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Declarations under Rule 4.17:

— *as to applicant's entitlement to apply for and be granted a  
patent (Rule 4.17(ii))*

Published:

— *with international search report (Art. 21(3))*  
— *before the expiration of the time limit for amending the  
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26 May 2017

(54) Title: *WUPA* NUCLEIC ACID MOLECULES THAT CONFER RESISTANCE TO COLEOPTERAN AND HEMIPTERAN PESTS

(57) Abstract: This disclosure concerns nucleic acid molecules and methods of use thereof for control of coleopteran and hemipteran pests through RNA interference-mediated inhibition of target coding and transcribed non-coding sequences in coleopteran and hemipteran pests. The disclosure also concerns methods for making transgenic plants that express nucleic acid molecules useful for the control of coleopteran and hemipteran pests, and the plant cells and plants obtained thereby.



## INTERNATIONAL SEARCH REPORT

International application No.

PCT/US 16/55300

<b>A. CLASSIFICATION OF SUBJECT MATTER</b> IPC(8) - C12N 1/21, 15/82, 15/113; A01N 63/00, 65/00; A01P 5/00 (2017.01) CPC - A01N 63/02; C12N 15/8279; C12N 15/8286; C12N 15/8285 According to International Patent Classification (IPC) or to both national classification and IPC		
<b>B. FIELDS SEARCHED</b> Minimum documentation searched (classification system followed by classification symbols) IPC(8): C12N 1/21, 15/82, 15/113; A01N 63/00, 65/00; A01P 5/00 (2017.01) CPC: A01N 63/02; C12N 15/8279; C12N 15/8286; C12N 15/8285 Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched CPC: A01N 63/02; C12N 15/8279; C12N 15/8286; C12N 15/8285 (text search) USPC: 514/44A, 4.5; 536/23.45; 424/93.2; 435/252.31, 252.33, 254.11 (text search) Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) Electronic data bases: PatBase; Google Patents; Google Scholar Search terms: RNA interference (RNAi, antisense, siRNA), coleopteran, hemipteran, Western corn rootworm (WCR, Diabrotica virgifera virgifera LeConte) transgenic plants, Bacillus thuringiensis cryotoxin		
<b>C. DOCUMENTS CONSIDERED TO BE RELEVANT</b>		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X ---- A	US 2012/0164205 A1 (BAUM et al.) 28 June 2012 (28.06.2012). Especially para [0014], [0015], [0019], [0025], [0033], [0051], [0098], [0110], [0193], [0305], claim 60, SEQ ID NO: 1086.	1, 2, 5-25, 53-58 ----- 3, 4
A	US 2011/0268691 A1 (SIEGFRIED et al.) 3 November 2011 (03.11.2011). Especially claims 1-18	1-25, 53-58
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/>		
* 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 10 March 2017		Date of mailing of the international search report <b>07 APR 2017</b>
Name and mailing address of the ISA/US Mail Stop PCT, Attn: ISA/US, Commissioner for Patents P.O. Box 1450, Alexandria, Virginia 22313-1450 Facsimile No. 571-273-8300		Authorized officer: Lee W. Young PCT Helpdesk: 571-272-4300 PCT OSP: 571-272-7774

## INTERNATIONAL SEARCH REPORT

International application No.

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## Box No. 1 Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
- a.  forming part of the international application as filed:  
 in the form of an Annex C/ST.25 text file.  
 on paper or in the form of an image file.
- b.  furnished together with the international application under PCT Rule 13ter.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
- c.  furnished subsequent to the international filing date for the purposes of international search only:  
 in the form of an Annex C/ST.25 text file (Rule 13ter.1(a)).  
 on paper or in the form of an image file (Rule 13ter.1(b) and Administrative Instructions, Section 713).
2.  In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.
3. Additional comments:

INTERNATIONAL SEARCH REPORT

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**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:  
-----Go to Extra Sheet for continuation-----

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 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.:  
Claims 1-25, 53-58 limited to SEQ ID NO: 1

- 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.

## INTERNATIONAL SEARCH REPORT

International application No.

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-----continuation of Box III (Lack of Unity of Invention)-----

This application contains the following inventions or groups of inventions which are not so linked as to form a single general inventive concept under PCT Rule 13.1. In order for all inventions to be examined the appropriate additional examination fees must be paid.

Group I+: Claims 1-25, 53-58, drawn to an isolated nucleic acid comprising at least one polynucleotide operably linked to a heterologous promoter.

The polynucleotide operably linked to a heterologous promoter will be searched to the extent that the polynucleotide encompasses SEQ ID NO: 1. It is believed that claims 1-25, 53-58 read on this first named invention and thus these claims will be searched without fee to the extent that they encompass SEQ ID NO: 1. Additional polynucleotide(s) will be searched upon payment of additional fees. Applicant must specify the claims that encompass any additional elected polynucleotides. Applicants must further indicate, if applicable, the claims which read on the first named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the "+" group(s) will result in only the first claimed invention to be searched/examined. An exemplary election would be: SEQ ID NO: 5 (claims 1-25, 53-58).

Group II+: Claims 26-30, 35-40, drawn to a method of controlling a coleopteran or hemipteran pest population by feeding an RNA agent.

