The *Journal of Cotton Science* 5:114-120 (2001)
http://journal.cotton.org, © The Cotton Foundation 2001

MOLECULAR BIOLOGY

Pink Bollworm Larvae Infection with a Double Subgenomic Sindbis (dsSIN) Virus to Express Genes of Interest

John J. Peloquin,* Thomas A. Miller, and Stephen Higgs

INTERPRETIVE SUMMARY

Pest control strategies that rely on genetic manipulation of pest insects depend on proper expression of foreign genes in the pest, as well as on experimental evaluation of genes introduced by transformation with various chimeric fusion constructs, following the work of Thomas et al. (2000). The evaluation of such constructs relies heavily on detection of gene products and demonstration of their effects *in vivo*.

A dependable genetic expression system that reliably can direct production of heterologous or engineered gene products at high levels would be of significant value to establish the presence and activity of the foreign proteins that are to be encoded in these constructs. It is also helpful to elucidate the biological effects of any engineered proteins in intact organisms. To this end, we report the expression of the heterologous gene, green fluorescent protein, from a viral vector in a major lepidopteran pest of cotton.

The double subgenomic expression vector in this study was based upon the Sindbis (SIN) alphavirus. These dsSIN viruses have been used to express a spectrum of genes: chloramphenicol acetyltransferase (Olson et al., 1994; Kamrud et al., 1995; Kamrud et al., 1997); luciferase (Johnson et al., 1999), a toxin gene from *Androctonus australis* Hector (Higgs et al., 1995a); green fluorescent protein from *Aequorea aequorea* Forskal (Higgs et al., 1996; Myles et al., 1999; Higgs and Lewis, 2000; Olson et al., 2000); and various heterologous viral genes (Olson et al., 1994; Powers et al., 1995; Higgs et al., 1998; Kamrud et al., 1998). The dsSIN viruses replicate in numerous cell lines (Powers et al., 1994; Gaines et al., 1996) and in species of mosquitoes [*Aedes aegypti* L., *Ae. triseriatus* Say, *Anopheles gambiae* Giles, *An. stephensi* Liston, *Armigeres subalbatus* Coquillett]; in Lepidoptera, buckeye butterfly (*Precis coenia* Hübner) and eastern tiger swallowtail (*Papilo glaucus* L.); Coleoptera, the red flour beetle [*Tribolium castaneum* Herbst]; Hemiptera, the large milkweed bug (*Oncopeltus fasciatus* Dallas); Crustacea, brine shrimp (*Artemia franciscana* Kellog) (Lewis et al., 1999). Proteins encoded by these viral genes are biologically active in mosquitoes and, when expressed in antisense orientation, can confer pathogen-derived resistance to prevent heterologous virus transmission by the vector mosquito (Olson et al., 1994; Powers et al., 1994; Higgs et al., 1998).

Although Sindbis infects and is naturally transmitted by mosquitoes, for this study it was speculated that this system might be useable to drive expression of various heterologous genes in the pink bollworm [*Pectinophora gossypiella* Saunders, Lepidoptera: Gelechiidae]. If so, genes could be tested prior to incorporation into complex and labor-intensive transformation experiments (Peloquin et al., 2000). This study reports the use of the virus TE/3'2J/GFP to infect and express green fluorescent protein in larvae of the pink bollworm. Though dsSIN viruses cannot themselves be employed as biological control agents, based upon the success of our feasibility study, we suggest that this virus is applicable to test the expression of more biologically relevant genes that may be involved in novel pest control strategies. Finally, due to regulatory and other concerns associated with production and containment of transgenic organisms, use of these viral expression constructs allows study of candidate genes for consideration of the production of transgenic insects without actually producing transgenic insects.

J.J. Peloquin and T.A. Miller, Dep. of Entomology, Univ. of California, Riverside, CA 92521, and S. Higgs, Dep. of Pathology and WHO Collaborating Cent. for Tropical Diseases, Univ. of Texas Medical Branch, 301 University Blvd., Galveston, TX 77555-0609. Received 12 Oct. 2000. *Corresponding author (peloquin@faculty.ucr.edu).

