In Vivo and In Vitro Activity of Venom From the Endoparasitic Wasp *Pimpla turionellae* (L.) (Hymenoptera: Ichneumonidae)

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The biological activity of venom from *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) was examined in vivo toward larvae and pupae of *Galleriae mellonella* L. (Lepidoptera: Pyralidae), and in vitro toward bacterial and fungal cultures, as well as cultured insect cells. Pupae of *G. mellonella* were far more susceptible to the venom than larvae. At low doses of venom [0.1 venom reservoir equivalents (VRE)], pupal abdominal mobility was inhibited within 30 min, and by 24 h, all pupae injected with venom concentrations >0.5 VRE were completely paralyzed. These same doses of venom resulted in an inhibition of adult emergence. Host larvae were far less sensitive to wasp venom as evidenced by all venom injected larvae remaining responsive to mechanical stimulation by 1 h post injection, even at concentrations equivalent to 1 venom reservoir. Eventually (>2 h at 25°C), venom-injected larvae became immobile, then flaccid, and all died within 24 h post-injection. At lower concentrations of wasp venom, the onset of paralysis was delayed by comparison to that evoked by 1 VRE, and few host larvae were able to pupate. Development of host larvae to adult emergence was also reduced in a dose-dependent manner, with eclosion completely prevented at high concentrations (>0.5 VRE) of venom. Venom doses <0.5 VRE did not appear to induce paralysis or alter larval development. When venom was incubated with bacterial or fungal cultures, no antimicrobial activity was detected. However, wasp venom was found to be cytotoxic and cytolytic to cultured cells derived from the cabbage looper, *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae) and the yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culcidae). Though both cell types displayed similar susceptibility in terms of LC_{50}s, the lepidopteran cells responded much more rapidly with regard to the onset of morphological changes and the timing of cell death. A possible mode of action for the venom is discussed. Arch. Insect Biochem. Physiol. 61:87–97, 2006. © 2006 Wiley-Liss, Inc.

KEYWORDS: wasp venom; insecticidal activity; paralysis; cytolytic; cytotoxic

INTRODUCTION

Parasitic Hymenoptera regulate their hosts in order to provide a suitable source of nutrition and dwelling for their offspring (Vinson and Iwantsch, 1980; Thompson, 1993; Jervis and Copeland, 1996). Host regulation includes alterations in physiology, biochemical profile, survival, reproduction, and endocrine and immune status (Tanaka and Vinson, 1991; Penacchio et al., 1995; Digilio et al., 2000; Richards and Parkinson, 2000; Moreau et al., 2002; Nakamatsu and Tanaka, 2003). Venoms of parasitoids are important resources of these regulatory substances. Although venom components can be fatal, sublethal doses exhibit various physiological and developmental effects including

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Abbreviations used: CFU = colony forming unit; CI = confidence intervals; LC_{50} = lethal concentration to kill 50% of population; LD = light-dark; LD_{50} = lethal dose to kill 50% of population; LD_{99} = lethal dose to kill 100% of population; LT_{50} = lethal time to kill 50% of population; MIC = minimal inhibitory concentration; RH = relative humidity; VRE = venom reservoir equivalent.

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paralysis and molt inhibition (Coudron and Brandt, 1996; Masler and Kovaleva, 1999; Parkinson and Weaver, 1999). Several recent studies have revealed the composition of parasitoid wasp venoms to be a mixture of amines, proteins, and enzymes, with specific components implicated in host processing (Doury et al., 1997; Parkinson et al., 2001; 2002a; 2002b; 2002c; 2003; Uçkan et al., 2004). The physiological effects caused by the venoms of wasps also vary depending on the host species and stage attacked (Drenth, 1974; Rivers at al., 1993; Digilio et al., 1998). Some wasps possess venom that paralyzes or kills the host, whereas others arrest or slow growth and development (Rivers et al. 1993; Parkinson and Weaver, 1999; Masler and Kovaleva, 1999; Coudron et al., 2000). Recently, antimicrobial activity of parasitoid venom has also been determined, which would be advantageous to the parasitoid progeny surviving and developing in an immunocompromised host (Dani et al., 2003).

