# COMPOSITIONS AND METHODS FOR CONTROL OF INSECT INFESTATIONS IN PLANTS

## CROSS REFERENCE TO RELATED APPLICATIONS

**[0001]** This application claims priority to U.S. Provisional Applications 60/560,842, filed Apr. 9, 2004, 60/565,632, filed Apr. 27, 2004, 60/579,062, filed Jun. 11, 2004, 60/603,421, filed Aug. 20, 2004, 60/617,261, filed Oct. 11, 2004, and Ser. No. 60/_____, filed Apr. 7, 2005, each incorporated herein by reference in its entirety.

## FIELD OF THE INVENTION

**[0002]** The present invention relates generally to genetic control of pest infestations in plants and in and on animals. More specifically, the present invention relates to the methods for modifying endogenous expression of coding sequences in the cell or tissue of a particular pest. More specifically, the present invention utilizes recombinant DNA technologies to post-transcriptionally repress or inhibit expression of a target coding sequence in the cell of a pest, by feeding to the pest one or more double stranded or small interfering ribonucleic acid (RNA) molecules transcribed from all or a portion of a target coding sequence, thereby controlling the infestation. Therefore, the present invention relates to sequence-specific inhibition of expression of coding sequences using double-stranded RNA (dsRNA) or small interfering RNA (siRNA) to achieve the intended levels of pest control.

**[0003]** Novel isolated and substantially purified nucleic acid molecules including but not limited to non-naturally occurring nucleotide sequences and recombinant DNA constructs for transcribing the dsRNA or siRNA molecules of the present invention are also provided that suppress or inhibit the expression of an endogenous coding sequence or a target coding sequence in the pest when introduced thereto. Transgenic plants that (a) contain nucleotide sequences encoding the isolated and substantially purified nucleic acid molecules and the non-naturally occurring recombinant DNA constructs for transcribing the dsRNA or siRNA molecules for controlling plant pest infestations, and (b) display resistance and/or enhanced tolerance to the insect infestations, are also provided. Compositions containing the dsRNA nucleotide sequences of the present invention for use in topical applications onto plants or onto animals or into the environment of an animal to achieve the elimination or reduction of pest infestation are also described.

## BACKGROUND OF THE INVENTION

**[0004]** The environment in which humans live is replete with pest infestation. Pests including insects, arachnids, crustaceans, fungi, bacteria, viruses, nematodes, flatworms, roundworms, pinworms, hookworms, tapeworms, trypanosomes, schistosomes, botflies, fleas, ticks, mites, and lice and the like are pervasive in the human environment, and a multitude of means have been utilized for attempting to control infestations by these pests. Compositions for controlling infestations by microscopic pests such as bacteria, fungi, and viruses have been provided in the form of antibiotic compositions, antiviral compositions, and antifungal compositions. Compositions for controlling infestations by larger pests such as nematodes, flatworm, roundworms, pinworms, heartworms, tapeworms, trypanosomes, schistosomes, and the like have typically been in the form of chemical compositions which can either be applied to the surfaces of substrates on which pests are known to infest, or to be ingested by an infested animal in the form of pellets, powders, tablets, pastes, or capsules and the like. The present invention is directed to providing an improved means for controlling pest infestation compared to the compositions known in the art.

**[0005]** Commercial crops are often the targets of insect attack. Substantial progress has been made in the last a few decades towards developing more efficient methods and compositions for controlling insect infestations in plants. Chemical pesticides have been very effective in eradicating pest infestations. However, there are several disadvantages to using chemical pesticidal agents. Chemical pesticidal agents are not selective. Applications of chemical pesticides are intended to control invertebrate pests that are harmful to various crops and other plants. However, because of the lack of selectivity, the chemical pesticidal agents exert their effects on non-target fauna as well, often effectively sterilizing a field for a period of time over which the pesticidal agents have been applied. Chemical pesticidal agents persist in the environment and generally are slow to be metabolized, if at all. They accumulate in the food chain, and particularly in the higher predator species. Accumulations of these chemical pesticidal agents results in the development of resistance to the agents and in species higher up the evolutionary ladder, act as mutagens and/or carcinogens often causing irreversible and deleterious genetic modifications. Thus there has been a long felt need for environmentally friendly methods for controlling or eradicating insect infestation on or in plants, i.e., methods which are selective, environmentally inert, non-persistent, and biodegradable, and that fit well into pest resistance management schemes.

**[0006]** Compositions that include *Bacillus thuringiensis* (B.t.) bacteria have been commercially available and used as environmentally safe and acceptable insecticides for more than thirty years. The insecticidal effect of Bt bacteria arises as a result of proteins that are produced exclusively by these bacteria that do not persist in the D environment, that are highly selective as to the target species affected, exert their effects only upon ingestion by a target pest, and have been shown to be harmless to plants and other non-targeted organisms, including humans. Transgenic plants containing one or more genes encoding insecticidal B.t. protein are also available in the art and are remarkably efficient in controlling insect pest infestation. A substantial result of the use of recombinant plants expressing Bt insecticidal proteins is a marked decrease in the amount of chemical pesticidal agents that are applied to the environment to control pest infestation in crop fields in areas in which such transgenic crops are used. The decrease in application of chemical pesticidal agents has resulted in cleaner soils and cleaner waters running off of the soils into the surrounding streams, rivers, ponds and lakes. In addition to these environmental benefits, there has been a noticeable increase in the numbers of beneficial insects in crop fields in which transgenic insect resistant crops are grown because of the decrease in the use of chemical insecticidal agents.

**[0007]** Antisense methods and compositions have been reported in the art and are believed to exert their effects through the synthesis of a single-stranded RNA molecule that in theory hybridizes in vivo to a substantially complementary sense strand RNA molecule. Antisense technology has been difficult to employ in many systems for three principle rea-