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Parasitoid-mediated transmission of an iridescent virus

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Abstract

We examined the interaction between an invertebrate iridescent virus (IIV) isolated from *Spodoptera frugiperda* (J.E. Smith) and the solitary ichneumonid endoparasitoid *Eiphosoma vitticolle* Cresson. In choice tests, parasitoids examined and stung significantly more virus infected than healthy larvae, apparently due to a lack of defense reaction in virus infected hosts. Parasitoid-mediated virus transmission was observed in 100% of the female parasitoids that stung a virus infected host in the laboratory. Each female parasitoid transmitted the virus to an average (±SE) of 3.7 ± 0.3 larvae immediately after stinging an infected larva. Caged field experiments supported this result; virus transmission to healthy larvae only occurred in cages containing infected hosts (as inoculum) and parasitoids (as vectors). The virus was highly detrimental to parasitoid development because of premature host death and lethal infection of the developing endoparasitoid. Female parasitoids that emerged from virus infected hosts did not transmit the virus to healthy hosts. We suggest that the polyphagous habits of many noctuid parasitoids combined with the catholic host range of most IIVs may represent a mechanism for the transmission of IIVs between different host species in the field.

Keywords: *Iridoviridae*; Virus transmission; Endoparasitoid; *Eiphosoma vitticolle*; *Spodoptera frugiperda*; Infection; Survival

1. Introduction

Invertebrate iridescent viruses (IIVs) (family *Iridoviridae*; genus *Iridovirus*) are nonoccluded icosahedral particles of approximately 130 nm diameter containing a dsDNA genome of approximately 140–210 kbp (Williams et al., 2000). These viruses have been isolated from insects including Lepidoptera, Coleoptera, Orthoptera, and Diptera and terrestrial isopods (Crustacea) and are generally associated with hosts that dwell in damp or aquatic habitats (Williams, 1998).

Patent lethal IIV infections are readily diagnosed because the host develops a characteristic opalescent lavender blue hue prior to death. Patent IIV infections are usually rare although epizootics have occasionally been reported (Fowler, 1989; Hernández et al., 2000; Ricou, 1975). In contrast, inapparent IIV infections have been detected by PCR, insect bioassay or electron microscopy and are reported to be prevalent in certain insect populations during certain periods (Tonka and Weiser, 2000; Williams, 1993, 1995). Such infections are not fatal but may cause a number of sublethal effects on the reproduction and longevity of covertly infected hosts (Marina et al., 1999).

The routes by which IIVs achieve transmission are unclear. Transmission via cannibalism of infected conspecifics has been demonstrated in tipulids, terrestrial isopods and mole crickets (Carter, 1973; Fowler, 1989; Grosholz, 1992). However, the extremely high infectivity of IIVs by injection has lead to the suggestion that parasites or insect parasitoids could transmit IIVs during the process of host penetration or oviposition, respectively (Hess and Poinar, 1985; Kelly, 1985; Mullens et al., 1999).

During a nucleopolyhedrovirus (SfMNPV) field trial in 1999, we observed a number of *Spodoptera frugiperda* (J.E. Smith) larvae patently infected by an IIV. Infected larvae occurred in control and nucleopolyhedrovirus sprayed plots alike indicating that this phenomenon was not due to contamination of the SfMNPV spray inoculum by an IIV. However, laboratory studies indicated

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that this IIV did not cause covert infections in *S. frugiperda* larvae, all infections were patent and lethal (O. Hernández, unpublished data). However, under specific conditions when larvae were inoculated just prior to pupation a very low prevalence of covert infection (2–5%) occurred in *S. frugiperda* adults (T. Williams, unpublished data).

Hymenopteran parasitoids are common and represent a significant factor of mortality in populations of *S. frugiperda* in maize, with a prevalence of parasitism typically between 15 and 30% (Martínez et al., 2000; Wheeler et al., 1989). We therefore set out to study the relationship between the IIV isolated from *S. frugiperda* larvae and the solitary ichneumonid endoparasitoid *Eiphosoma vitticolle* Cresson which is commonly found parasitizing *Spodoptera* spp. and a few other noctuid species in Mesoamerica (Ashley, 1979; Cave, 1995). This parasitoid attacks larvae in the second to fourth instar. The parasitoid larva emerges from the host and pupates in a cocoon approximately 12 days post-parasitism at 26 °C. The pupal stage lasts approximately 12 days for males and 13 days for females. Females begin reproduction at 6 days post-emergence and do not feed upon host fluids (Giraldo-Vanegas and García, 1995; M. López, unpublished data).

The particular objectives of the study were to determine whether: (i) parasitoids would discriminate between healthy and IIV infected *S. frugiperda* larvae, (ii) parasitoids that oviposited in infected hosts could transmit the virus to healthy hosts that were stung subsequently, (iii) parasitoids could develop in IIV infected hosts, (iv) parasitoids that emerged from IIV infected hosts could transmit the virus to healthy hosts, and (v) parasitoids could transmit IIV between infected and healthy hosts under field conditions.

2. Materials and methods

2.1. Insects and virus

A colony of the fall armyworm, *S. frugiperda*, was maintained on a semi-synthetic diet based on maize flour and soya modified from Mihm (1984). The colony of *E. vitticolle* was maintained using 6 day old *S. frugiperda* larvae from the laboratory colony. Parasitoids began reproducing at 6–8 days post-emergence. Honey solution was offered ad libitum as a food supplement. Both *S. frugiperda* and *E. vitticolle* colonies were started using healthy insects collected from maize fields within a 30 km radius of Tapachula, Chiapas, Mexico (14°54′N, 92°15′W).

