

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau



(43) International Publication Date
26 August 2010 (26.08.2010)

PCT

(10) International Publication Number
WO 2010/096249 A2

(51) International Patent Classification:

C12Q 1/70 (2006.01) *C12N* 7/02 (2006.01)
C12Q 1/68 (2006.01) *C12N* 15/11 (2006.01)

(21) International Application Number:

PCT/US2010/022325

(22) International Filing Date:

28 January 2010 (28.01.2010)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

12/378,683 18 February 2009 (18.02.2009) US

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(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, 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, IS, JP, KE, KG, KM, 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, PE, PG, PH, PL, PT, RO, RS, RU, 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, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (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, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Published:

— without international search report and to be republished upon receipt of that report (Rule 48.2(g))

— with sequence listing part of description (Rule 5.2(a))

(54) Title: **SOLENOPSIS INVICTA VIRUS**

(57) Abstract: A Unique *Solenopsis invicta* virus (SINV 3) has been identified and its genome sequenced. Oligonucleotide primers have been developed using the isolated nucleic acid sequences of the SINV 3. The virus is used as a biocontrol agent for control of fire ants.



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Solenopsis invicta Virus

BACKGROUND OF THE INVENTION

Field of the Invention

[0001] This invention relates to biological methods and products useful for the control of *Solenopsis invicta*. More specifically, the present invention is directed to a novel *Solenopsis invicta* virus, nucleic acids encoding the novel virus, biocontrol compositions, and methods of using the virus and/or biocontrol compositions for control of fire ants.

Description of the Related Art

[0002] Red imported fire ant, *Solenopsis invicta* (Buren), was first detected in the United States near Mobile, Alabama in the late 1920s (Loding, USDA Insect Pest Surv. Bull., Volume 9, 241, 1929). Since that time, it has spread to encompass more than 128 million hectares, from Virginia, south to Florida and west to California. (Williams et al., Am. Entomol., Volume 47, 146-159, 2001). Efforts to control, prevent, repair damage, and otherwise to mitigate the effects of this pest ant are estimated to exceed 3 billion dollars annually (Pereira, J. Agric. Urban Entomol., Volume 20, 123-130, 2004). Fire ants are known to destroy young citrus trees, growing crops, and germinating seeds. This has an economic impact on agriculture in infested areas. Telephone companies spend substantial amounts of money each year treating their electrical equipment to prevent fire ant invasion because fire ants accumulate at electrical contacts and can short out electrical equipment. Farm equipment can also be damaged by large fire ant mounds. Fire ants also present a danger to wildlife, such as ground nesting birds and animals. Furthermore, fire ants are known to excavate the soil from under roadways causing damage.

[0003] Fire ants also pose health care problems to millions of people stung each year-a significant number of which require medical care. Fire ant stings are also blamed for human deaths. Consequently, there is much interest in controlling these troublesome pests.

[0004] This interest has resulted in much research and resources being expended through the years to develop reagents and methods for controlling fire ants. While many useful insecticide formulations have resulted from this research, the problems associated with fire ants still exist because the relief gained by insecticide use is only temporary. Once the insecticide pressure is relaxed, fire ant populations invariably repopulate the areas. This reinfestation ability is attributed to the high reproductive capabilities, the efficient foraging behavior, and the ecological adaptability of the ants. While effective for controlling ants in relatively small defined areas, insecticides can create other problems. For example, some

insecticides, which are effective at controlling fire ants, can pose a significant threat to the environment, including birds and mammals.

[0005] Although considerable research effort has been brought to bear against the red imported fire ant, it remains the primary pest ant species in infested areas; initial eradication trials failed, yielding to the wide distribution of pesticide-based control products and a federally imposed quarantine to prevent further spread. Recently, much of the research effort has focused on elucidating basic life processes in an attempt to develop unique control measures, and fostering the development of self-sustaining methods of control, including biocontrol organisms and microbes (Williams et al., Am. Entomol., Volume 49, 150-163, 2003).

[0006] Despite intensive searches over the last 4 or 5 decades for viral infections of *S. invicta*, only recently were the first viral infections reported and characterized from this ant (Valles et al, Virology, Volume 328, 151-157, 2004; Valles and Strong, 2005; Valles et al., Virology, Volume 365, 457-463, 2007; J. Invert. Pathol., Volume 99, 74-81, 2008). *Solenopsis invicta* virus 1 (SINV-1) is a positive-strand, RNA virus with characteristics consistent with viruses in the Dicistroviridae (Mayo, Arch. Virol., Volume 147, 1655-1656, 2002). It possess a monopartite, single-stranded, 3'-polyadenylated, RNA genome that encodes 2 polyproteins. The 5'-proximal polyprotein contains sequences with identity to RNA-dependent RNA polymerase (RdRp), helicase, and cysteine protease proteins characteristic of single-stranded RNA viruses (Koonin, J. Gen. Virol., Volume 72, 2197-2206, 1991) and the 3'-proximal polyprotein contains sequences consistent with viral coat proteins which was confirmed by purification and N-terminal sequencing (Hashimoto and Valles, J. Invertebr. Pathol., Volume 99, 136-140, 2008). *Solenopsis invicta* virus 2 (SINV-2) is also a positive-strand RNA virus but with an unusual genome organization (Valles et al., 2007, *supra*). The SINV-2 genome is monopartite and polycistronic, with 4 open reading frames in the sense orientation (Valles et al., 2007, *supra*). SINV-1 and-2 have been associated inconsistently with colony death (Valles et al., 2004; 2007; Hashimoto and Valles, 2008, *supra*).

[0007] Use of positive-strand RNA viruses as insect control agents has been proposed (Scotti et al., Adv. Virus Res. Volume 26, 117-143, , 1981; Insect viruses: new strategies for pest control, 128-163. In Oakshott ,J. Whitten, M.J. (eds) Molecular approaches to fundamental and applied entomology. Springer Verlag, New York, NY et al, 1993) and successfully demonstrated for the olive fruit fly (Manousis and Moore, Appl. Environ. Microbiol., Volume 53, 142-148, 1987) and *Helicoverpa armigera* (Christian et al., J. Econ. Entomol.,

Volume 98, 1839-1847, 2005). However, production of pure virus has been limited to *in vitro* systems (cell culture). A cell line is not available for *S. invicta* which has hampered development and use of the *Solenopsis invicta* viruses as control agents against fire ants. Recently, successful *in vitro* expression of a positive-strand RNA virus was reported (Pal et al., J. Virol., Volume 81, 9339-9345, 2007; Boyapalle et al. Virology, Volume 375, 401-411, 2008). A clone of the *Rhopalosiphum padi* virus genome was expressed in a baculovirus expression system and found to be infectious and pathogenic to its aphid host (Pal et al., 2007; Boyapalle et al., 2008; both *supra*).

[0008] A dearth of natural enemies of the red imported fire ant have been found in the U.S. including a neogregarine (Pereira et al., J. Invertebr. Pathology, Volume 81, 45-48, 2002) and a fungus (Pereira et al., J. Invertebr. Pathology, Volume 84, 38-44, 2004).

[0009] United States Patent 6,660,290 discloses a non-sporulating mycelial stage of an insect-specific parasitic fungi for control of pests with fire ants listed as one of many examples of insects controlled by the biopesticide.

[0010] United States Patents 4,925,663; 5,683,689; 6,254,864; and 6, 403,085 disclose a biopesticide effective against fire ants that includes the fungus *Beauveria bassiana*.

[0011] There remains a need for biocontrol and/or microbial control agents that eliminate or at least reduce the spread of fire ant colonies using novel pathogens. The present invention described below is directed to a novel *Solenopsis invicta* virus useful for the control of fire ants which are different from prior art pathogens and their uses.

SUMMARY OF THE INVENTION

[0012] It is therefore an object of the present invention to provide a novel *Solenopsis invicta* virus (SINV-3) for biocontrol of *Solenopsis invicta*.

[0013] Another object of the present invention is to provide a novel *Solenopsis invicta* virus (SINV-3) identifiable by a primer selected from the group consisting of SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, and SEQ ID NO 33.

[0014] A further object of the present invention is to provide a nucleic acid sequence SEQ ID NO 1 for SINV-3.

[0015] Another object of the present invention is to provide a biocontrol method for controlling fire ants that includes applying a SINV-3 virus and a carrier that is a fire ant food source to form a biocontrol composition which is scattered near a fire ant colony.

[0016] Another object of the present invention is to provide a biocontrol composition comprising SINV-3 and an acceptable carrier.

[0017] Further objects and advantages of the present invention will become apparent from the following description.

BRIEF DESCRIPTION OF THE DRAWINGS

[0018] Figures 1(A-1(D) shows SEQ ID NO 1 of the present invention

[0019] Figures 2 (A) is a Northern blot of RNA prepared from purified SINV-3 particles (lane 1) and SINV-3-infected fire ant workers (lane 2). Numbers to the left of the blot indicate the size in nucleotides of single-stranded RNA standards.

Figure 2 (B) is an electron micrograph of a negative stain of purified SINV-3 used for the Northern analysis.

[0020] Figure 3 shows a genome organization of SINV-3. Positions of sequence motifs for helicase, protease, and RNA-dependent RNA-polymerase and their corresponding positions within the polyprotein of ORF 1 are indicated. The italicized numeral indicates the position of the first residue of the sequence provided. The lower right shows results of a silver-stained SDS-PAGE of purified SINV-3 particles. Two bands were consistently observed with a combined molecular mass of approximately 77,250 Da. The predicted molecular weight of the translated ORF 2 was approximately 73,186 Da.