Group II+ will be searched upon payment of additional fee(s). The method of controlling a coleopteran or hemipteran pest population may be searched, for example, to the extent that the polynucleotide encompasses SEQ ID NO: 89, for an additional fee and election as such. It is believed that claims 26-30, 35-37, read on this exemplary invention. Additional polynucleotide(s) will be searched upon the payment of additional fees. Applicants must indicate, if applicable, which claims read on this named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first named invention to be searched/examined. An exemplary election would be the polynucleotide comprises SEQ ID NO: 101 (claims 26, 28, 30, 38-40).

Group III+: Claims 31-34, 41-52, 59, 60 drawn to a method of producing a transgenic plant.

Group III+ will be searched upon payment of additional fee(s). The method of producing a transgenic plant may be searched, for example, to the extent that the polynucleotide encompasses SEQ ID NO: 1, for an additional fee and election as such. It is believed that claims 31-34, 41-52, 59, 60 read on this exemplary invention. Additional bioactive trigger polynucleotide(s) will be searched upon the payment of additional fees. Applicants must indicate, if applicable, which claims read on this named invention if different than what was indicated above for this group. Failure to clearly identify how any paid additional invention fees are to be applied to the '+' group(s) will result in only the first named invention to be searched/examined. An exemplary election would be the polynucleotide comprises SEQ ID NO: 5 (claims 31-34, 41-52, 59, 60).

The inventions listed as Groups I+, II+ and III+ do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons:

**Special Technical Features:**

Group I+ has the special technical feature of a composition comprising a polynucleotide operably linked to a heterologous promoter, not required by Groups II+ or III+.

Group II+ has the special technical feature of a method of controlling a coleopteran or hemipteran pest population by feeding an RNA agent, not required by Groups I+ or III+.

Group III+ has the special technical feature of a method of producing a transgenic plant, not required by Groups I+ or II+.

Among the inventions listed as Groups I+, II+, III+ are the specific wupA polynucleotide sequences recited therein. Each invention requires a specific wupA polynucleotide sequences, not required by any other inventions.

**Common Technical Features:**

The inventions of Group I+ share common technical feature a composition comprising a polynucleotide operably linked to a heterologous promoter.

The inventions of Group II+ share the common technical feature of a method of controlling a coleopteran or hemipteran pest population by feeding it a RNA agent.

The inventions of Group III+ share the common technical feature of a method of producing a transgenic plant.

Some inventions of Group III+ (claim 31) shares the common technical feature of claim 2.

Some inventions of Group III+ (claims 41 and 45) share the common technical feature of claim 1.

Some inventions of Groups II+ and III+ shares the common technical feature of a method for controlling a coleopteran pest population.

The inventions of Groups I+, II+ and III+ shares the common technical feature of wupA polynucleotide sequences.

-----continued on next sheet---

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However, said common technical features do not represent a contribution over the prior art, and is anticipated by US 2012/0164205 A1 to BAUM et al. (hereinafter "Baum").

As to claim 1, Baum teaches an isolated nucleic acid comprising at least one polynucleotide (para [0019]; Accordingly, in another aspect of the present invention, a set of isolated and purified nucleotide sequences as set forth in SEQ ID NOs: 1) operably linked to a heterologous promoter (para [0019]; synthesized denovo based on nucleotide sequences disclosed herein or known in the art as T7 phage RNA polymerase promoter sequences"; para [0019]; "A stabilized dsRNA, 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"), wherein the polynucleotide is selected from a fragment of at least 15 contiguous nucleotides of SEQ ID NO: 1 (SEQ ID NO: 3961; 1-GCAGGGAAGGGAAGTCTCAGCATCCGAGAGAAAAATTTGCAACAGTCAGCACTGAGTCCT-60 with 100% sequence identity to instant application SEQ ID NO: 1 nt 140-199).

As to claim 2, Baum teaches the polynucleotide of claim 1, wherein the polynucleotide is selected from a fragment of at least 15 contiguous nucleotides of SEQ ID NO: 1 (SEQ ID NO: 3961; nt 1-60 100% sequence identity).

As to the common technical feature of Group II+, a method of controlling a coleopteran or hemipteran pest population, Baum teaches (para [0018]; "In another aspect of the present invention, the method comprises the step of feeding to the pest one (or more) stabilized dsRNA molecules or its modified form such as an siRNA molecule"; Para [0193]; It is believed therefore that the dsRNA methods disclosed herein should be preferentially used in compositions and in plants to control coleopteran, dipteran, hemipteran, lygus, and piercing and sucking insects").

As to the common technical feature of Group III+, a method of producing a transgenic plant, Baum teaches (para [0022]; "The present invention also provides a recombinant DNA sequence for plant transformation constructed to contain at least one non-naturally occurring nucleotide sequence that can be transcribed into a single stranded RNA molecule. The single stranded RNA molecule forms a double stranded RNA molecule in vivo through intermolecular hybridization that, when provided in the diet of an invertebrate pest, inhibits the expression of at least one target gene in a cell of the invertebrate pest")

As the common technical features were known in the art at the time of the invention, they cannot be considered common special technical features that would otherwise unify the groups. The inventions lack unity with one another.

Therefore, Groups I+, II+ and III+ lack unity of invention under PCT Rule 13 because they do not share a same or corresponding special technical feature.