The iridescent virus was isolated from an infected fourth-instar *S. frugiperda* collected in a maize field 22 km west of Tapachula, Chiapas, Mexico. The isolate had been passaged once by injection into third instar *Galleria mellonella* L. held on semi-synthetic diet at 25 °C. When these larvae became patently infected 6–10 days later, they were separated from the diet, placed in microcentrifuge tubes and stored at −20 °C prior to use. Virus was purified and quantified by adding known quantities of latex spheres (460 nm diameter, Aldrich Chemical) followed by counting the number of virus and latex spheres using a scanning electron microscope as described previously (Constantino et al., 2001).

To infect early instar *S. frugiperda*, a fine needle was dipped in a suspension of 6×10^6 particles/ml containing 2 μl of Tween 80 as a wetting agent. Previous studies indicated that Tween 80 does not affect IIV activity (P. Christian, unpublished data). Larvae were inoculated at 4 days post-hatching and were visibly infected by 6 days post-inoculation. Because of the near absence of covert infection of *S. frugiperda* larvae by this virus (O. Hernández, T. Williams, unpublished data) in all cases we limited our study to patent, lethal IIV infections.

All insects used in the following experiments were reared in a bioclimatic chamber at 24.5 ± 0.5 °C, 65 ± 10% RH, 12 h:12 h L:D photoperiod.

2.2. Discrimination between infected and healthy hosts

Single mated *E. vitticolle* females, 6–8 days old, were offered 10 second instar *S. frugiperda* for a period of 10 min to confirm that they were reproductively active. The following day, each female was individually placed in a 50 cm clear polythene centrifuge tube containing a rectangular piece of maize leaf (1.0×0.5 cm) on which were placed two *S. frugiperda* larvae of identical size and stage. One of these larvae had been previously inoculated with IIV and had developed a patent infection. The behavior of the parasitoid was observed and the following activities noted: antennal examination of larvae, stinging of larvae, interval between exposure to the larvae and stinging. Parasitoids were observed for up to 5 min or until one of the larvae was stung. The procedure was repeated five times for each of 50 female parasitoids. Larvae that had been stung were dissected to determine the presence of a parasitoid egg. The number of larvae of each type that were stung and percent parasitism (defined by the presence of an egg) were subjected to χ^2 test whereas the mean interval between offering larvae and parasitoid stinging was averaged for each of the 50 females and analyzed by t test.

2.3. Transmission of virus by contaminated ovipositor

Mated female parasitoids of 6–8 days old were individually placed in 50 ml centrifuge tubes containing a single patently infected third instar larva and a rectangular piece of maize leaf as described above. After the infected larva had been stung, the female parasitoid was

immediately removed and placed in a clean tube containing 10 healthy third instar larvae and was allowed to oviposit in all 10 larvae. A small quantity of *S. frugiperda* feces was also placed in the tube which greatly facilitated parasitoid oviposition. Each time a larva was stung it was removed and placed individually in a plastic pot containing semi-synthetic diet and reared in the bioclimatic chamber previously mentioned. These larvae were checked for signs of infection at 7 days post-stinging. Larvae that did not acquire an obvious infection were allowed to develop until pupation and emergence of the moth or the parasitoid. This procedure was performed with 56 different female parasitoids at 0, 1, and 2 days post-stinging and 10 parasitoids at 3 and 4 days post-stinging. Control larvae were treated identically but were exposed to a parasitoid that had not stung a virus infected host.

2.4. Survival of parasitoids in infected hosts

Preliminary trials indicated that if IIV infection preceded parasitism, all parasitoids died prior to pupation ($N = 120$ observations). Consequently, the following experiment was performed to determine how the interval between parasitism and infection affected the probability of parasitoid survival and emergence. Batches of 25–30 larvae that had been parasitized as late second-instars 24 h previously, were assigned to one of the following treatments: (i) parasitized larva infected using the contaminated needle technique; (ii) parasitized larva pricked using a clean needle dipped in sterile water; (iii) parasitized larva not pricked. A group of nonparasitized larvae was used as an additional control. The interval between parasitism and pricking was 1, 3, 7, and 11 days. Parasitoid larvae began to emerge from the host and pupate at 12 days post-parasitism. Larvae were individually reared in plastic pots containing semi-synthetic diet. Following pupation, parasitoid pupae were weighed using an analytical balance.

The pupae of *E. vitticolle* develop inside a silk cocoon and are not readily visible unless dissected, which often results in death of the developing parasitoid. However, when adult parasitoids failed to emerge from the pupal stage, they were opened and examined under a dissecting microscope. A number of these pupae showed the opalescent blue coloration characteristic of IIV infection whereas others were darkened and dried. It was not possible to quantify the prevalence of infection because dissecting the pupal case usually killed the developing parasitoid. A random sample of 9 nonemerged pupae were homogenized in 500 μl sterile water and subjected to two steps of centrifugation at 600*g* and 16,000*g* each for 10 min. The resulting pellet was examined for signs of iridescence. An equal number of unemerged parasitoid pupae from treatments not involving virus were included as controls.

Following adult emergence, male parasitoids were individually stored at −20 °C whereas female parasitoids were used in the next experiment. The length of the central vein of the right wing of all parasitoids was measured post mortem using a dissecting microscope fitted with an eyepiece graticule, calibrated to a precision of 0.03 mm. The experiment was performed four times for the 1 and 3 days post-parasitism treatments and seven times for the 7 and 11 days post-parasitism treatments. The proportions of parasitoids that reached to pupal and adult stages were analyzed in GLIM (Numerical Algorithms Group, 1993) using binomial error structures. GLIM presents the results of such analyses in terms of χ^2 statistics. Where necessary, scaling was performed to accommodate minor overdispersion (Crawley, 1993). The results of scaled analyses are presented as F statistics with the scale parameter indicated. In all cases, the behavior of models was checked by examination of the distribution of residuals and fitted values. The weight and size of parasitoids was analyzed using GLIM with a normal error distribution. Parasitoid sex ratios were analyzed using contingency tables (χ^2 tests).