[0021] Figure 4 shows the alignment comparisons of predicted amino acid sequences of the RdRp of KFV, SINV-1, SINV-2, SINV-3 and Hepatitis A virus (HAV). The numbers on the left indicate the starting amino acids of aligned sequences. Identical residues in at least four of the five virus sequences are shown in reverse. Sequences motifs shown for RdRp (I-VIII) correspond to those identified and reviewed by Koonin and Dolja (Crit. Rev. Biochem. Molec. Biol., Volume 28, 375-430, 1993).

[0022] Figure 5 shows a phylogenetic analysis of the conserved amino acid sequences containing domains I to VIII of the putative RdRp from thirteen dicistroviruses (unaltered text font), seven iflaviruses (bold, italic), two plant RNA viruses (bold), four picornaviruses (italic), and four unassigned viruses (white text). Virus abbreviations, accession number of the virus RNA protein sequence, and amino acid residues of aligned sequences in a specific ORF (5' proximal ORF of dicistroviruses, otherwise an ORF number is specified) include:

Aphid lethal paralysis virus (APLV) [AD536531], 1661-1995; Black queen cell virus (BQCV) [NP620564], 1317-1585; Cricket paralysis virus (CPV) [NP647481], 1423-1697; *Drosophila* C virus (DCV) [AF014388], 1415-1693; Himetobi P virus (HiPV) [AB17037], 1441-1710; *Plautia stali* Intestine virus (PSIV) [NP620555], 1465-1739; *Rhopalosiphum padi* virus (RhPV) [AF022937], 1625-1916; *Triatoma* virus (TiV) [AF178440], 1446-1716; Acute bee paralysis virus (APV) [AAG13118], 1566-1837; *Homalodisca coagulata* virus (HoCV-1) [DQ288865], 1446-1716; Kashmir bee virus (KBV) [AY275710], 1594-1864; *Solenopsis invicta* virus-1 (SINV-1) [AY634314], 1052-1327; Taura syndrome virus (TaSV) [AF277675], 2628-2899; Infectious flacherie virus (IFV) [AB000906], 2618-2888; *Perina nuda* virus (PNV) [AF323747], 2628-2899; Sacbrood virus (SBV) [NC002066], 2522-2790; Deformed wing virus (DWV) [AJ489744], 2556-2826; *Ectropis ibliqua* picorna-like virus (EcOV) [AY365064], 2629-2900; Kakugo virus (KV) [AB070959], 2556-2826; Varroa destructor virus-1 (VDV-1) [AY251269], 2556-2826; Foot and Mouth disease virus (FMDV) [AF308157], 2011-2264; Hepatitis A virus (HAV) [NC001489], 1904-2161; Human parvovirus (HPV) [AJ005695], 1871-2117; Simian picornavirus 1 (SiPV-1) [AY064708], 2119-2368; Broad bean wilt virus 1 (BBWV) [NP951030], 405-657; Tomato ringspot virus (TRV) [ABG23688] RNA1, 406-672; Nora virus (NV) [DQ321720], ORF 2, 1763-2026; *Solenopsis invicta* virus 2 (SINV-2) ORF 4 [ABQ01575], 1814-2081; Kelp fly virus (KFV) [YP415507], 3015-3272; *Solenopsis invicta* virus 3 (SINV-3) ORF 1, 1848-2107.

[0023] Figure 6 is a graph showing SINV-3 genome equivalents determined for different developmental stages. Genome equivalents were interpolated from a standard curve generated simultaneously from a plasmid construct. Mean values are indicated by the bar and the internal bar signifies the minimum and maximum value observed for each group.

[0024] Figure 7 is a graph showing SINV-3 transmission to uninfected *S. invicta* fragment colonies. Worker ants were sampled on the week specified from each colony and tested for the presence of SINV-3 by QPCR.

[0025] Figure 8 is a graph showing mortality of *S. invicta* worker ants treated with 10^{10} SINV-3 particles (based on QPCR). Open circles are ants treated with SINV-3 and open squares are the control ants (treated with water). Asterisks indicate significant ($p < 0.05$) differences for each sample date from the control group by Student's t-test.

DETAILED DESCRIPTION OF THE INVENTION

[0026] Although viruses can be important biological control agents against insect populations (Lacey et al., Biol. Comtemp., Volume 21, 230-248, 2001), only recently have they been

shown to infect *Solenopsis invicta*. *Solenopsis invicta* viruses (SINV) represent the first infection of the red imported fire ant by this group of organisms. SINV-3, the virus of the present invention, represents the third virus to be discovered from *S. invicta* using the metagenomic approach (Valles et al, 2008, *supra*) and it possesses features consistent with placement within the order Picornavirales: 1. Non-enveloped particles with a diameter of approximately 30 nm; 2. A positive-sense, single-stranded RNA genome; 3. No production of subgenomic RNA; and 4. A polyprotein containing helicase, protease, and RdRp domains (Le Gall et al., Arch. Virol., Volume 153, 715-727, 2008). Although SINV-1, SINV-2, and the present invention SINV-3 are positive-strand RNA viruses infecting the same host (*S. invicta*), each is distinct phylogenetically (See Figure 5). Furthermore, they exhibit differences in genome organization; SINV-1 and SINV-3 are discistronic, while SINV-2 is polycistronic. The most important difference between SINV-1, SINV-2 and SINV-3 is apparent pathogenicity. SINV-1 and SINV-2 are similar to many positive-strand RNA viruses that infect honey bees, they are present as chronic, asymptomatic infections that may cause mortality under certain stressful conditions (Bailey, Ann. Appl. Biol., Volume 60, 43-48, 1967; Chen and Siede, Adv. Viral Res., Volume 70, 33-80, 2007). Transmission studies of SINV-1 and SINV-2 to uninfected ants were completed by regular feeding. However, mortality among recipient colonies was an occasional event (Valles et al., 2004; Valles unpublished). The virus of the present invention, SINV-3, showed transmission (Figure 7) associated with mortality (Figure 8) and a correspondingly high SINV-3 titer; dead ants typically contained greater than approximately 10^9 viral particles per ant. Further evidence of SINV-3 virulence is the presence of viral genome copies in all tissues of the ant (Table 3 below, Example 3). In the laboratory, SINV causes brood death of an entire colony and infection of healthy colonies (Valles et al., Virology, Volume 328, 151-157, 2004; Valles et al., J. Invert. Path., Volume 88, 232-237, 2005; both references herein incorporated in their entirety).

[0027] Published United States Patent Application 11/780,854, filed 07/20/2007, publication number US2008/0031856, published February 7, 2008 (Valles et al) describes two viruses, SINV-1 and SINV-1A, useful for the control of *S. invicta* (herein incorporated by reference in its entirety).

[0028] SINV-3 represents the third virus discovered that infects the red imported fire ant, *S. invicta*. SINV-3 is a unique virus with a genome that differs considerably from other positive-strand RNA viruses.

[0029] The present invention provides nucleic acids SEQ ID NO 1 (Figure 1(a)-1(d)) which

is the whole genome for SINV-3. The invention also provides nucleic acid sequences (SEQ ID NO 2-33; Table 4 in Example 3) capable of selectively hybridizing DNA, RNA, and cDNA sequences which can be derived from SEQ ID NO 1. These primers are useful for identifying any SINV-3 virus. To isolate SINV-3, RNA from fire ants, collected from a fire ant mound, was extracted from about 20-50 workers using TRIZOL reagent according to the manufacturer's directions (Invitrogen, Carlsbad, CA).

[0030] With the primers of the present invention and the teachings of the present specification, one of ordinary skill in the art could readily identify SINV-3 viruses of the present invention.

[0031] For purposes of the present invention, the term "fire ant" and "*Solenopsis invicta*" are used interchangeably to describe the common red fire ant, originating in South America, but now commonly found in the United States, and Puerto Rico. The term fire ant also is used to describe black fire ants and other hybrid fire ants or other ants that are infected by the viruses of the present invention.

[0032] For purposes of the present invention, the term "isolated" is defined as separated from other viruses found in naturally occurring organisms.

[0033] For purposes of the present invention, the term "composition" is used to describe a composition which contains the virus of the presently claimed invention, optionally a carrier and optionally a pesticide. The carrier component can be a liquid or a solid material and is an inert, non-repellent carrier for delivering the composition to a desired site. Liquids suitable as carriers include water, and any liquid which will not affect the viability of the viruses of the present invention. Solid carriers can be anything which the fire ant will feed on. Non-limiting examples of solid carriers of the present invention include materials such as corn cob grits, extruded corn pellets, boiled egg yolks, and frozen insects such as crickets.

[0034] Optional toxicants include Chlorfenapyr, Imidacloprid, Fipronil, Hydramethylnon, Sulfluramid, Hexaflumuron, Pyriproxyfen, methoprene, lufenuron, dimilin, Chlorpyrifos, and their active derivatives, Neem, azadiractin, boric acid based, etc. The toxicant acts as a stressor which may be required to initiate viral replication which in turn results in brood death in the fire ant colony.

[0035] The term "effective amount" or "amount effective for" as used herein means that minimum amount of a virus composition needed to at least reduce, or substantially eradicate fire ants in a fire ant colony when compared to the same colony or other colony which is untreated. The precise amount needed will vary in accordance with the particular virus composition used; the colony to be treated; the environment in which the colony is located.

The exact amount of virus composition needed can easily be determined by one having ordinary skill in the art given the teachings of the present specification. The examples herein show typical concentrations which will be needed to at least reduce the number of fire ants in a colony.

