Susceptibility of Five Plant Defoliators to Celery Looper (Lepidoptera: Noctuidae) Nuclear Polyhedrosis Virus[1]

Walter Solomon, Lavone Lambert and M. R. Bell

U. S. Department of Agriculture, Agricultural Research Service
Southern Insect Management Laboratory
Stoneville, MS 38776 U.S.A.

J. Entomol. Sci. 31(3): 306-314 (July 1996)

ABSTRACT Bioassays were conducted to determine the susceptibility of five lepidopteran defoliators to a nuclear polyhedrosis virus (NPV) isolated from the celery looper, *Anagrapha falcifera* (Kirby). Tobacco budworm, *Heliothis virescens* (F.), exhibited the greatest susceptibility, LC_{50} = 2,328PIB/ml, and velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), exhibited the least susceptibility, LC_{50} = 5,176,038 PIB/ml, after 7 d of feeding at the 5×10^4 PIB/ml dosage. Corn earworm, *Helicoverpa zea* (Boddie), soybean looper, *Pseudoplusia includens* (Walker), and beet armyworm, *Spodoptera exigua* (Hübner), expressed intermediate levels of susceptibility, LC_{50} - 11,742, 14,195, 14,614 respectively, after 7 d of feeding at the 5×10^4 PIB/ml dosage. These results were consistent at days 10 and 14 indicating the relative activity of the virus against each species.

KEY WORDS Nuclear polyhedrosis virus, soybean, lepidopterous pests, susceptibility

Several lepidopteran insect species are serious pests of soybean, *Glycine max* (L.) Merrill, as well as other economically important field crops. Soybean meal and oil are the most produced, traded, and utilized meal and oil in the world (Smith and Huyser 1987). Five major lepidopteran pests of soybeans in the U. S. are soybean loopers, *Pseudoplusia includens* (Walker), velvetbean caterpillar, *Anticarsia gemmatalis* (Hübner), corn earworm, *Helicoverpa zea* (Boddie), tobacco budworm, *Heliothis virescens* (F.), and beet armyworm, *Spodoptera exigua* (Hübner) (Smith and Huyser 1987). Soybean looper and velvetbean caterpillar overwinter in the tropics and migrate northward during spring and summer reaching economic damage levels in many areas of the southern United States annually. Soybean looper is more resistant to insecticides than other soybean insect pest species and is difficult to control (Turnipseed 1973). Recent studies show that resistance to insecticides by this pest is increasing (Leonard et al. 1990). Beet armyworm shows a preference for seedling soybeans in that they feed on plant terminals and consume all foliage. New growth usually develops, but plant development is delayed (Dietz et al. 1976). In recent years, beet armyworm has become a pest on soybean fruit forms during the reproductive period. Tobacco budworm and corn earworm are primarily pod feeders; velvetbean caterpillar is primarily a foliage feeder.

[1] Received 03 March 1995; Accepted for publication 16 May 1996.

Chemical insecticides have undesirable side effects in the biotic environment and many are becoming ineffective due to development of resistance by insect pests, alternative control measures need to be developed. Control of soybean lepidopteran defoliators with NPVs has been attempted with fair success (Ignoffo et al. 1978, Livingston et al. 1980, Moscardi et al. 1981, Richter and Fuxa 1984). NPVs, particularly the multiple-embedded NPVs, have considerable potential for the control of economically-damaging insect pests (Falcon 1976, Estrada 1986, Huber 1986). In 1985, a multiple-embedded NPV was isolated from celery looper collected from cabbage in central Missouri (Hostetter and Puttler 1991). The purpose of this study was to determine the relative levels of activity of the celery looper NPV on five lepidopteran insect pest species.

