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Quantification of Soil-to-Plant Transport of Recombinant Nucleopolyhedrovirus: Effects of Soil Type and Moisture, Air Currents, and Precipitation†

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Significantly more occlusion bodies (OB) of DuPont viral construct HzSNPV-LqhIT2, expressing a scorpion toxin, were transported by artificial rainfall to cotton plants from sandy soil (70:15:15 sand-silt-clay) than from silt (15:70:15) and significantly more from silt than from clay (15:15:70). The amounts transported by 5 versus 50 mm of precipitation were the same, and transport was zero when there was no precipitation. In treatments that included precipitation, the mean number of viable OB transported to entire, 25- to 35-cm-tall cotton plants ranged from 56 (clay soil, 5 mm of rain) to 226 (sandy soil, 50 mm of rain) OB/plant. In a second experiment, viral transport increased with increasing wind velocity (0, 16, and 31 km/h) and was greater in dry (−1.0 bar of matric potential) than in moist (−0.5 bar) soil. Wind transport was greater for virus in a clay soil than in silt or sand. Only 3.3×10^{-7} (clay soil, 5 mm rain) to 1.3×10^{-6} (sandy soil, 50 mm rain) of the OB in surrounding soil in experiment 1 or 1.1×10^{-7} (−0.5 bar sandy soil, 16-km/h wind) to 1.3×10^{-6} (−1.0 bar clay soil, 31-km/h wind) in experiment 2 were transported by rainfall or wind to cotton plants. This reduces the risk of environmental release of a recombinant nucleopolyhedrovirus (NPV), because only a very small proportion of recombinant virus in the soil reservoir is transported to vegetation, where it can be ingested by and replicate in new host insects.

Nucleopolyhedroviruses (NPV) are important agents of natural mortality for certain insects and crustaceans (5). In addition to their very specific host ranges, these viruses are characterized by their capability for causing epizootics with high prevalence and case-fatality rates in host insects (8). For these reasons, NPV have been researched extensively as environmentally safe, viral insecticides (24). To this end, epizootiology has contributed to improvements in their efficacy and methods of utilization (7).

NPV have succeeded in pest management in certain cases, but certain weaknesses, such as the slow death of the infected insect, have hindered their development as insecticides (8). Genetic modification has been pursued as a means to improve their lethal time (3). For example, survival times of the tobacco budworm, *Heliothis virescens*, and the bollworm, *Helicoverpa zea*, were reduced when infected with *Heliothis* NPV expressing highly specific toxin from the scorpion *Leiurus quinquestriatus hebraeus* (DuPont construct HzSNPV.LqhIT2) (16).

Epizootiology of DNA-recombinant NPV has assisted in their risk assessment for pesticide regulatory agencies. Knowledge of viral persistence, population growth, and spread can reduce their risk of environmental release, even when the possibility of harmfulness to nontarget organisms is difficult or impossible to ascertain (9). For example, *Autographa californica* NPV expressing toxin from the scorpion *Androctonus australis* produced smaller populations on leaves and in soil and was dispersed less by biotic agents than the wild-type, parental NPV (6, 20, 21). Similarly, the population density of HzSNPV-.LqhIT2 in soil was less than that of wild-type *Heliothis* NPV (11). The reduced population density and dispersal of the recombinant NPVs reduce the likelihood that they will contact nontarget organisms in the environment.

Ecology of NPV in soil is essential for their long-term insect control and risk assessment (7, 9). Soil is a major reservoir for NPV between hosts, particularly for long-term survival. These viruses, embedded in their proteinaceous occlusion bodies (OB), can persist in soil more than 5 years after release in a row crop (17) and up to 41 years after a natural epizootic in a forest ecosystem (30). These OB in soil then can be transported onto insect host plants, where they are ingested by their hosts to replicate and initiate new epizootics (12, 33, 34). Like its wild-type counterpart, the HzSNPV.LqhIT2 construct can persist in soil from one crop-growing season to the next, when it may have the opportunity to replicate in a new generation of host insects (11). A major gap in our knowledge of NPV ecology in soil is the transport of OB from soil to the insect's host plant. Rainfall (10, 13, 23, 29, 34) and air currents (13, 25, 31) have been implicated in soil-to-plant transport in a limited number of observational studies, but there has been little controlled experimentation with this phenomenon (9). The proportion of the soil population of NPV reaching foliage by these methods and the influence of environmental variables have not been quantified. In fact, there is little quantitative information on transport of any type of microorganism from soil to leaves, although wind speed and amount and intensity of rain are considered to be important factors (19).

