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 comprising a sequence from Domain III of the glycoprotein of dengue virus, and wherein the fused toxin peptide is toxic to mosquitoes.

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

inducing expression of 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 comprising a sequence from Domain III of the glycoprotein of dengue virus, and wherein the fused toxin peptide is toxic to mosquitoes; and

producing a modified plant expressing the fused toxin peptide.

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

inducing expression of 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 comprising a sequence 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

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.

[0005d] In a fourth aspect, the present disclosure refers to a modified plant prepared by the method of the second and third aspects.

[0005e] In a 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 comprising a sequence from Domain III of the glycoprotein of dengue virus, and wherein the fused toxin peptide is toxic to mosquitoes.

[0005f] In a sixth aspect, the present disclosure refers to a seed of the modified plant of the fifth aspect.

[0005g] In a seventh 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 comprising a sequence from 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. The targeted fusion peptide may be expressed in any suitable organism, including yeast or *E. coli*, and then extracted, isolated, or purified for application as a mosquitocidal toxin.

[0009] In some examples, a plant is engineered to produce the targeted fusion peptide in a manner that will ensure that a mosquito imbibes, consumes, is exposed to or otherwise takes up the peptide. In some examples, a nectar plant is engineered to express the targeted fusion peptide in nectar. Nectar is a critical component of the mosquito life cycle and is highly attractive to them. Mosquito males depend upon nectar or a supplied sugar source for their survival while female mosquitoes require nectar to power their blood quest flights. Non-nectar plants may also be engineered to express the targeted fusion peptide, so long as it is expressed in a manner that permits mosquitoes to consume or imbibe the peptide toxin.

[0010] The present disclosure also pertains to transgenic mosquitocidal plants producing the targeted fusion peptide. In some preferred examples, the plants are nectar plants, but they may be any suitable plants, including trees and shrubs. Preferred nectar plants that may be engineered as transgenic mosquitocidal plants include the common garden impatiens plant, a plant that grows with no required maintenance throughout the moist tropics but is also the top-selling commercial bedding plant worldwide. Studies have shown that impatiens (particularly *Impatiens walleriana*) excels in terms of mosquito attractiveness, nectar protein output and ability to be genetically transformed. In preferred examples, an impatiens plant is engineered to express a toxin, solely in nectar, which is nontoxic to honeybees but which effectively controls mosquitoes in outdoor garden trials. These transgenic plants serve as role models for safety, since they are non-crop plants, are specific to one pest species, and can be engineered to have no ability to spread the toxin transgene to the surrounding ecosystem.

[0011] In preferred examples, an exogenous genetic construct is used to express a targeted toxin peptide for isolation and purification, or to transform a plant into a transgenic mosquitocidal plant. The construct preferably includes different aspects. Plant-specific promoters, such as impatiens nectar promoters, are utilized. An insecticidal toxin peptide is expressed from the construct. A targeting peptide that will form a peptide fusion with the toxin peptide is also expressed, preferably to target mosquitoes by specific binding, such as binding to the gut epithelium. The targeted toxins are toxic to mosquitoes but not to nontarget insect species. These features accomplish the mosquitocidal aspects

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 comprising a sequence from Domain III of the glycoprotein of dengue virus, and wherein the fused toxin peptide is toxic to mosquitoes.

2. The mosquitocidal toxin of claim 1, wherein the mosquito targeting peptide targets *Aedes* mosquitoes.

3. The mosquitocidal toxin of claim 1, wherein the mosquito targeting peptide targets *Aedes aegypti* mosquitoes.

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

5. The mosquitocidal toxin of claim 1, wherein the toxin peptide is a peptide having toxicity against mosquitoes.

6. 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.

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

8. The mosquitocidal toxin of claim 1, wherein the fused toxin peptide lacks toxicity against other organisms.

9. The mosquitocidal toxin of claim 1, further comprising a carrier.

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

inducing expression of 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 comprising a sequence from Domain III of the glycoprotein of dengue virus, and wherein the fused toxin peptide is toxic to mosquitoes; and

producing a modified plant expressing the fused toxin peptide.

11. The method of claim 10, 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.

12. The method of claim 11, wherein the plant is *Impatiens walleriana*.

13. The method of claim 10, wherein the mosquito targeting peptide targets *Aedes* mosquitoes.

14. The method of claim 10, wherein the mosquito targeting peptide targets *Aedes aegypti* mosquitoes.

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

16. The method of claim 10, wherein the toxin peptide is a peptide having toxicity against mosquitoes.

17. The method of claim 10, wherein the toxin peptide is a peptide that lacks toxicity against mosquitoes without fusion to the mosquito targeting peptide.

18. The method of claim 10, wherein the toxin peptide is a Hv1a spider toxin peptide.

19. The method of claim 10, wherein the fused toxin peptide lacks toxicity against other organisms.

20. The method of claim 10, 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.

21. The method of claim 10, 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.

22. The modified plant prepared by the method of claim 10.

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

inducing expression of 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 comprising a sequence 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

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.

24. The modified plant prepared by the method of claim 23.

25. 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 comprising a sequence from Domain III of the glycoprotein of dengue virus, and wherein the fused toxin peptide is toxic to mosquitoes.

26. The modified mosquitocidal plant of claim 25, 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.

27. The modified mosquitocidal plant of claim 26, wherein the plant is *Impatiens walleriana*.

28. The modified mosquitocidal plant of claim 25, wherein the mosquito targeting peptide targets *Aedes* mosquitoes.

29. The modified mosquitocidal plant of claim 25, wherein the mosquito targeting peptide targets *Aedes aegypti* mosquitoes.

30. The modified mosquitocidal plant of claim 25, wherein the mosquito targeting peptide binds to gut epithelium of *Aedes aegypti* mosquitoes.

31. The modified mosquitocidal plant of claim 25, wherein the toxin peptide is a peptide having toxicity against mosquitoes.

32. The modified mosquitocidal plant of claim 25, wherein the toxin peptide is a peptide that lacks toxicity against mosquitoes without fusion to the mosquito targeting peptide.

33. The modified mosquitocidal plant of claim 25, wherein the toxin peptide is a Hv1a spider toxin peptide.

34. The modified mosquitocidal plant of claim 25, wherein the fused toxin peptide lacks toxicity against other organisms.

35. The modified mosquitocidal plant of claim 25, 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.

36. A seed of the modified plant of claim 25.

37. 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 comprising a sequence from 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.