGTGC
GTGCCTCCGTGCACATCAAGCTGCCCAAGCTGGCTGCCGACAAGGCCAAGCTGG
AGGAGATCGCATCCAAGTACCACCTGCAGGTGCGCGGAACCCGCGGCGAGCAC
ACCGAGGCTGAGGGCGGCGTCTACGACATCTCCAACAA
- Sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA) gene - targeting the SERCA gene may affect the muscles of an insect. The SERCA gene is involved in calcium uptake in the endoplasmic reticulum. The cDNA sequence for the SERCA gene (which corresponds to the RNA sequence) is provided in SEQ ID NO.5 below (231bp):
TTCCTTGAATTCGAAATTACTGGCTCCACCTACGAACCCATTGGTGACGTTTACC
TGAAGGGACAGAAGATCAAGGCCGCTGAATTCGATGCTCTGCACGAACTTGGTA
CCATTTGCGTTATGTGCAATGACTCCGCTATTGATTTCAACGAATTCAAACAGGC
TTTCGAAAAGGTCGGTGAGGCCACTGAAACCGCTCTTATCGTCCTCGCTGAGAA
AATGAACCCCTTC
- Glutathione Transferase (GTT) gene - targeting the GTT gene may affect the gut of an insect. The GTT gene is important in the detoxification of compounds. The cDNA sequence for the Glutathione S-transferase gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 6 below (300bp):
GAAAGCAGATGAGGCTCTGCTCAAGAAGCTGGAGGAAGCTCTGCACTTCCTCAA
CACATTCCTCGAAGGTCAGAAGTACGCTGCGGGTGACAAACTGACCTTGGCAGA
CCTCAGTCTCGTGGCGACTGTGTCCACTATAGACGCCGTCGACATCAGCCTGAA
GGAATATCCCAATGTTGAAAAGTGGTTCGAGCTGGTGAAAGCGACTGCCCCGGG
ATACCAGGAAGCAAATGAAGCTGGCCTTAAAGCATTCAGAGCTATGGTAGCGC
AGTTAAAAGCTAAAACTGAATTGTAAGTGTA
- Acetylcholinesterase (AchE) gene - targeting the AchE gene may affect the body wall and appendages of an insect. The AchE gene is important in growth and development. The cDNA sequence for the AchE gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 7 below (310bp):
GAGTGGAGACTCAACGAAGATCAATTGGCCGGTGCACACGGCGTCCGGGCGTG
AATACCTGTCCTTAGCAGTCAACTCCAGCTCCATAGGCCACGGGCTGAGGGTCA
AGGAGTGCGCCTTCTGGCAGAAGTACTTGCCACAGTTGATGGCTGCCACCAATA
AGCCAGAACCTCCGAAGAATTGCACGAACAGCGCAGCGCCCGTCAAGGTCCCG
TACGAAATCTTCGGCGTGGGCGTCGTGATAGCTACGGGCTTAGCCAAGACAACG
TGGTTCAAGTACATCATATGAGTTCATTATGTGGTCTAAGAG
- Juvenile hormone esterase gene - The cDNA sequence for the juvenile hormone esterase gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 8

below (309bp):

CCACCAAGATCTACACGGACCAGAATATTTGGTCAGCAAGAATGCCATCGTCAT

CACATTTAATTACAGATTGAACGTCTTCGGTTTCCTGTCCATGAATACGACAAAA

ATCCCCGGCAACGCTGGTCTCCGAGACCAGGTGACCCTGTTGCGCTGGGTGCAG

AGAAATGCTAAGCATTTCGGAGGAGACCCCAACAACGTCACCATAGCGGGGCA

GAGCGCTGGTGCAGCAGCCGCGCATCTATTGACTCTGTCTAAAGCTGCTAAAGG

TCTTTTCAAAAGAGCAATCTTGATGAGCGGAACAGGAAT

- Methoprene-tolerant (Met) receptor gene - the Met receptor is an intracellular receptor in the insect. The cDNA sequence for the Met receptor gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 9 below (375bp):

GCCGCATAGATGGCATTCTAAGGCGTTCCGATAAAGCCACATCAAATGGTGTTC

AGGATGAGCAAATTATAAGAAGGCAAAGAGTAAGAACTAATAGAACATTTTCA

TCCAGTGGGAATGATGTTGTTTTTATTGGTATGATTCATGTTCTATCCAGTGCAA

TGCCACCTCGAATTCTACCCCCTACAGCCTATTCAGAATACTGGACGAGACATTT

GATTGATGGTCGTATCGTTCAGTGTGACCAGAGTATATCATTAGCAATTGGCTA

CATGACAGAAGAAGTTACTGGAACATCTGCTTTCGTCTTCATGCACAAAGATGA

TGTCCGCTGGGTAATTTGTGTATTACGACAAATGTATGATGAGAGCCGGG

- Rab4b GTPase gene - Rab4b GTPase is a GTPase hydrolase enzyme. The cDNA sequence for the Rab4b GTPase gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 10 below (313bp):

AGGACATGGAGGAATCAAGAGAAGTCACTTTTACAGAAGCTAGTCAATTTGCCC

AAGAAAATGAATTGATGTTTCTTGAAACCAGTGCTAAAACAGGTGAAAATGTAG

AAGAAGCTTTCTTGAAATGTTCCAAAACAATTTTGGCTAAAATTGAAACAGGTG

AATTAGATCCCGAGCGAATAGGTTCAGGCATTCAATATGGGACTGGGACCTCTA

AAAGGCTTAGCGCGCCCAAGAAACCTGCAAGAAGTCCATCTGATTGTGCTTGTC

ATGTATAACATTATTTCTTGGATACATAGAATGCATGATCTGC

- Prophenoloxidase gene - targeting the prophenoloxidase gene may affect the foregut of an insect. The prophenoloxidase gene is involved in detoxification of compounds. The cDNA sequence for the prophenoloxidase gene (prophenoloxidase subunit 2) (which corresponds to the RNA sequence) is provided in SEQ ID NO. 11 below (242bp):

ACGATTCCGTTCGAACAGACGTTCCGTGACCTCTCTGTTCAGAGCAACGACCCT

CGCCGCCCCAACTTGGCCGAGTTCAACTTCTGCGGTTGCGGCTGGCCCCAGCAC

ATGTTGGTCCCCAAGGGTACTGAGGCGGGCGCCGCCTACCAGTTGTTCGTTATG

CTTTCGAACTACGATCTTGACAGCGTGGAACAACCTGACGGCTCACAGTTGAGC

TGCGTCGAAGCTTCCAGTTTCTGCGG

- Cathepsin L gene - targeting the cathepsin L gene may affect the cuticle and epidermis of an insect. The cathepsin L gene plays an important role in the moulting process. The cDNA sequence for the cathepsin L gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 12 below (280bp):

GGTGGAGGACAAGTTCCGCATGAAGATCTACCTGGAGAACAAGCACCGCATCG

CCAAGCACAACCAGCGCTTCGAGCAGGGCGCCGTCAGCTACAAGCTGCGCCCC

AACAAGTACGCCGACATGCTCAGCCACGAGTTCGTGCACGTCATGAACGGCTTC

AACAAGACCCTCAAGCACCCGAAGGCCGTGCACGGCAAGGGTCGCGAGTCCCG

GCCCGCCACGTTCATCGCGCCGGCGCACGTCACCTACCCCGACCACGTGGACTG
GCGCAAGAAGGGC

- NV2 gene - targeting the NV2 gene may affect the mitochondria of an insect. The NV2 gene plays an important role in the electron transport chain (ETC). The cDNA sequence for the NV2-1 gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 13 (374bp) below:

GCCACAAGCGTGGTGCCATGATCCCACTGCTCGACCTGGCTCAGCGTCAGGCTG
GAGGTTGGCTGCCGATATCTGCCATGCATAAAGTAGCTGAAATCCTCAAACTTC
CTCGCATGAGAGTTTATGAGGTTGCTACGTTCTACACTATGTTTATTAGACGACC
AATCGGCAAGTACCATATCCAAGTTTGCACGACAACTCCTTGCTGGCTCCGAGG
TTCTGATGCTATCCTGAAAGCTCTCACTGAGGGTACACAATGCCATGTTGGAGG
AAACAGCCCTTGTGGCAAGTTCTCTATTTCTGAGGTTGAATGCCTTGGTGCCTGT
GTTAATGCTCCTATGATTCAAGTCAACGATGATTACTACGAAGACCTG

[0100] The cDNA sequence for the NV2-2 gene (which corresponds to the RNA sequence) is provided in SEQ ID NO. 14 (255bp) below:

GGTTGAATGCCTTGGTGCCTGTGTTAATGCTCCTATGATTCAAGTCAACGATGAT
TACTACGAAGACCTGTCAGTAGATGACACAAAGGAAATTATTGAAAAGCTCAA
AAGGGACGAGAAACCGAAAGCTGGCCCTAGGAGCGGCAGATTCGCCTCAGAAC
CCTTGGGAGGACTCACTTCTCTCACCGAAGAACCTACAGGCCCCGGTTTTGGAC
TACAACCCGGCCTCAAGGCCTAGAACAAAAAGTTTCCGTT

[0101] The experiments below detail the use of the Rieske gene, the VDAC gene, GFP and the Control (MEGAscript®) in the composition. However, it is expected that any of the above sequences may be used for the composition.

Preparation of Double Stranded RNA (dsRNA)

[0102] The Rieske gene sequence was cloned into pGEM-T-Easy vector, sequenced and used for *in vitro* dsRNA synthesis. The T7 promoter sequences were added to gene specific primers (provided below). The T7 DNA template (300 bp) for dsRNA synthesis was synthesised using PCR. The dsRNA synthesis was performed using MEGAscript T7 Transcription Kit and purified by Trizol extraction protocol. The dsRNA concentration was analysed using NanoDrop 1000 and integrity was checked by agarose gel electrophoresis (1%). The primer sequences used were as follows (T7 sequence underlined):

SEQ ID NO. 15: TAATACGACTCACTATAGGGAGTCGGGGTAGCTGATGTCG

SEQ ID NO. 16: TAATACGACTCACTATAGGGAGGCAAGTTCATCGGTGGTT

[0103] The Rieske and VDAC sequences were also synthesised by an *in vitro* transcription method by AgroRNA company (Seoul, South Korea). The Rieske sequence is 300 bp (conserved region of sequence) and the VDAC sequence is 232 bp (conserved region of sequence), as outlined above.

EXAMPLE 1 - BENTONITE - POLYETHYLENEIMINE - dsRNA (BEN-PEI-dsRNA) COMPOSITION

[0104] In general, bentonite - polyethyleneimine (BEN-PEI) was prepared first by mixing dispersed bentonite in excess PEI solution overnight (~16 hrs) followed by washing away of excess PEI and dispersing the collected BEN-PEI. dsRNA was mixed with the dispersed BEN-PEI at varied dsRNA:BEN-PEI mass ratios, which was subjected to the gel test to determine the best loading ratio of dsRNA and BEN-PEI. Polyethyleneimine (PEI) was purchased from Sigma Aldrich, and had a molecular weight of 60,000 and 800.

Preparation of BEN-PEI

[0105] BEN-PEI was prepared, using PEI with both a molecular weight of 60,000 and a molecular weight of 800. First, 1 g of bentonite (supposed Cation Exchange Capacity (CEC) was 100 meq/100g) was dispersed in 20 mL deionised water by stirring overnight. Next, 0.5 g of a 50% PEI solution (molecular weight of 60,000 or 800) was dispersed in 10 mL deionized water and the pH was adjusted to 6-7 using 1.0 M HCl while stirring. While vigorously stirring, the dispersed PEI solution was then added dropwise into the bentonite solution. The bentonite PEI solution was stirred overnight at 50 °C.

[0106] The bentonite - polyethyleneimine (BEN-PEI) complex was then collected and washed five times using high-speed centrifugation (10000 g, 5 min; 20000 g, 10 min; 20000 g, 15 min; 20000 g, 15 min; 20000 g, 15 min). The washed BEN-PEI complex was dispersed in 100 mL deionised water.

[0107] The mass concentration was 11.7 mg/ml for BEN-PEI (60,000 molecular weight) and 8.3 mg/ml for BEN-PEI (800 molecular weight). Mass concentration was determined by centrifugation (4,000 rpm, 20 min) of 10 mL sonicated bentonite or bentonite-PEI (BEN-PEI) suspension. Resulting supernatant was discarded and the pellet vacuum-dried (2 hrs), followed by air-drying (16 hrs). The dried pellet was weighed for calculation of the mass concentration.

dsRNA Loading onto BEN-PEI

[0108] The mass loading ratio, i.e. the ratio of complete dsRNA binding with Ben-PEI was determined by mixing 500 ng MEGAscript control dsRNA (500 bp) with BEN-PEI (60,000 and 800 molecular weight) at mass ratios of 1:1, 1:10, and 1:20 up to 1:140 and 1:150. The resulting mixtures were subjected to orbital shaking (20 min) followed by gel electrophoresis (1.0%).

[0109] As illustrated in Figures 1A and 1B, as the amount of BEN-PEI relative to dsRNA increased (i.e. the mass ratio from 1:1 to 1:100), the intensity of the bottom band for free dsRNA became weaker in intensity, indicating the formation of more BEN-PEI-dsRNA complexes. Complete binding of dsRNA with BEN-PEI was shown to be around -1:80 (Figure 1A, PEI MW 60,000) and -1:115 (Figure 1B, PEI MW 800). At these ratios, all dsRNA was associated with Ben-PEI, leaving no free dsRNA to migrate down the gel. This illustrates that BEN-PEI is able to completely load dsRNA at a dsRNA:BEN-PEI mass ratio of 1:80-1:115, depending on the molecular weight of PEI; thus the dsRNA loading capacity is about 1 wt% in the solid system.

pH Dependent Release of dsRNA from BEN-PEI-dsRNA compositions

[0110] pH release testing was performed by preparing BEN-PEI-dsRNA solutions at the mass ratio with complete dsRNA association (as discussed in the preceding paragraph: 1:100 for BEN-PEI-dsRNA (in which PEI has a molecular weight of 60,000) and 1:120 for BEN-PEI-dsRNA (in which PEI has a molecular weight of 800)). The resulting suspensions were centrifuged at 13,000 rpm for 15 min and the supernatant electrophoresed (1.0%) to check for the absence of free dsRNA before discarding the supernatant. The pellets were resuspended in 20 µL aqueous solutions ranging from pH 2-12.5, and orbital shaken for 30 min. Gel electrophoresis (1.0%) was then used to examine the release of dsRNA from the BEN-PEI-dsRNA composition, and the results are provided in Figure 2.

[0111] As illustrated in Figures 2A and 2B, the dsRNA release was pH dependent and was substantially released from the compositions at pH 11. However, the pH in the final mixed solution would be lower than the initial value. This means that dsRNA release would start at a pH lower than 11.0 but higher than 10.5.

EXAMPLE 2 - BENTONITE - ETHOXYLATED POLYETHYLENEIMINE - dsRNA (BEN-etPEI-dsRNA or BReP) COMPOSITION

[0112] A different polymer was used in the composition instead of PEI: 80% ethoxylated branched polyethyleneimine (etPEI). This polymer has a molecular weight of 70,000 and is available from Sigma Aldrich.

