



- (51) **International Patent Classification:**
C12N 15/82 (2006.01) C07K 14/435 (2006.01)
- (21) **International Application Number:**
PCT/US2014/047204
- (22) **International Filing Date:**
18 July 2014 (18.07.2014)
- (25) **Filing Language:** English
- (26) **Publication Language:** English
- (30) **Priority Data:**
61/856,137 19 July 2013 (19.07.2013) US
61/899,000 1 November 2013 (01.11.2013) US
61/980,800 17 April 2014 (17.04.2014) US
- (71) **Applicant:** MONSANTO TECHNOLOGY LLC
[US/US]; 800 North Lindbergh Boulevard, Mail Zone E1NA, St. Louis, Missouri 63167 (US).
- (72) **Inventors:** BEATTIE, Jodi Lynn; 800 North Lindbergh Boulevard, Mail Zone E1NA, St. Louis, Missouri 63167 (US). CRAWFORD, Michael John; 800 North Lindbergh Boulevard, Mail Zone E1NA, St. Louis, Missouri 63167 (US). EADS, Brian Donovan; 800 North Lindbergh Boulevard, Mail Zone E1NA, St. Louis, Missouri 63167 (US). FLAGEL, Lex Evan; 800 North Lindbergh Boulevard, Mail Zone E1NA, St. Louis, Missouri 63167 (US). KAPOOR, Mahak; 800 North Lindbergh Boulevard, Mail Zone E1NA, St. Louis, Missouri 63167 (US). TAYLOR, Christina Marie; 800 North Lindbergh Boulevard, Mail Zone E1NA, St. Louis, Missouri 63167 (US).
- (74) **Agents:** LANSBERY, Kristan et al.; 555 12th Street, NW, Washington, District of Columbia 20004-1206 (US).

(81) **Designated States** (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BN, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IR, IS, JP, KE, KG, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PA, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SA, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) **Designated States** (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, KM, ML, MR, NE, SN, TD, TG).

Published:

- with international search report (Art. 21(3))
- before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))
- with sequence listing part of description (Rule 5.2(a))

(88) **Date of publication of the international search report:**
23 April 2015

(54) **Title:** COMPOSITIONS AND METHODS FOR CONTROLLING *LEPTINOTARSA*

(57) **Abstract:** Disclosed herein are methods of controlling insect pests, in particular *Leptinotarsa* spp. which infest crop plants, and methods of providing plants resistant to such pests. Also disclosed are polynucleotides and recombinant DNA molecules and constructs useful in such methods, insecticidal compositions such as topical sprays containing insecticidal double-stranded RNAs, and solanaceous plants with improved resistance to infestation by *Leptinotarsa* spp. Further disclosed are methods of selecting target genes for RNAi-mediated silencing and control of *Leptinotarsa* spp.



INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/047204

A. CLASSIFICATION OF SUBJECT MATTER
INV. C12N15/82 C07K14/435
ADD.

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C12N C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

EPO-Internal, Sequence Search, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2013/010691 A1 (DEVGEN NV [BE]; BEGHYN MYRIAM [BE]; BOGAERT THIERRY [BE]; FELDMANN PAS) 24 January 2013 (2013-01-24) the whole document example 5 figures 24,26	1-15
X	WO 2007/074405 A2 (DEVGEN NV [BE]; RAEMAEEKERS ROMAAN [BE]; FELDMANN PASCALE [BE]; PLAETIN) 5 July 2007 (2007-07-05) paragraph [0233] - paragraph [0307]; example 3 ----- -/-	1-15



Further documents are listed in the continuation of Box C.



See patent family annex.

* Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search

7 November 2014

Date of mailing of the international search report

10/03/2015

Name and mailing address of the ISA/

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040,
Fax: (+31-70) 340-3016

Authorized officer

Blanco Urgoiti, B

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2014/047204

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2007/035650 A2 (MONSANTO TECHNOLOGY LLC) 29 March 2007 (2007-03-29) the whole document page 76 - page 77; example 7; table 6; sequence 736 page 86; table 11 -----	16-20
X	J. K. COLBOURNE ET AL: "The Ecoresponsive Genome of <i>Daphnia pulex</i> ", SCIENCE, vol. 331, no. 6017, 3 February 2011 (2011-02-03), pages 555-561, XP055151455, ISSN: 0036-8075, DOI: 10.1126/science.1197761 clone CBIB7954 -& DATABASE EMBL [Online]	16,17
X	27 February 2008 (2008-02-27), "CBIB7954.fwd CBIB_Daphnia_pulex_Chosen_One_Library_2 Daphnia_pulex_cDNA_clone_CBIB7954_5',_mRNA sequence.", XP002732239, retrieved from EBI accession no. EM_EST:FE348695 Database accession no. FE348695 sequence -----	16

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2014/047204

Box No. II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

see additional sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fees, this Authority did not invite payment of additional fees.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-20(partially)

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.
- ☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

This International Searching Authority found multiple (groups of) inventions in this international application, as follows:

1. claims: 1-20(partially)

Method for controlling a Leptinotarsa species infestation of a plant contacting the said Leptinotarsa species with a polynucleotide that is antisense or dsRNA to Leptinotarsa gene of SEQ ID NO:730; method wherein the dsRNA comprises a strand comprised in SEQ ID NOs: 989 or 988; plant with improved resistance; insecticidal composition; recombinant DNA construct; vectors; and transgenic solanaceous plant thereof.