[0036] In the method of using the viruses of the present invention, to reduce or eradicate a population of fire ants, the compositions are delivered to the fire ants by spreading the composition at or near the fire ant colonies. The amount of composition used is an effective amount for producing the intended result, whether to reduce or eradicate the population of fire ants. The composition is prepared by homogenizing approximately 300 workers from a SINV infected colony in an equal volume of water and placing the resulting homogenate on a carrier.

[0037] Initial blast analysis (Altschul et al., Nucleic Acids Research, Volume 25, 3389-3402, 1997) of expressed sequence tags (ESTs) from a *Solenopsis invicta* expression library had indicated weak identity of EST 16A4 (Genbank accession number: EH413252) with the polyprotein of *Acyrtosiphum pisum* virus (Valles et al., 2004, *supra*). Subsequent re-analysis of this EST sequence revealed significant identity with a portion of the kelp fly virus genome (Hartley et al., J. Virol., Volume 79, 13385-13398, 2005). EST 16A4 was therefore used as the primary sequence from which oligonucleotide primers were designed and RACE (3' and 5') reactions conducted to acquire additional sequence and to confirm that this sequence was of viral origin. Subsequent sequence acquisition and analysis confirmed the presence of a new virus from *S. invicta* ants which is SINV-3 (*Solenopsis invicta* virus-3). The 10,386 nucleotide genome is monopartite, bicistronic (non-overlapping), and single-stranded. The genome size was confirmed by Northern analysis in which a band was observed at approximately $10,968 \pm 270$ nucleotides; no subgenomic RNA was evident. Blastp analysis of ORF 1 resulted in recognition of a conserved domain for RNA-independent RNA polymerase (RdRp) characteristic of positive-strand RNA viruses (Koonin and Dloja, 1993, *supra*). These viruses invariably possess 8 common sequence motifs in the RdRp (Koonin, J. Gen. Virol., Volume 72, 2197-2206, 1991); all eight of these motifs were present in SINV-3 (See Figures 2 and 3). Further, sequence motifs IV, V, and VI were reported to be unequivocally conserved throughout this class of viruses exhibiting 6 invariant amino acid residues (Koonin and Dolja, 1993, *supra*). These core RdRp motifs were shown by site-directed mutagenesis to be crucial to the activity of the enzyme (Sankar and Porter, J. Biol. Chem., Volume 267, 10168-10176, 1992). SINV-3 possessed all 6 of these characteristic residues D¹⁹²⁰, D¹⁹²⁵ (motif IV), G¹⁹⁷⁹, T¹⁹⁸³ (motif V), and D²⁰²⁸, D²⁰²⁹ (motif

VI).

[0038] Alignment of SINV-3 ORF 1 with nonstructural polyproteins of positive-strand RNA viruses revealed domains for helicase and protease. Three sequence motifs are conserved among positive-strand helicases, designated A, B, and C (Gorbalenya et al., FEBS Lett., Volume 262, 145-148, 1990). The consensus sequence for motif A, GX₄GK (Gorbalenya et al., 1990, *supra*), thought to be responsible for nucleotide binding, was found in the translated ORF 1 of SINV-3 at amino acid position 396 (G³⁹⁶). Motifs B and C were also identified based on the presence of conserved residues (Q⁴⁴¹, D⁴⁴⁷-motif B; and N⁴⁹⁷ preceded by a stretch of hydrophobic residues) (Gorbalenya et al., 1990, *supra*).

[0039] Characteristic motifs for a 3C-like protease, but not a 2A protease, were also detected in the translated ORF 1 of SINV-3. Amino acids thought to form the catalytic triad of the 3C-like protease, H¹²⁵⁸, D¹³⁰⁹, and C¹³⁸¹ were present in SINV-3 ORF 1 (Koonin and Dolja, 1993, *supra*; Ryan and Flint, J. Gen. Virol., Volume 78, 699-723, 1997; Luke et al., J. Gen. Virol., Volume 89, 1036-1042, 2008). Furthermore, the consensus GxCG sequence motif was present at amino acids 1379 to 1382.

[0040] Phylogenetic analysis of SINV-3 RdRp showed a close relationship with KFV (Figure 5). Many of the unique features exhibited by KFV are also shared by SINV-3, including a smaller virion size (approximately 27.3 ± 1.3 nm, Figure 2B) with apparent surface projections (Scotti et al., J. Gen. Virol., Volume 30, 1-9, 1976; Hartley et al., 2005) and higher buoyant density (1.39 ± 0.02 g/ml). Another similarity is the presence of only 2 major capsid proteins (VP1 and VP2) as opposed to four which is typical of *Dicistroviridae* or iflaviruses. Despite a close phylogenetic relationship between KFV and SINV-3, their genome structures were different. KFV genome is monocistronic and SINV-3 is dicistronic. They also exhibited poor identity (<10%) among their structural proteins.

[0041] SINV-3 was found to infect all stages of *S. invicta*, including the eggs (See Figure 6). QPCR of different stages shows that immature ants contained significantly lower quantities of SINV-3 compared with adults (workers, alates, and queens).

[0042] The following examples are intended only to further illustrate the invention and are not intended to limit the scope of the invention as defined by the claims.

EXAMPLE 1

[0043] A one-step reverse transcriptase polymerase chain reaction (RT-PCR) was used to identify SINV-3-infected *S. invicta* ants. A 20 ml scintillation vial was plunged into a fire ant mound in the field for several minutes to collect a sample of the worker caste. The ants were returned to the laboratory and RNA was extracted from about 10-20 ants using TRIZOL

reagent according to the manufacturer's directions (Invitrogen, Carlsbad, CA). cDNA was synthesized and subsequently amplified using the One-Step RT-PCR kit (Invitrogen) with oligonucleotide primers p705-SEQ ID NO 2 and p707 ID NO 4 (Table 4). Samples were considered positive for the virus when a visible amplicon (about 72 nucleotides) was present after separation on about a 1.2% agarose gel stained with ethidium bromide. RT-PCR was conducted in a PTC 100 thermal cycler (MJ Research, Waltham, MA) under the following optimized temperature regime:

- 1 cycle at about 45°C for about 30 minutes;
- 1 cycle at about 94° C for about 2 minutes;
- 35 cycles at about 94° C for about 15 seconds;
- 1 cycle at about 63° C for about 15 seconds;
- 1 cycle at about 68° C for about 15 seconds; and
- a final elongation step of about 68°C for about 5 minutes.

[0044] SINV-3 was purified by discontinuous and isopycnic centrifugation. Briefly, approximately 50 grams of a mixture of worker and brood ants were homogenized in about 150 ml of NT buffer (10 mMTris-HCl, pH about 7.4, approximately 100 mM NaCl) in a Waring blender on high speed for approximately 2 minutes. The mixture was filtered through about 8 layers of cheese cloth and then extracted with an equal volume of chloroform for approximately 10 minutes with constant shaking. The mixture of centrifuged for about 5 minutes at approximately 5,000 X g and the supernatant collected by pipette. The supernatant was layered onto a discontinuous CsCl gradient (about 1.2 and about 1.5 g/ml) which was centrifuged at about 190,000 X g for about 2 hours in a Ti50.1 rotor. A whitish band visible near the interface was removed and brought to a density of approximately 1.3 grams/ml CsCl. This sample was then centrifuged at approximately 330,000 X g for about 16 hours in a Ti70.1 rotor. A whitish band at about 1.39 ± 0.02 grams/ml was collected. The sample was negatively stained with about 2% phosphotungstic acid, about pH 7, and examined with a Hitachi H-600 transmission electron microscope (Hitachi, Pleasanton, CA) at an accelerating voltage of approximately 75 kV. Uninfected worker ants were prepared and examined in the same manner and served as controls.

[0045] Particles purified from SINV-3 –infected fire ants exhibited similar characteristics and migrated to a density of approximately 1.39 ± 0.02 grams/ml CsCl. No corresponding particles were observed in samples prepared from uninfected fire ants. Electron microscopic examination of negatively stained samples from SINV-3-infected fire ants revealed isometric particles with apparent projections and a diameter of approximately 27.3 ± 1.3 nm (Figure 2B).

EXAMPLE 2

[0046] A series of nine 5' RACE reactions were conducted to obtain the upstream sequence of SINV-3 genome using the 5' RACE system (Invitrogen, Carlsbad, CA) and primer walking. cDNA was synthesized for about 50 minutes at about 48 degrees C with approximately 2.5 µg of total RNA extracted with Trizol from purified SINV-3 particles (as described above in Example 1) with a gene-specific oligonucleotide primer (GSP, Table 1), the RNA template was degraded with RNase H, and the cDNA was purified. The 3' end of the cDNA was polycytidylated with terminal deoxynucleotidyl transferase and dCTP. The tailed cDNA was then amplified with a nested, GSP (3' end) and an abridged anchor primer (AAP). Gel purified amplicons were ligated into pCR4-TOPO vector, transformed into TOP10 competent cells (Invitrogen, Carlsbad, CA) and sequenced by the Interdisciplinary Center for Biotechnology Research (University of Florida). (See Tables 1 and 2)

[0047] Two 3'RACE reactions were conducted with the GeneRacer kit (Invitrogen). cDNA was synthesized from total RNA (approximately 1 µg) using the GeneRacer Oligo dT primer. Amplicons were cloned and sequenced as described for 5' RACE. Two 3' RACE reactions were required because a region of high adenine density (nucleotides 8847-8874) was binding the oligo dT primer upstream of the 3' poly A tail (Table 1).