The purpose of the present study was to quantify, under

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carefully controlled conditions, the effects of soil type, soil moisture, precipitation, and air currents on soil-to-plant transport of a recombinant NPV. We also determined the proportion of the soil population of NPV that was transported onto plants under these variables.

MATERIALS AND METHODS

Virus and insect. The virus, provided by DuPont Agricultural Products (Wilmington, Del.), was a variant of the *Heliothis* or *Helicoverpa* NPV genetically modified to express an insect-specific neurotoxin from the scorpion *L. quinquestriatus hebraeus* (HzSNPV.LqhIT2). The insects used in bioassays were neonatal *H. virescens* shipped as eggs from DuPont.

Soil type-precipitation experiment. The experiments were conducted in a greenhouse in order to control the necessary variables. This experiment tested the effects of soil type, precipitation, and soil-precipitation interactions on viral transport. There were nine treatments (three soil types × three precipitation rates) with virus in the soil plus a control for each soil type with no virus, and there were four replications per treatment.

Ingredient soils were mixed by weight to provide experimental soil types with clay-silt-sand ratios of 70:15:15, 15:70:15, and 15:15:70, respectively. The following ingredient soils were analyzed for content at the Louisiana State University Agricultural Center Soil Laboratory: (i) Quickrete Play Sand (Quickrete Co., Atlanta, Ga.), 4.8% silt, 1.0% clay, and 94.2% sand; (ii) white clay (Southern Pottery, Baton Rouge, La.), 9.2% silt, 90.0% clay, and 0.8% sand; and (iii) silt from a field near Baton Rouge, 81.0% silt, 15.2% clay, and 3.8% sand. Samples of the final soil mixtures then were analyzed again at the Soil Laboratory with the following results: the clay mixture was 70.3% clay, 15.3% silt, and 14.4% sand, with 1.22% organic matter and a pH of 6.8. The silt mixture was 15.8% clay, 69.5% silt, and 14.7% sand, with 1.66% organic matter and a pH of 7.5. The sand mixture was 14.6% clay, 14.5% silt, and 70.9% sand, with 1.14% organic matter and a pH of 7.2. After the three soil types were prepared, the moisture of each was determined with a Jet-Filled Tensiometer (model 2725 ARL; Soil Moisture Equipment Corp., Santa Barbara, Calif.) embedded in the soil for 24 h. Distilled water was added to soil to result in a water potential of −0.5 bar in every treatment, which was confirmed with another 24-h tensiometer reading.

For each replication of each treatment, HzSNPVLqhIT2 was mixed into 68 kg of the appropriate soil type at 2,500 viral OB/g of soil. This amount of soil was spread out on a 1.5-m by 1.5-m area, and the OB were suspended in 200 ml of distilled water and then sprayed evenly over the entire soil surface with a CO_2 sprayer. The soil was then mixed thoroughly by hand to evenly distribute the virus.

For each treatment or replication, a cotton plant was grown in a 3.8-liter pot, which in turn was placed in the center of a 1.2-m by 1.2-m flat. All plants in the experiment were 25 to 35 cm high, assigned in a manner such that all treatments within a replication included plants of a similar height. Untreated soil was added to the flat to a height of 3.8 cm below the top edge of the pot. This soil layer was covered with plastic, and the virus-treated soil then was layered onto the untreated soil so that the entire flat contained virus-treated soil to a depth of 2.5 cm, up to the top edge of the pot with the cotton plant. A control for each soil type was set up in an identical manner, except that no virus was added to the soil.