2. claims: 1-20(partially)

Method for controlling a Leptinotarsa species infestation of a plant contacting the said Leptinotarsa species with a polynucleotide that is antisense or dsRNA to Leptinotarsa gene of SEQ ID NO:807; method wherein the dsRNA comprises a strand comprised in any of SEQ ID NOs: 855-860; plant with improved resistance; insecticidal composition; recombinant DNA construct; vectors; and transgenic solanaceous plant thereof.

3-731. claims: 1-20(partially)

Invention 3 being idem to invention 2, but related to Leptinotarsa gene of SEQ ID NO:1; and corresponding dsRNA chosen from the sequences of SEQ ID NOs:831-1085,1095-1104, and/or 1110-1114;...; and invention 731 being idem to invention 2, but related to Leptinotarsa gene of SEQ ID NO:729; and corresponding dsRNA chosen from the sequences of SEQ ID NOs:831-1085,1095-1104, and/or 1110-1114.

732-807. claims: 1-20(partially)

Invention 732 being idem to invention 2, but related to Leptinotarsa gene of SEQ ID NO:731; and corresponding dsRNA chosen from the sequences of SEQ ID NOs:831-1085,1095-1104, and/or 1110-1114;...; Invention 807 being idem to invention 2, but related to Leptinotarsa gene of SEQ ID NO:806; and corresponding dsRNA chosen from the sequences of SEQ ID NOs:831-1085,1095-1104, and/or 1110-1114.

808-830. claims: 1-20(partially)

Invention 808 being idem to invention 2, but related to Leptinotarsa gene of SEQ ID NO:808; and corresponding dsRNA chosen from the sequences of SEQ ID NOs:831-1085,1095-1104, 1110-1114, 1118, 1119 and/or 1124; ...; invention 830 being

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

idem to invention 2, but related to Leptinotarsa gene of SEQ ID NO:830; and corresponding dsRNA chosen from the sequences of SEQ ID NOs:831-1085,1095-1104, 1110-1114, 1118, 1119 and/or 1124

831-838. claims: 1-20(partially)

Invention 831 being idem to invention 2, but related to Leptinotarsa gene of SEQ ID NO:1087; and corresponding dsRNA chosen from the sequences of SEQ ID NOs:831-1085,1095-1104, and/or 1110-1114;...; invention 838 being idem to invention 2, but related to Leptinotarsa gene of SEQ ID NO:1094; and corresponding dsRNA chosen from the sequences of SEQ ID NOs:831-1085,1095-1104, and/or 1110-1114.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No

PCT/US2014/047204

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2013010691 A1	24-01-2013	US 2012297501 A1 WO 2013010691 A1	22-11-2012 24-01-2013
WO 2007074405 A2	05-07-2007	AU 2006329563 A1 BR PI0615791 A2 CA 2622660 A1 CN 104357446 A EP 1929020 A2 EP 2275562 A2 EP 2275563 A2 EP 2281896 A2 EP 2295584 A2 JP 2009508481 A US 2009300796 A1 WO 2007074405 A2	05-07-2007 16-06-2009 05-07-2007 18-02-2015 11-06-2008 19-01-2011 19-01-2011 09-02-2011 16-03-2011 05-03-2009 03-12-2009 05-07-2007
WO 2007035650 A2	29-03-2007	AR 055169 A1 AT 548459 T AU 2006292362 A1 BR PI0616181 A2 CA 2622687 A1 CA 2812343 A1 CN 101310020 A CN 101365795 A CN 101365796 A CN 102409050 A CR 9821 A EC SP088291 A EP 1924696 A2 EP 2330207 A2 EP 2426208 A1 EP 2431473 A1 EP 2439278 A1 EP 2439279 A1 SG 166097 A1 SV 2009002845 A TW 200804596 A UA 98445 C2 US 2007124836 A1 US 2012137387 A1 US 2014013471 A1 UY 29800 A1 WO 2007035650 A2 ZA 200803338 A	08-08-2007 15-03-2012 29-03-2007 16-06-2009 29-03-2007 29-03-2007 19-11-2008 11-02-2009 11-02-2009 11-04-2012 01-08-2008 28-04-2008 28-05-2008 08-06-2011 07-03-2012 21-03-2012 11-04-2012 11-04-2012 29-11-2010 19-02-2009 16-01-2008 25-05-2012 31-05-2007 31-05-2012 09-01-2014 30-04-2007 29-03-2007 28-01-2009