[0048] Northern analysis was conducted to determine the genome size and possibly whether subgenomic RNA was produced following the glyoxal denaturation procedure of Sambrook and Russel (Molecular Cloning, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001; herein incorporated by reference in its entirety) . Total RNA (approximately 2 to 8 µg) prepared with Trizol from purified SINV-3 particles was denatured with glyoxal and separated on a 1% agarose gel. Electrophoresis was conducted at about 60 volts for about 1.5 hours in BPTE buffer (approximately 30 mM Bis-Tris; 10 mM PIPES; 1 mM EDTA; pH 6.5). Gel separated nucleic acids were transferred onto a Nytran Supercharged Nylon membrane by downward blotting in approximately 0.01 M NaOH and approximately 3 M NaCl. After neutralization and blocking in approximately 6X SSC (approximately 0.9 M NaCl, approximately 0.09 M sodium citrate) the blot was pre-hybridized in approximately 0.5 M sodium phosphate (pH approximately 7.2), approximately 7% SDS , approximately 1 mM EDTA for about 2 hours. The 499 nucleotide probe was synthesized by PCR using oligonucleotide primers p762 SEQ ID NO 22 and p764 SEQ ID NO 24 (Table 4), ³²P-labeled dCTP, and a clone from the 3' end of the genome as template. The probe was added to the hybridization chamber and incubated for about 16 hours under high stringency conditions at approximately 68 degrees C. After hybridization, the blot was dried

and exposed to x-ray film for about 12 hours. Molecular weight of the SINV-3 genome was interpolated with two sets of RNA standards electrophoresed concurrently.

[0049] Blastp analysis was conducted with conserved regions of SINV-3 RdRp and helicase of ORF 1. Representative viruses exhibiting significant e-scores ($<10^{-5}$) were included in the phylogenetic analysis and multiple-alignment with SINV-3 using Vector NTI Advance software (Version 10.1.1, Invitrogen). Multiple alignments were carried out for the deduced amino acid sequences of the non-structural and structural polyproteins of viral genomes. Specifically, conserved regions of the RdRp (domains-I to VIII) and helicase (domains A, B, and C) were aligned and subsequently used to construct an unrooted radial phylogenetic tree using the neighbor-joining method (Saitou and Nei, Mol. Biol. Evol., Volume 4, 406-425, 1987) in ClustalX (Thompson et al., Nucleic Acids Res., Volume 22, 4673-4680, 1994). The statistical significance of branch order was estimated by performing 1000 replications of bootstrap re-sampling of the original aligned amino acid sequences. Trees were generated with TreeView (Page, Computer Applications in the Biosciences, Volume 12, 357-358, 1996).

[0050] The genome of *Solenopsis invicta* virus 3 was constructed by compiling sequences from a series of nine successive 5'RACE reactions, two 3' RACE reactions, and the sequence of EST 16A4 (Accession number:EH413252; Tables 1 and 2). The SINV-3 genome was found to be approximately 10,386 nucleotides in length, excluding the poly(A) tail present on the 3' end. This genome size was consistent with Northern analysis results of total RNA extracted from purified SINV-3 (Figure 2A). Northern blotting yielded a band at approximately $10,968 \pm 270$ nucleotides. No hybridization was observed in RNA extracted from fire ants determined to be free of SINV-3 by RT-PCR as described above in Example 1.

[0051] The SINV-3 genome sequence was A/U rich (approximately 70.9% A/U; approximately 29.1 % G/C). Analysis of the genome revealed 2 large open reading frames (ORFs) in the sense orientation (out of frame with each other) with an untranslated region (UTR) at each end and between the two ORFs (Figure 3). The 5' proximal ORF (ORF) commenced at the first canonical (AUG) start codon present at nucleotide position 92 and ended at a UGA stop codon at nucleotide 7,832 which encoded a predicted protein of approximately 299,095 Da (2,580 amino acids). The 3' proximal ORF (ORF 2), commenced at nucleotide position 8,308 (canonical AUG start codon), terminated at nucleotide position 10,261 (UAA stop codon) and encoded a predicted protein at 73,186 Da (651 amino acids). No large ORFs were found in the inverse orientation suggesting that the SINV-3 genome was a positive-strand RNA virus. The 5',3' and intergenic UTRs were comprised of

approximately 91, 123, and 473 nucleotides, respectively. Blastp analysis (Altschul et al., 1997, *supra*) of the translated ORF 1 recognized a conserved domain (GenBank cd01699) for RNA-dependent RNA polymerase (RdRp, Figures 2 and 3) which is encoded in the genomes of all RNA viruses with no DNA stage (Koonin and Dolja, 1993, *supra*). The most significant expectation scores from blastp analysis of the RdRp region of ORF 1 were to Kelp Fly virus (KFV, 10^{-58}) with corresponding identities of approximately 35.1 and 25.2 %, respectively. Helicase and protease sequence motifs were also present in ORF 1 upstream of the RdRp (Figure 4). Blastp analysis of ORF 2 did not yield any sequences with significant identity. Silver stained SDS-PAGE of purified SINV-3 particles yielded 2 bands (band 1L 41,000 Da; Band 2: 36,250 Da) with a combined molecular mass of approximately 77,250 Da which was similar in mass predicted by ORF 2 (approximately 73,186 Da; Figure 3).

[0052] Phylogenetic analysis of the conserved amino acid sequences containing domains I to VIII of the RdRp (Figures 4 and 5) from dicistroviruses, iflaviruses, plant RNA viruses, picornaviruses, and 4 unassigned positive-strand RNA viruses revealed a trichotomous phenogram with SINV-3 and Kelp Fly virus comprising a unique cluster (Figure 5). The close relationship between SINV-3 and KFV is supported by a significant expectation score and sequence identity when the SINV-3 ORF 1 and KFV ORF were compared by blastp analysis.

Table 1. Strategy used to acquire the genome of SINV-3. Successive 5' and 3' RACE reactions were conducted,, the corresponding portion of the genome acquired, and oligonucleotide primers used for cDNA synthesis and PCR amplification are indicated.

Reaction	Regions acquired (nts, 5'→3')	Size (nts)	Oligonucleotide primers for:	
			cDNA synthesis	PCR amplification
5' RACE	1-1167	1168	p763 SEQ ID NO 23	p767 SEQ ID NO 25/AAP
5' RACE	1038-1582	545	p722 SEQ ID NO 9	p762 SEQ ID NO 22/AAP
5' RACE	1338-2697	1360	p734 SEQ ID NO 12	p755 SEQ ID NO 20/APP
5' RACE	2423-3789	1367	p735 SEQ ID NO 13	p754 SEQ UD NO 19/AAP
5' RACE	3610-4773	1164	p707 SEQ ID NO 4	p750 SEQ ID NO 17/APP
5' RACE	4585-5784	1200	p710 SEQ ID NO 6	p739 SEQ ID NO 15/APP
5' RACE	5636-6333	698	p734 SEQ ID NO 12	p731 SEQ ID NO 11
5' RACE	6292-6773	482	p722 SEQ ID NO 9	p720 SEQ ID NO 8/APP
5' RACE	6677-7194	518	p707 SEQ ID NO 4	p706 SEQ ID NO 3/APP
3'RACE	7164-8869	1706	Oligo dT	p705 SEQ ID NO 2/3' PRIMER
3'RACE	8743-10411	1669	Oligo dT	p789 SEQ ID NO 32/3' PRIMER
EST 16A4	7118-7812	695	N/A	N/A

Table 2. Genome sequencing strategy for SINV-3. Oligonucleotide primers used to amplify overlapping regions of the SINV-3 genome. The clone designation and corresponding region of the genome sequence are indicated.

Clone Designation	Oligonucleotide primers (orientation)	Genome Region amplified
12B/178/2	P791 (F)/p767(R)	1-1167
12B/157/6	P764 (F)/p756 (R)	1039-2624
12B/157/5	P769 (F)/p752(R)	2003-3696
12B/157/4	P770 (F)/p748(R)	2938-4683
12B/157/3	P771(F)/p738(R)	4042-5746
12B/157/2	P772(F)/p734(R)	5038-6442
12B/157/8	P730(F)/p708(R)	6296-7271
12B/174/1	P705(F)/p713(R)	7164-8605
12B/180	P775(F)/p787(R)	8038-9730
12B/157/1	P789(F)/3'-primer(R)	8743-10411