ABSTRACT

Genetic transformation of insects depends on clear expression of marker genes for selection and effective expression of heterologous proteins. An established framework of genetic studies is helpful in the design of reliable transforming constructs. The background these studies provide is most often not available for insects other than *Drosophila*. Accordingly, a reliable method to test simply the design of expression constructs would be of great value. This study shows that the Sindbis double subgenomic viral vector, which has been engineered successfully to direct mosquito cells to express green fluorescent protein, also will direct the expression of green fluorescent protein in vivo in pink bollworm larvae [*Pectinophora gossypiella* Saunders, Lepidoptera: Gelechiidae]. Expression of green fluorescent protein was not uniform within all tissues of the infected larvae, and certain tissues expressed fluorescence more strongly than others did. Additionally, expression was much greater in larvae than in adult tissues. Such viral constructs drive expression of heterologous proteins in a rapid and dependable manner and in a wide range of species. These properties can be used to evaluate the integrity of protein-expressing constructs before any labor-intensive transformation efforts are undertaken. Finally, as these virus-infected insects are not transgenic, the extensive containment necessary for transgenic arthropods is not needed.

Genetic modification of insect pests is now possible and under development for use in sterile insect technique and other pest control strategies. Proper expression of foreign genes from transforming DNA constructs in the pest insect and the experimental evaluation of the genes introduced by transformation with various chimeric fusion constructs (Thomas et al., 2000) are central to any such use of genetic technology. Presently, the evaluation of such constructs relies on detection of gene products and demonstration of their effects *in vivo*, but only after a laborious transformation process that itself is subject to quarantine precautions consistent with production of a transgenic organism. A dependable system for genetic expression that reliably can direct production of heterologous or engineered gene products at high levels without requiring genetic transformation of an animal, and hence elaborate and expensive regulatory and containment procedures, would be of significant value. If this system could be achieved in an intact insect, it would allow study of expression at the organismic level, rather than merely at the cellular or tissue level. This study demonstrates such a system, reporting green fluorescent protein expression from a viral vector in a major cotton pest.

The double subgenomic expression vector in this study was based upon the Sindbis (SIN) alphavirus. Such viruses have expressed a spectrum of genes: chloramphenicol acetyltransferase (Olson et al., 1994; Kamrud et al., 1995; Kamrud et al., 1997); luciferase (Johnson et al., 1999), a toxin gene from *Androctonus australis* Hector (Higgs et al., 1995a); green fluorescent protein from *Aequorea aequorea* Forskal (Higgs et al., 1996; Myles et al., 1999; Higgs and Lewis, 2000; Olson et al., 2000); and various heterologous viral genes (Olson et al., 1994; Powers et al., 1995; Higgs et al., 1998; Kamrud et al., 1998). The dsSIN viruses replicate in numerous cell lines (Powers et al., 1994; Gaines et al., 1996) and in species of mosquitoes [*Aedes aegypti* L., *Ae. triseriatus* Say, *Anopheles gambiae* Giles, *An. stephensi* Liston, *Armigeres subalbatus* Coquillett]; in Lepidoptera, buckeye butterfly (*Precis coenia* Hübner) and eastern tiger swallowtail (*Papilo glaucus* L.); Coleoptera, the red flour beetle [*Tribolium castaneum* Herbst]; Hemiptera, the large milkweed bug (*Oncopeltus fasciatus* Dallas); Crustacea, brine shrimp (*Artemia franciscana* Kellog) (Lewis et al., 1999). Proteins encoded by these viral genes are biologically active in mosquitoes and, when expressed in antisense orientation, can confer pathogen-derived resistance to prevent heterologous virus transmission by the vector mosquito (Olson et al., 1994; Powers et al., 1994; Higgs et al., 1998).