Preparation of dsRNA-etPEI

[0113] First, 35% w/w (density = 1.05 g/mL) of 80%-ethoxylated branched PEI (etPEI) solution was diluted 1,000 times with deionised water and the pH was adjusted to 7-7.5 using 1.0 M HCl while stirring. The mass concentration of etPEI was then adjusted to 0.35 mg/mL.

[0114] Rieske dsRNA (500 ng, insect specific gene, 300 bp, 1.1 µg/uL) was added to etPEI solutions with N:P ratios (N: amine in PEI and P: phosphate in dsRNA) of 1, 2, 3, 4, 5, 10 and 20 (denoted NP1, NP2, NP3, NP4, NP5, NP10 and NP20 herein). The mixtures were then incubated on ice for 15 minutes with gradual mixing by inversion. Gel electrophoresis was used to examine if there is free dsRNA in the dsRNA-etPEI mixed solutions, and these results are provided in Figure 3.

[0115] The image provided in Figure 3 confirmed that some free dsRNA was present in the NP2 (dsRNA-etPEI) solution and a very minimal amount of free dsRNA was present in the NP3 (dsRNA-etPEI) solution. Complete binding of dsRNA with etPEI occurred in the NP4 and NP5 solutions. For the N:P molar ratio of 3, 4, and 5, the dsRNA/etPEI mass ratio was nearly 1.41, 1.05, and 0.84, respectively.

pH Dependent Release of dsRNA from dsRNA-etPEI

[0116] NP3 and NP4 (dsRNA-etPEI) solutions were prepared, and then mixed in the same volume of buffer. The buffer solutions had a pH of from 8.0 to 10.5. The mixtures were shaken for 30 minutes, and then gel electrophoresis was used to examine the release of dsRNA from the dsRNA-etPEI complexes. The results are provided in Figures 4A (for NP3) and 4B (for NP4).

[0117] As shown in Figures 4A and 4B, there was some free dsRNA in NP3 complexes while almost no free dsRNA in NP4. Both NP3 and NP4 dsRNA-etPEI complexes released a substantial amount of loaded dsRNA at pH 9.5 and 10. NP3 dsRNA-etPEI complexes started to release dsRNA at probably pH 8.5-9.0 while NP4 dsRNA-etPEI complexes started to release dsRNA at pH 9.5. This experiment illustrates that dsRNA release started at pH 9.0-9.5, and that a substantial amount of dsRNA was released at pH 9.5-10 and most released at pH 10.5.

pKa of etPEI

[0118] The pKa of 80% ethoxylated branched polyethyleneimine (etPEI) was determined. First, solutions of 0.1 mol/L HCl, etPEI, and NaOH were prepared. 10 mL 0.1 mol/L HCl and 10 mL 0.1 mol/L etPEI were mixed, and the pH recorded. To the etPEI solution was added 0.5 mL 0.1 mol/L NaOH while stirring and the pH recorded; this step was repeated until the pH began to increase sharply. To the etPEI solution was added 0.1 mL 0.1 mol/L NaOH while stirring and the record pH recorded; this step was repeated until the pH increase slowed down. The

previous two steps were then repeated until the pH of the solution was 11-11.5. The results are provided in Figure 5. This illustrates that etPEI has a pKa of about 9.0-9.5.

Preparation of BEN-etPEI-dsRNA (BReP) Composition

[0119] The prepared dsRNA-etPEI samples were immobilised with bentonite (BEN) at different BEN:etPEI mass ratios, and then the dsRNA release from the BEN-dsRNA-etPEI (BReP) at different pH values was examined.

Preparation of BReP

[0120] First, bentonite (0.5 g) was dispersed in 100 mL deionised water via stirring for 5 minutes, and this suspension was then diluted to a final mass concentration of 5 μg/μL. Next, dsRNA-etPEI mixtures at a N:P ratio of 3, 4 and 5 (i.e. NP3, NP4 and NP5) were prepared as described above.

[0121] The bentonite suspension was mixed with the dsRNA-etPEI mixtures (NP3, NP4 and NP5) at a bentonite:etPEI mass ratio of 6:1 to form BEN-etPEI-dsRNA complexes (at NP3, NP4 and NP5 - i.e. BReP3, BReP4 and BReP5). The mixtures were incubated on ice for 15 minutes with gradual mixing by inversion and then gel electrophoresis was used to examine for the presence of free dsRNA.

[0122] As illustrated in Figure 6A and 6B, at pH 8.0-8.5 BReP3 released some dsRNA (Figure 6A), BReP4 released a little amount of dsRNA (Figure 6B) and BReP5 released almost no dsRNA (Figure 6B). In comparison with the gel images in Figures 3, 4A and 4B, there is much more free dsRNA in BReP3 and BReP4 than in NP3 and NP4. Without wishing to be bound by theory, it is believed that this is because bentonite sheets carry negative charges which can neutralise some positive charges in etPEI, and neutralisation of positive charges in etPEI can result in release of dsRNA. This effect is expected to decrease at higher N:P ratios in etPEI-dsRNA (such as NP5), and for BReP5 it appears that there are enough positive charges in etPEI to ameliorate this effect.

pH Dependent Release of dsRNA from BReP

[0123] BReP4 and BReP5 suspensions were prepared, as described above. The suspensions were then mixed with the same volume of buffer. The buffer solutions had a pH from 8.5 to 10.0. The mixtures were shaken for 30 minutes, and then gel electrophoresis was used to examine the release of dsRNA from the BReP4 and BReP5 complexes. The results are provided in Figures 6 and 7.

[0124] As shown in Figure 7, a small amount of free dsRNA was present in the BReP4 complex and in dsRNA-etPEI (NP4). The pH dependant release of dsRNA from BReP4 seemed to start at pH 8.5, consistent with that shown in Figure 6B. The release became more significant at pH 9.0 and 9.5, with the majority of the dsRNA released at pH 10.0.

[0125] As shown in Figure 8, the amount of free dsRNA present in the BReP5 and dsRNA-etPEI (NP5) was minimal. The near total release of dsRNA from BReP5 occurred abruptly at pH 9.5, with an almost complete release at pH 10.0.

[0126] BReP6 was also prepared to test the pH dependent release behaviour. BReP6 was as prepared above for BReP4 and BReP5, except that the N:P ratio (N: amine in etPEI and P: phosphate in dsRNA) in the etPEI -dsRNA was 6 (i.e. NP6). As shown in Figure 9, no amount of free dsRNA was observed in the BReP6 and in dsRNA-etPEI (NP6) preparations. The minimal release of dsRNA from BReP6 started at pH 10.0.

Summary

[0127] BReP4, BReP5 and BReP6 were able to carry a large amount of dsRNA. For example, the mass ratio BEN:dsRNA:etPEI was 6:1.41:1, 6:1.05:1, 6:0.84:1, and 6:0.70:1 for BReP3, BReP4, BReP5 and BReP6, respectively. This means that the dsRNA wt% was 16.7%, 13.0%, 10.7% and 9% in BReP4, BReP5, and BReP6, respectively.

[0128] dsRNA in BReP5 and BReP6 were almost completely associated with BEN-etPEI to form BEN-dsRNA-etPEI complexes, which were stable at pH 6-9, without any obvious release of loaded dsRNA. dsRNA started to release at pH 9.5 and 10.0, for BReP5 and BReP6, respectively. There was a small amount of free dsRNA in BReP4 at pH 7.0-8.5. For BReP4, the release of dsRNA started at pH 8.5-9.0, and completed at pH 10.0.