Immediately after the soil was added to each flat, one of three rates of precipitation (0, 5, or 50 mm) was applied in 1 min by an overhead sprinkler system designed to water evenly over the entire flat. The low rate of 5 mm/min has successfully contributed to artificial epizootics in a similar greenhouse microcosm in previous research (21). The leaves were allowed to dry, and then they all were excised from the plant. The entire procedure was repeated for each replication or treatment. The experimental area was decontaminated with 0.525% NaClO after all treatments were run in one replication.

All of the leaves from each plant were weighed and then homogenized for 2 min in a Sorvall Omni-Mixer (DuPont Instruments, Newtown, Conn.) in distilled water at a ratio of 3 g of plant tissue to 10 ml of water. Aliquots were fed to neonatal *H. virescens* larvae by droplet-feeding bioassay (14). The larvae then were transferred to 30-ml individual cups containing tobacco budworm artificial diet (Southland Products, Lake Village, Ark.) and kept at 26.7°C until they died or pupated. Death due to nuclear polyhedrosis was confirmed by detection of OB under phase-contrast microscopy.

A standard bioassay curve was determined in order to convert vegetation bioassay percentages of mortality to concentrations of viral OB in the leaf samples. Known amounts of leaf tissue were homogenized as described above, and known amounts of the virus were added to the homogenate, which then was homogenized for an additional 2 min. The homogenate was then bioassayed as described above. The bioassay standard curve had two replications, with eight doses and 50 insects per dose per replication.

TABLE 1. Percentage of infection of *Helicoverpa zea* in bioassays of leaves from cotton plants[a]

Soil type[b]	No-virus control (50 mm of precipitation)	Mean % infection (± SE) versus artificial precipitation (mm)			Overall mean[c,d]
		0	5	50	
Sandy	0	0	38.0 ± 5.89	32.5 ± 7.90	23.5a
Silt	0	0	13.5 ± 3.00	23.0 ± 3.46	12.2b
Clay	0	0	4.5 ± 1.91	10.0 ± 4.00	4.8c
Overall mean[d]		0b	18.7a	21.8a	

[a] Cotton plants were set in one of three soil types inoculated with HzSNPV-.LqhIT2 and subjected to different amounts of artificial precipitation.
[b] Proportions of sand, silt, and clay, respectively, were 70:15:15 for the sandy soil type, 15:70:15 for the silt, and 15:15:70 for the clay.
[c] Exclusive of the no-virus control.
[d] Means in a column (soil type) or row (precipitation amounts) followed by the same letter are not significantly different ($P < 0.05$; Tukey HSD).

Soil type-air current-soil moisture experiment. The soil type-air current-soil moisture experiment tested the effects of soil type, wind velocity, soil moisture, and soil-wind-moisture interactions on viral transport. There were 18 treatments (three soil types × three wind velocities × 2 soil moisture levels) with virus in the soil plus a control with no virus for each soil type at the low moisture level, and there were four replications per treatment.

The same three soil types were used as in the soil type-precipitation experiment. Distilled water was added to each batch of soil to establish soil water potentials at either −0.5 (moist) or −1.0 (dry) bar, as described above. A floor model, 41-cm-diameter electric fan provided the wind or air current at velocities of 16.1 and 30.6 km/h, as determined with a model 05–005 anemometer (Science First, Buffalo, N.Y.) at 100 cm from the fan.

For each treatment or replication, a potted cotton plant was set in a flat, and soil was added as described above, except that the plant was set at the middle of one edge of the flat instead of the center. The fan faced the flat at 30 cm outside the far edge of the flat and was run at the appropriate velocity for 2 min. The remainder of the experiment (collection of leaves, bioassay, data analysis, and decontamination) was the same as in the soil type-precipitation experiment.

Statistical analysis. The main experimental data were tested by the general linear models (GLM) analysis of variance, with the Tukey Studentized range honestly significant difference (HSD) test for comparison among means (SAS/STAT user's guide, vol. 2, GLM-VARCOMP, version 6, 4th ed. SAS Institute, Cary, N.C., 1990). Two analyses were run for each experiment, one each with bioassay percentage of mortality or number of OB per plant as the dependent variable. The virus-plant standard curve data were subjected to probit analysis with MicroProbit 3.0 (T. C. Sparks and A. Sparks, Lily Research Laboratories, Greenfield, Ind.).