Materials and Methods

All bioassays were conducted at the Southern Insect Management Laboratory, Stoneville, MS. Celery looper NPV was obtained from a previously purified stock solution provided by M. R. Bell, USDA-ARS, Stoneville, MS. Polyhedral inclusion body (PIB) concentrations were determined and quantified with a phase contrast microscope using a Neubauer hemacytometer (Vail et al. 1971, 1973). The stock solution was diluted with sterile distilled water to obtain five concentrations for testing: 0 (control, water only), 1×10^3, 5×10^3, 1×10^4, and 5×10^4 PIB/ml. The concentrations, or dosages of virus, were incorporated within a measured amount of insect diet by methods similar to those described by Dulmage et al. (1976). The virus dosages were dispersed in the diet by blending 10 ml of each stock solution in 290 ml of diet that was precooled to 45°C to obtain the final concentrations for testing listed above. Viral dosages were mixed within the media, instead of surface applied, because some insect species to be tested burrow into the diet when feeding and would be less exposed to a surface-applied virus. Diet consisted of a soyflour-wheat germ media with low agar content (King and Hartley 1985). Approximately 5 ml of diet was dispensed into each 30-ml plastic cup and the cups were covered with a sterile cloth. The cups were stored in plastic bags at room temperature under a laminar flow hood for 24 h to remove surface moisture on the diet which could trap and drown small larvae.

Insect eggs and diet, specific for each insect species, were supplied by G. G. Hartley, USDA-ARS, Stoneville, MS. Insect egg sheets were sterilized with a formaldehyde solution (3% active ingredient) for 10 min to eliminate contamination with other pathogens and placed in sterile flasks. The egg sheets were held in environmental control chambers at 26.5 ± 1 °C and 60-70% RH with a photoperiod of 14:10 (L:D) h (King and Hartley 1985). Upon hatching, one neonate larva was placed via a sterile artist paintbrush in each diet cup. Thirty-six insects (replicates) of each species were used for each dosage of virus. A randomized complete block (RCB) design was used with one cup of each treatment placed at random on a tray. There were 36 trays (blocks) within the environmental control chamber maintained at 26.5 ± 1°C and 60-70% RH with a photoperiod of 14:10 (L:D) h. Insects were allowed to feed ad libitum on the diet and were observed daily for mortality which was defined as the failure of an insect to respond to tactile stimulation and/or obvious lysis of the integument. Treatments had a factorial structure of five species by five dosages. Mortality was measured daily on the same 36 insects. The test was repeated three times. This bioassay was used to determine relative levels of insect susceptibility to the celery looper NPV.

Mortality data for each experiment and species were subjected to probit analysis (Finney 1971), based on the log transformed dosage and calculated by the Statistical Analysis System using Probit Procedure (SAS Institute 1988). Median lethal concentrations (LC_{50}) calculated from the probit analysis were analyzed by SAS using ANOVA for a RCB design with experiment being the block and treatment the five species. Means were compared using least significant differences (LSD) at $P \leq 0.05$ (SAS Institute 1988). Probit analysis also was performed on data for each species of insect, combined over experiment using SAS Probit Procedure (SAS Institute 1988) and treating experiment as a block effect.

Results and Discussion

Results are reported for 7, 10 and 14 d. Probit analysis fit the data well on all days reported as can be seen from χ^2 values of the slope (Table 1). The ANOVA performed on the data in Table 1 yielded significant differences in the LC values among insect species (Table 2). Velvetbean caterpillar required the highest dosage of virus to reach LC_{50} levels on all days reported. Tobacco budworm required the lowest dosage of virus to reach LC_{50} levels on all days reported. Corn earworm, soybean looper, and beet armyworm all required similar intermediate levels of virus to achieve LC_{50} levels on all days reported. This trend also was seen for LC_{70} and LC_{90} levels on all days reported. The same trend was seen in the probit analysis when combined over experiment for the five larval pests at 7, 10, and 14 d (Table 3). Mortality at 7 d for the velvetbean caterpillar was 10%, compared to mortality for tobacco budworm, soybean looper, beet armyworm, and corn earworm at 98, 78, 77, and 79%, respectively, at the 5×10^4 PIB/ml dosage (Fig. 1). Mortality at 7 d decreased as dosage decreased; this trend was found on all days reported. Day 10 mortality for the velvetbean caterpillar was 46%, while tobacco budworm, soybean looper, beet armyworm, and corn earworm had 99, 90, 87, and 87% mortality, respectively, at the 5×10^4 PIB/ml dosage (Fig. 2). Mortality at 14 d for Velvetbean caterpillar was 72%, while mortality for, tobacco budworm, soybean looper, beet armyworm, and corn earworm was 100, 95, 95, and 91%, respectively, at the 5×10^4 PIB/ml dosage (Fig. 3). Results indicate the virus to have the best relative activity against the tobacco budworm and least relative activity against the velvetbean caterpillar at 7, 10 and 14 d with soybean looper, beet armyworm, and corn earworm exhibiting intermediate responses (Figs. 1-3).

