CLAIMS

1. A chimeric ribonucleic acid (RNA) molecule, comprising a double-stranded RNA (dsRNA) region which comprises a first sense ribonucleotide sequence of at least 50 contiguous nucleotides in length and a first antisense ribonucleotide sequence of at least 50 contiguous nucleotides in length, whereby the first sense ribonucleotide sequence and the first antisense ribonucleotide sequences are capable of hybridising to each other to form the dsRNA region, wherein

i) the first sense ribonucleotide sequence consists of, covalently linked in 5' to 3' order, a first 5' ribonucleotide, a first RNA sequence and a first 3' ribonucleotide,

ii) the first antisense ribonucleotide sequence consists of, covalently linked in 5' to 3' order, a second 5' ribonucleotide, a second RNA sequence and a second 3' ribonucleotide,

iii) the first 5' ribonucleotide basepairs with the second 3' ribonucleotide to form a terminal basepair of the dsRNA region,

iv) the second 5' ribonucleotide basepairs with the first 3' ribonucleotide to form a terminal basepair of the dsRNA region,

v) between 5% and 40% of the ribonucleotides of the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence, in total, are basepaired in a non-canonical basepair,

vi) the dsRNA region does not comprise 20 contiguous canonical basepairs,

vii) the RNA molecule is capable of being processed in a plant, arthropod or fungal cell or *in vitro* whereby the first antisense ribonucleotide sequence is cleaved to produce short antisense RNA (asRNA) molecules of 20-24 ribonucleotides in length,

viii) the RNA molecule or at least some of the asRNA molecules, or both, are capable of reducing the expression or activity of a target RNA molecule in a plant, arthropod or fungal cell, and

ix) the RNA molecule is capable of being made enzymatically by transcription *in vitro* or in a plant, arthropod or fungal cell, or both.

2. The chimeric RNA molecule of claim 1, wherein the first sense ribonucleotide sequence is covalently linked to the first antisense ribonucleotide sequence by a first linking ribonucleotide sequence which comprises a loop sequence of at least 4 nucleotides, or between 4 and 1,000 ribonucleotides, or between 4 and 200 ribonucleotides, or between 4 and 50 ribonucleotides, or at least 10 nucleotides, or between 10 and 1,000 ribonucleotides, or between 10 and 200 ribonucleotides, or

between 10 and 50 ribonucleotides, in length, whereby the first linking ribonucleotide sequence is covalently linked to either the second 3' ribonucleotide and the first 5' ribonucleotide, or to the first 3' ribonucleotide and the second 5' ribonucleotide, so that the sequences are comprised in a single, contiguous strand of RNA.

3. The chimeric RNA molecule of claim 2, wherein the loop sequence in the RNA molecule comprises one or more binding sequences which are complementary to an RNA molecule which is endogenous to the plant, arthropod or fungal cell, and/or the loop sequence in the RNA molecule comprises an open reading frame which encodes a polypeptide.

4. The chimeric RNA molecule according to any one of claims 1 to 3, wherein between 5% and 40% of the ribonucleotides of the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence of the dsRNA, in total, are basepaired in G:U basepairs.

5. The chimeric RNA molecule according to any one of claims 1 to 4, wherein the first antisense ribonucleotide sequence is fully complementary to a region of the target RNA and the first sense ribonucleotide sequence is different in sequence to the region of the target RNA by the substitution of C nucleotides in the region of the target RNA with U nucleotides.

6. The chimeric RNA molecule according to any one of claims 1 to 5 which comprises one or more of:

- a second sense ribonucleotide sequence and the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence are linked by a first linking ribonucleotide sequence comprising a loop sequence of at least 4 nucleotides in length, whereby the first linking ribonucleotide sequence is covalently linked to the first 3' ribonucleotide and the second 5' ribonucleotide, and the RNA molecule further comprises a second linking ribonucleotide sequence which comprises a loop sequence of at least 4 nucleotides in length and which is covalently linked to the second 3' ribonucleotide and the second sense ribonucleotide sequence;
- a second antisense ribonucleotide sequence and wherein the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence are linked by a first linking ribonucleotide sequence comprising a loop sequence of at least 4 nucleotides in length, whereby the first linking ribonucleotide sequence is covalently

linked to the second 3’ ribonucleotide and the first 5’ ribonucleotide, and the RNA molecule further comprises a second linking ribonucleotide sequence which comprises a loop sequence of at least 4 nucleotides in length and which is covalently linked to the first 3’ ribonucleotide and the second antisense ribonucleotide sequence;