EXAMPLE 3

[0053] Tissue tropism of SINV-3 was examined by dissecting different tissues or groups of tissues and quantifying the number of SINV-3 genome equivalents in each respective preparation by quantitative PCR (QPCR). This experiment was conducted for mated queens, workers, and 4th instar larvae. Ice-chilled fire ant workers and queens from SINV-3-positive colonies were placed on a glass microscope slide, and head, thorax, and abdomen were separated with a surgical blade under a Leica MS5 dissecting microscope. The head and thorax were placed directly in Trizol and the abdomen was immersed in 1mM Tris-HCL, pH approximately 8.0, for further dissection. Tissues were isolated and removed from the abdomen with micro-dissecting forceps in the following order: crop, poison sac, midgut, hindgut, Malpighian tubules, fat body (queens only), ovaries (queens only), and the remaining abdominal carcass. Fourth instar larvae from SINV-3 positive colonies were dissected to the following groups: Malpighian tubules, midgut, and remaining carcass. Six replicates were conducted for each stage using individual queens per replicate and pooled groups of workers and larvae (n=10). RNA was extracted with Trizol. cDNA was synthesized from the SINV-3 genome region corresponding to the RdRp with total RNA using SuperScript III Reverse Transcriptase (SsRT; Invitrogen) and a gene-specific primer p707 SEQ ID NO 4 (Table 4). In an approximately 0.5 ml PCR tube, approximately 2 µl of primer p707 SEQ ID NO 4 (approximately 1 µM), approximately 1 µl of a dNTP mix (approximately 10 mM), and approximately 10 µl of total RNA (approximately 50 ng) were mixed and heated to about 65 degrees C for about 5 minutes in a PTC 100 thermal cycler, followed by incubation on ice for about 1 minute. Then, approximately 4 µl of first-strand

buffer (approximately 250 mM Tris-HCl, pH about 8.3, approximately 375 mM KCl, approximately 15 mM MgCl₂), approximately 2.75 µl of DEPC-treated water, and approximately 0.25 µl SsRT (approximately 200 U/µl) were added. The mixture was incubated at about 55 degrees C for about 30 minutes, followed by inactivation of SsRT by heating to about 70 degrees C. for about 15 minutes. This cDNA was used to perform QPCR. [0054] QPCR was conducted on an ABI PRISM 7000 Sequence Detection System interfaced to the ABI prism 7000 SDS software (Applied Biosystems, Foster City, CA) in an approximately 25 µl reaction volume. The reaction contained approximately 12.5 µl of SYBR Green SuperMix (with UDG and ROX, Invitrogen), approximately 0.4 µl each of approximately 10 µM SINV-3-specific primers p705 and p707, approximately 3mM MgCl₂, approximately 1 µl of the cDNA synthesis reaction, and approximately 10.7 µl of DEPC-water. QPCR conditions consisted of one cycle at approximately 50 degrees C for about 2 minutes, and approximately 95 degrees C for about 10 minutes, followed by about 40 cycles at approximately 95 degrees C for about 15 sec, approximately 64 degrees C for about 15 sec, approximately 72 degrees C for about 1 minute. The non-template control for QPCR included a complete cDNA synthesis reaction devoid of RNA template. A standard curve was constructed from a plasmid clone of the corresponding SIN-3 genome region using a copy number range of approximately 5-5 x 10⁷ copies. Reaction efficiencies were determined by regressing C_T vales against the template copy number (log) and calculated according to the formula $[E=(10^{-1/\text{slope}})-1]$ (Klein et al., 1999). Reaction efficiencies reoutinely exceeded approximately 95%.

[0055] Experiments were conducted to quantify the SINV-3 infection in different developmental stages of *S. invicta*. Samples of eggs (n=20), queens (n=11), workers (n=18), early (1st -2nd instars), larvae (n=5) and late 93rd – 4th instars) larvae 9n=5) were taken from SINV-3-positive colonies. RNA was extracted from the specimens with Trizol reagent. The RNA concentration was determined spectrophotometrically. The number of SINV-3 genome equivalents in different developmental stages and tissues was quantified by QPCR as described above.

[0056] SINV-3 genome was detected in all tissues of *S. invicta* queens, workers and larvae examined by QPCR (Table 3). The tissues comprising the largest percentage of SINV-3 genome equivalents was the carcass for queens (approximately 27%) and workers (approximately 50.4%) and midgut (approximately 34.6%) for the larvae. Based on these data, SINV-3 infection appears to be systemic. SINV-3 was detected in *S. invicta* eggs, workers, larvae, and alate stages (Figure 6).

Table 3. Distribution of SINV-3 genome equivalents among different tissues and tagma of worker, larval (4th instars), queen *S. invicta* ants.

Tissue/tagma	Percent of total SINV-3 genome equivalents for stage*		
	Queen	Worker	Larvae
Remaining Carcass ¹	27.0±3.7	50.4±6.7	34.4±6.8
Midgut	9.0±7.5	33.6±3.1	34.6±6.7
Malpighian tubules	8.0±5.5	3.0±1.2	31.0±9.9
Hindgut	4.2±1.9	2.7±0.7	
Poison Sac	8.2±4.1	2.0±1.0	
Head	13.1±11.1	4.5±1.1	
Thorax	4.0±1.6	2.0±0.4	
Crop	7.3±3.6	1.5±0.3	
Ovary	12.6±7.7		
Fat Body	6.9±5.3		

*Mean (±SE) number of SINV-3 genome equivalents was approximately $1.46 \pm 2.83 \times 10^6$ for workers, $1.39 \pm 3.22 \times 10^9$ for larvae, and $2.0 \pm 2.67 \times 10^9$ for queens.

¹For queens and workers the remaining carcass is comprised of remaining tissues in the abdomen, for larvae the carcass is comprised of remaining tissues of the entire body carcass.

Table 4. Oligonucleotide primers used throughout the studies.

Oligonucleotide Designation	Oligonucleotide (5'-3')	Genome Position	Orientation	SEQ ID NO
p705	CTGCTGGTATGATGGCAACAGATCCTTCTGT	7164-7194	→	2
p706	ACAGAAAGGATCTGTTGCCATCATCATACCAGCAG	7164-7194	←	3
p707	AAGGAGTTTGTGTATTAGTTGCAATGCCAGAATCT	7201-7235	←	4
p708	ATGCTCTAGCTATGGGATTCAATACACGGGA	7241-7271	←	5
p710	CATCATTCTTGAACATTAGTTATAGGATGTTTCGAC	7718-7753	←	6
p713	CGGGAACAAATGGATCAGAACCGATATCCACCA	8574-8605	←	7
p720	CTTTATTAATAATGCCTTCAAGGGCAGCTT	6744-6773	←	8
p722	GATGAGGCCCTTTTGAAAGATTTCATTGAGGT	6816-6847	←	9
p730	CAAAATTTGAACTATTGAAACATTATCTTTTATA	6296-6331	→	10
p731	TATAAAAGATAATGTTCAATAGTTTCAAAATTTGG	6296-6331	←	11
p734	ATGTTCAAGGAGTATCATCAGTTGCATAACAAA	6411-6442	←	12
p735	CTCTTCTCCCCATAATGCAGCTTCTTCTGAATTG	6462-6496	←	13
p738	ACGAGCTGTGAACCTACCAAGAATCCAACGTT	5715-5746	←	14
p739	TAACATCCTACAGCACAAATTTTCATCCCAAGCA	5752-5784	←	15
p748	GCAGTTGTAAGTTTCCAACCATIAGTAACCGTCAAT	4648-4683	←	16
p750	TTGAATATGCAATTGAATTGAACGTCACAACTCTA	4738-4773	←	17
p752	AATCCATTTACATCGGGTAAAGTAAGAACTTCTGCT	3660-3696	←	18
p754	ATACAATTTTCATCATTAGCAATCATATAAACTGGA	3754-3789	←	19
p755	GGTGAAAGATACATTTTCCAATCAAAATGCAAAAAG	2663-2697	←	20
p756	CCAATTCAAAAGGATTATTGAAAGAAACTCTATGAA	2589-2624	←	21
p762	ATGTTCAATATCTGCAGCAGCACAAATTTAAATATGC	1502-1537	←	22
p763	ATTACAGTTGCAAAATACCAATTCAAAATGGACATGGA	1546-1582	←	23
p764	TGATAATTTCTTGGTATTCCTAATTATAAAATTTGCT	1039-1075	→	24
p767	ATTTCACTTGTATTCTTTAGGACAATCACGA	1135-1167	←	25
p769	GCAAAATGCTACTAATCTTAAATTTGGATCAGAGG	2003-2036	→	26
p770	TGATGGTGATTATGTTTATATTCTGAGCATAAAATTC	2938-2975	→	27
p771	AGAAGAGCTTTATGATGCTGAAAATTTGTAATACTGTC	4042-4079	→	28
p772	AGTTGAAAATGAAACAGTTGAAATTTTAGGTATAACTCA	5038-5076	→	29
p775	CATAATATTACTGATGTTGTGGTTTCTTCAAAACC	8038-8072	→30	30
p787	CATGATTTTGTGTTCAATAGGTTTCAAAATATGTTCTCAT	9689-9730	←	31
p789	ATGGTTAGACAGGGTTCTTCAACAACCAATCTACTGATA	8743-8782	→	32
p791	TTTAAAATAGGAAATTAAGTCCAGTAAGGTTACTG	1-37	→	33

EXAMPLE 4

[0057] SINV-3-uninfected laboratory-reared newly mated queen monogyne colonies were identified by RT-PCR. These colonies were at an early stage of establishment, comprised of

approximately 1 ml of brood and approximately 100 workers. Three colonies were infected by feeding them a purified preparation (see above) of SINV-3 in an approximately 10% sucrose solution. QPCR was conducted on the purified preparation to determine the concentration of viral particles. The preparation was diluted in approximately 10% sucrose to achieve a concentration of approximately 1×10^6 SINV-3 particles per $\mu\text{l/ml}$. This solution was placed into a small glass test tube with a cotton stopped end. The ant colonies were allowed to feed on the virus preparation for about three days and then the glass test tube was removed. Afterward, the ants were fed unadulterated approximately 10% sucrose, water, frozen crickets (*Acheta domesticus*), and egg yolk (hard-boiled) *ad libitum*. Worker ants (n=10) from each colony were examined for the presence of SINV-3 by extracting total RNA and conducting QPCR on 0, 7, 14, and 21 days after exposure to the SINV-3 preparation.

[0058] Mortality tests were conducted with a SINV-3 purified preparation. Worker ants (n=30) from a single SINV-3-negative field colony were individually dipped into an aqueous solution of SINV-3 particles (approximately 1×10^{10} genome equivalents/ μl). The ants were subsequently dried of excess water by placing them on a paper towel briefly. They were then held in small soufflé cups (30 ml) with water and approximately 10% sucrose. Mortality was monitored every two days. Control ants were dipped into water only.