The solitary endoparasitic wasp, *Pimpla turionellae* L. (Hymenoptera: Ichneumonidae) envenomates and oviposits into prepupae and pupae of a number of lepidopteran species including the greater wax moth, *Galleria mellonella* L. (Lepidoptera: Pyralidae). Venom from this endoparasitic wasp contains several mid- to high-range molecular weight proteins, quantified previously as 0.04 µg protein per venom sac (Uçkan et al., 2004), as well as noradrenaline, apamin, and melittin (Uçkan et al., 2004). The latter are consistent with the non-specific paralytic action of the venom in multiple life stages of lepidopteran hosts (Kansu and Uğur, 1984). There have been no detailed studies on the activity of venom from *P. turionellae*, although an inhibition of haemocyte pseudopodia formation and encapsulation by venom has been reported (Osman, 1978). Kılınçer (1975) has stated that venom of this parasitoid is lethal and paralysis seemed to be induced by the neurotoxic effect of venom on the abdominal muscles in pupae of *G. mellonella*. However, there is still scarce information on the diversity of the bioactive effects of *P. turionellae* venom towards hosts.

The present study was aimed at examining the biological activity in vivo and in vitro of venom from *P. turionellae*. Venom was examined for its toxicity toward larvae and pupae of *G. mellonella*. We also investigated the antimicrobial activity of venom towards bacterial and fungal cultures. The toxicity of wasp venom was also evaluated in vitro using cultured cells (BTI-TN-5B1-4) from the cabbage looper, *Trichoplusia ni* Hubner (Lepidoptera: Noctuidae) and larvae of the yellow fever mosquito, *Aedes aegypti* (L.) (Diptera: Culcidae).

MATERIALS AND METHODS

Insect Rearing and Venom Isolation

P. turionellae was maintained as laboratory culture on pupae of the greater wax moth, *G. mellonella* at 25 ± 1°C, 60 ± 5% RH and a photoperiod of 12:12 h LD. Venom reservoir contents were isolated from honey- and host-fed females as previously described (Uçkan et al., 2004). A venom reservoir equivalent (VRE) was defined as the reservoir material obtained from one wasp. To examine the dose-dependent effect of venom on host larvae and pupae, venom reservoirs obtained from 1, 2, 10, and 20 females were placed separately in microcentrifuge tubes (1 ml) each containing 100 µl of ice-cold physiological saline. The final concentration in each tube was adjusted to venom reservoir equivalents (VRE) of 0.05, 0.1, 0.5, and 1 in 5 µl of saline, respectively. Venom samples of 0.02, 0.01, and 0.005 VRE/5 µl used additionally in host pupa assays were adjusted by placing one female reservoir content in microcentrifuge tubes (1 ml) containing 250, 500, and 1,000 µl physiological saline. Venom reservoirs in tubes were gently ruptured with forceps. Then, the tubes were centrifuged at 3,000*g* for 10 min and the pellets were removed. Venom solutions were used immediately.

Insecticidal Assays

Injections were performed between the last two lateral abdominal segments of pupae by using a 10-µl Hamilton microsyringe (Hamilton, Reno,

NV) in each set of experiments. The injected volume was 5 µl per pupae in all experimental treatments for various VRE units given above. Injections to 5 host larvae were also performed through the basal membrane of the hind leg of larvae. The injected volume was 5 µl per larvae. Control insects were injected with 5 µl of physiological saline (Control I) or an empty injection (Control II) only was performed to test whether or not insects were affected from trauma associated with injection. Injections were always carried out on pupae weighing 90 ± 3 mg and last instars weighing 160 ± 3 mg to avoid variability of the injected amount of venom solution into hosts. Each set of experiments was replicated three times for pupae and larvae. Control and treated insects were held at 25 ± 2°C, 60 ± 5% RH under a photoperiod of 12:12 h LD, and observed for response to mechanical stimulus at the end of first 30, 60, and 120 minutes. The response was assessed by the presence of abdominal mobility for larvae and pupae when insects were disturbed by forceps. Subsequently, insects were observed daily (30 days for pupae and 90 days for larvae) to record survival, adult emergence ratio (the ratio of host larvae and pupae reached to adult stage), and the development period from injection to emergence. Mortality data were derived from survival rates, and the lethal doses to kill 50% (LD_{50}s) and 99% (LD_{99}s) (VRE/insect) of the population with 95% confidence intervals (CI) were calculated by using probit analysis (PriProbit, PriProbitNM (C) 1998-2000 Masayuki Sakuma, Kyoto University, Kyoto, Japan) after Abbott's correction (Abbott, 1925) for natural mortality.

