SUMMARY

[0005] The present disclosure pertains to targeted insecticidal proteins that are toxic to mosquitoes but not to non-target insect species. The present disclosure also relates to plants engineered to produce the toxins. In particular examples, the transgenic plants express an insecticidal toxin protein that includes a targeting peptide to ensure specificity against mosquitoes. The insecticidal toxin may be produced in nectar made by the plants. These plants represent an ecologically sensitive, cost-effective and long-lived approach which leverages the mosquito population's critical requirement for nectar feeding.

[0005a] In a first aspect, the present disclosure refers to a mosquitocidal toxin, comprising:

a fused toxin peptide, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide of SEQ ID NO: 1 from Domain III of the glycoprotein of dengue virus, wherein the mosquito targeting peptide targets *Aedes aegypti* mosquitoes, and wherein the fused toxin peptide is toxic to *Aedes aegypti* mosquitoes.

[0005b] In a second further aspect, the present disclosure refers to a composition comprising the mosquitocidal toxin disclosed herein and a carrier.

[0005c] In a third aspect, the present disclosure refers to a method for producing a modified plant expressing mosquitocidal toxins, comprising:

producing a plant;

~~inducing expression of~~introducing an exogenous gene construct encoding a fused toxin peptide in target cells of the plant by transforming at least one target cell of the plant with the exogenous gene construct, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide of SEQ ID NO: 1 from Domain III of the glycoprotein of dengue virus, wherein the mosquito targeting peptide targets *Aedes*

aegypti mosquitoes, and wherein the fused toxin peptide is toxic to *Aedes aegypti* mosquitoes; and

expressing the fused toxin peptide in the target cells of the plant to produce

~~producing~~ a modified plant expressing the fused toxin peptide.

[0005d] In a fourth aspect, the present disclosure refers to a method for producing a modified plant expressing mosquitocidal toxins in nectar of the plant, comprising:

producing a plant;

~~inducing expression of~~introducing an exogenous gene construct encoding a fused toxin peptide in cells of the plant producing nectar by transforming at least one cell of the plant producing nectar with the exogenous gene construct, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide of SEQ ID NO:1 from Domain III of the glycoprotein of dengue virus, wherein the toxin peptide is a Hv1a spider toxin peptide, and wherein the plant is *Impatiens walleriana*, and wherein the exogenous gene construct comprises a promoter specific to *Impatiens walleriana*; and

expressing the fused toxin peptide in the nectar of the plant to produce

~~producing~~ a modified plant expressing the fused toxin peptide in the nectar of the modified plant and not expressing the fused toxin peptide in non-nectar tissues of the modified plant.

~~**[0005e]** In a fifth aspect, the present disclosure refers to a modified plant prepared by the methods disclosed herein.~~

[0005~~f~~e] In a ~~sixth~~ fifth aspect, the present disclosure refers to a modified mosquitocidal plant, wherein the modified plant expresses an exogenous gene construct encoding a fused toxin peptide in target cells of the plant, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide of SEQ ID NO:1 from Domain III of the glycoprotein of dengue virus, wherein the mosquito targeting peptide targets *Aedes aegypti* mosquitoes and wherein the fused toxin peptide is toxic to *Aedes aegypti* mosquitoes.

[0005~~g~~f] In a ~~seventh~~ sixth aspect, the present disclosure refers to a seed of the modified plant of the ~~sixth~~ fifth aspect, wherein a plant produced by the seed will express the exogenous gene construct.

[0005~~h~~g] In a further aspect, the present disclosure refers to a modified mosquitocidal *Impatiens walleriana* plant, wherein the modified plant expresses an exogenous gene construct encoding a fused toxin peptide in cells of the plant producing nectar, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide of SEQ ID NO:1 from Domain III of the glycoprotein of dengue virus, wherein the toxin peptide is a Hv1a spider toxin peptide, and wherein the modified plant fails to express the fused toxin peptide in non-nectar tissues of the modified plant.

[0006] Mosquito populations are critically dependent on nectar as a food source. Males use nectar and other sugar sources as their sole source of nutrition while females depend on it to energize their blood quest flights, for preparation for overwintering, and other purposes. Leveraging this fact, sugar baits dosed with pesticides have proven to be a viable control measure against mosquitoes. However, it would be preferable to avoid the use of pesticides altogether. Appropriate and effective delivery mechanisms for a mosquitocidal peptide would enable a biosafe mosquito control strategy.

[0007] Toxic peptides targeted to specific organisms have been produced. The specificity of antimicrobial peptides has been altered using targeting peptides and the fusions have been produced in high yield in *E. coli*. Chemically synthesized fusion peptides specifically toxic to *Staphylococcus aureus* and *Streptococcus mutans* have been produced. Further, transgenic plants expressing targeted fusion peptides were shown to be specifically resistant to *Fusarium* root rot fungus and aphids.

[0008] The present disclosure pertains to a targeted fusion peptide having a targeting peptide that is specific to mosquitoes and a toxin peptide that are fused together. The targeting peptide ensures that the fusion peptide is taken up, or bound in some fashion that induces toxicity of the toxin, by mosquitoes only. Unless the fusion peptide is taken up or bound in this fashion, the toxin peptide will lack toxicity. Accordingly, targeting the peptide to mosquitoes results in a toxin that has no effect against non-mosquito insects.

WHAT IS CLAIMED IS:

1. A mosquitocidal toxin, comprising:

a fused toxin peptide, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide of –SEQ ID NO: 1 from Domain III of the glycoprotein of dengue virus, wherein the mosquito targeting peptide targets *Aedes aegypti* mosquitoes, and wherein the fused toxin peptide is toxic to *Aedes aegypti* mosquitoes.