[0059] QPCR was conducted to separately quantify the plus (genomic) and minus (replicative) RNA strands of SINV-3. cDNA was synthesized from the SINV-3 plus strand with oligonucleotide primer p707 (SEQ ID NO 4) and minus strand with oligonucleotide primer p705 (SEQ ID NO 2) as described above in example 3. After cDNA synthesis, the RNA templates were digested with RNase A and RNase H at approximately 37 degrees C for about 30 minutes. QPCR was conducted as described in Example 3 with oligonucleotide primers p705 (SEQ ID NO 2) and p707 (SEQ ID NO 4).

[0060] SINV-3 was successfully transmitted to uninfected workers by feeding (Figure 7). SINV-3 genome was detected within approximately 7 days of providing uninfected *S. invicta* ants a sucrose solution containing purified SINV-3. SINV-3 was detectable for at least 21 days after treatment indicating sustained infection among recipient colonies. Furthermore, minus (replicative) strand of SINV-3 was detected in worker ants indicating replication of the virus.

[0061] *S. invicta* worker ants that were dipped in a solution containing 10^{10} SINV-3 genome equivalents exhibited significantly higher cumulative mortality compared with worker ants dipped in water only (Figure 8). No significant differences in mortality were observed

between SINV-3 exposed and unexposed worker ants during the first 6 days of the experiment. However, significant mortality was observed on days 8 through 16. Interestingly, no significant mortality was detected among worker ants injected with purified preparations of SINV-3 compared with the control (data not shown).

[0062] The foregoing detailed description is for the purpose of illustration. Such detail is solely for that purpose and those skilled in the art can make variations without departing from the spirit and scope of the invention.

We claim:

1. A purified *Solenopsis invicta* virus identifiable by a primer selected from the group consisting of SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, and SEQ ID NO 33.

2. A purified *Solenopsis invicta* having SEQ ID NO 1.

3. A biocontrol composition comprising:

- a. an effective amount of a *Solenopsis invicta* virus 3 preparation to at least reduce the number of fire ants in a colony, and
- b. a carrier.

4. The composition of claim 3 wherein said carrier is a food source for said ants.

5. The composition of claim 4 wherein said food source is selected from the group consisting of insects, cooked egg yolk, corn cob grits, soybean oil, extruded corn pellets, and mixtures thereof.

6. The composition of claim 3 wherein said virus is identifiable by a primer selected from the group consisting of SEQ ID NO 2, SEQ ID NO 3, SEQ ID NO 4, SEQ ID NO 5, SEQ ID NO 6, SEQ ID NO 7, SEQ ID NO 8, SEQ ID NO 9, SEQ ID NO 10, SEQ ID NO 11, SEQ ID NO 12, SEQ ID NO 13, SEQ ID NO 14, SEQ ID NO 15, SEQ ID NO 16, SEQ ID NO 17, SEQ ID NO 18, SEQ ID NO 19, SEQ ID NO 20, SEQ ID NO 21, SEQ ID NO 22, SEQ ID NO 23, SEQ ID NO 24, SEQ ID NO 25, SEQ ID NO 26, SEQ ID NO 27, SEQ ID NO 28, SEQ ID NO 29, SEQ ID NO 30, SEQ ID NO 31, SEQ ID NO 32, and SEQ ID NO 33.

7. A biocontrol method comprising:

- a. spreading a composition of claim 3 at or near a fire ant colony.

8. The biocontrol method of claim 7 wherein said composition is the composition of claim 4.

9. The biocontrol method of claim 7 wherein said composition is the composition of claim 5.

10. 10. The biocontrol method of claim 8 wherein said composition is the composition of claim 6.

1 ttttaaaata ggaataataa gtccagtaag gttactggca tttctatitt aactcaaagc
 61 cttccgattc gggtttgcga gaatcaacgc catgtctgaa aagacacaaa ctttcgttca
 121 aaatgaaact catgttttag acatgacttc tgatttttaa tcagacttat cacttgaaaa
 181 agtgacttca tcagttgaac aaactgatga cttagttagt aaatcatta ataataatga
 241 cttagatatt aaggatcttt ctttttaag gaacttactt ttaagtactt tgcaatatatt
 301 aggtattgct aaatttgtag ctattaatat tactttaagt atcttaagta ttttaattgtt
 361 acttataaac tcttgtagta agtttactcg tattgttaat ttaagtagtc atattttaaa
 421 tattataact actttaggct tatacttcca ggatctctct atggagattg aagaaataac
 481 ccaaaacttt gaaaatgaat tcggaactta tgatgatgat aaaattttat ctactatat
 541 taaaatttgt aacttaccta atcgtaagaa tgtatatgaa tatatatctt taaatgattt
 601 aaaatataa ataaaacttc cggatatctt tttttatgaa cttaaaaatg atatactttc
 661 aaaaaataa aatttacact tatggatttt ccaaaaattt actgatgaat ttcttgctat
 721 gtggtttggt gttcaacctt atcgatttc aaatcttcgt gaaatgttag taatatctcg
 781 tcaaggattt attcctaaag atttatttaa tgaatttcga aaattatga atatgggtgt
 841 ttcgtttata atttcttca ttcaatctaa attatttgat gaaccttta aaaagagaga
 901 ttgtacccaa gctttaaaag atgcttctgt tatctcttct ccatttgata ctctctggaa
 961 tcttatttct aaacaagttt gtgataattc tgctgaagag cgatttactc aaactatatt
 1021 ggatttttaca tctgaatttg ataatttctt tggatttcca aattataaat ttgctaaaaa
 1081 tcaaaaattg gtaaatacta tttctaatac tcttgatgct tgtgctaaat ttattcgtga
 1141 ttgtcctaaa gataaacaaa ctgaaaattt tcctcttcaa ggattacata ctgcaacagt
 1201 taaacgtcgt aatgaaaatt taactaatgt tatgccaaa tttgctcgtc aggaaccatt
 1261 tgttgttctt tttcaaggac ctggtggtat tggtaaaact catcttggtc aacaattggc
 1321 tactaaatgt gtaaatctt tttatcaga tcacgaagat gattatatgg aaatttcacc
 1381 tgatgatataa tattggcctc ctcttcttgg acaacgtgtt gcattttttg atgaagctgg
 1441 taatttgaat gatttaactg aggatcttct tttcagaaat attaaaaagta ttgttcacc
 1501 tgcataattt aattgtgctg ctgctgatat agaacataaa atttcccat gtccatttga
 1561 attagtgtt gcaactgtaa atactgattt agatactctt caatctaaaa tttcttcaac
 1621 atttggtcaa gcatctgtt ttccaatttg gcgtcgttg atgtgttcag agtgttcag
 1681 gaatgaaaaa gaattgggac cttttaatta taaaaatcct tctggtcac gtcttgatta
 1741 tagtcatatt actatgaatt atatgtcata tgatgataaa actcaaaaat tggctttaga
 1801 aaaagaaatt aattttgata ctttgtttga tatgattcgt ttaagattta gaaagaaaca
 1861 acaagaacat gatactaaaa tttctattct taataatgaa attcaacgtc aatctaattc
 1921 taaacaacat ttttctgtat gtttatatgg tgaacctggg caaggtaaaa catataatct
 1981 taataaatta ataacaactt ttgcaaatgc aactaatctt aaaattggat cagaggaaaa
 2041 accttctatt catatttttg atgattatat taaagatgaa aaatgatgaa attgtcttaa
 2101 atttatggat atttataata ataaattacc taataattct gtaatatatt ctgcaactaa
 2161 tgtttatcct aaaactcatt tctttccaac atttttctta actaatttaa tatatgcttt
 2221 tattcaacct tttaaacaag ttggacttta tcgcagattg ggatttgatg gttatactga

Fig. 1A

Fig. 1B

2281 tattcctaatt tcttctgtta atgctccaat ttttgtacaa aattttaaat tttatgaacg
 2341 taaacaacat atttgttatt ttctttccct tgaatttctt aaaaataataa tttgttatat
 2401 cttcttcttt ttatacttcc cgtaaatt tataaagaaa attgatttaa ttgaaataaa
 2461 ggatgtgaac aaatatgttt atgatatgata tataaacctt ttatctcttt ctaaaacaaat
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 2641 taataaaaaat tcctatgaaa atcttttgca ttttgattgg aaatgtatc tttcacctag
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Fig. 1C

4561 aggaacaata attaagcatt ctggtgataa attttatgga agcgtaaaac attctcatac
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 5461 aatgaaagaa ttatatcgag taactaaaaa gagtgatgtt ataaatttag attcaaacgg
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Fig. 1D

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Fig. 1E

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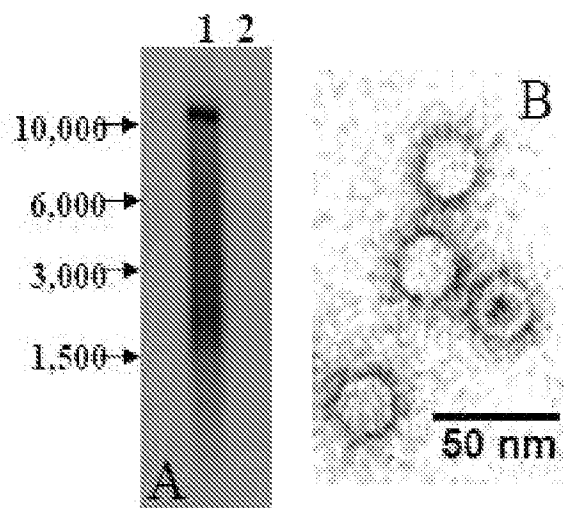