Physicochemical Properties

[0129] Scanning Electron Microscope (SEM) images were recorded in a JEOL JSM-6300 (JEOL, Tokyo, Japan) to investigate the morphology and particle size of bentonite and bentonite-etPEI samples (prepared analogously to the bentonite-PEI samples discussed above). All samples were dropped on silica wafers to avoid immersion into carbon film during coating. As illustrated in Figures 10A (for bentonite) and 10B (for BEN-etPEI), BEN-etPEI forms large stacked aggregate (which is negative for the on-leaf residence).

[0130] The particle size distribution and zeta potential of bentonite, BEN-etPEI-dsRNA (NP5, as described above), etPEI and etPEI-dsRNA (NP5) were measured on a Nanosizer Nano ZS instrument (Malvern Instruments). Figure 11 shows the particle size distribution of bentonite, etPEI, etPEI-dsRNA complex, and BEN-etPEI-dsRNA and the z-average value of the particle

sizes are ~450, -7.4, -750 and -2110 nm, respectively. The zeta potentials of bentonite, BEN-etPEI complex, etPEI (pH adjusted to ~7), etPEI-dsRNA, and BEN-etPEI-dsRNA were measured and were -32, -25, 18, 0.5, and 1.3 mV, respectively.

EXAMPLE 3 - NUCLEASE PROTECTION ASSAY

[0131] Rieske dsRNA (500 ng) was loaded on etPEI at a N:P ratios (N: amine in PEI and P: phosphate in dsRNA) of 5 (NP5), as mentioned above. NP5 complexes were then loaded on bentonite at mass ratio of 1:6 to make BReP5. The final volume of the mixture was adjusted to 10 µl using diethylpyrocarbonate (DEPC) water. High salt Nuclease A (2 µl) was added and the mixture incubated at 37 °C for 20 minutes. A buffer at pH 10 (8 µl) was added to release and check dsRNA release from BReP5 complexes. Solutions were loaded on agarose gel (1%) and the gel image recorded. As shown in Figure 12, naked dsRNA was degraded in the presence of Nuclease A. In sharp contrast, dsRNA in BReP5 was protected from the degradation by Nuclease A, and released at pH 10.

EXAMPLE 4 - INSECT FEEDING ASSAY USING BReP5

Insect Rearing

[0132] Neonates of *Helicoverpa armigera* were fed on artificial diet for 4 days and were subsequently used in leaf feeding assay. The larvae were reared under controlled conditions of 27 ± 2 °C, 65 ± 5 % relative humidity and 16:8 h of light and dark cycle.

Leaf Feeding Assay

[0133] *Nicotiana tabacum* (1 month old plant) leaves were selected for the insect feeding assay. The assay included four groups of randomly apportioned insects and leaves, with 8 replicates for each group. The groups were: DEPC water (negative control), BEN-etPEI, naked Rieske dsRNA and BReP5.

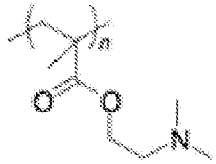
[0134] To perform the assay, the single tender leaf was placed on a moist filter paper in a petri-plate. A composition was spread on both sides of each leaf using a paint brush. The compositions used were: naked dsRNA: 50 µg/100 µL DEPC water; BReP5: corresponding to 50 µg dsRNA/100 µL; BEN-etPEI: at 6:1 bentonite:etPEI - the same mass as of BReP5 in 100 µL; or water: 100 µL. Four day old larvae were transferred to petri-plates and fed with the first fresh leaves with respective treatments for three days, and then repeated with second fresh leaves treated similarly for another three days. After the sixth day of treatment feeding, larvae were shifted to standard artificial diet for further growth.

[0135] As illustrated in Figure 13, no mortality was observed in DEPC water only treated group. In the group treated with BEN-etPEI only, one larva was dead and one larva was obviously affected in growth. Comparatively, naked dsRNA treatment group showed a single mortality. In contrast, BReP5 treatment showed mortality in four larvae with one larvae also showing retarded growth. The statistical data are also presented in Figure 14 and shows the mortality rate in BReP5-treated group is obviously higher than in other groups.

EXAMPLE 5 - BENTONITE - pDMAEMA - dsRNA (BEN-pDMAEMA-dsRNA) COMPOSITION

pDMAEMA

[0136] pDMAEMA is poly(2-dimethylaminoethyl methacrylate), of the structure shown below. pDMAEMA is a pH-sensitive polymer with a pKa of 7.0-7.5. pDMAEMA can be readily synthesised by reversible addition fragmentation chain transfer (RAFT) radical polymerization with a predetermined molecular weight and narrow molecular weight distribution. In one embodiment, pDMAEMA has an average molecular weight of 15,000 Dalton. However, in this example the pDMAEMA has a molecular weight of 33,000. pDMAEMA may be prepared according to the method outlined in Fournier D et al., (2007) Macromolecules, 40, 915-920.



Preparation of dsRNA-pDMAEMA

[0137] pDMAEMA powder was dissolved in deionised water and the pH was adjusted to 6-6.5 using 1.0 M HCl while stirring. The mass concentration of pDMAEMA was then adjusted to 4 mg/mL.

[0138] Control dsRNA (500 ng, MEGAscript RNAi kit, 1μg/(μL) was added to pDMAEMA solutions with N:P ratios (N: amine in pDMAEMA and P: phosphate in dsRNA) of 0.5, 1, 2, and 3 (denoted NP1/2, NP1, NP2, and NP3 herein). The mixture was incubated at room temperature (RT) for 5 minutes with gradual mixing by inversion. Gel electrophoresis was used to examine if there is free dsRNA in the dsRNA-pDMAEMA solutions, and results are provided in Figure 15.

[0139] The image provided in Figure 15 confirms that some free dsRNA was present in the NP1/2 (dsRNA-pDMAEMA) solution. Complete binding of dsRNA with pDMAEMA occurred in

the NP1, NP2 and NP3 solutions.

[0140] An N:P ratio of 1 means that the mass ratio of pDMAEMA:dsRNA is about 1:2. This is because the molecular weight of the monomeric unit in pDMAEMA is 157 (and this unit has only one nitrogen atom), and the average molecular weight of a single base-sugar-phosphate unit in DNA is about 330 (this unit includes one phosphorous atom). Ben-pDMAEMA-dsRNA may have approximately 63 wt% of Ben, 13 wt% of pDMAEMA and 25 wt% of dsRNA.

Preparation of dsRNA-pDMAEM-BEN

[0141] Bentonite (0.5 g) was dispersed in 80 mL deionised water via stirring for 5 minutes, and this suspension was then diluted to a final mass concentration of 5 µg/µL. The dsRNA was added into the pDMAEMA solution at pH 6.0 to make dsRNA-pDMAEMA mixtures at the N:P ratio of 1 (NP1). The bentonite suspension was then mixed with the dsRNA-pDMAEMA mixtures (NP1) at a bentonite:pDMAEMA mass ratio of 5:1 to form a BEN-pDMAEMA-dsRNA complexes (at NP1 - i.e. BPR5). The mixtures were incubated on ice for 15 minutes with gradual mixing by inversion.

pH Dependent Release of dsRNA from dsRNA-pDMAEMA-BEN

[0142] A series of buffer solutions of BPR5 (as prepared above) were prepared at pH 8, 8.5, 9, 9.5, 10, 10.5 and 11. Each of the solutions was prepared with same volume buffer. The mixture was incubated at room temperature (RT) for 5 minutes with gradual mixing by inversion, and then gel electrophoresis was run to examine the release of dsRNA from the dsRNA-pDMAEMA-bentonite complexes. The result is provided in Figure 16.