2. The mosquitocidal toxin of claim 1, wherein the mosquito targeting peptide binds to gut epithelium of *Aedes aegypti* mosquitoes.

3. The mosquitocidal toxin of claim 1, wherein the toxin peptide is a peptide that lacks toxicity against mosquitoes without fusion to the mosquito targeting peptide.

4. The mosquitocidal toxin of claim 1, wherein the toxin peptide is a Hv1a spider toxin peptide.

5. The mosquitocidal toxin of claim 1, wherein the fused toxin peptide lacks toxicity against other -species of mosquitoes.

6. A composition comprising the mosquitocidal toxin of claim 1 and a carrier.

7. A method for producing a modified plant expressing mosquitocidal toxins, comprising:

producing a plant;

~~inducing expression of~~introducing an exogenous gene construct encoding a fused toxin peptide in target cells of the plant by transforming at least one target cell of the plant with the exogenous gene construct, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide of SEQ ID NO:1 from Domain III of the glycoprotein of dengue virus, wherein the mosquito targeting peptide targets *Aedes aegypti* mosquitoes, and wherein the fused toxin peptide is toxic to *Aedes aegypti* mosquitoes; and

expressing the fused toxin peptide in the target cells of the plant to produce

~~producing~~ a modified plant expressing the fused toxin peptide.

8. The method of claim 7, wherein the plant is a nectar plant, the target cells of the plant are nectar-producing cells, and the modified plant expresses the fused toxin peptide in nectar of the plant.

9. The method of claim 8, wherein the plant is *Impatiens walleriana*.

10. The method of claim 7, wherein the mosquito targeting peptide binds to gut epithelium of *Aedes aegypti* mosquitoes.

11. The method of claim 7, wherein the toxin peptide is a peptide that lacks toxicity against mosquitoes without fusion to the mosquito targeting peptide.

12. The method of claim 7, wherein the toxin peptide is a Hv1a spider toxin peptide.

13. The method of claim 7, wherein the fused toxin peptide lacks toxicity against other species of mosquitoes.

14. The method of claim 7, wherein the exogenous gene construct comprises a promoter specific to the plant, a gene encoding the mosquito targeting peptide, and a gene encoding the toxin peptide.

~~15. The method of claim 7, wherein the step of inducing expression of the exogenous gene construct comprises transforming at least one cell of the plant with the exogenous gene construct to produce a modified plant expressing the fused toxin peptide.~~

~~16. The modified plant prepared by the method of claim 7.~~

~~17.~~15. A method for producing a modified plant expressing mosquitocidal toxins in nectar of the plant, comprising:

producing a plant;

~~inducing expression of~~introducing an exogenous gene construct encoding a fused toxin peptide in cells of the plant producing nectar by transforming at least one cell of the plant producing nectar with the exogenous gene construct, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide ~~–~~of SEQ ID NO:1 from Domain III of the glycoprotein of dengue virus, wherein the toxin peptide is a Hv1a spider toxin peptide, and wherein the plant is *Impatiens walleriana*, and wherein the exogenous gene construct comprises a promoter specific to *Impatiens walleriana*; and

expressing the fused toxin peptide in the nectar of the plant to produce

~~producing~~ a modified plant expressing the fused toxin peptide in the nectar of the modified plant and not expressing the fused toxin peptide in non-nectar tissues of the modified plant.

~~18. The modified plant prepared by the method of claim 17.~~

~~19.~~16. A modified mosquitocidal plant, wherein the modified plant expresses an exogenous gene construct encoding a fused toxin peptide in target cells of the plant, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide ~~–~~of SEQ ID NO:1 from Domain III of the glycoprotein of dengue virus, wherein the mosquito targeting peptide targets *Aedes aegypti* mosquitoes, and wherein the fused toxin peptide is toxic to *Aedes aegypti* mosquitoes.

~~20.~~17. The modified mosquitocidal plant of claim 16~~19~~, wherein the plant is a nectar plant, the target cells of the plant are nectar-producing cells, and the modified mosquitocidal plant expresses the fused toxin peptide in nectar of the plant.

~~21.~~18. The modified mosquitocidal plant of claim 17~~20~~, wherein the plant is *Impatiens walleriana*.

~~22.~~19. The modified mosquitocidal plant of claim 16~~19~~, wherein the mosquito targeting peptide binds to gut epithelium of *Aedes aegypti* mosquitoes.

~~23.~~20. The modified mosquitocidal plant of claim 16~~19~~, wherein the toxin peptide is a peptide that lacks toxicity against mosquitoes without fusion to the mosquito targeting peptide.

~~24.~~21. The modified mosquitocidal plant of claim 16~~19~~, wherein the toxin peptide is a Hv1a spider toxin peptide.

~~25.~~22. The modified mosquitocidal plant of claim 16~~19~~, wherein the fused toxin peptide lacks toxicity against other ~~-~~species of mosquitoes.

~~26.~~23. The modified mosquitocidal plant of claim 16~~19~~, wherein the exogenous gene construct comprises a promoter specific to the nectar of the plant, a gene encoding the mosquito targeting peptide, and a gene encoding the toxin peptide.

~~27.~~24. A seed of the modified plant of claim 16~~19~~, wherein a plant produced by the seed will express the exogenous gene construct.

~~28.~~25. A modified mosquitocidal *Impatiens walleriana* plant, wherein the modified plant expresses an exogenous gene construct encoding a fused toxin peptide in cells of the plant producing nectar, wherein the fused toxin peptide comprises a mosquito targeting peptide fused to a toxin peptide, wherein the mosquito targeting peptide is a peptide ~~-~~of SEQ ID NO:1 from Domain III of the glycoprotein of dengue virus, wherein the toxin peptide is a Hv1a spider toxin peptide, and wherein the modified plant fails to express the fused toxin peptide in non-nectar tissues of the modified plant.