FIG. 2

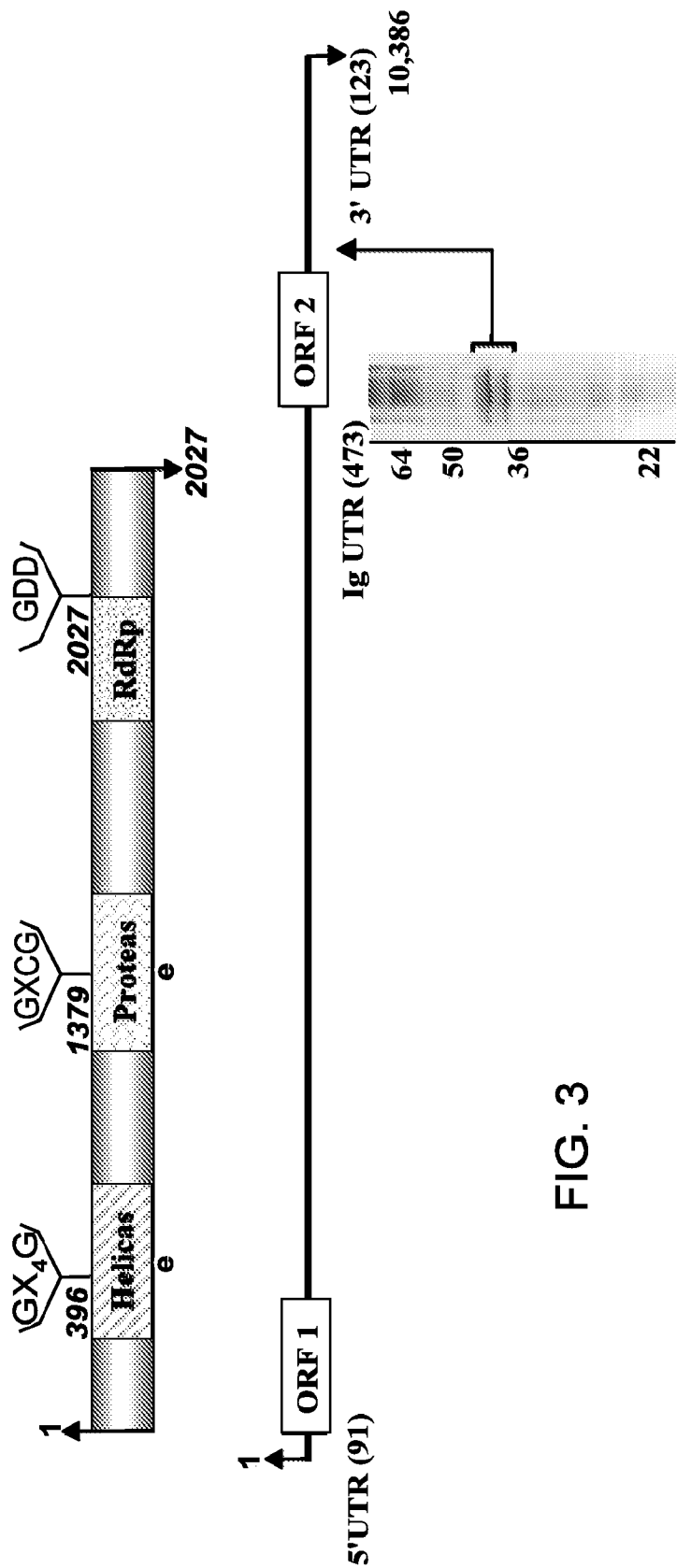
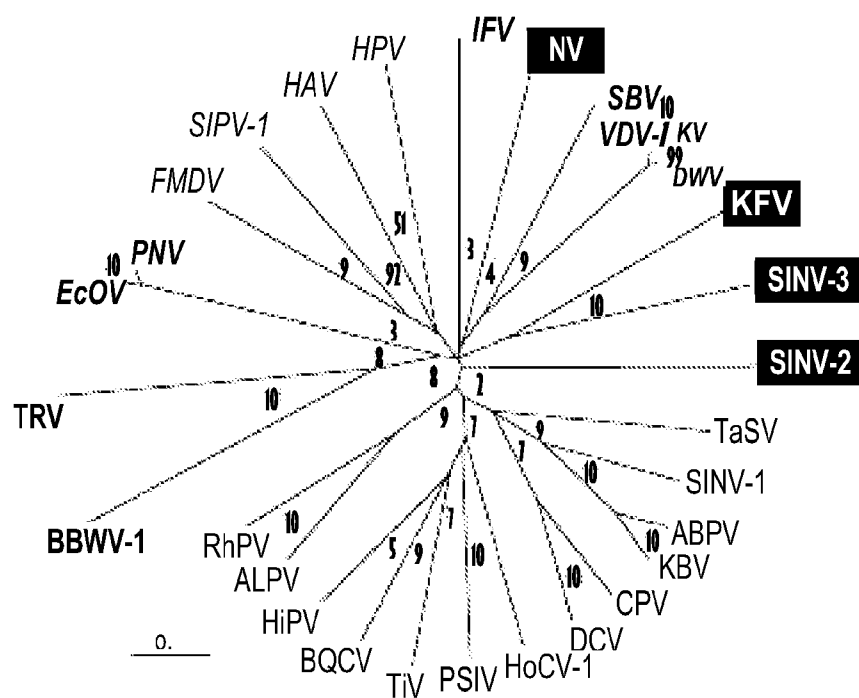


FIG. 3

	I	II	III	IV
KFV	3015	IKDEKLIKQKT..YKGEQFSAADFLLLILEPKYLGQFLAKAVK...YDKETAVGMDPIIDFHE-13-VDFVSWKKIPA		
SINV-3	1848	MEMEKLKPSN..FIPRFFTAQDLNGVLMERNILGEFTARALA...WDENCAVGCNPIYATFK-13-QDYKNFDRITPK		
SINV-2	1815	RFINKVLGDETTPTTRSVTCMNYYIILAMERYTMRPFMSAMHRAADGTSMTGPGINPEGPEWS-14-PDVSNNWDGFLFA		
SINV-1	1052	IKDERRP IELVDALKTRVFSNGPMDEFNFAFRKYFLGFLAHLMENR.IDNEVAIGTINVYSRDWT-15-GDFSNFDSLSNA		
HAV	1904	PKDELRLP LENVLESKTRAI DACPLDYSILCRMYGCPAISYFHLNPGFHTGVAIGCIDPDQRQWDE-14-IDFSAFDASLSLP		
		V	VII	VIII
KFV	3140	NGTMAAGCVATAPLNSVINNFLM-25-QISYGDDKWI ST.D..LDWTFQMV1-26-PLDQISLISRYPR...KLPSCV		
SINV-3	1973	HGCMFSCCVPTAPLNSKVNDIMI-27-RLFYGDDVILAVDDSIADIFACQ1-26-TIETLSFISFFRP.LKHQENF		
SINV-2	1951	SRGITSGFPCTAEVNTLAHILLI-24-AILYGGDILLTIHDDILHLFAGK1-25-PLSQCCFLKSSWR...QLLPGY		
SINV-1	1184	THSQPSCNPATITPLNCLINSIGL-36-LISYGDDNVIINIHP LISHLFAMM1-27-TLEEVSF LKRGFIFN..EERN C		
HAV	2035	CGSMFSCSPCTADLNSIHNNVNL-22-..CYGDDVLI VFSR.DVQIDNLDL-27-PVSELTFLKRSFN...LVEDR		