2.5. Transmission of virus by parasitoids that emerged from infected hosts

To determine the incidence of virus contamination of parasitoids that had emerged from virus infected hosts, following adult emergence, male parasitoids were individually stored at −20 °C whereas female parasitoids that emerged from hosts injected at 7 and 11 days post-parasitism were individually placed in perspex and aluminium cages 30 cm high × 30 cm wide × 45 cm long with a cotton gauze door for ventilation. These females were allowed to mate with healthy males from the laboratory colony. Honey was provided ad libitum. Six days after adult emergence each female parasitoid was offered 10 healthy second instar *S. frugiperda*. Following a period of 10 min exposure to parasitism, these larvae were individually reared in plastic pots with semi-synthetic diet and checked periodically to determine the presence of IIV infection. The process was repeated at 24 h intervals a total of five times for each of 14 parasitoids that emerged from virus infected hosts, i.e., each female parasitoid was exposed to a total of 50 healthy hosts. These procedures were also performed with control parasitoids that emerged from hosts that had not been inoculated with virus.

Following these procedures, female parasitoids were individually homogenized in 500 μl sterile water and 8.6 μl volumes of the homogenate were injected into third instar *G. mellonella*. These larvae were reared on semi-synthetic diet and checked for signs of virus infection at 8 and 12 days post-injection. The bioassay was repeated with male parasitoids that had been frozen shortly after adult emergence.

2.6. Transmission of virus by parasitoids in the field

To determine whether parasitoid mediated transmission of IIV occurred under field conditions, we performed two caged experiments in a field of maize located approximately 10 km west of the town of Tapachula, Chiapas, Mexico. The climate in this region is warm (36 °C day, 23 °C night) and humid (70–90% RH). The first experiment was performed during the months of August and September 2001 when average precipitation was ~300 mm/month. The second experiment was performed in November 2001, when rainfall was <150 mm/month.

2.6.1. Experiment 1, 5 plants/cage

Field cages were constructed of three wooden struts, 1.2 m in length, which were driven into the ground in a triangular arrangement, resembling a native American tepee. White polyester gauze was stapled to the struts and soil was placed on the area of the material that touched the soil surface to prevent the escape of insects. Each cage covered five maize plants approximately 20 cm tall (~15 days post-emergence) growing in an "X" design and separated from one another by a distance of at least 30 cm (Fig. 1). Each cage was randomly assigned to one of the following treatments: (i) IIV infected *S. frugiperda* larvae and healthy *S. frugiperda* larvae + parasitoids, (ii) infected larvae + healthy larvae without parasitoids, (iii) healthy larvae + parasitoids, (iv) healthy larvae without parasitoids. Twelve cages were assigned to each treatment. In all cases, healthy second and third instar larvae (5 larvae/plant) were placed on each of the plants within the cage. In the treatment involving virus infected larvae, three patently infected third instar larvae were placed on the central plant located between the plants infested with healthy larvae. In this case, the central plant was not infested with healthy larvae (Fig. 1). Immediately after infesting the plants with *S. frugiperda* larvae, three male-female pairs of 6 day old *E. vitticolle* adults were released into each of the cages assigned to parasitoid treatments.

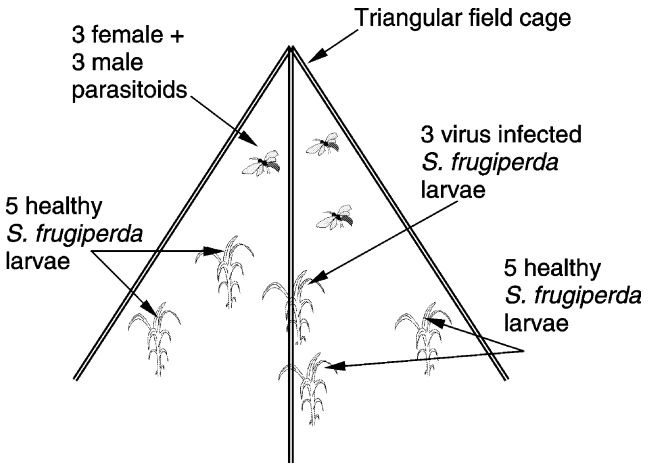

Fig. 1. Diagram of field cage used to study parasitoid transmission of iridescent virus. Five maize plants/cage were used in Experiment 1. For treatments involving virus, the central plant was infested with 3 infected *S. frugiperda* larvae and the surrounding plants were each infested with 5 healthy larvae/plant. For treatments involving parasitoids, 3 male–female pairs of *E. vitticolle* were released into each cage. Three pairs of maize plants/cage were planted in a line in Experiment 2. For treatments with virus, 3 infected larvae were placed on the central plant whereas 10 healthy larvae were placed on the plants at either side of the central plant. See text for additional details.

After 48 h, plants were cut, individually placed in plastic bags and transported to the laboratory where they were dissected and all living healthy *S. frugiperda* larvae were individually placed in plastic pots containing semi-synthetic diet. These larvae were placed in the bioclimatic chamber and examined periodically for signs of IIV infection and parasitoid pupation until 17 days after collection.