[0143] As shown in Figure 16, NP1 dsRNA-pDMAEMA-bentonite complexes started to release dsRNA probably at pH 8.0, and that a substantial amount of dsRNA was released at a pH of from 8.5-11.

EXAMPLE 6 - NUCLEASE PROTECTION ASSAY

[0144] Control dsRNA (500 ng) was loaded on pDMAEMA at an N:P ratio (N: amine in pDMAEMA and P: phosphate in dsRNA) of 1 (NP1), as mentioned above. NP1 complexes were then loaded on bentonite at a mass ratio of 1:5 to make BPR5. The final volume of the mixture was adjusted to 10 µL using diethylpyrocarbonate (DEPC) water. High salt Nuclease A (0.125 ng) was added and the mixture incubated at 37 °C for 20 minutes. A buffer at pH 10 (8 µl) was added to release and check dsRNA release from BPR5 complexes. Solutions were loaded on agarose gel (1%) and the gel image recorded. As shown in Figure 17, naked dsRNA was degraded in the presence of Nuclease A. In sharp contrast, dsRNA in BPR5 was protected

from the degradation by Nuclease A, and released at pH 10.

EXAMPLE 7 - FEEDING BIO-ASSAY

[0145] Newly emerged neonates of *Helicoverpa armigera* were fed on water, bentonite-pDMAEMA (bentonite polymer or BP), Green Fluorescent Protein (GFP - negative control), Rieske, VDAC, bentonite-pDMAEMA-Rieske (BP-Rieske) and bentonite-pDMAEMA-VDAC (BP-VDAC). BP-Rieske and BP-VDAC were prepared as outlined above for dsRNA-pDMAEMA-BEN, with a N:P ratio of 1 (nitrogen in pDMAEMA : phosphorous in dsRNA), and a mass ratio of bentonite:pDMAEMA of 5:1.. Solutions were applied onto the surface of artificial diet cubes (~1 cm2) such that 60 µg dsRNA was applied to each cube (a volume of 100 µL), and the solutions were allowed to percolate into the diet cubes. The resultant cubes were subsequently fed to newly emerged neonates of *Helicoverpa armigera.* Solutions were fed to the larvae three times with an interval of 24 hours between consecutive feeding. New artificial diet cubes were used at the time of application of the dsRNA solutions.

[0146] During the feeding experiment, 15 larvae from each of the treatment groups were pooled together in liquid nitrogen 3 days after their first dsRNA exposure (DPE). The gene silencing effects were analyzed in terms of relative transcripts.

RT-PCR Analysis

[0147] To evaluate the effectiveness of the nanoparticle-based RNAi method in silencing both Rieske and VDAC genes through *H. armigera* larval feeding, dsRNAs for Rieske (300 bp - conserved region of sequence) and VDAC (232 bp - conserved region of sequence) were synthesized through AgroRNA company (Seoul, South Korea).

[0148] Total RNA was extracted from 15 pooled larvae and TriSure reagent (Bioline) and DNAse (Thermo Fisher) treatments were performed as per the manufacturer's instructions. Real time reaction was carried out in 20µL using 100ng of total RNA. A gene encoding ribosomal protein (L27, RPL27) was used as an internal reference. Real time PCT (RT-PCR) was performed with a sensifast SYBR No-ROX one step kit (Bioline), and the $2^{-\Delta\Delta CT}$ method was used to evaluate the transcript levels of Rieske and VDAC relative to GFP in the *H. armigera* fed on the artificial diets. Primer pairs without overlapping with dsRNA regions were synthesized for examining the suppression of gene transcript by quantitative real-time PCR. The sequences of primers used were as follows.

SEQ ID NO. 17 (Rieske): CCGCAAACGAGATTAGCACC

SEQ ID NO. 18 (Rieske): ACTTCGGCGGCTACTACTG

SEQ ID NO. 19 (VDAC): GACTGCGGTATTAGCATGAAG

SEQ ID NO. 20 (VDAC): CAGAGAACACTTGAAGATACTG

SEQ ID NO. 21 (RPL27): ACAGGTATCCCCGCAAAGTGC

SEQ ID NO. 22 (RPLS27): GTCCTTGGCGCTGAACTTCTC

[0149] Figures 18 and 19 show that feeding *H. armigera* larvae with either bentonite-pDMAEMA-Rieske (BP-Rieske) or bentonite-pDMAEMA-VDAC (BP-VDAC) effectively triggered RNAi in the larvae. The BP-Rieske and BP-VDAC repressed the transcript level by 42.8% and 79.4% respectively compared to GFP dsRNA fed larvae. However, naked VDAC dsRNA repressed the transcript level by 22.6% and naked Rieske dsRNA did not suppress the transcript level significantly.

[0150] In the present specification and claims (if any), the word 'comprising' and its derivatives including 'comprises' and 'comprise' include each of the stated integers but does not exclude the inclusion of one or more further integers.

[0151] Reference throughout this specification to 'one embodiment' or 'an embodiment' means that a particular feature, structure, or characteristic described in connection with the embodiment is included in at least one embodiment of the present invention. Thus, the appearance of the phrases 'in one embodiment' or 'in an embodiment' in various places throughout this specification are not necessarily all referring to the same embodiment. Furthermore, the particular features, structures, or characteristics may be combined in any suitable manner in one or more combinations.

[0152] In compliance with the statute, the invention has been described in language more or less specific to structural or methodical features. It is to be understood that the invention is not limited to specific features shown or described since the means herein described comprises preferred forms of putting the invention into effect. The invention is, therefore, claimed in any of its forms or modifications within the proper scope of the appended claims (if any) appropriately interpreted by those skilled in the art.

ADVANTAGES

[0153] Advantages of the present invention may include:

- dsRNA of a large size can be included in the composition;
- the dsRNA in the composition may be protected from nucleases and U.V. light;
- the molecule having a plurality of positively charged groups may act as a 'pH control' switch to release the dsRNA from the composition at an alkaline pH (for example, for Lepidopteran insects this occurs in the midgut);
- after being sprayed on an insect food, the dsRNA should be viable for weeks;

- the composition has the capacity to cause mortality of insects feeding on food sources sprayed with or comprising the composition;
- dsRNA is highly specific to a target organism and is ecologically friendly. The composition should not affect species that are not targeted by the composition;
- the composition may be prepared easily at relatively low cost;
- the composition may have a high dsRNA loading capacity;
- the composition may have a low toxicity; and
- the composition may be readily dispersed for spraying, drip-feeding or application via irrigation.

SEQUENCE LISTING

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REFERENCES CITED IN THE DESCRIPTION

Cited references

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- **TENLLADO, F.J.R. DIAZ-RUIZ**Double-stranded RNA-mediated interference with plant virus infection.Journal Virology, 2001, vol. 75, 12288-12297 [0009]
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SAMMENSÆTNING

PATENTKRAV

1. Sammensætning, der omfatter dobbeltstrenget RNA, som er adsorberet på et lerkompleks, hvor lerkomplekset indbefatter:
   - et kationisk ler; og
   - et molekyle, der indbefatter en flerhed af positivt ladede grupper, idet de positivt ladede grupper er positivt ladede i en opløsning med en pH-værdi fra 5,0 til 7,0; og
     (i) de positivt ladede grupper har en pKa, der er større end 7,5; eller
     (ii) molekylet er en polymer, der er eller indbefatter et polydialkylaminoalkylmethacrylat;

   hvor sammensætningen indbefatter fra 0,5 til 30 vægt-% dobbeltstrenget RNA, og hvor masseforholdet mellem ler og molekyle er fra 1:1 til 10:1.