Proteins encoded by these viral genes are biologically active and present a good test of the activity of a particular heterologously expressed protein. Sindbis infects and is naturally transmitted by mosquitoes. For this study it was speculated that this system could drive expression of various heterologous genes in the pink bollworm and let us test genes prior to committing to complex and labor-intensive transformation experiments (Peloquin et al., 2000). This study reports that virus TE/3'2J/GFP infects and expresses green fluorescent protein in larvae of the pink bollworm. Though dsSIN viruses cannot themselves be employed as biological control agents, this study suggests use of this virus to test

the expression of more biologically relevant genes related to novel pest control strategies. Finally, due to the regulatory and other concerns associated with production and containment of transgenic organisms, these viral expression constructs allow study of candidate genes for production of transgenic insects without actually producing transgenic insects, which are subject to substantial quarantine and regulatory constraints.

MATERIALS AND METHODS

C.M. Rice of the University of Washington (St. Louis, MO) supplied the vector pTE/3'2J. TE/3'2J/GFP virus was produced by insertion of the green fluorescent protein gene (ClonTech, Palo Alto, CA) into this DNA vector (Higgs et al., 1996). Pink bollworm larvae were inoculated with approximately 0.5 to 1.0 mL virus suspension injected into the larval hemocoel with glass capillary needles and an apparatus, as described by Rosen and Gubler (1976). L-15 medium (BioWhittaker, Walkersville, MD) was inoculated into control larvae. Immediately after inoculation, larvae were returned to an artificial diet and maintained at 28°C within a biosafety level-3 containment laboratory (BSL-3, NIH online at http://bmbl.od.nih.gov/sect3bsl3.htm). Samples of larvae were removed each day and examined for fluorescence, using either an Olympus IMT-2 fitted with fluorescein isothiocyanate (FITC) filters (Sigma Chemicals, St. Louis, MO), or an SZX stereomicroscope (Olympus, Melville, NY) fitted with a green fluorescent protein filter set. Images were recorded on 100ASA Kodak Elite slide film. Developed slides were scanned electronically to produce plates for publication. In addition to experimental and control larvae, numerous uninoculated larvae were examined (both whole and dissected) to determine whether natural autofluorescence occurred.

To determine whether virus had replicated in larvae, individual larvae were first screened for fluorescence as described above, then designated as *negative*, *weak positive*, or *strong positive*. Viral assays were performed as described by Higgs et al. (1997). Each larva was triturated in 1 mL of Leibovitz L-15 medium, filtered through a 0.2-µm syringe filter, then diluted in 10-fold steps in a 96-well tissue culture plate (Corning. Corning, NY). A suspension of Vero cells was added to each well, and the plate was incubated 7 d at 37°C. Wells were examined and scored for cytopathic effect. Virus was quantified in this endpoint assay as the tissue culture infectious dose 50% endpoint (TCID50) following Rhodes and Van Rooyen (1953, p. 65-68). Viral titer was expressed as $\log_{10}$TCID50 mL^{-1}.

RESULTS

Green fluorescent protein expression was first seen in larvae examined 72 h following inoculation. The distribution of green fluorescent protein increased with incubation time but was restricted to certain organ types. No green fluorescent protein was observed in either control or uninfected larvae. Although we could not quantify green fluorescent protein expression, we did grade levels on an arbitrary scale of brightness from weak to strong. Green fluorescent protein expression, cytopathology, and virus titer results that describe the treatments are compared in Table 1. The virus titer is a representation of the amount of virus present in the injected larva at 10 d post-inoculation.

The relative level of green fluorescent protein expression was correlated with virus titer and an elevated cytopathology index, which demonstrated that the expression of green fluorescent protein was dependent upon the titer to which the virus replicated. As is seen in Table 1, virus did not replicate equally in all larvae.