FIG. 4



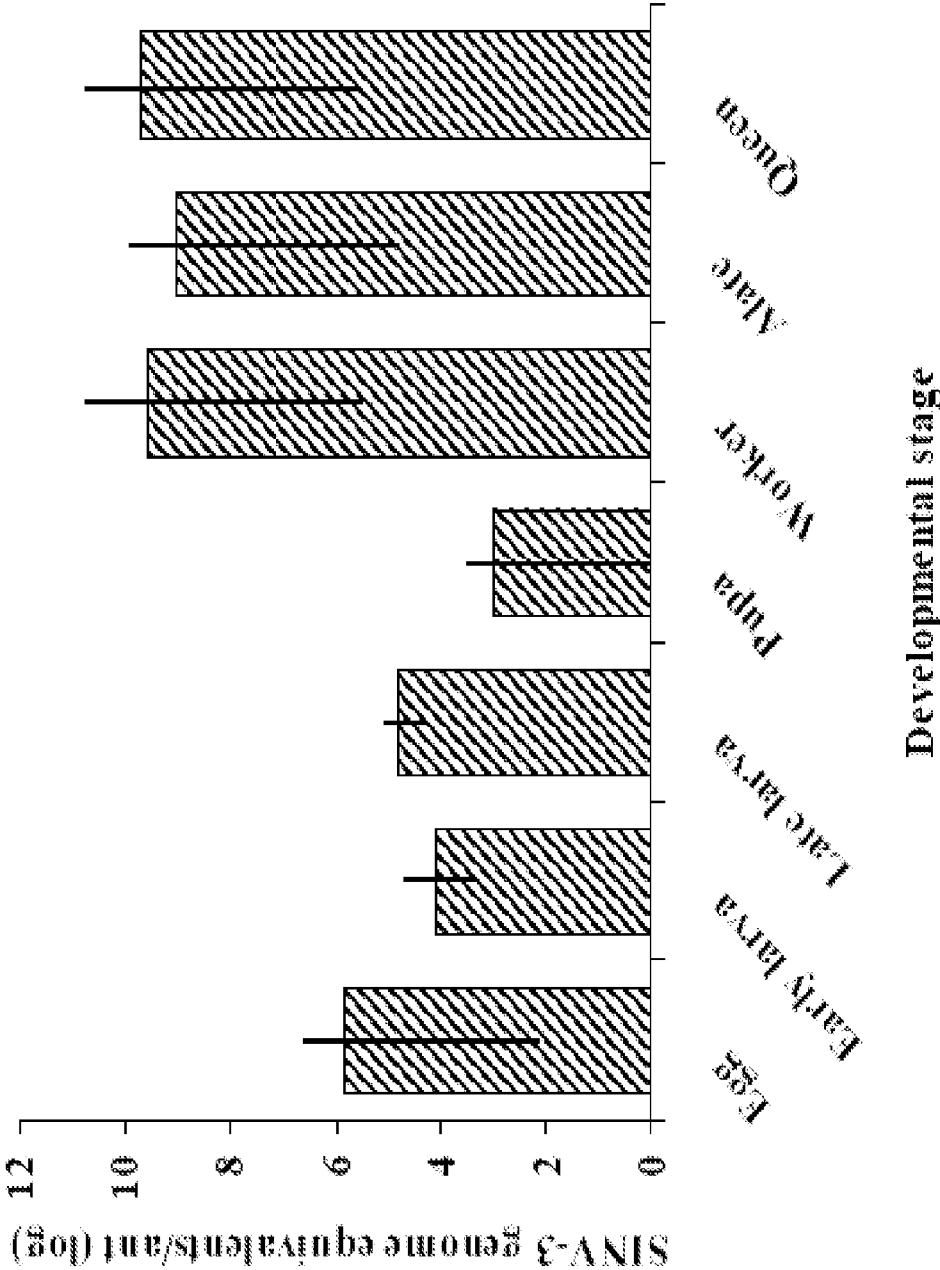


FIG. 6

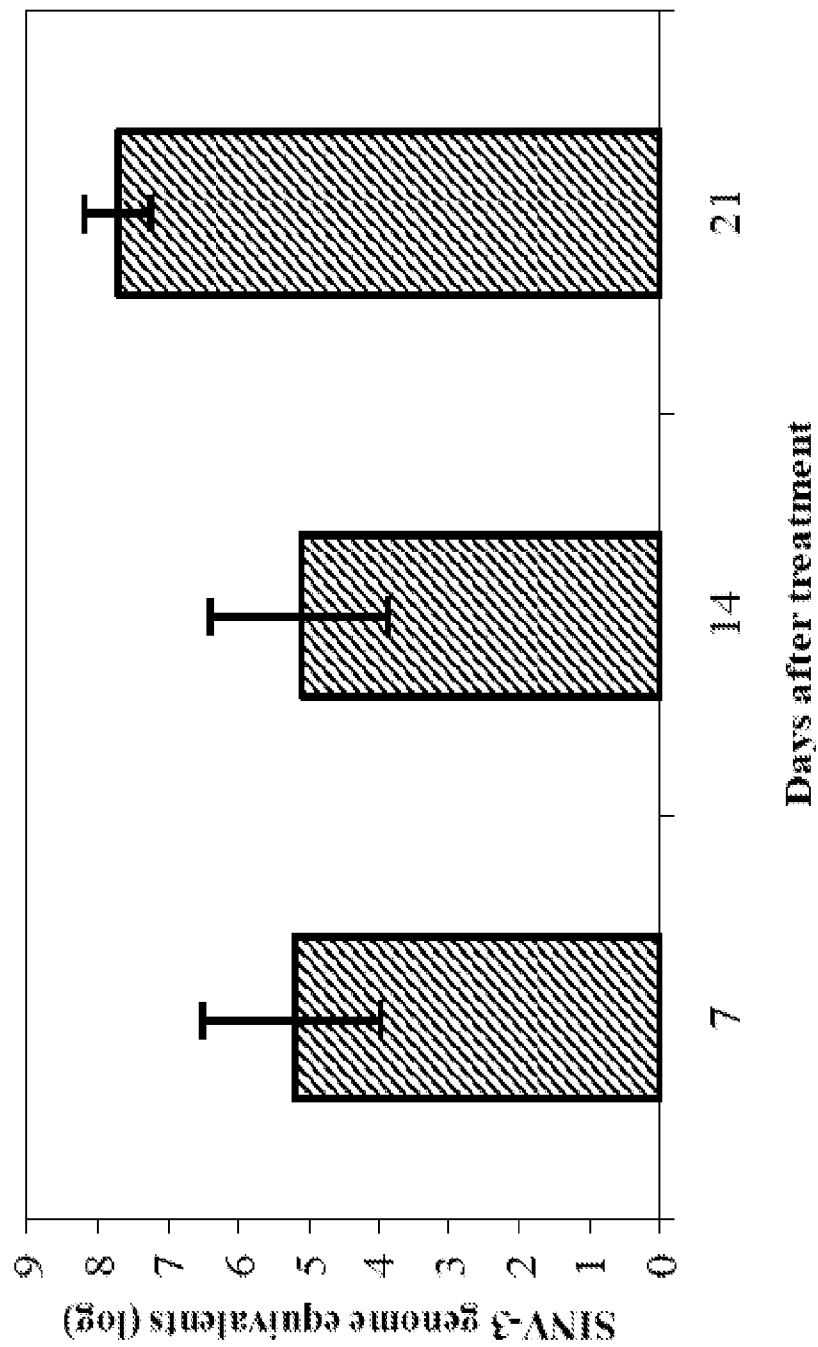


FIG. 7

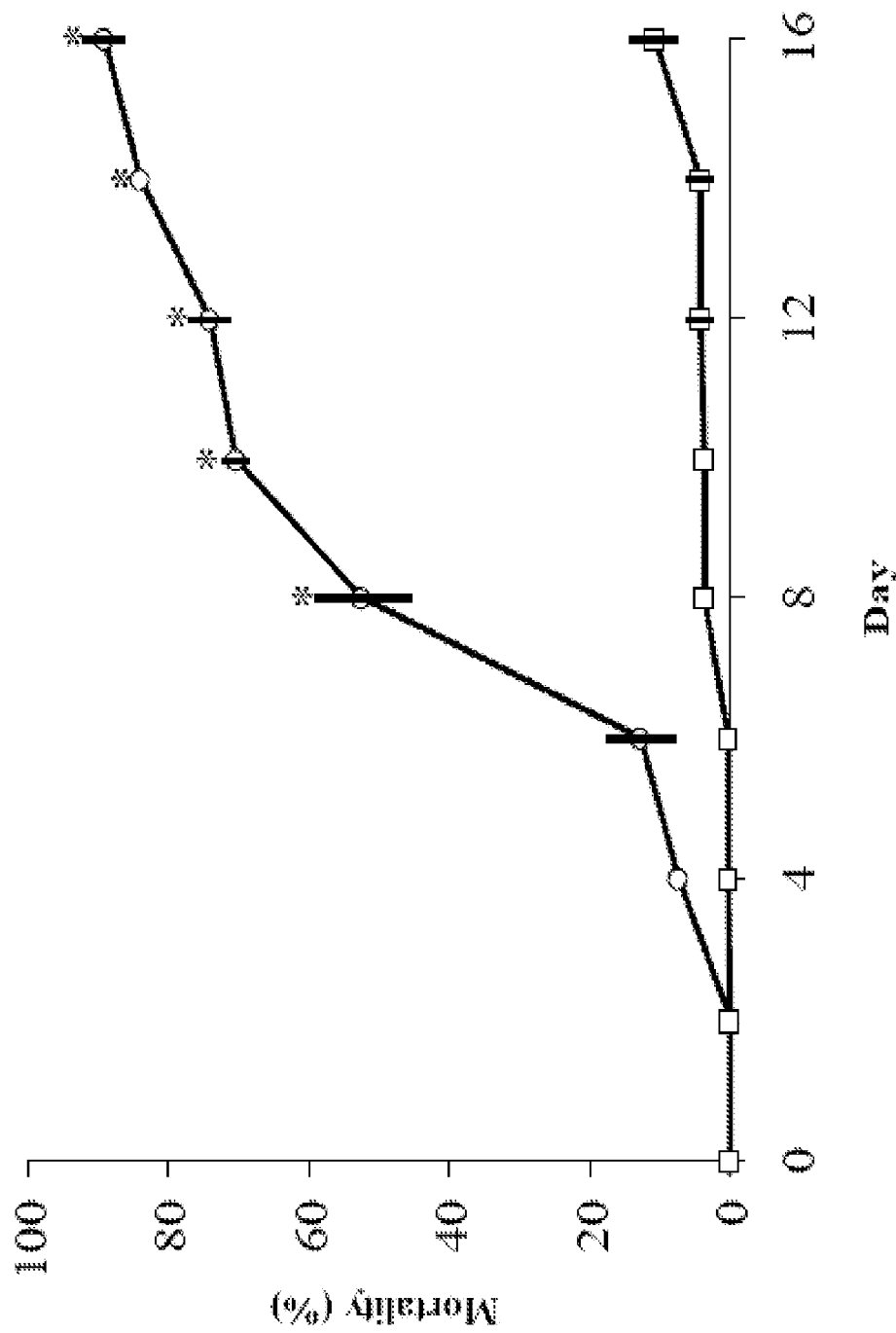


FIG. 8