2. Sammensætning ifølge krav 1, hvor sammensætningen er til afgivelse af dobbeltstrenget RNA til et insekt med en basisk pH-værdi i fordøjelseskanalen, og hvor lerkomplekset er konfigureret til at frigive det dobbeltstrengede RNA ved den basiske pH-værdi.

3. Sammensætning ifølge krav 1 eller krav 2, hvor sammensætningen er en plantebeskyttelsessammensætning.

4. Sammensætning ifølge krav 3, hvor det dobbeltstrengede RNA er insektdræbende dobbeltstrenget RNA, og det insektdræbende dobbeltstrengede RNA via RNA-interferens er i stand til at beskytte en plante mod insekter; for eksempel hvor det dobbeltstrengede RNA er insektdræbende for et insekt af ordenen *Diptera*, *Lepidoptera* eller *Coleoptera*.

5. Sammensætning ifølge et hvilket som helst af kravene 1 til 4, hvor det dobbeltstrengede RNA som mål har mindst én i gruppen bestående af: et insekts elektrontransportkæde (ETC), en spændingsafhængig kanal hos et insekt, en muskel hos et insekt, en tarm hos et insekt, en kropsvæg og/eller vedhæng hos et insekt, en intracellulær receptor hos et insekt, et GTPase-hydrolaseenzym hos et

insekt, en juvenil hormon-esterase hos et insekt, et insekts mitokondrier og et insekts kutikula og/eller epidermis.

6. Sammensætning ifølge et hvilket som helst af kravene 1 til 5, hvor det kationiske ler er bentonit.

7. Sammensætning ifølge et hvilket som helst af kravene 1 til 6, hvor molekylet, der indbefatter en flerhed af positivt ladede grupper, er en polymer med to eller flere positivt ladede nitrogenatomer.

8. Sammensætning ifølge krav 7, hvor polymeren er polyætylenbaseret, hvor ætylengruppen i den polyætylenbaserede polymer er substitueret med en aminogruppe og eventuelt substitueret med en elektrontiltrækkende gruppe vicinalt til aminogruppen.

9. Sammensætning ifølge krav 7 eller krav 8, hvor forholdet mellem nitrogen i polymeren og fosforen i det dobbeltstrengede RNA er mindst 4; og hvor masseforholdet mellem ler og polymer er fra 3:1 til 8:1.

10. Præparat, der indbefatter sammensætningen ifølge et hvilket som helst af kravene 1 til 9.

11. Præparat ifølge krav 10, hvor præparatet indbefatter en vandig fase og en fast fase, der indbefatter sammensætningen ifølge et hvilket som helst af kravene 1 til 9; og hvor præparatet kan påsprøjtes en plante.

12. Fremgangsmåde til afgivelse af dobbeltstrenget RNA til et insekt med en basisk pH-værdi i fordøjelseskanalen, hvilken fremgangsmåde omfatter administration af sammensætningen ifølge et hvilket som helst af kravene 1 til 9 eller præparatet ifølge krav 10 eller krav 11 til insektnæring, hvor det dobbeltstrengede RNA er til anvendelse med insekter; eventuelt hvor insektnæringen er en plante, en plantedel, syntetisk insektnæring, insektlokkemad eller en vandfælde.

13. Fremgangsmåde til beskyttelse af en afgrøde mod et insekt med en basisk pH-værdi i fordøjelseskanalen, hvilken fremgangsmåde omfatter det trin, at

sammensætningen ifølge et hvilket som helst af kravene 1 til 9 eller præparatet ifølge krav 10 eller krav 11 administreres til en afgrøde, hvor det dobbeltstrengede RNA er til anvendelse med insekter; eventuelt hvor sammensætningen ifølge et hvilket som helst af kravene 1 til 9 eller præparatet ifølge krav 10 eller krav 11 administreres til afgrøden ved påsprøjtning, dråbevis tilføring eller overrisling.

14. Fremgangsmåde til fremstilling af sammensætningen ifølge et hvilket som helst af kravene 1 til 9, hvilken fremgangsmåde indbefatter følgende trin:
    a. adsorbering af det dobbeltstrengede RNA på molekylet, der indbefatter en flerhed af positivt ladede grupper; og
    b. adsorbering af det dobbeltstrengede RNA/molekyle på leret.

15. Kit, der omfatter:
    a. et lerkompleks som defineret i krav 1; og
    b. dobbeltstrenget RNA;

    hvor det dobbeltstrengede RNA kan adsorberes på lerkomplekset til dannelse af sammensætningen ifølge et hvilket som helst af kravene 1 til 9.