RESULTS

Soil type-precipitation experiment. Precipitation clearly transported the NPV from soil onto cotton plants (Tables 1 to 3). The percentages of mortality due to and the amounts of HzSNPV.LqhIT2 transported from soil to cotton plants did not differ significantly between 5 versus 50 mm of precipitation (Tables 1 and 3). However, both amounts of artificial rainfall transported significantly more OB than zero precipitation, in which there was no viral contamination of plants. There was no viral contamination of control plants, which were subjected to 50 mm of precipitation.

Soil type clearly affected viral transport due to precipitation (Tables 1 to 3). Significantly greater percentages of mortality and OB transport resulted from artificial rainfall applied to cotton plants in sandy soil than in silt, and significantly more occurred in silt than in clay (Tables 1 and 3). The interaction between soil type and precipitation also was significant for the percentage of infection and the number of OB transported

TABLE 2. General linear models procedure analysis of variance indicating sources of variation in the soil type-precipitation experiment

Data set	Degrees of freedom	Sum of squares	F	Probability $> F$
% Infection (Table 1)				
Soil type	2	2,122.7	66.6	0.0001
Precipitation	2	3,340.7	104.8	0.0001
Replication	3	30.6	0.6	0.5972
Soil type × precipitation	4	1,302.7	20.4	0.0001
Error	24	382.4		
No. of OB (Table 3)				
Soil type	2	62,289.0	16.9	0.0001
Precipitation	2	176,162.1	47.9	0.0001
Replication	3	18,107.6	3.3	0.0382
Soil type × precipitation	4	32,504.9	4.4	0.0081
Error	24	44,130.9		

(Table 2), probably because there was less difference in plant contamination between amounts of rainfall in sandy soil than in silt or clay (Tables 1 and 3).

Only a small proportion of HzSNPV.LqhIT2 in soil was transported by precipitation onto cotton plants. The 1.44 m^2 of soil surrounding each plant to a depth of 3.8 cm weighed 68 kg with 2,500 viral OB/g, for a total viral population of 1.7×10^8 OB/flat. Thus, the proportion of OB transported by precipitation from soil to a 25- to 35-cm-high plant ranged from 3.3×10^{-7} (clay soil, 5 mm of precipitation) to 1.3×10^{-6} (sandy soil, 50 mm of precipitation) (Table 3). If one assumes that viral OB in only the top 0.2 cm of soil are subject to possible transport by precipitation, then the proportion of OB transported to foliage of cotton plants still ranged only from 6.2×10^{-6} to 2.5×10^{-5}. In spite of the small proportion of soil NPV transported, this was still sufficient to initiate infection rates of 4.5 to 38.0% in first-instar *H. virescens*.

Soil type-air current-soil moisture experiment. Air currents also transported the NPV from soil onto cotton plants (Tables 4 to 6). The percentage of mortality due to and amounts of HzSNPV.LqhIT2 transported from soil to cotton plants were significantly greater in a 30.6-km/h wind than at 16.1 km/h (Tables 4 and 6); there was no viral contamination of plants without air currents. There was no viral contamination of control plants, which were subjected to a wind velocity of 30.6 km/h.

Soil type and moisture clearly affected viral transport due to air currents (Tables 4, 5, and 6). Significantly greater mortality and OB transport resulted in artificial wind applied to cotton plants in clay than in silt or in sandy soil (Tables 4 and 6), the opposite of soil effects in the precipitation experiment (Tables 1 and 3). Furthermore, the percentage of mortality and the number of OB on plants were significantly greater in dry (−1.0 bar) than in moist (−0.5 bar) soil (Tables 4 and 6). All two-way interactions (soil type × moisture; soil type × wind; moisture × wind) were significant (Table 5). The three-way interaction (soil type × moisture × wind) was significant when the number of OB per plant was the dependent variable, but not when the percentage of mortality was the dependent variable.

