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(71) Applicants *(for all designated States except US)*: **PIONEER HI-BRED INTERNATIONAL, INC.** [US/US]; 7100 N.w. 62nd Avenue, Johnston, Iowa 50131-1014 (US). **E. I. DUPONT DE NEMOURS & COMPANY** [US/US]; 1007 Market Street, Wilmington, Delaware 19898 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **ABAD, Andre R.** [FR/US]; 1030 Belle Mar Drive, West Des Moines, Iowa 50266 (US). **CHUN, Gary C.** [US/US]; 20 West Kapok Drive, Newark, Delaware 19702 (US). **FLANNAGAN, Ronald D.** [US/US]; 512 N.w. Norton Circle, Grimes, Iowa 50111 (US). **HERRMANN, Rafael** [IL/US]; 1803 Wyckwood Court, Wilmington, Delaware 19803 (US). **LU, Albert L.** [CA/US]; 14 Charles Pointe, Newark, Delaware 19702 (US). **MCCUTCHEN, Billy F.** [US/US]; 600 W. 24th Street, Cameron, Texas 76520 (US). **RICE, Janet A.** [US/US]; 126 Median Drive, Wilmington, Delaware 19803 (US). **SCHEPERS, Eric J.** [US/US]; 99 Water Wheel Drive, Port Deposit, Maryland 21904 (US).

(74) Agent: **MOXON, Claire L.**; Pioneer Hi-Bred International, Inc., 7250 N.w. 62nd Avenue, Johnston, Iowa 50131-0552 (US).

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(54) Title: INSECT-SPECIFIC PROTEASE RECOGNITION SEQUENCES

(57) Abstract: Compositions and methods for protecting a plant from an insect pest are provided. Nucleic acid molecules encoding insect protoxins or insect toxins modified to comprise at least one proteolytic activation site that is sensitive to an insect gut protease are provided. Cleavage of a modified insect protoxin at the proteolytic activation site by an insect gut protease produces an active insect toxin in the gut of the insect pest. Cleavage of a modified insect toxin of the invention at a proteolytic activation site results in the production of an active insect toxin in the insect gut that displays improved pesticidal activity relative to the insect toxin that lacks the proteolytic activation site. Methods of using the modified insect protoxin and modified insect toxin nucleic acid sequences and the polypeptides they encode to protect a plant from an insect pest are provided. Particular embodiments of the invention further provide modified insect protoxin and modified insect toxin compositions and formulations, expression cassettes, and transformed plants, plant cells, and seeds. Insect gut proteases and the nucleic acid molecules that encode them are also disclosed herein.

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A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/325 C12N9/64
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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)
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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 practical, search terms used)
EPO-Internal, BIOSIS, FSTA, EMBASE, WPI Data, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 2004/003148 A2 (DU PONT [US]) 8 January 2004 (2004-01-08) page 2, paragraph 2; claims the whole document	1-25
X	WO 02/34774 A2 (DU PONT [US]; ABAD ANDRE R [US]; DUCK NICHOLAS B [US]; FENG XIANG [US]) 2 May 2002 (2002-05-02) the whole document	1-25
X	US 2003/120054 A1 (CHEN ERIC [US] ET AL) 26 June 2003 (2003-06-26) the whole document	1-25

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[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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7 November 2006	01/12/2006

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL – 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Madruga, Jaime

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C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	BOWN D P ET AL: "Characterisation of cysteine proteinases responsible for digestive proteolysis in guts of larval western corn rootworm (Diabrotica virgifera) by expression in the yeast Pichia pastoris" INSECT BIOCHEMISTRY AND MOLECULAR BIOLOGY, ELSEVIER SCIENCE LTD, GB, vol. 34, April 2004 (2004-04), pages 305-320, XP002335995 ISSN: 0965-1748 the whole document figure 2	26,27
P,X	WO 2005/063996 A2 (PIONEER HI BRED INT [US]; DU PONT [US]; ABAD ANDRE [US]; FLANNAGAN RON) 14 July 2005 (2005-07-14) the whole document	1-14, 16-27
P,X	WO 2005/066349 A2 (PIONEER HI BRED INT [US]; DU PONT [US]; ABAD ANDRE [US]; DONG HUA [US]) 21 July 2005 (2005-07-21) the whole document	1-14, 16-25
A	OPPERT BRENDA: "PROTEASE INTERACTIONS WITH BACILLUS THURINGIENSIS INSECTICIDAL TOXINS" ARCHIVES OF INSECT BIOCHEMISTRY AND PHYSIOLOGY, ALAN R. LISS, NEW YORK, NY, US, vol. 42, no. 1, September 1999 (1999-09), pages 1-12, XP008070748 ISSN: 0739-4462 cited in the application the whole document	
A	KOIWA H ET AL: "A plant defensive cystatin (soyacystatin) targets cathepsin L-like digestive cysteine proteinases (DvCALs) in the larval midgut of western corn rootworm (Diabrotica virgifera virgifera)" FEBS LETTERS, ELSEVIER, AMSTERDAM, NL, vol. 471, no. 1, 7 April 2000 (2000-04-07), pages 67-70, XP004336986 ISSN: 0014-5793 the whole document	

-/--

INTERNATIONAL SEARCH REPORT

International application No
PCT/US2006/021902

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	RUKMINI ET AL: "Bacillus thuringiensis crystal delta-endotoxin: Role of proteases in the conversion of protoxin to toxin" BIOCHIMIE, MASSON, PARIS, FR, vol. 82, February 2000 (2000-02), pages 109-116, XP002209172 ISSN: 0300-9084 the whole document	
A	SCHNEPF E ET AL: "BACILLUS THURINGIENSIS AND ITS PESTICIDAL CRYSTAL PROTEINS" MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 62, no. 3, September 1998 (1998-09), pages 775-806, XP000986964 ISSN: 1092-2172 cited in the application the whole document	
A	CRICKMORE N ET AL: "Revision of the nomenclature for the Bacillus thuringiensis pesticidal crystal proteins" MICROBIOLOGY AND MOLECULAR BIOLOGY REVIEWS, AMERICAN SOCIETY FOR MICROBIOLOGY, US, vol. 62, no. 3, September 1998 (1998-09), pages 807-813, XP002132141 ISSN: 1092-2172 cited in the application the whole document	

INTERNATIONAL SEARCH REPORT

Information on patent family members

International application No
PCT/US2006/021902

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 2004003148 A2	08-01-2004	AU 2003279760 A1	19-01-2004
		BR 0312064 A	09-08-2005
		CA 2490548 A1	08-01-2004
		EP 1531664 A2	25-05-2005
		MX PA04012647 A	23-03-2005
WO 0234774 A2	02-05-2002	AT 338820 T	15-09-2006
		AU 2870502 A	06-05-2002
		BR 0114891 A	28-06-2005
		CA 2426793 A1	02-05-2002
		EP 1356054 A2	29-10-2003
		HU 0302523 A2	28-10-2003
		MX PA03003666 A	25-01-2005
		US 2002151709 A1	17-10-2002
		US 2003177528 A1	18-09-2003
US 2003120054 A1	26-06-2003	US 2006085870 A1	20-04-2006
WO 2005063996 A2	14-07-2005	AR 047167 A1	11-01-2006
		CA 2551011 A1	14-07-2005
		EP 1709068 A2	11-10-2006
WO 2005066349 A2	21-07-2005	AR 047350 A1	18-01-2006
		CA 2551102 A1	21-07-2005
		EP 1699816 A2	13-09-2006