DRAWINGS

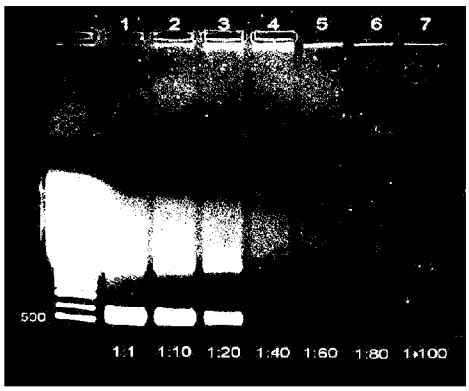

Figure 1A

Figure 1B

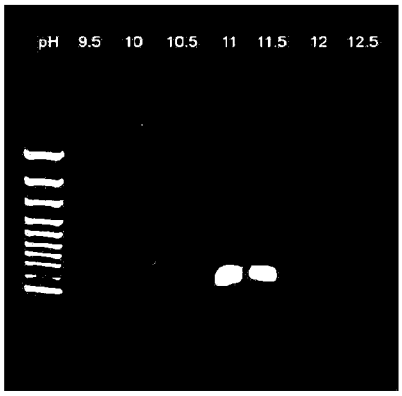

Figure 2A

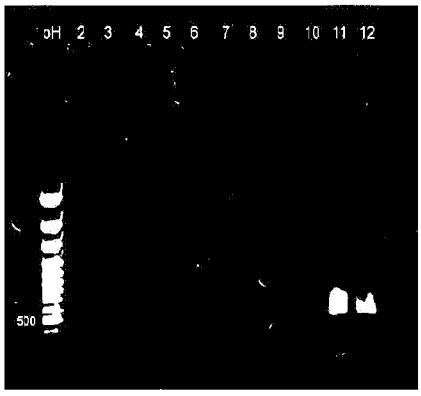

Figure 2B

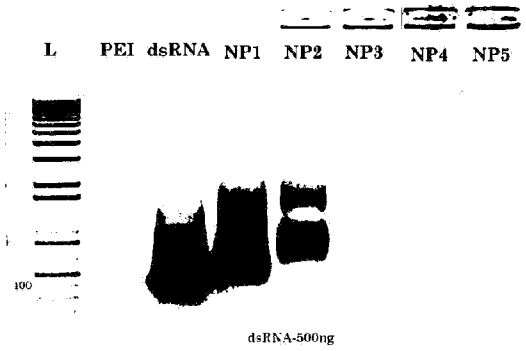

Figure 3

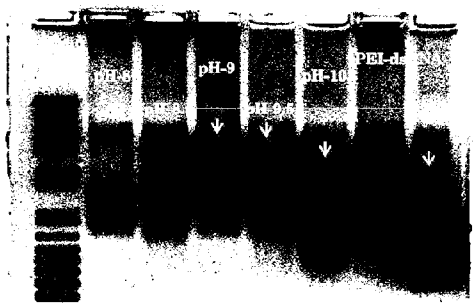

Figure 4A

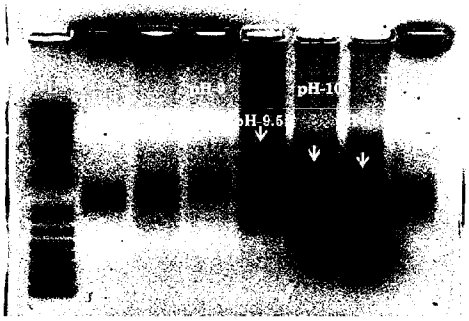

Figure 4B

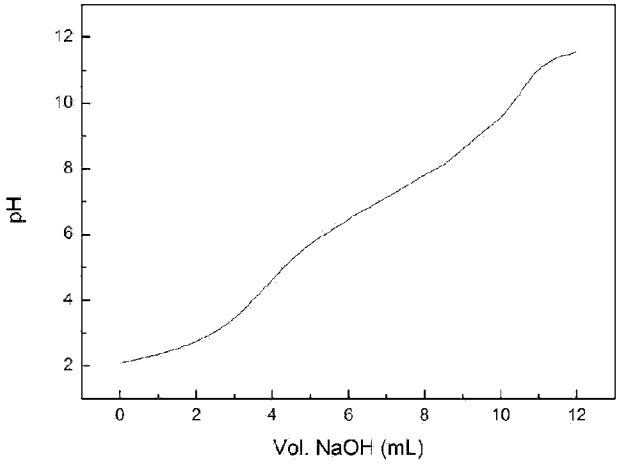

Figure 5

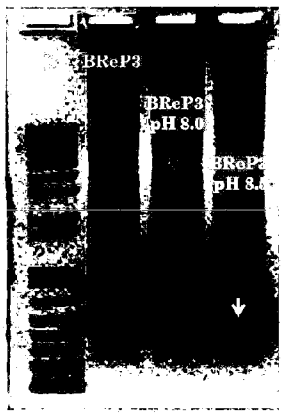

Figure 6A

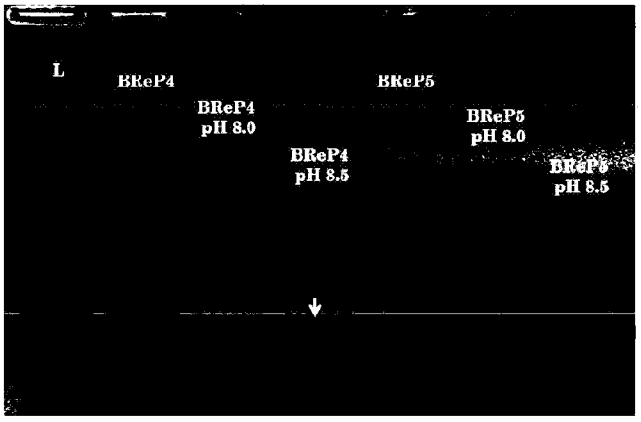

Figure 6B

Figure 7

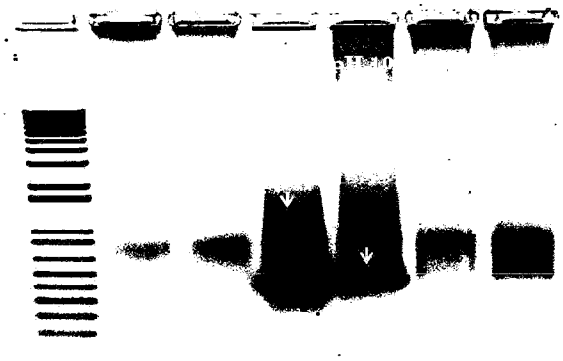

Figure 8

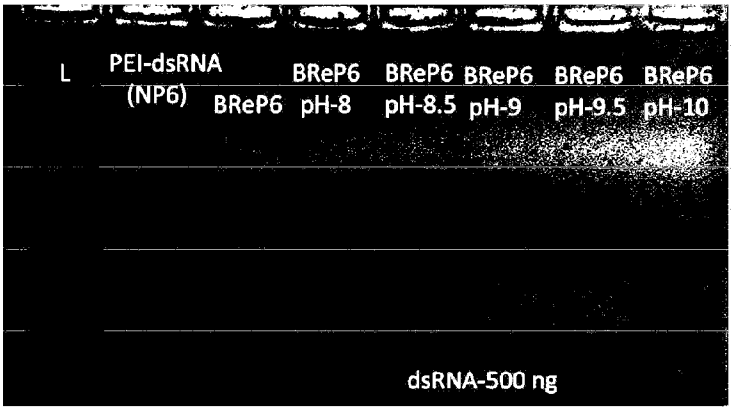

Figure 9

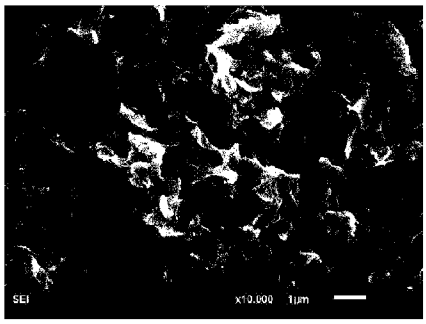

