Deletion genotypes reduce occlusion body potency but increase occlusion body production in a Colombian *Spodoptera frugiperda* nucleopolyhedrovirus population

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Abstract. The Colombian field isolate (SfCOL-wt) of *Spodoptera frugiperda* nucleopolyhedrovirus (SfMNPV) is a mixture of different genotypes. To evaluate the insecticidal properties of the different variants in SfCOL-wt a plaque assay was performed and ten distinct genotypes were identified (called A through J). SfCOL-A was the most prevalent (71±9%) and showed a *Pst*I restriction profile identical to that of SfCOL-wt. The remaining nine genotypes presented genomic deletions of 3.8-21.8 Kb, located between nucleotides 11.436 and 33.883 in the Nicaraguan genotype of SfMNPV (SfMNPV-B). These deletions affected the region between open reading frames (ORFs) *sf20* and *sf33*. The potency of SfCOL-A occlusion bodies (OBs) was 4.4-fold higher than SfCOL-wt OBs, whereas the speed of kill of SfCOL-A was similar to that of SfCOL-wt. Deletion genotype OBs were similarly or less potent than SfCOL-wt but six deletion genotypes were faster killing than SfCOL-wt. The potency of mixtures of OBs and co-occluded mixed genotype OBs were consistently reduced in two-genotype mixtures involving equal proportions of SfCOL-A and one of three deletion genotypes (SfCOL-C, -D or –F). Speed of kill and OB production were improved only when the certain genotype mixtures were co-occluded, although OB production was higher in the SfCOL-wt isolate than in any of the component genotypes or mixtures thereof. We conclude that the SfCOL-wt population is structured to maximize the production of OBs in each infected host.

Key words: SfMNPV, Colombia, Wild-type, Genotypes, Mixtures of OBs, Phenotype.

Introduction

Previous studies on *Spodoptera frugiperda* multiple nucleopolyedrovirus (SfMNPV) as a potential biological control agent in Colombia identified the SfCOL isolate as the most insecticidal of a total of 38 field isolates from Colombia or Nicaragua (SfNIC) (Barrera et al., 2011). SfMNPV populations have been found to be composed of different genotypes (Harrison et al., 2008; Simón et al., 2004). Previous studies have addressed that interactions between genotypes determined the transmissibility of the wild-type populations (Muñoz et al., 1998; Simón et al., 2005). Evaluating interactions between genotypes can be highly advantageous during the process of selecting the active material for the development of virus-based biological insecticides.

The objectives of the present study were to determine the genotypic diversity present in the SfCOL isolate and evaluate the contribution of the component genotypes to the insecticidal properties of the natural isolate.

Material and Methods

Individual genotypes present within SfCOL-wt (Barrera et al., 2011) were isolated by plaque assay following the protocol described by Simón et al. (2004). 248 well isolated plaques were picked individually and injected into fourth instars *S. frugiperda* for viral amplification. OBs were purified and DNA was extracted and analyzed with the restriction endonucleases *Pst*I. Physical maps were constructed by comparison of co-migrating and genotype-specific fragments, and confirmed by sequencing the polymorphic fragments. Relative proportion of the complete genotype SfCOL-A was determined by qPCR. The *egt* gene was used as an indicator gene for this genotype, as was the only gene absent in all deleted genotypes and present only in the complete SfCOL-A genotype, which permitted its use as.

OB and co-occluded mixtures, involving equal proportions of SfCOL-A and one of three deletion genotypes (SfCOL-C, -D or –F), were produced as described by Simón et al. (2005). The insecticidal activity of the SfCOL strain, individual genotypes and OB and co-occluded mixtures was compared with that of SfCOL strain in a renewed insect colony obtained from larvae collected in maize fields close to Bogota, Colombia. The median lethal concentration (LC_{50}), mean time to death (MTD) and OB productivity (OBs/larva) were determined. Virus induced mortality was subjected to probit analysis using the PoloPlus program (LeOra-Software, 1987). Time mortality data were subjected to Weibull survival analysis using GLIM program (Crawley, 1993). OB production was determined by counting in cohorts of 24 overnight-starved second instars inoculated with the LC_{90}.

Results and discussion

The complete SfCOL-A genotype accounted the majority of genotypes in SfCOL-wt

Ten different genotypes (named SfCOL-A to -J) were identified by analysis of plaques using *Pst*I endonuclease (Fig.1). SfCOL-A genotype with the complete genome showed a *Pst*I restriction profile identical to that of SfCOL-wt, suggesting that it is present at high frequency in the population. qPCR analysis confirmed that SfCOL-A accounted for 71±9% of the genotypes.