2.6.2. Experiment 2, 3 pairs of plants/cage

The second experiment was identical to the first, except in the following details. A total of 6 maize plants were planted in 3 pairs in a line separated by 30 cm within each cage. The same treatments were applied as in experiment 1, except that each pair of maize plants was infested with 10 healthy larvae or, in the case of the treatment involving virus infected larvae, 3 patently infected larvae were placed on the central pair of plants. A small quantity of *S. frugiperda* feces taken from nearby naturally infested plants was sprinkled into the whorl of each plant in order to stimulate parasitoid activity. Parasitoids were removed after 48 h inside the cages and the *S. frugiperda* larvae were left to feed on maize plants for the following 6 days, after which each pair of plants was cut, bagged, transported to the laboratory for examination and healthy *S. frugiperda* larvae were individually reared on semi-synthetic diet and periodically checked for infection and parasitism as described above. The experiment involved 14 cages assigned to each of the treatments involving parasitoids and 10 cages assigned to each of the treatments involving host larvae without parasitoids (total 48 cages).

3. Results

3.1. Discrimination between infected and healthy hosts

Parasitoids examined and stung significantly more infected larvae than healthy hosts (Table 1). As a result, the percentage parasitism of infected hosts was approximately double that of healthy hosts (Table 1). This was apparently because healthy larvae responded vigorously to the touch of parasitoid antennae or ovipositor, waving the upper half of their bodies energetically from side to side at an average rate of 1.2 ± 0.1 times/s

Table 1
Host discrimination and attack time of *E. vitticolle* simultaneously offered a healthy and a virus-infected *S. frugiperda* larva of the same stage and size

	Type of host		P^a
	Healthy	Infected	
Number hosts offered	250	250	
Number of hosts examined[b]	92	134	0.005
Number hosts stung	66	107	<0.002
Percentage parasitism[c]	17.6%	33.6%	<0.001
Mean attack time (s) ± SE	147 ± 14	152 ± 11	N.S.

[a] Probability determined by χ^2 test (df = 1) for number of hosts examined or attacked and percent parasitism and t test (df = 80) for comparison of mean attack time.
[b] The duration of antennal examination was extremely brief (~1 s) in all cases.
[c] Determined by dissection of hosts.

for a mean duration of 16 ± 3.5 s following the touch of the parasitoid (behavior quantified in 8 larvae). In contrast, virus infected larvae were lethargic and showed no equivalent responses to the parasitoid attack. There was no significant difference in the mean interval between exposure and attack by parasitoids which was around 2 min 30 s for both healthy and infected hosts alike (Table 1).

3.2. Transmission by contaminated ovipositor

All of the 56 female parasitoids that had previously stung an infected host transmitted the virus to healthy larvae. Each female parasitoid transmitted the virus to an average of 3.7 ± 0.3 larvae immediately after stinging an infected larva. This number fell markedly to an average of 0.2 ± 0.07 and 0.17 ± 0.07 at 1 and 2 days after stinging the infected larva, respectively. No transmission was observed in larvae exposed to parasitoids at 3 and 4 days after stinging the infected host. These figures represent 36.7, 2.0, and 1.8% of the larvae that were stung by the parasitoid at 0, 1, and 2 days after stinging the infected host, respectively (Fig. 2). Percentage emergence of *S. frugiperda* adults varied from 25.5 to 53.2% during the course of the experiment whereas percentage emergence of adult parasitoids varied between 19.1 and 29% during the experiment. Approximately 20% of larvae died of unknown causes (Fig. 2). None of the control larvae developed signs of IIV infection.

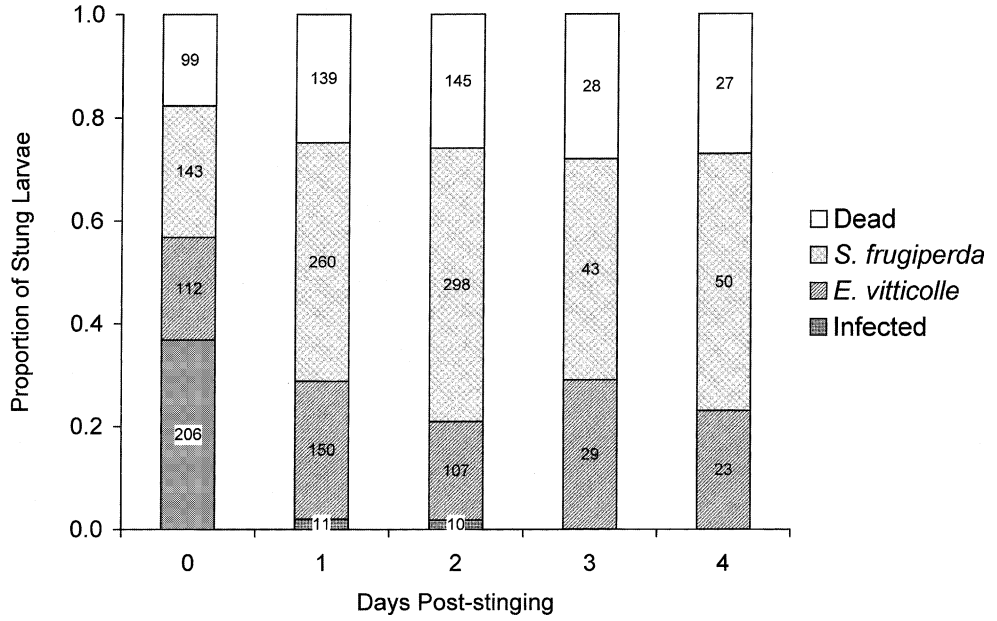

Fig. 2. Virus transmission by female parasitoids that stung an infected *S. frugiperda* larva. Each parasitoid was allowed to parasitize 10 healthy hosts immediately after stinging the infected larvae and the same number of healthy hosts at daily intervals thereafter. The fate of these larvae was monitored and is shown as the proportion of those hosts that developed signs of iridescent virus disease, emergence of a parasitoid or moth or those that died from unknown causes. Figures within columns indicate sample size (number of host larvae that gave rise to each result). There were 56 replicates at 0, 1, and 2 days and 10 replicates at 3 and 4 days post-stinging.