Green fluorescent protein expression in injected larvae was extensive, though more concentrated in certain tissues. The larval ventral nerve tract can be seen brightly fluorescing with green fluorescent protein (Fig. 1a) along with a punctate distribution of fluorescence in epidermis-associated tissues and what appears to be a fat body (Fig. 1c). The hind gut and Malpighian tubules were also highly fluorescent (Fig. 1b). Because no green fluorescent protein was seen in uninfected or L-15 inoculated larvae, these are not shown in the figure; however, nonfluorescent tissues can be seen as the dark background in all figures.

Fluorescence of green fluorescent protein in pupae was not obvious, if present at all. We also did not see fluorescence typical of that expected for green fluorescent protein expression in the adults, even when dissected. For comparison, the flight muscles of Culicidae were high expressors of green fluorescent protein, whereas in pink bollworm, we

Table 1. Treatment and virus titer results compared. The virus titer is a representation of the amount of virus present in the injected larva at 10 d post-inoculation.

Treatment	Virus titer (logs) (TCID50)
Virus injected and strong green fluorescent protein	7.0
Virus injected and good green fluorescent protein	6.0
Virus injected and moderate green fluorescent protein	6.5
Virus injected and weak green fluorescent protein	6.0
Virus injected and weak green fluorescent protein	5.5
Virus injected and no green fluorescent protein	0.0 (uninfected)
Virus injected and no green fluorescent protein	5.0
Virus injected and no green fluorescent protein	3.5
Pupa from injected larva and no green fluorescent protein	0.0 (uninfected)
Pupa from injected larva and green fluorescent protein	6.0
Uninfected (L-15 inoculated)	0.0
Uninfected (L-15 inoculated)	0.0

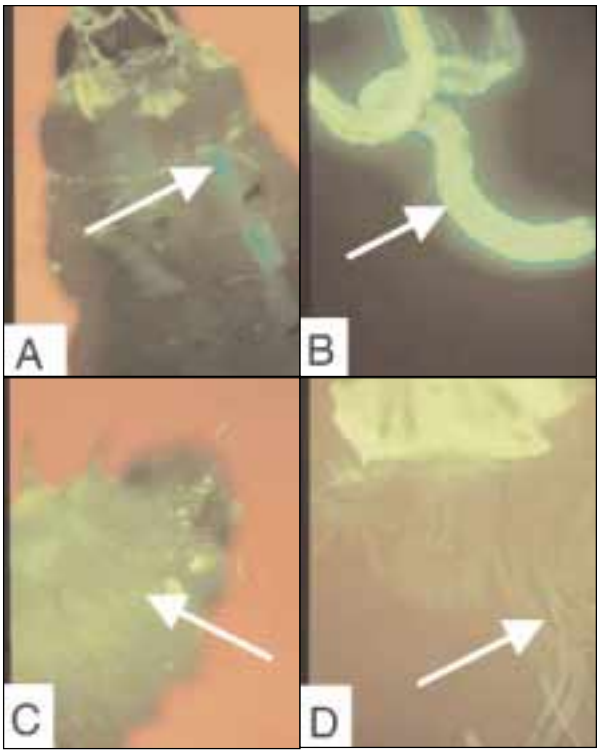

Figure 1. Digitized slide photographs of fourth instar pink bollworm larvae and dissected adults illuminated under conditions for viewing green fluorescent protein. Plates A and C are shown with red background to provide better contrast. Plate A shows green fluorescent protein expression in the anterior of a larva viewed ventrally. The white arrow points to the ventral nerve cord, the tissues thereof strongly express green fluorescent protein. Plate B shows larval Malphighian tubules and gut (the latter marked by a white arrow), both strongly expressing green fluorescent protein. Plate C is a ventral view of the anterior of a larvae that exhibits green fluorescent protein expression. A white arrow points to an area of punctate green fluorescent protein expression. Plate D shows a dissected adult flight muscle attached to a piece of integument. Note that flight muscle does not express green fluorescent protein. The arrow in this plate is pointing to a dissected muscle fiber.

saw no such expression, even in dissected flight muscle (Fig. 1d). The insects were killed before they could reproduce, so there was no examination for transovarial transmission of virus or expression of green fluorescent protein in offspring.