The results of this bioassay show susceptibility by all five of the defoliators tested with the celery looper NPV. As more integrated pest management (IPM) systems are developed for agroecosystems, the value and use of viruses for control or suppression of insect pest population levels increases. Further testing is warranted with the celery looper NPV for possible use in an IPM system for

Table 1. Results of probit analysis by experiment for five larval pests fed celery looper nuclear polyhedrosis virus.

		Day 7						Day 10						Day 14					
		LC_{50} (μ)			Slope (1/σ)			LC_{50} (μ)			Slope (1/σ)			LC_{50} (μ)			Slope (1/σ)		
Exp	Insect	(μ)	(95 C.I.)		(1/σ)	SE	χ^2	(μ)	(95 C.I.)		(1/σ)	SE	χ^2	(μ)	(95 C.I.)		(1/σ)	SE	χ^2
1	CEW**	9,963	1,986	27,369	0.388	0.100	14.97*	4,762	1,358	10,067	0.458	0.102	19.96*	3,551	1,797	5,831	0.489	0.094	27.09*
1	VBC	2,031,635	142,124	1.06	0.270	0.130	4.27+	47,543	17,907	396,636	0.342	0.090	14.13*	24,891	13,536	64,595	0.449	0.100	19.92*
1	SBL	10,687	6,988	16,958	0.692	0.108	40.78*	7,810	3,257	13,374	0.600	0.133	20.20*	6,057	1,330	11,107	0.644	0.176	13.36*
1	BAW	15,353	8,960	24,064	0.690	0.153	20.22*	5,522	1,893	9,528	0.665	0.154	18.47*	3,688	1,893	5,548	0.839	0.175	22.78*
1	TBW	1,834	1,006	2,745	0.705	0.125	31.78*	931	–	–	4.180	14779	8.0E-8	832	–	–	3.920	14,854	6.97E-8
2	CEW	17,988	10,485	28,802	0.682	0.168	16.39*	13,164	7,717	20,248	0.751	0.171	19.17*	9,554	4,943	14,513	0.789	0.191	17.01*
2	VBC	1,813,642	–†	–	0.288	0.391	0.54	75,956	26,571	2,541,346	0.297	0.095	9.63*	8,353	4,223	14,307	0.574	0.121	22.28*
2	SBL	12,323	1,663	22,756	0.547	0.192	8.07*	6,101	1,369	11,960	0.571	0.146	15.20*	3,248	1,053	6,019	0.643	0.135	22.61*
2	BAW	7,894	3,355	12,689	0.720	0.183	15.36*	2,278	684	4,309	0.473	0.111	17.98*	162	0.021	949	0.305	0.131	5.43+
2	TBW	2,271	1,437	3,189	0.865	0.144	35.78*	560	127	935	0.918	0.269	11.63*	754	–	–	3.711	14,999	6.15E-8
3	CEW	7,276	4,149	11,940	0.636	0.104	37.01*	3,438	1,046	6,775	0.622	0.126	24.20*	1,549	18.31	6,781	0.462	0.135	11.66*
3	VBC	11,682,837	–	–	0.324	0.328	0.98	41,534	26,486	86,244	0.714	0.143	24.86*	16,388	9,792	25,924	0.745	0.176	17.92*
3	SBL	19,575	12,553	35,151	0.587	0.108	29.14*	5,694	3,659	8,220	0.743	0.124	35.53*	2,276	1,256	3,450	0.643	0.111	33.06*
3	BAW	20,597	11,875	49,805	0.420	0.085	23.87*	6,957	1,388	17,244	0.387	0.101	14.53*	3,091	846	6,369	0.383	0.096	15.76*
3	TBW	2,879	1,024	5,326	0.686	0.132	26.79*	974	496	1,457	1.220	0.282	18.54*	942	–	–	4.211	15,087	7.81E-8

** CEW - corn earworm, VBC - velvetbean caterpillar, SBL - soybean looper, BAW - beet armyworm, TBW - tobacco budworm.
μ and σ are the mean and standard deviation, respectively, of the tolerance distribution based on log dose.
$\chi 2$ test for significant slope are denoted by + or * for $P \leq 0.05$ or $P \leq 0.01$, respectively.
–† Confidence intervals (CI) are missing when little or no mortality or 100% mortality occurs at the first dose. Probit does not fit this response.