3.3. Survival of parasitoids in infected hosts

IIV Infections were only observed in *S. frugiperda* larvae that had been inoculated with virus. All of the parasitoids developing in hosts that were injected with virus at 1 and 3 days post-parasitism died prior to pupation (Fig. 3A). This was due to premature death of the virus-infected host at 10–12 days post-parasitism. Parasitoid development to the pupal stage was reduced by two thirds in hosts injected with virus at 7 days post-parasitism, compared to parasitized control larvae or parasitized larvae injected with water ($F_{2,18} = 17.1$, $P < 0.001$, scale parameter = 1.89). In contrast, pupation of developing parasitoids was only slightly reduced when virus was injected at 11 days post-parasitism and this effect was not significant ($F_{2,18} = 1.88$, $P = 0.18$, scale parameter = 1.85) (Fig. 3A).

Pupal mortality varied from 28 to 36% in parasitoids that pupated in the treatment of larvae that had been injected with water and noninjected controls whereas 61.3 and 48.5% of pupae that had developed in hosts injected with virus at 7 days ($\chi^2 = 8.1$, df = 2, $P < 0.02$) and 11 days ($\chi^2 = 6.73$, df = 2, $P < 0.04$) post-parasitism died during the pupal stage, respectively. The combined effect of reduced pupation and elevated pupal mortality caused a significant reduction in the emergence of adult parasitoids from hosts that were injected with virus at 7 days ($\chi^2 = 48.4$, df = 2, $P < 0.001$) and 11 days ($\chi^2 = 12.6$, df = 2, $P < 0.002$) post-parasitism compared to parasitoids that developed in larvae that had been injected with water and noninjected controls (Fig. 3B).

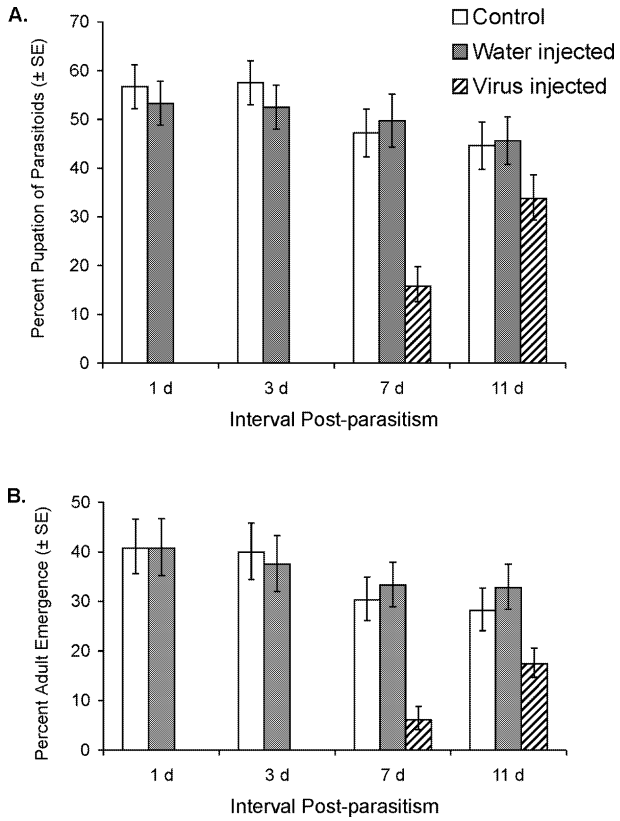

Fig. 3. Survival of parasitoids in host *S. frugiperda* larvae that were inoculated with iridescent virus at 1, 3, 7, and 11 days post-parasitism. Control larvae were not inoculated, or were inoculated with water. Graphs indicate (A) percentage parasitoid survival to the pupal stage and (B) percentage emergence of adult parasitoids. Groups of 25–30 larvae/treatment were used in each replicate. There were 4 replicates at 1 and 3 days post-parasitism and 7 replicates at 7 and 11 days post-parasitism.

Many of the parasitoid larvae that developed in infected hosts themselves developed an opalescent blue coloration and died prior to pupation. This coloration, a characteristic sign of IIV infection, was particularly evident along the central ventral and lateral epidermis of the parasitoid pupa. When pupae were homogenized and subjected to centrifugation, a brilliant blue iridescent pellet was obtained in 6/9 pupae tested from the treatments with the virus whereas control pupae from nonvirus treatments never produced an iridescent pellet.

The sex ratio of parasitoids at emergence was consistently male biased (0.74–0.82 proportion male) and did not differ significantly between virus and control treatments ($\chi^2 = 4.26$, df = 2, $P = 0.12$, data pooled for parasitoids that emerged from hosts injected at 7 and 11 days post-parasitism).

The mean weight of female parasitoid pupae was 47.4 ± 1.2 mg whereas male parasitoid pupae were significantly lighter with a mean weight of 36.9 ± 0.9 mg ($F_{1,37} = 52.7$, $P < 0.001$; all treatments pooled). Virus infection of the host caused virtually no change in the mean weight of male ($F_{2,17} = 0.02$, $P = 0.98$) or female ($F_{2,16} = 0.08$, $P = 0.92$) parasitoid pupae compared to parasitoids that emerged from control hosts that were not injected or those injected with water (data pooled for pupae from hosts treated at 7 and 11 days post-parasitism). Mean wing length was greater in female (6.63 ± 0.03 mm) than in male (5.84 ± 0.03 mm) parasitoids ($F_{1,37} = 337$, $P < 0.001$) but did not differ significantly between treatments ($F_{2,35} = 0.49$, $P = 0.62$; data pooled for parasitoids from hosts treated at 7 and 11 days post-parasitism).