As in the precipitation experiment, only a small proportion of HzSNPV.LqhIT2 in soil was transported by wind onto cotton plants. The 1.44 m^2 of soil in the flat to a depth of 3.8 cm again contained a total of 1.7×10^8 OB. Thus, the proportion of OB transported by wind from soil to a 25- to 35-cm-high plant ranged from 1.1×10^{-7} (−0.5 bar sandy soil, 16.1-km/h wind) to 1.3×10^{-6} (−1.0 bar clay, 30.6-km/h wind) (Table 6). If one assumes that viral OB in only the top 0.2 cm of soil are subject to possible transport by wind, then the proportion of OB transported to foliage of cotton plants still ranged only from 2.1×10^{-6} to 2.6×10^{-5}. Thus, the greatest proportion of OB transported by artificial wind was almost identical to the greatest proportion transported by artificial precipitation. In spite of the small proportion of soil NPV transported, this was

TABLE 3. Mean number of OB of HzSNPV.LqhIT2 per entire cotton plant[a]

Soil type[b]	No-virus control (50 mm of precipitation)	Mean no. of OB (± SE) vs artificial precipitation (mm)			Overall mean[c,d]
		0	5	50	
Sandy	0	0	219.2 ± 116.0	225.6 ± 60.6	148.2a
Silt	0	0	126.7 ± 46.1	169.7 ± 29.2	98.8b
Clay	0	0	55.5 ± 15.2	83.6 ± 20.2	46.4c
Overall mean[d]		0b	133.8a	159.6a	

[a] Cotton plants were set in one of three soil types inoculated with NPV and subjected to different amounts of artificial precipitation. Bioassay percentage of mortality for the leaves of each plant (Table 1) was converted to viral OB per gram of plant by means of the standard curve $Y = 2.349X + 0.945$ (slope SE = 0.147, $\chi^2 = 7.197$ [not significant]), where Y is the probit percentage of mortality in the bioassay and X is the log OB per gram of dried leaf tissue. The total dry weight of the leaves on each plant was then use to calculate the number of OB per plant.

[b] Proportions of sand, silt, and clay, respectively, were 70:15:15 for the sandy soil type, 15:70:15 for the silt, and 15:15:70 for the clay.

[c] Exclusive of the no-virus control.

[d] Means in a column (soil type) or row (precipitation amount) followed by the same letter are not significantly different ($P < 0.05$, Tukey HSD).

TABLE 4. Percentage of infection of *Helicoverpa zea* in bioassays of leaves from cotton plants[a]

Soil type[b]	Soil moisture[c]	No-virus control[d]	Mean % infection (± SE) vs artificial wind velocity (km/h)			Overall mean for soil type[e]
			0	16.1	30.6	
Sandy	−0.5		0	0.5 ± 1.00	3.5 ± 1.91	5.8b
	−1.0	0	0	13.5 ± 3.79	17.5 ± 3.00	
Silt	−0.5		0	3.0 ± 1.15	9.0 ± 5.03	7.8b
	−1.0	0	0	14.0 ± 6.32	20.5 ± 4.12	
Clay	−0.5		0	4.0 ± 0	10.5 ± 5.26	10.9a
	−1.0	0	0	20.0 ± 8.16	31.0 ± 1.15	
Overall mean[e]			0c	9.2b	15.3a	

[a] Cotton plants were set in soils of different types and moisture levels, which were inoculated with HzSNPV.LqhIT2 and subjected to different velocities of artificial wind.

[b] Proportions of sand, silt, and clay, respectively, were 70:15:15 for the sandy soil type, 15:70:15 for the silt, and 15:15:70 for the clay.

[c] Soil moisture is expressed as bar matric potential. Overall mean bioassay mortality percentages for soil moisture levels were 3.4% for −0.5 bar and 12.9% for −1.0 bar (significantly different at $P < 0.0001$) (Table 2).

[d] Subjected to a 30.6-km/h wind velocity.

[e] Overall means in a column (soil type) or row (wind velocities) followed by the same letter are not significantly different ($P < 0.05$; Tukey HSD).