DISCUSSION

This study further demonstrates the likely universal utility of Sindbis virus-derived vectors for driving expression of heterologous proteins in a wide range of organisms, including intact insects. Though Sindbis virus originally was isolated from mosquitoes (Hurlbut, 1953; Taylor et al., 1955), this study shows that a vector derived from this virus is usable in Lepidoptera. Though both Diptera and Lepidoptera are holometabolous orders, they are highly divergent and probably have not shared a common ancestor for at least 245 million yr; insects identifiable as Diptera have been reported from upper Permian fossil strata (Riek, 1977). Additionally, Sindbis virus is infective in mammalian and other vertebrate cells (Frolov et al., 1999), which suggests the utility of Sindbis-derived vectors for driving expression of heterologous proteins in a wide host range of insects and other animals.

Infection with Sindbis virus-derived vector and subsequent expression of heterologous green fluorescent protein was accomplished by intrahemocoelic injection. Expression of green fluorescent protein was seen in larval tissues of pink bollworm injected as larvae, but not in tissues of adults that had been injected as larvae. Presumably, at the time of injection all larval tissues were bathed with hemolymph and, thus,

should have been available to infection with the high titer virus injected into the hemolymph. Yet, the study results show that certain tissues were more fluorescent than others were. A number of reasons may contribute to this pattern of expression. The virus may have a greater tropism for certain tissues (Bowers et al., 1995). It may be that protein expression overall in adult pink bollworm is at a relatively low level, and that cellular resources needed to produce visible amounts of the heterologous proteins were simply unavailable in the adult insect. The promoters or other elements of the vector may not have been active in the adult-stage pink bollworm. It may be most likely that imaginal disk tissues present in the larva at the time of injection may not have been accessible or susceptible to infection with the virus, so no adult structures would be observed to express the virus or green fluorescent protein. For whatever reason, green fluorescent protein expression from the Sindbis-derived vector demonstrated marked variation, although green fluorescent protein expression was seen easily in larvae injected with the green fluorescent protein-encoding virus.

As seen in titration data, the virus replicated to different levels in different individuals sampled at the same time post-inoculation, probably owing to variability in the initial site of infection. Although virus was inoculated into the thoracic region, the tissues in which the infection became established might have varied. Tissues differ in susceptibility to infection; so if, for example, nerve tissue predominantly were exposed, one might anticipate a more productive infection to result than if connective tissue were targeted. Expression of green fluorescent protein was correlated with the titer to which virus replicated, and there seemed to be a threshold effect (5 $\log_{10}$ TCID50/mL^{-1}) below which green fluorescent protein was not detectable.

In inoculated larvae that failed to develop a productive infection, no green fluorescent protein fluorescence was observed. A similar threshold effect has been observed in which Scotox protein was not expressed at lethal concentrations until virus titer reached 5.5 $\log_{10}$ TCID50/mL^{-1} (Higgs et al., 1995b). For safety reasons, the number of infected larvae were minimized in this study by using L-15 medium for controls rather than using a non-green-fluorescent-protein dsSIN virus. From numerous experiments it is known that such viruses are never associated with natural fluorescence. Therefore, the authors are confident that the fluorescence described was expressed as a result of infection with the TE3’2J/GFP virus.

Though injecting pink bollworm with virus was relatively simple and presented little technical difficulty, it also may be possible to infect larvae through simple oral administration of the virus (Higgs et al., 1999). This latter method would eliminate needle-stick hazards and reduce the effort involved in infecting pink bollworm larvae to simply spiking a small amount of artificial diet with a sufficient amount of virus, then putting larvae on a piece of diet so treated. If such oral administration of viral vector were successful, it would be interesting to see how similar the expression patterns of heterologous proteins would be between larvae infected by oral administration vs. injection.

ACKNOWLEDGMENT

Supported by grants from the California Cotton Pest Control Board. All work was performed under the guidelines and regulations of the authors institutions and governmental regulatory agencies.