3.4. Transmission of virus by parasitoids that emerged from infected hosts

Parasitism of healthy larvae by female parasitoids ($N = 14$) that emerged from hosts that had been infected with IIV at 7 and 11 days post-parasitism was observed in 24% ($N = 169$) of the 700 healthy larvae offered. However, these parasitoids did not transmit the virus to any of the healthy larvae that were offered to them for oviposition. When parasitoids were homogenized and injected into *G. mellonella*, evidence of IIV contamination was only seen in male parasitoids and was more prevalent in those that developed in hosts

Table 2
Results of a bioassay for the presence of iridescent virus contamination of male and female parasitoids that emerged from virus infected hosts inoculated at 7 and 11 days post-parasitism

Treatment	Sex of parasitoid			
	Female		Male	
	Number tested	Number positive	Number tested	Number positive
Host infected at 7 days post-parasitism	3	0	6	6
Host infected at 11 days post-parasitism	7	0	17	4
Controls	10	0	10	0

The presence of virus was detected by individually homogenizing each parasitoid and injecting the homogenate into third instar *G. mellonella* larvae that subsequently developed signs of infection if virus was present in the parasitoid homogenate.

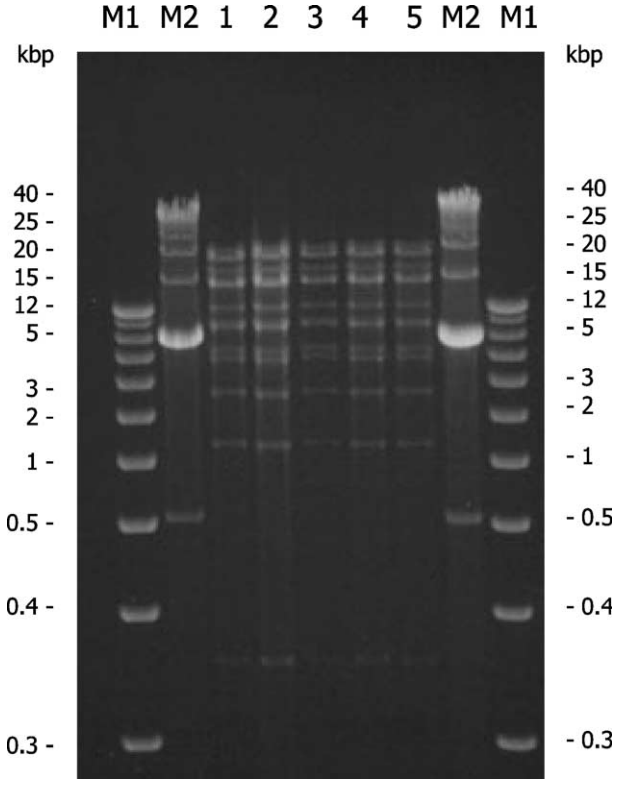

Fig. 4. Restriction endonuclease analysis of viral DNA purified from the original iridescent virus isolated from *S. frugiperda* larvae (lane 1), and infected *G. mellonella* larvae that had been injected with samples of adult male parasitoids that emerged successfully from virus inoculated hosts (lanes 2–5). M1 and M2 DNA fragment size markers in kbp (M1 = 5 Kb DNA ladder, M2 = 1 Kb + DNA ladder, Gibco BRL).

infected at 7 days than at 11 days post-parasitism (Table 2). A sample of 4 of these infected *G. mellonella* larvae was subjected to *Pst*I restriction enzyme analysis and was found to be identical to the original IIV isolate from *S. frugiperda* (Fig. 4).

3.5. Transmission of virus by parasitoids in the field

3.5.1. Experiment 1 (5 plants/cage)

An average (±SE) of 11.5 ± 0.7 healthy larvae were recovered from each cage after 48 h in the field, and this did not differ according to treatment ($F_{3,44} = 0.39$, $P = 0.76$). Parasitoid activity was very low in this experiment. Of the 12 replicate cages used for each treatment, parasitized hosts were only recovered from 1 cage assigned to the treatment involving virus infected larvae + parasitoids and 3 cages assigned to the treatment involving healthy larvae + parasitoids (Table 3). However, in the single cage assigned to the virus infected larvae + parasitoids treatment in which parasitoid activity was detected, 5/6 recovered healthy larvae subsequently developed IIV infection and the remaining larva died of parasitism. None of the larvae from any other treatments developed signs of IIV infection indicating that transmission of the virus did not occur due to migration of virus infected larvae to the surrounding plants followed by acts of aggression or cannibalism between infected and healthy larvae (Table 3).

3.5.2. Experiment 2 (3 pairs of plants/cage)

An average of 5.1 ± 0.3 healthy larvae were recovered from each cage after 6 days in the field and this did not differ according to treatment ($F_{3,44} = 1.61$, $P = 0.2$). Parasitoid activity was detected in 7 of the 14 cages assigned to each of the treatments involving parasitoids (Table 3). Of the 19 healthy larvae recovered from the 7 cages assigned to the treatment involving virus infected larvae + parasitoids in which parasitoid activity was detected, total of 3 larvae from 3 different cages subsequently developed IIV infection. As in the previous experiment, none of the larvae in any other treatment developed signs of IIV infection (Table 3).

