dsRNA mediated approaches to genetic control have been tested in the fruit fly *Drosophila melanogaster* (Kennerdell and Carthew, 1998; Kennerdell and Carthew, 2000). Kennerdell and Carthew (1998) describe a method for delivery of dsRNA involved generating transgenic insects that express double stranded RNA molecules or injecting dsRNA solutions into the insect body or within the egg sac prior to or during embryonic development.

[0012] Research investigators have previously demonstrated that double stranded RNA mediated gene suppression can be achieved in nematodes either by feeding or by soaking the nematodes in solutions containing double stranded or small interfering RNA molecules and by injection of the dsRNA molecules. Rajagopal et. al. (2002) described failed attempts to suppress an endogenous gene in larvae of the insect pest *Spodoptera litura* by feeding or by soaking neonate larvae in solutions containing dsRNA specific for the target gene, but were successful in suppression after larvae were injected with dsRNA into the hemolymph of 5th instar larvae using a microapplicator. Recently, Yadav et al. (2006) reported that host-generated dsRNA produced in a plant can protect such plants from infection by nematodes. Similarly, U.S. Patent App. Pub. No. 2003/0150017 prophetically described a preferred locus for inhibition of the lepidopteran larvae *Helicoverpa armigera* using dsRNA delivered to the larvae by ingestion of a plant transformed to produce the dsRNA. WO 2005/110068 teaches providing, in the diet of corn rootworm (CRW), CRW-specific dsRNA directed to essential CRW genes. The dsRNA is provided in the CRW diet in-vitro and in-planta, with the result that CRW larvae are stunted or killed after feeding on the diet, and this effect was demonstrated for several different genes.

[0013] Therefore, there has existed a need for identifying efficacious nucleotide sequences for use in improved methods of modulating gene expression by repressing, delaying or otherwise reducing gene expression within a particular coleopteran pest for the purpose of controlling pest infestation or to introduce novel phenotypic traits.

SUMMARY OF THE INVENTION

[0014] In one aspect, the invention provides a method of inhibiting expression of a target gene in a coleopteran pest. In certain embodiments, the method comprises modulating or inhibiting expression of one or more target genes in a coleopteran pest that causes cessation of feeding, growth, development, reproduction and/or infectivity and eventually result in the death of the insect. The method comprises introduction of partial or fully, stabilized double-stranded RNA (dsRNA), including its modified forms such as small interfering RNA (siRNA) sequences, into the cells or into the extracellular environment, such as the midgut, within a coleopteran pest body wherein the dsRNA enters the cells and inhibits expression of at least one or more target genes and wherein the inhibition exerts a deleterious effect upon the coleopteran pest. The methods and associated compositions may be used for limiting or eliminating coleopteran pest infestation in or on any pest host, pest symbiont, or environment in which a pest is present by providing one or more compositions comprising the dsRNA molecules described herein in the diet of the pest. The method will find particular benefit for protecting plants from insect attack. In one embodiment, the pest is defined as comprising a digestive system pH within the range of from about 4.5 to about 9.5, from about 5 to about 9, from about 6 to about 8, and from about pH 7.0.

[0015] In another aspect, the present invention provides exemplary nucleic acid compositions that are homologous to at least a portion of one or more native nucleic acid sequences in a target pest. In certain embodiments, the pest is selected from among *Diabrotica* sp. including Western Corn Rootworm (WCR, *Diabrotica virgifera* or *Diabrotica virgifera virgifera*), Southern Corn Rootworm (SCR, *Diabrotica undecimpunctata howardi*), Mexican Corn Rootworm (MCR, *Diabrotica virgifera zea*), Brazilian Corn Rootworm (BZR, *Diabrotica balteata, Diabrotica viridula, Diabrotica speciosa*), Northern Corn rootworm (NCR, *Diabrotica barberi*), *Diabrotica undecimpunctata*; as well as Colorado Potato Beetle (CPB, *Leptinotarsa decemlineata*), Red Flour Beetle (RFB, *Tribolium castaneum*), and Mexican Bean Beetle (*Epilachna varivestis*). In other embodiments the pest is selected from among Lepidopteran insects including European Corn Borer (ECB, *Ostrinia nubilalis*), Black Cutworm (BCW, *Agrotis ipsilon*), Corn Earworm (CEW, *Helicoverpa zea*), Fall Armyworm (FAW, *Spodoptera frugiperda*), Cotton Ball Weevil (BWV, *Anthonomus grandis*), silkworms (*Bombyx mori*) and *Manduca sexta*, and from Dipteran insects including *Drosophila melanogaster, Anopheles gambiae*, and *Aedes aegypti*. Specific examples of such nucleic acids provided by the invention are given in the attached sequence listing as SEQ ID NO:1 through SEQ ID NO:906.

[0016] In yet another aspect, the invention provides a method for suppression of gene expression in a coleopteran pest such as a corn rootworm or related species that comprises the step of providing in the diet of the pest a gene suppressive amount of at least one dsRNA molecule transcribed from a nucleotide sequence as described herein, at least one segment of which is complementary to an mRNA sequence within the cells of the pest. The method may further comprise observing the death, inhibition, stunting, or cessation of feeding of the pest. A dsRNA molecule, including its modified form such as an siRNA molecule, fed to a pest in accordance with the invention may be at least from about 80, 81, 82, 83, 84, 85, 86, 87, 88 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, or about 100% identical to a RNA molecule transcribed from a nucleotide sequence selected from the group consisting of SEQ ID NO:1 through SEQ ID NO:906. In particular embodiments, the nucleotide sequence may be selected from the group consisting of SEQ ID NO:697, SEQ ID NOs:813-819, SEQ ID NO:841, and SEQ ID NO:874.

[0017] Accordingly, in another aspect of the present invention, a set of isolated and purified nucleotide sequences as set forth in SEQ ID NO:1 through SEQ ID NO:906 is provided. The present invention provides a stabilized dsRNA molecule or the expression of one or more miRNAs for inhibition of expression of a target gene in a coleopteran pest expressed from these sequences and fragments thereof. A stabilized dsRNA, including a miRNA or siRNA molecule can comprise at least two coding sequences that are arranged in a sense and an antisense orientation relative to at least one promoter, wherein the nucleotide sequence that comprises a sense strand and an antisense strand are linked or connected by a spacer sequence of at least from about five to about one thousand nucleotides, wherein the sense strand and the antisense strand may be a different length, and wherein each of the two coding sequences shares at least 80% sequence identity, at least 90%, at least 95%, at least 98%, or 100%