REFERENCES

Bowers, D.F., B.A. Abell, and D.T. Brown. 1995. Replication and tissue tropism of the alphavirus Sindbis in the mosquito *Aedes albopictus*. Virology 212:1-12.

Frolov, I., E. Agapov, T.A. Hoffman Jr., B.M. Prágai, M. Lippa, S. Schlesinger, and C.M. Rice. 1999. Selection of RNA replicons capable of persistent noncytopathic replication in mammalian cells. J. Virol. 73:3854-3865.

Gaines, P.J., K.E. Olson, S. Higgs, A.M. Powers, B.J. Beaty, and C.D. Blair. 1996. Pathogen-derived resistance to dengue type 2 virus in mosquito cells by expression of the premembrane coding region of the viral genome. J. Virol. 70:2132-2137.

Higgs, S., and D.L. Lewis. 2000. The green fluorescent protein marker for transgenic insects. p. 93-108. *In* A.M. Handler and A.A. James (ed.) Insect transgenesis: Methods and applications. CRC Press, Boca Raton, FL.

Higgs, S., K.E. Olson, P. Gaines, A. Powers, C. Blair, J. Carlson, and B.J. Beaty. 1995a. Pathogen-derived resistance in mosquito cells by expression of the PrM Coding regions of dengue-2 and yellow fever 17D virus in sense and antisense orientations. *In* Keystone Symp. Toward the Genetic Manipulation of Insects, Tamarron, CO. 17-23 Mar. 1995. J. Cell. Biochem. Suppl. 0(21A):223.

Higgs, S., K.E. Olson, K.I. Kamrud, A.M. Powers, and B. Beaty 1997. Viral expression systems and viral infections in insects. p. 459-483. *In* C.B. Beard and C. Louis (ed.) The molecular biology of disease vectors: A methods manual. Chapman and Hall, London.

Higgs, S., K.E. Olson, L. Klimowski, A.M. Powers, J.O. Carlson, R.D. Possee, and B.J. Beaty. 1995b. Mosquito sensitivity to a scorpion neurotoxin expressed using an infectious Sindbis virus vector. Insect Mol. Biol. 4:97-103.

Higgs, S., C.T. Oray, K. Myles, K.E. Olson, and B.J. Beaty. 1999. Infecting larval arthropods with a chimeric, double subgenomic Sindbis virus vector to express genes of interest. Biotechniques 27:908-911.

Higgs, S., J.O. Rayner, K.E. Olson, B.S. Davis, B.J. Beaty, and C.D. Blair. 1998. Engineered resistance in *Aedes aegypti* to a West African and a South American strain of yellow fever virus. Am. J. Trop. Med. Hyg. 58:663-670.

Higgs, S., D. Traul, B.S. Davis, K.I. Kamrud, C.L. Wilcox, and B.J. Beaty. 1996. Green fluorescent protein expressed in living mosquitoes without the requirement of transformation. Biotechniques 21:660-664.

Hurlbut, H.S. 1953. The experimental transmission of coxsackie-like viruses by mosquitoes. J. Egypt. Med. Assoc. 36:495-498.

Johnson, B.W., K.E. Olson, T. Allen-Miura, A. Rayms-Keller, J.O. Carlson, C.J. Coates, N. Jasinskiene, A.A. James, B.J. Beaty, and S. Higgs. 1999. Inhibition of luciferase expression in transgenic *Aedes aegypti* mosquitoes by Sindbis virus expression of antisense luciferase RNA. Proc. Natl. Acad. Sci. 96:13399-13403.

Kamrud, K.I., K.E. Olson, S. Higgs, and B.J. Beaty. 1995. Use of the Sindbis virus replicon system to express heterologous genes in C6/36 cells and whole mosquitoes. *In* Keystone Symposium. Toward the Genetic Manipulation of Insects, Tamarron, CO. 17-23 Mar. 1995. J. Cell. Biochem. Suppl. 0(21A): 230.