4. Discussion

The ability of the endoparasitoid *E. vitticolle* to transmit an IIV to larvae of *S. frugiperda* was clearly demonstrated in laboratory studies. The phenomenon was also observed in a number of the field cages containing parasitoids with healthy and infected hosts. This confirms previous speculation that endoparasitoids may act as vectors for these viruses by stinging hosts with a contaminated ovipositor (Williams, 1998). The basis for this ability lies in the extremely high infectivity of IIV particles by injection. For example, typical infective

Table 3
Parasitism and virus transmission by parasitoids in field cages containing 5 plants (Experiment 1) or 3 pairs of maize plants (Experiment 2) infested with healthy *S. frugiperda* larvae with and without parasitoids, or virus infected and healthy *S. frugiperda* larvae with and without parasitoids

Treatment	Number of cages	Number of cages in which parasitism was observed	Number of larvae parasitized/total larvae sampled	Number of cages in which virus transmission was observed	Number of larvae that became infected/total larvae sampled
Experiment 1 *5 Plants/cage*					
Healthy + infected larvae + parasitoids	12	1	1/6 (17%)	1	5/6 (83%)
Healthy larvae + parasitoids	12	3	4/54 (7%)	0	0
Healthy + infected larvae, no parasitoids	12	0	0	0	0
Healthy larvae, no parasitoids	12	0	0	0	0
Experiment 2 *3 Pairs of plants/cage*					
Healthy + infected larvae + parasitoids	14	7	7/37 (19%)	3	3/19 (16%)
Healthy larvae + parasitoids	14	7	7/28 (25%)	0	0
Healthy + infected larvae, no parasitoids	10	0	0	0	0
Healthy larvae, no parasitoids	10	0	0	0	0

dose (ID_{50}) values for *Invertebrate iridescent virus 6* (IIV-6) injected into *G. mellonella* range from 1 to 3 particles per larva (Constantino et al., 2001; Marina et al., 2000). Laboratory assays indicated that the infectivity of the IIV isolate from *S. frugiperda* was very similar to that of IIV-6 (N. Hernández, unpublished data).

Adult female parasitoids that emerged from IIV infected hosts did not transmit the virus to hosts that they parasitized subsequently. However, IIV contamination of male parasitoids, detected by *G. mellonella* bioassay, was common. This may have been due to the differences in the treatment of male and female parasitoids. Females were tested for their ability to transmit the virus to healthy hosts and were killed and tested for IIV contamination at the age of 12 days, whereas male parasitoids were killed by freezing within 24 h of adult emergence. The virus particles that contaminated females may have been lost during cleaning or inactivated by low humidity during the transmission tests whereas no such processes occurred in male specimens. Male parasitoids might be capable of passing virus to females during mating, but this was not tested in our study.

Interestingly, the sluggish behavior of IIV infected larvae left them more prone to parasitism than healthy conspecifics. Healthy larvae were consistently observed to wave the upper half of their bodies frenetically in response to the touch of a parasitoid, whereas infected hosts were lethargic and unresponsive and were easily attacked by female parasitoids. The fact that *E. vitticolle* did not avoid parasitism of infected hosts that were clearly unsuitable for progeny development suggests that the reproduction of this parasitoid is time-limited rather than egg-limited; it is therefore advantageous to parasitize each and every host encountered irrespective of its condition. Optimal foraging theory predicts that parasitoids with egg-limited reproduction suffer high fitness costs by failure to discriminate against suboptimal hosts whereas time such costs are negligible in time-limited parasitoids (Godfray, 1994). The sluggish behavior commonly observed in insects with patent IIV infections may also make them vulnerable to parasitism, predation or cannibalism, creating inherent opportunities for virus transmission.

Nevertheless, the virus had a number of detrimental effects on parasitoid reproduction. Parasitoid survival to adulthood was severely reduced in hosts infected by IIV, even when infection occurred just prior to parasitoid pupation (11 days post-parasitism). IIV infection of the host appeared to have no adverse effects on the size or weight of parasitoids, possibly because parasitoid survival was only possible when the host was infected shortly before parasitoid pupation, by which time parasitoid development was nearly complete.

The reason for the decrease in parasitoid survival in infected hosts appears to be due to premature death of the host and virus infection of the developing parasitoid. However, it was not possible to quantify the proportion of developing parasitoids that acquired a lethal IIV infection because dissection of parasitized hosts or pupal cocoons resulted in death of the parasitoid. IIV infection of parasitic hymenopterans has not been previously reported although an IIV has been isolated from the Indian honey bee *Apis cerana* F. (Bailey et al., 1976) and IIV-like particles have been observed in the ant *Formica lugubris* Zetterstedt (Steiger et al., 1969). The observation of IIV infection of the endoparasitoid was not a surprise. Earlier observations with the eulophid ectoparasitoid *Euplectrus plathypenae* Howard developing on IIV infected *S. frugiperda* larvae revealed that the developing ectoparasitic larvae became brilliant blue and died before completing their development (O. Hernández and T. Williams, unpublished data).

Parasitoids in field cages were reluctant to engage in parasitic activity. In many cases they attempted to climb up the sides and escape through the top of the cage, and often became trapped in the folds of the gauze material. Careful observations made from dawn until dusk and even during the night indicated that parasitic activity tended to occur in the morning (8.00–11.00 h) and the late afternoon (16.00–18.00 h). Very high humidity following rainfall inhibited parasitoid activity. In this respect, the first field cage experiment was performed during the rainy season whereas the second experiment was performed at the start of the dry season.

Nevertheless, we consider that the modest number of cages in which virus transmission was observed is evidence that parasitoid mediated vectoring of IIV from infected to healthy *S. frugiperda* larvae occurs under field conditions. None of the healthy larvae became infected in any of the other treatments involving infected larvae without parasitoids or healthy larvae with parasitoids indicating that transmission of virus did not occur due to cannibalism or through healthy larvae consuming foliage contaminated by the feces of infected hosts.