TABLE 5. GLM procedure analysis of variance indicating sources of variation in the soil type, soil-moisture-wind experiment

Data set	Degrees of freedom	Sum of squares	F	Probability $> F$
% Infection (Table 4)				
Soil type	2	316.3	20.4	0.0001
Soil moisture	1	1,643.6	211.7	0.0001
Wind velocity	2	2,857.3	184.0	0.0001
Replication	3	226.0	9.7	0.0001
Soil type × moisture	2	68.1	4.4	0.0175
Soil type × wind	4	213.3	6.9	0.0002
Soil moisture × wind	2	833.8	53.7	0.0001
Soil type × moisture × wind	4	43.6	1.4	0.2465
Error	51	396.0		
No. of OB (Table 6)				
Soil type	2	8,820.1	8.7	0.0006
Soil moisture	1	53,808.4	106.1	0.0001
Wind velocity	2	221,408.8	218.2	0.0001
Replication	3	15,095.9	9.9	0.0001
Soil type × moisture	2	4,070.4	4.0	0.0241
Soil type × wind	4	8,355.4	4.1	0.0058
Soil moisture × wind	2	26,920.7	26.5	0.0001
Soil type × moisture × wind	4	5,236.5	2.6	0.0482
Error	51	25,877.7		

still sufficient to initiate infection rates of 0.5 to 31.0% in first-instar *H. virescens*.

DISCUSSION

The results showed conclusively that some physical force is required to transport NPV onto cotton plants, because no virus was detected on plants in any of the controls in the precipitation or wind experiments. NPV transport increased with increasing wind velocity, but there was no difference in transport between different amounts of rain, although rain intensity has been positively correlated with splash transport of fungal phytopathogens (22). It is possible that a less intense rainfall than 5 mm in 2 min would have resulted in significantly less viral transport. Once OB were splashed onto the plant, additional precipitation probably did not wash them away, because several studies have indicated that rain does not remove viral OB from leaf surfaces (18).

Previous research quantifying soil-to-plant transport of microorganisms generally has not incorporated the control of abiotic factors as independent variables. An observational study implicated road dust in dispersal of *Neodiprion sertifer* NPV up to 30 m or more in a forest ecosystem (25), and a *Pseudoplusia includens* NPV exhibited a dose response in terms of viral epizootics in the insects infesting soybean after one-time application of the virus to soil (33). Other research has dealt primarily with phytopathogens, especially fungi. For example, dust dispersed by wind was a primary source of inoculum for *Colletotrichum truncatum* (4), and rain splash and wind interacted in the transport of *Colletotrichum gloeosporioides*, a commercial mycoherbicide (32). Soil surface microtopography (i.e., roughness) greatly influenced splash transport of fungal phytopathogens, with splash distance increasing as roughness declined (22).

It was interesting that the type of soil in the current research affected efficiency of soil-to-plant transport differently in wind than in rain. Rain splash transported more virus from sand than silt and more from silt than clay. In a wind, the order was the opposite: clay was best for transport, then silt, then sand. Dry soil was better than moist soil, probably because the former is more conducive to formation of airborne dust. The reasons for these opposite effects may relate to NPV adhesion to soil particles and to the size of those particles. Inorganic soil particulates include coarse sand (0.2 to 2.0 mm in diameter), fine sand (0.02 to 0.2 mm), silt (0.002 to 0.02 mm), and clay (<0.002 mm) (2, 26). If NPV in our research was adhering to clay particles, which have a dominant role in the ecology of soil microorganisms (2), this may explain the efficient transport of NPV from dry clay soil by wind. Soil compaction probably did not affect transport in our research, because the experiments were run immediately after treated soil was added loosely to the flats.

Previous research of NPV surface characteristics and soil interactions is sparse. Surface characteristics of NPV polyhedra are largely unknown and complex, though they may behave as hydrophobic entities (27). Polyhedra of cypovirus (cytoplasmic polyhedrosis virus), which have an amino acid composition similar to polyhedra of NPV (28), may adsorb to soil particles mainly by coulomb force, depending on pH and the inorganic fractions of soil (15). Negative as well as positive adsorption of OB to soil particles is possible; it cannot be assumed that adhesion explains all microbial responses in soil (26).

