

dsRNA. Diet was replaced with fresh diet containing topically-applied dsRNA after 7 days. The number of surviving insects were assessed at regular intervals. The percentage of surviving larvae were calculated relative to day 0 (start of assay).

[0018] FIG. 6-LD. Effects of *E. coli* strains expressing dsRNA target LD010 on survival of larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, over time. The two bacterial strains were tested in separate artificial diet-based bioassays: (a) AB309-105; data points for pGBNJ003 and pGN29 represent average mortality values from 5 different bacterial clones, (b) BL21(DE3); data points for pGBNJ003 and pGN29 represent average mortality values from 5 different and one single bacterial clones, respectively. Error bars represent standard deviations.

[0019] FIG. 7-LD. Effects of different clones of *E. coli* strains (a) AB309-105 and (b) BL21(DE3) expressing dsRNA target LD010 on survival of larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, 12 days post infestation. Data points are average mortality values for each clone for pGN29 and pGBNJ003. Clone 1 of AB309-105 harbouring plasmid pGBNJ003 showed 100% mortality towards CPB at this timepoint. Error bars represent standard deviations.

[0020] FIG. 8-LD. Effects of different clones of *E. coli* strains (a) AB309-105 and (b) BL21(DE3) expressing dsRNA target LD010 on growth and development of larval survivors of the Colorado potato beetle, *Leptinotarsa decemlineata*, 7 days post infestation. Data points are % average larval weight values for each clone (one clone for pGN29 and five clones for pGBNJ003) based on the data of Table 10. Diet only treatment represents 100% normal larval weight.

[0021] FIG. 9-LD. Survival of larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, on potato plants sprayed by double-stranded RNA-producing bacteria 7 days post infestation. Number of larval survivors were counted and expressed in terms of % mortality. The bacterial host strain used was the RNaseIII-deficient strain AB309-105. Insect gene target was LD010.

[0022] FIG. 10-LD. Growth/developmental delay of larval survivors of the Colorado potato beetle, *Leptinotarsa decemlineata*, fed on potato plants sprayed with dsRNA-producing bacteria 11 days post infestation. The bacterial host strain used was the RNaseIII-deficient strain AB309-105. Data figures represented as percentage of normal larval weight; 100% of normal larval weight given for diet only treatment. Insect gene target was LD010. Error bars represent standard deviations.

[0023] FIG. 11-LD. Resistance to potato damage caused by larvae of the Colorado potato beetle, *Leptinotarsa decemlineata*, by double-stranded RNA-producing bacteria 7 days post infestation. Left, plant sprayed with 7 units of bacteria AB309-105 containing the pGN29 plasmid; right, plant sprayed with 7 units of bacteria AB309-105 containing the pGBNJ003 plasmid. One unit is defined as the equivalent of 1 ml of a bacterial suspension at OD value of 1 at 600 nm. Insect gene target was LD010.

[0024] FIG. 12-LD. Survival of *L. decemlineata* adults on potato leaf discs treated with dsRNA. Young adult insects were fed double-stranded-RNA-treated leaf discs for the first two days and were then placed on untreated potato foliage. The number of surviving insects were assessed regularly; mobile insects were recorded as insects which were alive and appeared to move normally; moribund insects were recorded

as insects which were alive but appeared sick and slow moving—these insects were not able to right themselves once placed on their backs. Target LD002 (SEQ ID NO: 168); Target LD010 (SEQ ID NO: 188); Target LD014 (SEQ ID NO: 198); Target LD016 (SEQ ID NO: 220); gfp dsRNA (SEQ ID NO: 235).

[0025] FIG. 13-LD. Effects of bacterial produced target double-stranded RNA against larvae of *L. decemlineata*. Fifty μ l of an OD 1 suspension of heat-treated bacteria expressing dsRNA (SEQ ID NO: 188) was applied topically onto the solid artificial diet in each well of a 48-well plate. CPB larvae at L2 stage were placed in each well. At day 7, a picture was taken of the CPB larvae in a plate containing (a) diet with bacteria expressing target 10 double-stranded RNA, (b) diet with bacteria harbouring the empty vector pGN29, and, (c) diet only.

[0026] FIG. 14-LD Effects on CPB larval survival and growth of different amounts of inactivated *E. coli* AB309-105 strain harbouring plasmid pGBNJ003 topically applied to potato foliage prior to insect infestation. Ten L1 larvae were fed treated potato for 7 days. Amount of bacterial suspension sprayed on plants: 0.25 U, 0.08 U, 0.025 U, 0.008 U of target 10 and 0.25 U of pGN29 (negative control; also included is Milli-Q water). One unit (U) is defined as the equivalent bacterial amount present in 1 ml of culture with an optical density value of 1 at 600 nm. A total volume of 1.6 ml was sprayed on to each plant. Insect gene target was LD010.

[0027] FIG. 15-LD Resistance to potato damage caused by CPB larvae by inactivated *E. coli* AB309-105 strain harbouring plasmid pGBNJ003 seven days post infestation. (a) water, (b) 0.25 U *E. coli* AB309-105 harbouring pGN29, (c) 0.025 U *E. coli* AB309-105 harbouring pGBNJ003, (d) 0.008 U *E. coli* AB309-105 harbouring pGBNJ003. One unit (U) is defined as the equivalent bacterial amount present in 1 ml of culture with an optical density value of 1 at 600 nm. A total volume of 1.6 ml was sprayed on to each plant. Insect gene target was LD010.

[0028] FIG. 1-PC: Effects of ingested target dsRNAs on survival and growth of *P. cochleariae* larvae. Neonate larvae were fed oilseed rape leaf discs treated with 25 μ l of topically-applied solution of 0.1 μ g/ μ l dsRNA (targets or gfp control). After 2 days, the insects were transferred onto fresh dsRNA-treated leaf discs. At day 4, larvae from one replicate for every treatment were collected and placed in a Petri dish containing fresh untreated oilseed rape foliage. The insects were assessed at days 2, 4, 7, 9 & 11. (a) Survival of *E. varivestis* larvae on oilseed rape leaf discs treated with dsRNA. The percentage of surviving larvae was calculated relative to day 0 (start of assay). (b) Average weights of *P. cochleariae* larvae on oilseed rape leaf discs treated with dsRNA. Insects from each replicate were weighed together and the average weight per larva determined. Error bars represent standard deviations. Target 1: SEQ ID NO: 473; target 3: SEQ ID NO: 478; target 5: SEQ ID NO: 483-; target 10: SEQ ID NO: 488; target 14: SEQ ID NO: 493; target 16: SEQ ID NO: 498; target 27: SEQ ID NO: 503; gfp dsRNA: SEQ ID NO: 235.

[0029] FIG. 2-PC: Survival of *P. cochleariae* on oilseed rape leaf discs treated with different concentrations of dsRNA of (a) target PC010 and (b) target PC027. Neonate larvae were placed on leaf discs treated with 25 μ l of topically-applied solution of dsRNA. Insects were transferred to fresh treated leaf discs at day 2. At day 4 for target PC010 and day 5 for target PC027, the insects were transferred to untreated leaves. The number of surviving insects were assessed at days 2, 4, 7,