

[0237] The sequences of the degenerate primers used for amplification of each of the genes are given in Table 2-LD, which displays *Leptinotarsa decemlineata* target genes including primer sequences and cDNA sequences obtained. These primers were used in respective PCR reactions with the following conditions: 10 minutes at 95° C., followed by 40 cycles of 30 seconds at 95° C., 1 minute at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C. The resulting PCR fragments were analyzed on agarose gel, purified (QIAquick Gel Extraction kit, Cat. Nr. 28706, Qiagen), cloned into the pCR8/GW/topo vector (Cat. Nr. K2500 20, Invitrogen), and sequenced. The sequences of the resulting PCR products are represented by the respective SEQ ID NOs as given in Table 2-LD and are referred to as the partial sequences. The corresponding partial amino acid sequence are represented by the respective SEQ ID NOs as given in Table 3-LD, where the start of the reading frame is indicated in brackets.

B. dsRNA Production of the *Leptinotarsa decemlineata* Genes

[0238] dsRNA was synthesized in milligram amounts using the commercially available kit T7 Ribomax™ Express RNAi System (Cat. Nr. P1700, Promega). First two separate single 5' T7 RNA polymerase promoter templates were generated in two separate PCR reactions, each reaction containing the target sequence in a different orientation relative to the T7 promoter.

[0239] For each of the target genes, the sense T7 template was generated using specific T7 forward and specific reverse primers. The sequences of the respective primers for amplifying the sense template for each of the target genes are given in Table 8-LD. The conditions in the PCR reactions were as follows: 4 minutes at 95° C., followed by 35 cycles of 30 seconds at 95° C., 30 seconds at 55° C. and 1 minute at 72° C., followed by 10 minutes at 72° C. The anti-sense T7 template was generated using specific forward and specific T7 reverse primers in a PCR reaction with the same conditions as described above. The sequences of the respective primers for amplifying the anti-sense template for each of the target genes are given in Table 8-LD. The resulting PCR products were analyzed on agarose gel and purified by PCR purification kit (Qiaquick PCR Purification Kit, Cat. Nr. 28106, Qiagen) and NaClO₄ precipitation. The generated T7 forward and reverse templates were mixed to be transcribed and the resulting RNA strands were annealed, DNase and RNase treated, and purified by sodium acetate, following the manufacturer's instructions. The sense strand of the resulting dsRNA for each of the target genes is given in Table 8-LD. Table 8-LD displays sequences for preparing ds RNA fragments of *Leptinotarsa decemlineata* target sequences and concatemer sequences, including primer sequences.

C. Screening dsRNA Targets Using Artificial Diet for Activity Against *Leptinotarsa decemlineata*

[0240] Artificial diet for the Colorado potato beetle was prepared as follows (adapted from Gelman et al., 2001, J. Ins. Sc., vol. 1, no. 7, 1-10): water and agar were autoclaved, and the remaining ingredients (shown in Table 2 below) were added when the temperature dropped to 55° C. At this temperature, the ingredients were mixed well before the diet was aliquoted into 24-well plates (Nunc) with a quantity of 1 ml of diet per well. The artificial diet was allowed to solidify by cooling at room temperature. Diet was stored at 4° C. for up to three weeks.

TABLE 2

Ingredients for Artificial diet	
Ingredients	Volume for 1 L
water	768 ml
agar	14 g
rolled oats	40 g
Torula yeast	60 g
lactalbumin	30 g
hydrolysate	
casein	10 g
fructose	20 g
Wesson salt mixture	4 g
tomato fruit powder	12.5 g
potato leaf powder	25 g
b-sitosterol	1 g
sorbic acid	0.8 g
methyl paraben	0.8 g
Vanderzant vitamin mix	12 g
neomycin sulfate	0.2 g
aureomycin	0.130 g
rifampicin	0.130 g
chloramphenicol	0.130 g
nystatin	0.050 g
soybean oil	2 ml
wheat germ oil	2 ml

[0241] Fifty µl of a solution of dsRNA at a concentration of 1 mg/ml was applied topically onto the solid artificial diet in the wells of the multiwell plate. The diet was dried in a laminar flow cabin. Per treatment, twenty-four Colorado potato beetle larvae (2nd stage), with two insects per well, were tested. The plates were stored in the insect rearing chamber at 25±2° C., 60% relative humidity, with a 16:8 hours light:dark photoperiod. The beetles were assessed as live or dead every 1, 2 or 3 days. After seven days, for targets LD006, LD007, LD010, LD011, and LD014, the diet was replaced with fresh diet with topically applied dsRNA at the same concentration (1 mg/ml); for targets LD001, LD002, LD003, LD015, and LD016, the diet was replaced with fresh diet only. The dsRNA targets were compared to diet only or diet with topically applied dsRNA corresponding to a fragment of the GFP (green fluorescent protein) coding sequence (SEQ ID NO: 235).

[0242] Feeding artificial diet containing intact naked dsRNAs to *L. decemlineata* larvae resulted in significant increases in larval mortalities as indicated in two separate bioassays (FIGS. 1LD-2LD).

[0243] All dsRNAs tested resulted ultimately in 100% mortality after 7 to 14 days. Diet with or without GFP dsRNA sustained the insects throughout the bioassays with very little or no mortality.

[0244] Typically, in all assays observed, CPB second-stage larvae fed normally on diet with or without dsRNA for 2 days and molted to the third larval stage. At this new larval stage the CPB were observed to reduce significantly or stop altogether their feeding, with an increase in mortality as a result.

D. Bioassay of dsRNA Targets Using Potato Leaf Discs for Activity Against the *Leptinotarsa decemlineata*

[0245] An alternative bioassay method was employed using potato leaf material rather than artificial diet as food source for CPB. Discs of approximately 1.1 cm in diameter (or 0.95 cm²) were cut out off leaves of 2 to 3-week old potato plants using a suitably-sized cork borer. Treated leaf discs