The current results should be useful in epizootiology and risk assessment of baculoviruses. For example, in previous research, the percentage of silt in soil was negatively correlated with naturally occurring epizootics of nuclear polyhedrosis in populations of *Spodoptera frugiperda* infesting pastures over a

TABLE 6. Mean number of OB of HzSNPV.LqhIT2 per entire cotton plant[a]

Soil type[b]	Soil moisture[c]	No-virus control[d]	Mean no. of OB (± SE) vs artificial wind velocity (km/h)			Overall mean for soil type[e]
			0	16.1	30.6	
Sandy	−0.5		0	18.5 ± 36.9	67.3 ± 33.9	61.2b
	−1.0	0	0	132.4 ± 37.0	149.2 ± 39.5	
Silt	−0.5		0	63.0 ± 19.2	101.6 ± 55.7	71.6b
	−1.0	0	0	116.7 ± 18.1	148.1 ± 17.7	
Clay	−0.5		0	58.6 ± 7.7	107.8 ± 19.8	88.1a
	−1.0	0	0	133.4 ± 29.2	228.9 ± 52.9	
Overall mean[e]			0c	87.1b	133.8a	

[a] Cotton plants were set in soils of different types and moisture levels, which were inoculated with the virus and subjected to different velocities of artificial wind. Bioassay percentage of mortality for the leaves of each plant (Table 4) was converted to viral OB per gram of plant by means of the standard curve $Y = 2.349X + 0.945$ (slope SE = 0.147, $\chi^2 = 7.197$ [not significant]), where Y is the probit percentage of mortality in the bioassay and X is the log OB per gram of dried leaf tissue. The total dry weight of the leaves on each plant was then used to calculate the number of OB per plant.

[b] Proportions of sand, silt, and clay, respectively, were 70:15:15 for the sandy soil type, 15:70:15 for the silt, and 15:15:70 for the clay.

[c] Soil moisture is expressed as bar matric potential. Overall mean OB per gram of plant for soil moisture levels were 46.3 OB/g for −0.5 bar and 101.0 OB/g for −1.0 bar (significantly different at $P = 0.0001$) (Table 5).

[d] Subjected to a 30.6-km/h wind velocity.

[e] Overall means in column (soil type) or row (wind velocities) followed by the same letter are not significantly different ($P < 0.05$; Tukey HSD).

2-year period (10). This finding is consistent with results of the current research, under the safe assumption that the *S. frugiperda* NPV in pasture soils was subject to both wind and rain during the 2 years. Our results also are pertinent to environmental risk assessment of recombinant NPV. The proportion of HzSNPV.LqhIT2 in soil that was transported to cotton plants was so small—less than 0.003% from the top 0.2 cm of soil under the best conditions, and far less under more stringent conditions—that this reduces the probability of the recombinant virus replicating in host insects, which, in turn, reduces the probability that it will come into contact with nontarget organisms. Of course, the small proportion of HzSNPV.LqhIT2 transported still was sufficient to infect up to 38% of *H. zea* larvae under the best conditions (Table 1). However, we used larvae that were <1 day old for our bioassays, the most susceptible stage of the insect. The susceptibility of *H. zea* to HzSNPV decreases by 250× in larvae between the ages of 3 and 8 days (1). Furthermore, HzSNPV.LqhIT2 does not accumulate in soil from season to season in the cotton agroecosystem (11). Although HzSNPV.LqhIT2 is unlikely to be used in pest management in cotton, the current results are relevant to epizootiology and risk assessment of NPV in general, because the insertion of a scorpion toxin gene does not alter the polyhedrin protein comprising the OB (3).

Further research is necessary to further elucidate abiotic soil-to-plant transport of NPV, particularly the effect of plant height and the thresholds of soil inocula necessary to initiate epizootics. It also would be helpful to learn the dimensions of the soil "universe"—the distance from the plant and soil depth—from which transport to a particular plant is likely.

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