Table 2. Comparisons of LC means, presented in untransformed format, for selected control values between the five larval pests.*

	DAY 7			DAY 10			DAY 14		
Insect	LC_{50}	LC_{70}	LC_{90}	LC_{50}	LC_{70}	LC_{90}	LC_{50}	LC_{70}	LC_{90}
VBC**	5,176,038 a	28,062,045 a	331,764,800 a	55,011 a	249,843 a	2,635,887 a	16,544 a	44,624 a	200,077 a
CEW	11,742 b	31,284 b	147,593 b	7,121 b	16,460 b	59,138 b	4,884 ab	11,249 ab	40,629 ab
SBL	14,195 b	34,214 b	122,988 b	6,535 b	15,166 b	51,777 b	3,860 ab	8,715 b	28,245 b
BAW	14,614 b	40,306 b	193,364 b	4,919 b	15,314 b	87,203 b	2,313 b	6,644 b	38,455 b
TBW	2,328 c	4,733 c	13,296 c	821 c	1,181 c	2,107 c	842 c	961 c	1,164 c

* Means within a column followed by the same letter are not significantly different based on LSD comparisons ($P \leq 0.05$) using log transformed data.
** VBC - velvetbean caterpillar, CEW - corn earworm, SBL - soybean looper, BAW - beet armyworm, TBW - tobacco budworm.

Table 3. Comparison of probit analysis, combined over experiment, for five larval pests.

		LC_{50} (μ)			Slope (1/σ)		
Day	Insect	(μ)	95 C.I.		(1/σ)	SE	χ^2
7	CEW**	12736	7384	18273	0.585	0.098	35.14 *
7	VBC	858381	192348	3.397E10	0.461	0.180	6.55 +
7	SBL	16204	12933	20659	0.685	0.072	89.32 *
7	BAW	14016	10071	18579	0.632	0.096	42.81 *
7	TBW	2428	1882	3014	0.743	0.070	109.74 *
10	CEW	6686	5164	8544	0.586	0.058	100.57 *
10	VBC	67637	41111	150488	0.423	0.065	41.90 *
10	SBL	6955	5548	8645	0.670	0.062	114.81 *
10	BAW	5170	3703	6925	0.507	0.057	77.64 *
10	TBW	796	578	988	1.163	0.180	41.78 *
14	CEW	3971	2853	5267	0.522	0.056	84.62 *
14	VBC	14662	10679	20287	0.545	0.068	64.29 *
14	SBL	3782	2953	4722	0.642	0.060	113.30 *
14	BAW	1985	1070	3049	0.468	0.065	51.59 *
14	TBW	851	–	–	3.973	8520.657	2.17E-8

** CEW - corn earworm, VBC - velvetbean caterpillar, SBL- soybean looper, BAW - beet armyworm, TBW - tobacco budworm.

χ^2 tests for significant slope are denoted by + or * for $P \leq 0.05$ or $P \leq 0.001$, respectively.

(μ) adjustments were made to the intercept (μ) of tolerance distribution based on Log dose to account for the block effect of experiments.

control or suppression of economically important population levels of larval pests.