- a second sense ribonucleotide sequence and a second antisense ribonucleotide sequence, wherein the second sense ribonucleotide sequence and the second antisense ribonucleotide sequences are capable of hybridising to each other to form a second dsRNA region, and the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence are linked by a first linking ribonucleotide sequence comprising a loop sequence of at least 4 nucleotides in length, whereby the first linking ribonucleotide sequence is covalently linked to the first 3’ ribonucleotide and the second 5’ ribonucleotide, and the RNA molecule optionally comprises a second linking ribonucleotide sequence which comprises a loop sequence of at least 4 nucleotides in length and which is covalently linked to the second 3’ ribonucleotide and the second sense ribonucleotide sequence or which covalently links the second sense ribonucleotide sequence and the second antisense ribonucleotide sequence; and

- a second sense ribonucleotide sequence and a second antisense ribonucleotide sequence and, wherein the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence are linked by a first linking ribonucleotide sequence comprising a loop sequence of at least 4 nucleotides in length, whereby the first linking ribonucleotide sequence is covalently linked to the second 3’ ribonucleotide and the first 5’ ribonucleotide, and the RNA molecule further comprises a second linking ribonucleotide sequence which comprises a loop sequence of at least 4 nucleotides in length and which is covalently linked to the first 3’ ribonucleotide and the second antisense ribonucleotide sequence, or which covalently links the second sense ribonucleotide sequence and the second antisense ribonucleotide sequence.

7. The chimeric RNA molecule of claim 6, wherein one or more or all of:

a) the second sense ribonucleotide sequence and the second antisense ribonucleotide sequence each comprise at least 20 contiguous nucleotides in length;

b) the first and second sense ribonucleotide sequences are covalently linked by an intervening ribonucleotide sequence which is unrelated in sequence to the target RNA molecule, or which is related in sequence to the target RNA molecule, or the first and second sense ribonucleotide sequences are covalently linked without an intervening ribonucleotide sequence;

c) the first and second antisense ribonucleotide sequences are covalently linked by an intervening ribonucleotide sequence which is unrelated in sequence to the complement of the target RNA molecule, or which is related in sequence to the complement of the target RNA molecule, or the first and second antisense ribonucleotide sequences are covalently linked without an intervening ribonucleotide sequence;

d) between 5% and 40% of the ribonucleotides of the second sense ribonucleotide sequence and the second antisense ribonucleotide sequence, in total, are either basepaired in a non-canonical basepair or are not basepaired, wherein the second dsRNA region does not comprise 20 contiguous canonical basepairs, and wherein the RNA molecule is capable of being processed in a plant, arthropod or fungal cell or *in vitro* whereby the second antisense ribonucleotide sequence is cleaved to produce short antisense RNA (asRNA) molecules of 20-24 ribonucleotides in length;

e) each linking ribonucleotide sequence is independently between 4 and 2000 nucleotides in length, or between 4 and 1200 nucleotides in length, or between 4 and 200 nucleotides in length, or between 4 and 50 nucleotides in length;

f) the chimeric RNA molecule further comprises a 5' leader sequence or a 3' trailer sequence, or both.

8. A chimeric RNA molecule comprising a first RNA component and a second RNA component which is covalently linked to the first RNA component,

wherein the first RNA component comprises a first double-stranded RNA (dsRNA) region, which comprises a first sense ribonucleotide sequence and a first antisense ribonucleotide sequence which are capable of hybridising to each other to form the first dsRNA region, and a first intervening ribonucleotide sequence of at least 4 nucleotides which covalently links the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence,

wherein the second RNA component comprises a second sense ribonucleotide sequence, a second antisense ribonucleotide sequence and a second intervening ribonucleotide sequence of at least 4 ribonucleotides which covalently links the second sense ribonucleotide sequence and the second antisense ribonucleotide sequence, wherein the second sense ribonucleotide sequence hybridises with the second antisense ribonucleotide sequence in the RNA molecule,