Kamrud, K.I., K.E. Olson, S. Higgs, J.O. Carlson, and B.J. Beaty. 1998. Use of the Sindbis replicon system for expression of LaCrosse virus envelope proteins in mosquito cells. Arch. Virol. 143:1365-1377.

Kamrud, K.I., K.E. Olson, S. Higgs, A.M. Powers, J.O. Carlson, and B.J. Beaty. 1997. Detection of expressed chloramphenicol acetyltransferase in the saliva of *Culex pipiens* mosquitoes. Insect Biochem. Mol. Biol. 27:423-429.

Lewis, D.L., M.A. DeCamillis, C.R. Brunetti, G. Halder, V.A. Kassner, J.E. Selegue, S. Higgs, and S.B. Carroll. 1999. Ectopic gene expression and homeotic transformations in arthropods using recombinant Sindbis viruses. Curr. Biol. 9:1279-1287.

Myles, K.M., K.E. Olson, R.C. Seabaugh, S. Higgs, J.O. Carlson, and B.J. Beaty. 1999. Development of a double subgenomic Sindbis virus that expresses green fluorescent protein in the midgut of *Aedes aegypti* following per Os infection. Am. J. Trop. Med. Hyg. 61:401.

Olson, K.E., S. Higgs, C.S. Hahn, C.M. Rice, J.O. Carlson, and B.J. Beaty. 1994. The expression of chloramphenicol acetyltransferase in *Aedes albopictus* (C6/36) cells and *Aedes triseriatus* mosquitoes using a double subgenomic recombinant Sindbis virus. Insect Biochem. Mol. Biol. 24:39-48.

Olson, K.E., K.M. Myles, R.C. Seabaugh, S. Higgs, J.O. Carlson, and B.J. Beaty. 2000. Development of a Sindbis virus expression system that efficiently expresses green fluorescent protein in midguts of *Aedes aegypti* following per Os infection. Insect Mol. Biol. 9:57-65.

Peloquin, J.J., S.T. Thibault, R. Staten, and T.A. Miller. 2000. Germ-line transformation of pink bollworm (Lepidoptera: Gelechiidae) mediated by the *piggyBac* transposable element. Insect Mol. Biol. 9:323-333.

Powers, A.M., K.I. Kamrud, K.E. Olson, S. Higgs, J.O. Carlson, and B.J. Beaty. 1995. Intracellular immunization of C6/36 mosquito cells and *Aedes triseriatus* mosquitoes to bunyaviruses using a Sindbis virus expression system. *In* Keystone Symposium on Toward the Genetic Manipulation of Insects, Tamarron, CO. 17-23 Mar. 1995. J. Cell. Biochem. Suppl. 0(21A):230.

Powers, A.M., K.E. Olson, S. Higgs, J.O. Carlson, and B.J. Beaty. 1994. Intracellular immunization of mosquito cells to LaCrosse virus using a recombinant Sindbis virus vector. Virus Res. 32:57-67.

Rhodes, A.J., and C.E. van Rooyen. 1953. Textbook of virology for students and practitioners of medicine. Williams and Wilkins Co., Baltimore, MD.

Riek, E.F. 1977. Four-winged Diptera from the upper Permian of Australia. Proc. Linnean Soc. N.S.W. 101:250-255.

Rosen, L., and D. Gubler. 1976. The use of mosquitoes to detect and propagate dengue viruses. Am. J. Trop. Med. Hyg. 23:1153-1160.

Taylor, R.M., H.S. Hurlbut, T.H. Work, J.R. Kingsbury, and T.E. Frothingham. 1955. Sindbis virus: A newly recognized arthropod-transmitted virus. Am. J. Trop. Med. Hyg. 4:844-846.

Thomas, D.D., C.A. Donnelly, R.J. Wood, and L.S. Alphey. 2000. Insect population control using a dominant, repressible, lethal genetic system. Science 287:2474-2476.