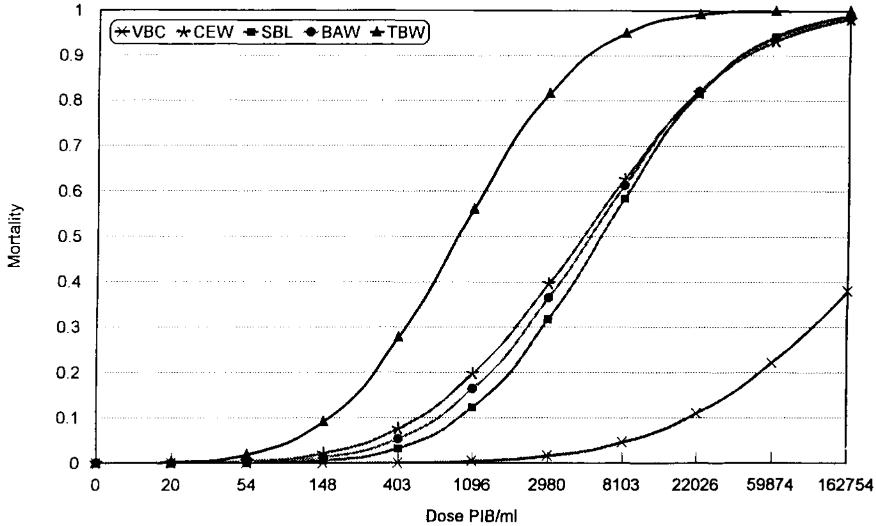

Fig. 1. Dose/mortality trends, produced by probit analysis, of five larval pests fed celery looper virus at day seven.

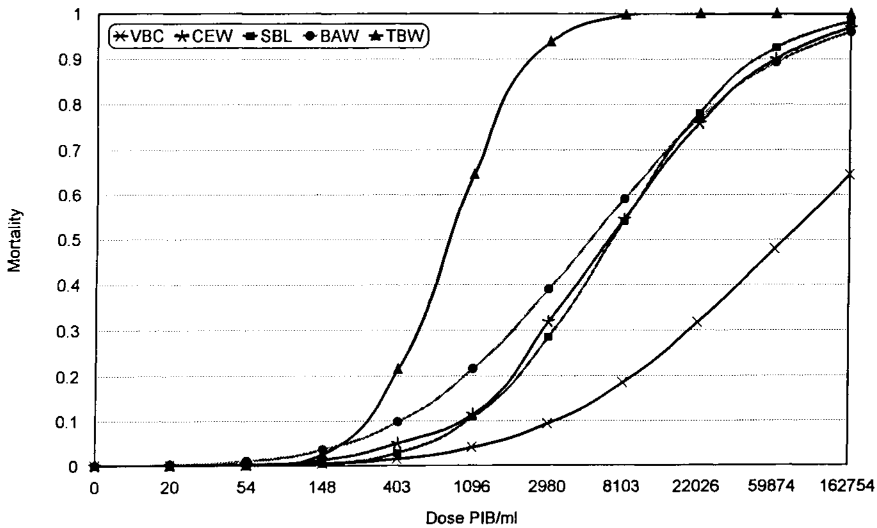

Fig. 2. Dose/mortality trends, produced by probit analysis, of five larval pests fed celery looper virus at day ten.

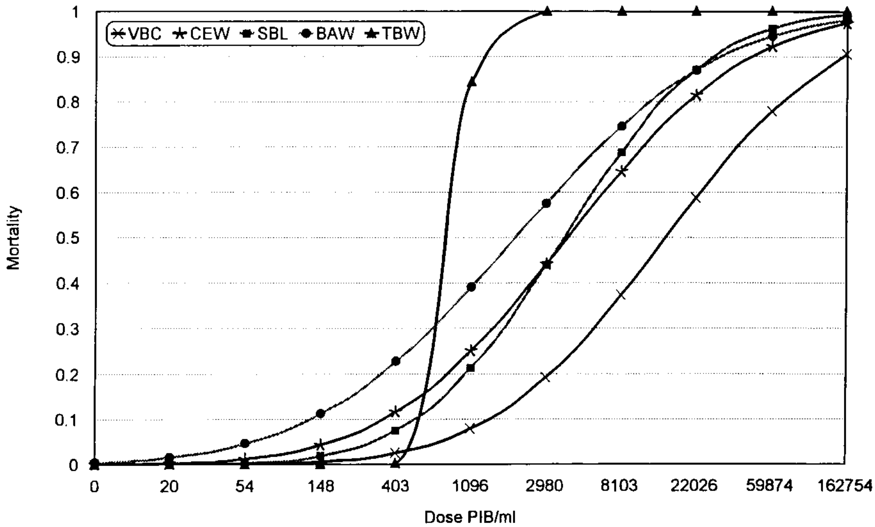

Fig. 3. Dose/mortality trends, produced by probit analysis, of five larval pests fed celery looper viruses at day fourteen.

Acknowledgments

This research was supported by the Southern Insect Management Laboratory, Stoneville, MS, D. D. Hardee, research leader. Special thanks is extended to D. Boykin, statistician, USDA-ARS, Stoneville, MS, for her suggestions and advice on the statistical portion of this article. I would also like to thank G. Tillman and G. Snodgrass, research entomologists, USDA-ARS, Stoneville, MS, for their assistance in editing and continued interest in my research.