Unlike the IIV isolates infecting *Simulium variegatum* Meigen populations in Great Britain, and the mayfly *Ecdyonurus torrentis* Kimmins in the Czech Republic (Tonka and Weiser, 2000; Williams, 1995), the IIV isolated from *S. frugiperda* larvae in Mexico did not induce abundant covert infections. This isolate is currently being characterized in more detail but initial results suggest that it is a possible member of the oligoiridovirus complex described by Williams and Cory (1994) within the genus *Iridovirus*. The only other member of this complex is IIV-6 (the identity of IIV-21 and IIV-28 is uncertain and they are possibly strains of IIV-6) (Webby and Kalmakoff, 1998). An IIV has also been reported at a low prevalence in *S. frugiperda* populations in Argentina but has not been characterized (Vera et al., 1995).

In a previous study of an IIV infecting aquatic larvae of the midge *Culicoides variipennis sonorensis* Wirth and Jones, 24/25 patently infected midge larvae were found to be parasitized by a mermithid nematode whereas 4/25 larvae were parasitized but showed no signs of IIV disease (Mullens et al., 1999). It was assumed that nematodes introduced the virus into the host during the act of penetration. Laboratory tests confirmed this; IIV inoculum caused 40–100% infection in the presence of preparasitic nematodes whereas the same concentration of IIV inoculum caused no visible infections in the absence of nematodes.

Recent analysis of the δDNA polymerase gene has indicated that iridoviruses are phylogenetically related to ascoviruses infecting lepidopteran larvae (Stasiak et al., 2000). Ascoviruses are also of low infectivity per os, but are highly infectious by injection (Govindarajan and Federici, 1990) and can be effectively transmitted from diseased to healthy noctuid hosts via the female parasitoid's contaminated ovipositor (Hamm et al., 1985). The ability of parasitoids to act as vectors has also been observed in a number of baculoviruses (Beegle and Oatman, 1975; Brooks, 1993; Caballero et al., 1990, 1991; Eller et al., 1988; Hochberg, 1991b; Levin et al., 1979; Young and Yearian, 1990). In almost all cases, these studies have been restricted to laboratory observations although evidence from field experiments supports the idea that female parasitoids can indeed vector baculoviruses (Fuxa and Richter, 1994; Hochberg, 1991b). Moreover, in certain baculoviruses, males that emerged from virus-infected hosts have been shown capable of limited virus dissemination by transferring virus from their feet to plant surfaces that are subsequently consumed by the host insect (Beegle and Oatman, 1975; Sait et al., 1996).

In dually parasitized and infected hosts, there may be intense interspecific competition for host resources (Hochberg, 1991a). As a consequence, there may exist clear benefits to each organism to evolve mechanisms for reducing such competition. For parasitoids, this may involve avoiding oviposition in diseased hosts (Stark et al., 1999; Versoi and Yendol, 1982). In contrast, viruses may employ physiological mechanisms to interfere with the development of juvenile parasitoids, such as the toxic factors produced by baculoviruses (Hotchkin and Kaya, 1985; Kaya and Tanada, 1973) and an entomopoxvirus (Kyei-Poku and Kunimi, 1998). Moreover, as recently pointed out, although certain insect viruses may benefit from parasitoid mediated vectoring, the costs of reduced virus progeny production in parasitized hosts may be greater than any benefits in terms of transmission opportunities arising through emergence of virus-contaminated parasitoids that subsequently transmit the virus to healthy hosts (Williams and Christian, 2002). This is because only half the parasitoid population

(females) is likely to transmit the disease and because the probability of successful transmission via parasitoids that emerge from infected hosts may be highly uncertain (Escribano et al., 2000; Sait et al., 1996; Vail, 1981). As such, baculoviruses, iridoviruses, and even ascoviruses may have toxin producing capabilities targeted at interfering with the development of insect parasitoids or parasites that compete for host resources (Williams and Christian, 2002).

Clearly, in the case of the IIV from *S. frugiperda*, the virus directly infected the developing parasitoids and thereby eliminated parasitoid competition for host resources. This reflects the marked differences in the host specificity of IIVs compared to the narrow host range of most insect baculoviruses, for example (Ward and Kalmakoff, 1991). However, host range in nature is likely to be determined to a large degree by the mechanism of transmission. Preliminary tests with another endoparasitoid commonly found parasitizing Mesoamerican *S. frugiperda* populations, the ichneumonid *Ophion flavidus* Brullé, indicated that this species could also act as a vector for the IIV (M. López, unpublished data). In contrast, after parasitizing an IIV infected host, the ectoparasitoid *Eu. plathypenae* was not capable of transmitting the virus to healthy hosts, presumably because the ovipositor did not pierce the host integument during the act of oviposition (O. Hernández and T. Williams, unpublished data). Both *E. vitticolle* and *O. flavidus* attack a number of different noctuid host larvae, including soil dwelling *Agrotis* spp. and may potentially transmit the virus from one type of host to another. Indeed, the IIV from *S. frugiperda* has not appeared in subsequent field trials involving sampling of very large numbers of *S. frugiperda* larvae. The origin of the outbreak detected in 1999 remains uncertain, but may be a completely different host species feeding on another plant.

In conclusion, we have demonstrated parasitoid mediated virus transmission of an invertebrate iridescent virus in 100% of the female parasitoids that stung a virus infected host in the laboratory. This result was supported by field cage studies in maize. The virus was highly detrimental to parasitoid development because of premature host death and lethal infection of the developing endoparasitoid. Female parasitoids that emerged from virus infected hosts did not transmit the virus to healthy hosts. We suggest that the polyphagous habits of many noctuid parasitoids combined with the catholic host range of many IIVs may represent a mechanism for the transmission of IIVs between different host species in the field. This hypothesis merits detailed empirical testing.

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