Figure 10A

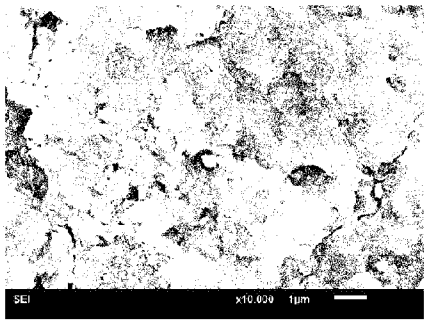

Figure 10B

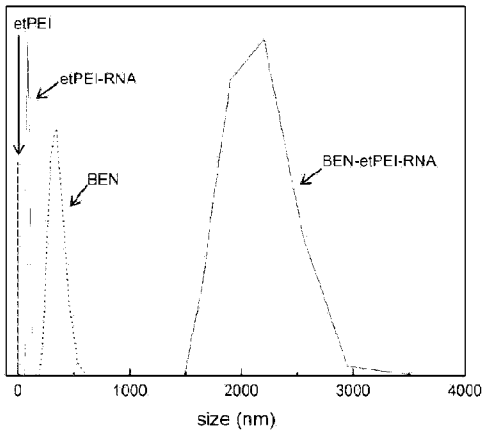

Figure 11

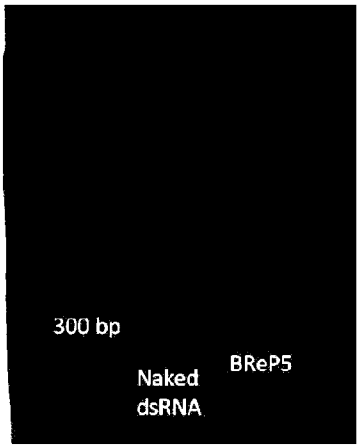

Figure 12

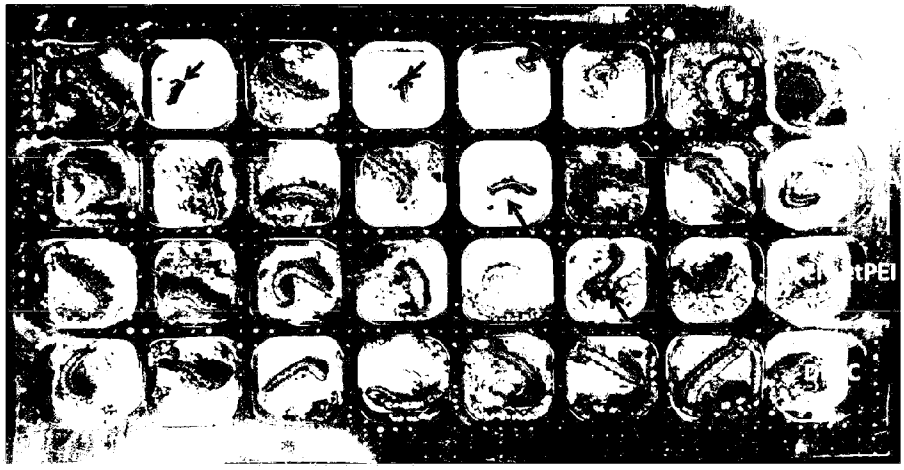

Figure 13

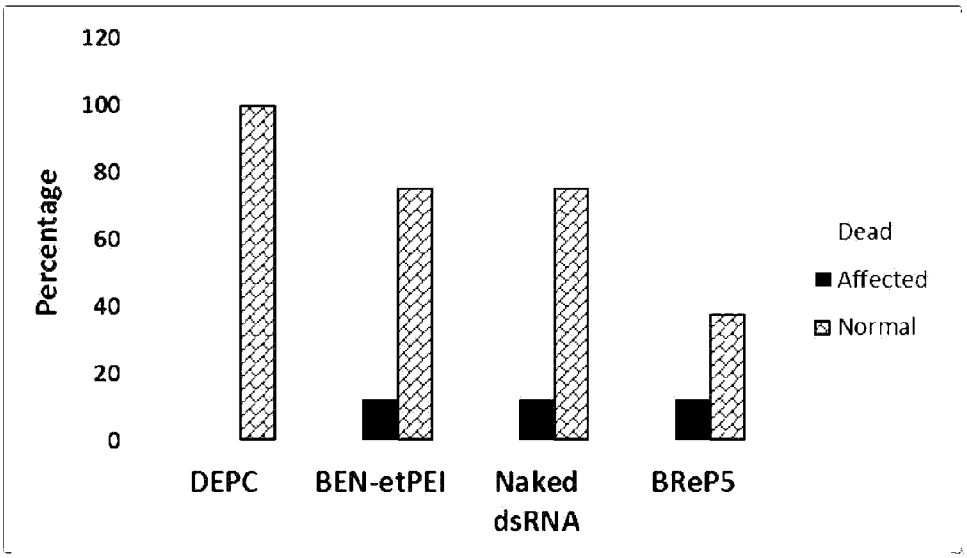

Figure 14

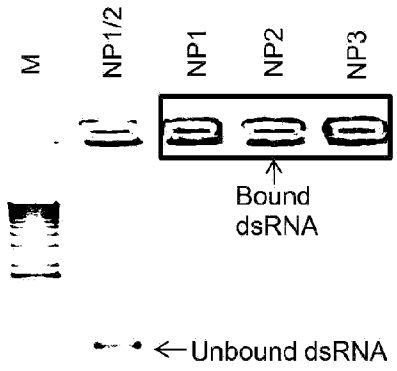

Figure 15

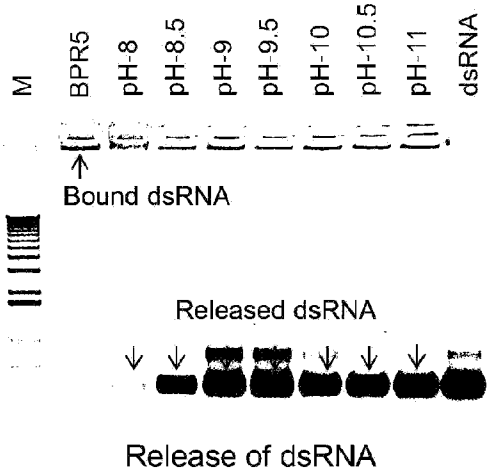

Figure 16

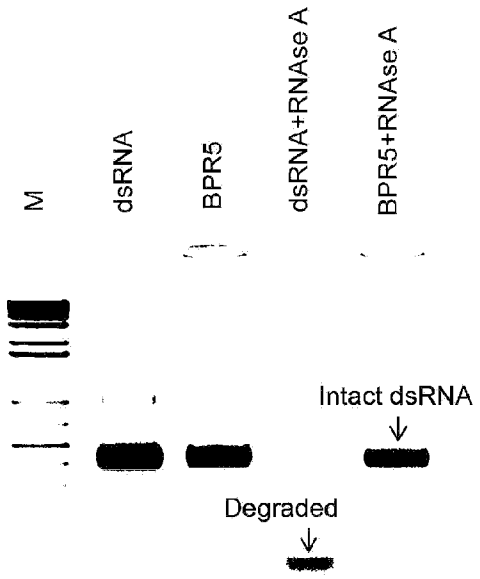

Figure 17

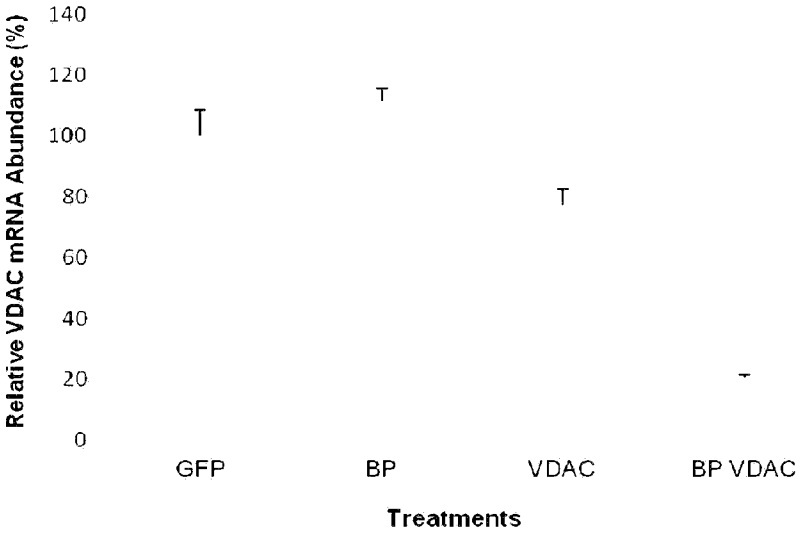

Figure 18

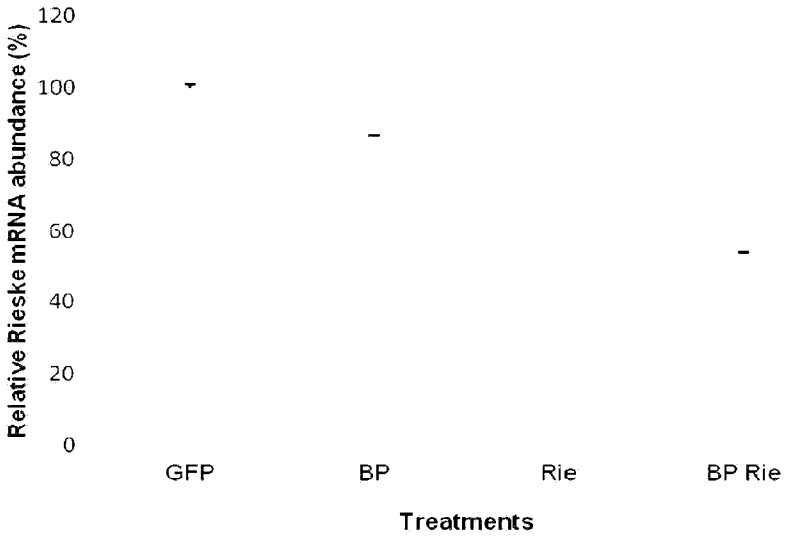
Relative Rieske mRNA abundance (%)
120
100
80
60
40
20
0
GFP
BP
Rie
BP Rie
Treatments

Figure 19