References Cited

Dietz, L. L., J. W. Van Duyn, J. R. Bradley, R. L. Rabb, W. M. Brooks and L. A. Falcon. 1976. Problems associated with the use of arthropod viruses in pest control. Annu. Rev. Entomol. 21: 305-324.

Dulmage, H. T., A. J. Martinez and T. Pena. 1976. Bioassay of *Bacillus thuringiensis* (Berliner) δ-endotoxin using the tobacco budworm. U. S. Dep. Agric. Tech Bull. 1528.

Estrada, R. 1986. VPN virus successful in Guatamala. IPM Practitioner 8: 16.

Falcon, L. A. 1976. Problems associated with the use of arthropod viruses in pest control. Annu. Rev. Entomol. 21: 305-324.

Finney, D. J. 1971. Probit Analysis, 3rd ed. Cambridge University Press, London.

Hostetter, D. L. and B. Puttler. 1991. A new broad host spectrum nuclear polyhedrosis virus isolated from a celery looper, *Anagrapha falcifera* (Kirby), (Lepidoptera: Noctuidae). Environ. Entomol. 20: 1480-1488.

Huber, J. 1986. Use of baculoviruses in pest management programs, pp. 181-202. *In* R. R. Granados and B. A. Federici [eds.], The biology of baculoviruses, Vol. 2, Practical application for insect control. CRC, Boca Raton.

Ignoffo, C. M., K. D. Hostetter, K. D. Biever, C. Garcia, G. D. Thomas, W. A. Dickerson, and R. Pinnell. 1978. Evaluation of an entomopathogenic bacterium, fungus, and virus for control of *Heliothis zea* on soybeans. J. Econ. Entomol. 71: 165-168.

King, E. G. and G. G. Hartley. 1985. *Diatraea saccharalis*, pp. 265-270. *In* P. Singh and R. F. Moore [eds.], The handbook of insect rearing. Elsevier, Amsterdam.

Leonard, B. R., D. J. Boethel, A. N. Sparks, M. B. Layton, J. S. Mink, A. M. Pavloff, E. Burris and J. B. Graves. 1990. Variations in repsonse of soybean looper (Lepidoptera: Noctuidae) to selected insecticides in Louisiana. J. Econ. Entomol. 83: 27-34.

Livingston, J. M., P. J. McLeod, W. C. Yearian and S. Y. Young. 1980. Laboratory and field evaluation of a nuclear polyhedrosis virus of the soybean looper, *Pseudoplusia includens*. J. Georgia Entomol. Soc. 15: 194-199.

Moscardi, F., G. E. Allen and G. L. Greene. 1981. Control of the velvetbean caterpillar by nuclear polyhedrosis virus and insecticides and the impact of treatments on the natural incidence of the entomopathogenic fungus *Nomuraea rileyi*. J. Econ. Entomol. 74: 480-485.

Richter, A. R. and J. R. Fuxa. 1984. Timing, formulation, and persistence of a nuclear polyedrosis and a microsporidium for control of the velvetbean caterpillar (Lepidoptera: Noctuidae) in soybeans. J. Econ. Entomol. 77: 1299-1306.

SAS Institute. 1988. SAS Stat user's guide, version 6.03 ed. SAS Institute, Cary, NC.

Smith, K. J. and W. Huyser. 1987. World distribution and significance of soybean, pp. 1-21. *In* J. R. Wilcox (ed.), Soybeans: improvement, production, and uses. Am. Soc. Agron. Madison, WI.

Turnipseed, S. G. 1973. Soybeans: improvement, production, and uses, pp. 545-572. *In* B. E. Caldwell [ed.], WI: American Society of Agronomy Publishers.

Vail, P. V., S. J. Anderson and D. L. Jay. 1973. New procedures for rearing cabbage loopers and other lepidopterous larvae for propagation of a nuclear polyhedrosis virus. J. Environ. Entomol. 2: 339-344.

Vail, P. V., T. Whitaker, H. Toba and A. N. Kishaba. 1971. Field and cage tests with polyhedrosis viruses for control of the cabbage looper. J. Econ. Entomol. 64: 1132-1136.