wherein in the first RNA component,

i) the first sense ribonucleotide sequence consists of at least 20 contiguous ribonucleotides covalently linked, in 5' to 3' order, a first 5' ribonucleotide, a first RNA sequence and a first 3' ribonucleotide,

ii) the first antisense ribonucleotide sequence consists of at least 20 contiguous ribonucleotides covalently linked, in 5' to 3' order, a second 5' ribonucleotide, a second RNA sequence and a second 3' ribonucleotide,

iii) the first 5' ribonucleotide basepairs with the second 3' ribonucleotide,

iv) the second 5' ribonucleotide basepairs with the first 3' ribonucleotide,

v) between 5% and 40% of the ribonucleotides of the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence, in total, are basepaired in a non-canonical basepair, and

vi) the first dsRNA region does not comprise 20 contiguous canonical basepairs,

wherein the chimeric RNA molecule is capable of being processed in a plant, arthropod or fungal cell or *in vitro* whereby the first antisense ribonucleotide sequence is cleaved to produce short antisense RNA (asRNA) molecules of 20-24 ribonucleotides in length, and wherein

(a) the chimeric RNA molecule or at least some of the asRNA molecules, or both, are capable of reducing the expression or activity of a target RNA molecule in the plant, arthropod or fungal cell, or

(b) the first antisense ribonucleotide sequence comprises a sequence of at least 20 contiguous ribonucleotides which is at least 50% identical in sequence, or at least 90% identical in sequence, or 100% identical in sequence, to a region of the complement of the target RNA molecule, or

(c) both (a) and (b).

9. The chimeric RNA molecule of claim 8, wherein the ribonucleotides of the first sense ribonucleotide sequence and the first antisense ribonucleotide sequence which are basepaired in a non-canonical basepairs are basepaired in G:U basepairs, and/or the first dsRNA region does not comprise 12 contiguous canonical basepairs.

10. The chimeric RNA molecule according to any one of claims 1 to 9, wherein one or more of:

a) at least 20 contiguous ribonucleotides of the first antisense ribonucleotide sequence are all capable of basepairing to nucleotides of a first region of the target RNA molecule;

b) the chimeric RNA molecule comprises two or more antisense ribonucleotide sequences, and sense ribonucleotide sequences based paired thereto, which antisense sequences are each complementary to a region of a target RNA molecule, and wherein:

- the two or more antisense ribonucleotide sequences are complementary to different regions of the same target RNA molecule; or,
- the two or more antisense ribonucleotide sequences are complementary to regions of different target RNA molecules;

c) the chimeric RNA molecule comprises:

- a hairpin RNA (hpRNA) structure having a 5' end, a sense ribonucleotide sequence which is at least 50 nucleotides in length, an antisense ribonucleotide sequence which is fully base paired with the sense ribonucleotide sequence over at least 21 contiguous nucleotides, an intervening loop sequence and a 3' end; or,
- a single strand of ribonucleotides having a 5' end, at least one sense ribonucleotide sequence which is at least 50 nucleotides in length, an antisense ribonucleotide sequence which is fully base paired with each sense ribonucleotide sequence over at least 21 contiguous nucleotides, at least two loop sequences and a 3' end;

d) between 10% and 30%, between 15% and 30%, or between 16% and 25%, of the ribonucleotides of the sense ribonucleotide sequence and the antisense ribonucleotide sequence, in total, are basepaired in a non-canonical basepair;

e) at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, at least 97%, or 100% of the non-canonical basepairs are G:U basepairs;

f) less than 25%, less than 20%, less than 15%, less than 10%, less than 5%, less than 1% or none, of the ribonucleotides in the dsRNA region are not basepaired;

g) every one in four to every one in six ribonucleotides in the dsRNA region form a non-canonical basepair or are not basepaired;

h) the dsRNA region:

- does not comprise 8 contiguous canonical basepairs,
- comprises at least 8 contiguous canonical basepairs, or
- at least 8 but not more than 12 contiguous canonical basepairs;

i) all of the ribonucleotides in the dsRNA region, or in each dsRNA region, are base-paired with a canonical basepair or a non-canonical basepair;

j) one or more ribonucleotides of the sense ribonucleotide sequence or one or more ribonucleotides of the antisense ribonucleotide sequence, or both, are not basepaired;

k) the antisense RNA sequence is less than 100% identical, or between 80% and 99.9% identical, or between 90% and 98% identical, or between 95% and 98% identical, in sequence to the complement of a region of the target RNA molecule; or,

the antisense RNA sequence is 100% identical in sequence to a region of the target RNA molecule;

l) the sense and/or antisense ribonucleotide sequence is at least 100, at least 200, at least 300, at least 400, at least 500, at least 600, at least 700, at least 800, at least 900, at least 1,000, or 100 to 1,000, or 50 to 1000 nucleotides, or 50 to 500 nucleotides, in length;

m) the number of ribonucleotides in the sense ribonucleotide sequence is between 90% and 110% of the number of ribonucleotides in the antisense ribonucleotide sequence;

n) the number of ribonucleotides in the sense ribonucleotide sequence is the same as the number of ribonucleotides in the antisense ribonucleotide sequence;

o) the chimeric RNA molecule further comprises a 5' extension sequence which is covalently linked to the first 5' ribonucleotide or a 3' extension sequence which is covalently linked to the second 3' ribonucleotide, or both;

p) the chimeric RNA molecule further comprises a 5' extension sequence which is covalently linked to the second 5' ribonucleotide or a 3' extension sequence which is covalently linked to the first 3' ribonucleotide, or both;

q) the chimeric RNA molecule comprises two or more dsRNA regions which are the same or different;

r) when the RNA molecule is expressed in a plant, arthropod or fungal cell, more asRNA molecules are formed that are 22 and/or 20 ribonucleotides in length when compared to processing of an analogous RNA molecule which has a corresponding dsRNA region which is fully basepaired with canonical basepairs.

11. The chimeric RNA molecule according to any one of claims 1 to 5, 8 or 9, wherein the chimeric RNA molecules does not comprise 12 contiguous canonical basepairs.

12. The chimeric RNA molecule according to any one of claims 1 to 5 or 8 to 10, wherein the cell is a plant cell.

13. An isolated and/or exogenous polynucleotide encoding a chimeric RNA molecule according to any one of claims 1 to 12, or a vector comprising the polynucleotide.

14. A host cell, or a non-human organism comprising the cell, comprising one or more or all of a chimeric RNA molecule according to any one of claims 1 to 12, small RNA molecules produced by processing of the chimeric RNA molecule, or a polynucleotide or a vector of claim 13, wherein the host cell is a fungal cell, an arthropod cell or a plant cell, and wherein the non-human organism is a fungus, arthropod or plant.

15. The host cell or non-human organism of claim 14 which is a plant cell or a plant.

16. The host cell or non-human organism of claim 14 which is a fungal cell, such as a yeast cell.

17. A method of producing a chimeric RNA molecule according to any one of claims 1 to 12, the method comprising expressing the polynucleotide or vector of claim 13 in a host cell or cell-free expression system.

18. A composition comprising one or more of a chimeric RNA molecule according to any one of claims 1 to 12, small RNA molecules produced by processing of the chimeric RNA molecule, a polynucleotide or vector of claim 13, a host cell or non-human transgenic organism according to any one of claims 14 to 16, and one or more suitable carriers.

19. A method for reducing or down-regulating the level and/or activity of a target RNA molecule in a plant, arthropod or fungal cell or a plant, arthropod or fungus, the method comprising delivering to the cell or plant, arthropod or fungus, one or more of a chimeric RNA molecule according to any one of claims 1 to 12, small RNA molecules produced by processing of the chimeric RNA molecule, a polynucleotide or vector of claim 13, a host cell or non-human transgenic organism according to any one of claims 14 to 16, or a composition of claim 18.

20. A method of reducing damage caused by a pest or pathogen to a non-human organism, the method comprising delivering to the pest or pathogen, or contacting the

pest or pathogen with, one or more of a chimeric RNA molecule according to any one of claims 1 to 12, small RNA molecules produced by processing of the chimeric RNA molecule, a polynucleotide or vector of claim 11, a host cell or non-human transgenic organism according to any one of claims 14 to 16, or a composition of claim 18, wherein when delivered to the pest or pathogen the RNA molecule, or the small RNA molecules produced by processing of the chimeric RNA molecule, or both, have a deleterious effect on the pest or pathogen.