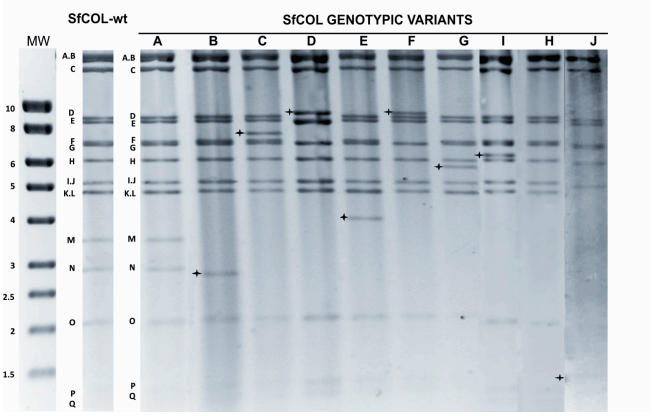

Fig. 1. REN patterns of SfCOL-wt and SfCOL variants DNA digested with *Pst*I. ✦ marks the polymorphic fragments of each genotype.

All the others genotypes displayed deletions of 3.8-15.1 kb affecting orfs s*f20* to *sf34* (nt 17.7740 to 35.302 in the SfMNPV-B genome; Simón et al., 2011), that. This region of variability among the genotypes, which included ORFs that encoded non-essential proteins with auxiliary functions, was also indentified in Missouri (Harrison et al., 2008) and Nicaragua (Simón et al., 2004) SfMNPV isolates.

Deletion genotypes reduce pathogenicity but increase OB productivity

SfCOL-A was approximately 4.4-fold more potent (in terms of concentration mortality-metrics) than SfCOL-wt (Table 1), indicating that the other genotypes diminish the pathogenicity of the population. SfCOL-wt and pure genotypes SfCOL-A, -B, -D and -G were the lower virulent viruses, which in the case of SfCOL-wt was related with a higher productivity.

Table 1. Estimated LC_{50} values, relative potencies, mean time to death (MTD) and OB productivity (OBs/larva) values of SfCOL-wt, pure genotypes and OB and co-occluded mixtures in *S. frugiperda* second instars from Colombia. Values followed by different letters indicated significant differences.

Virus	LC_{50} (OBs/ml)	Relative Potency	*P* value	MTD (h)	Productivity (OBs/larva)
SfCOL-wt *vr* SfCOL genotypes assay					
SfCOL	$1.03x10^5$	1.0	-	167ab	$2.95x10^6$a
A	$2.34x10^4$	4.4	0.001	178b	$5.40x10^5$b
B	$1.99x10^5$	0.5	0.102	154bcd	$6.60x10^5$b
C	$9.02x10^4$	1.1	0.740	151cd	$2.90x10^5$bc
D	$3.31x10^5$	0.3	0.005	158ab	$3.50x10^5$bc
E	$1.32x10^5$	0.8	0.538	140d	$8.00x10^5$b
F	$2.53x10^5$	0.4	0.025	124e	$5.60x10^5$cd
G	$7.17x10^5$	1.4	0.357	160ab	$2.50x10^5$bc
H	$2.85x10^5$	0.4	0.012	125e	$2.60x10^5$bc
I	$1.95x10^5$	0.5	0.110	134de	$4.40x10^5$bcd
J	$4.60x10^5$	0.2	<0.001	126de	$1.20x10^5$e
OB and co-occluded mixtures assay					
SfCOL	$1.09x10^5$	1.0	-	142a	$1.63x10^7$a
A	$2.69x10^4$	3.7	0.005	144a	$1.07x10^6$b
OB mixtures					
(A)+(C)	$4.44x10^4$	2.5	0.010	161a	$1.20x10^5$c
(A)+(D)	$9.02x10^5$	1.2	0.580	160a	$1.10x10^5$c
(A)+(F)	$7.84x10^5$	1.4	0.320	142a	$1.70x10^5$c
Co-occluded mixtures					
(A+C)	$7.17x10^5$	1.4	0.357	142a	$8.70x10^6$b
(A+D)	$2.85x10^5$	0.4	0.012	115b	$1.04x10^6$b
(A+F)	$1.95x10^5$	0.5	0.110	120b	$1.25x10^6$bc

The potency of OBs and co-occluded mixtures were consistently reduced in two-genotype mixtures involving equal proportions of SfCOL-A and one of three deletion genotypes (Table 1). Speed of kill and OB production were improved only when the certain genotype mixtures were co-occluded, although OB production was higher in the SfCOL-wt isolate than in any of the component genotypes or mixtures thereof. Deleted genotypes reduced occlusion body potency but increased occlusion body production, suggesting that SfCOL-wt is structured to maximize the transmissibility.

In conclusion, the SfCOL-wt field isolate comprises a high genotypic diversity of which SfCOL-A was the most pathogenic and was as virulent as SfCOL-wt. Genotypic mixtures reduced speed of kill but also reduced OB pathogenicity which is undesirable for the development of a biological insecticide. SfCOL-wt seems to be structured to maximize the likelihood of transmission. While SfCOL-A due to its highest pathogenicity is better suited to be developed as a bioinsetcicide to control *S. frugiperda* in Colombia.

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