Antimicrobial Assays

Antimicrobial activity was measured by using liquid growth inhibition assays (Bulet et al., 1993) with *Staphylococcus aureus* (gram positive, ATCC-29213), *Escherichia coli* (gram negative, ATCC-35218), and *Candida albicans* (ATCC-14053). Twenty microliters of bacterial (10^6 CFU/well) or fungal (10^5 CFU/well) suspensions were added to each well of a 96-well plate containing 90 µl Müller-Hinton broth. Crude venom was tested for antimicrobial activity by adding 10-µl aliquots of venom solution (1-10^{-5} VRE/well in physiological saline) to wells containing either bacteria or fungi. Each plate was incubated at 37°C for 24 h, and microbial growth was assessed by an increase in absorbance at 600 nm in order to determine the minimal inhibitory concentration (MIC) for each microorganism (Bulet et al., 1993). Measurements of microbial growth in the presence of physiological saline or no-treatment served as controls. All assays were replicated three times.

Cell Assays

To evaluate whether venom from *P. turionellae* displayed cytotoxicity, venom assays were performed in vitro using the well-characterized cell line BTI-TN-5B1-4 (also known as High Five™) derived from *T. ni*, by the methods of Rivers et al. (1999). Cells were grown (approximately 2–3 days) to confluency at 27°C in 96-well microtiter plates containing TC-100 (Sigma Chemical Co., St. Louis, MO) with 10% fetal bovine serum (Sigma) (100 µl/well). Following the addition of wasp venom (in distilled water), cell viability was assessed using trypan blue dye exclusion staining (Rivers et al., 1993). Cell responses (i.e., morphological changes) were monitored continuously with a Sony CCD camera mounted on a phase-contrast inverted microscope and connected to a Macintosh G4 containing a Scion CG-7 frame grabber (Scion Corp., Frederick, MD). Changes in cell shape and membrane integrity (i.e., swelling and lysis) were determined from captured images following the criteria of Trump and Berezesky (1995). The experiments were repeated using cells (Aag2) derived from larvae of the yellow fever mosquito, *A. aegypti* (Shih et al., 1988). Mosquito cells were maintained in Schneider's medium (Sigma) containing 10% fetal bovine serum at 27°C.

In a parallel set of experiments, the concentration of crude venom required to kill 50% of the cells (LC_{50}) was determined using 5–6 venom concentrations at 27°C as described (Rivers et al., 1993). Mortality was assessed at 24-h intervals for 4 days and analyzed using probit analysis (Finney,

1971). The time required to kill 50% of the cells (LT_{50}) was calculated in a similar manner using an LC_{99} dose of venom for each cell line at 10 time intervals (0–24 h). The time required to kill 50% of the cells was calculated by probit analysis (Finney, 1971).

RESULTS

Insecticidal Activity

Injection of venom into the hemocoel of *G. mellonella* resulted in a host response that was dose- (F = 59.231; d.f. = 8; $P < 0.001$) and developmental stage-dependent (F = 60.007; d.f. = 1; $P < 0.001$) in terms of the onset of paralysis, adult emergence, and induction of death (Tables 1 and 2). The pupal stage appeared to be more sensitive to venom than the larval stage: a greater percentage of paralysis and mortality was observed in this natural host stage than with larvae (Tables 1 and 2).

Pupae of *G. mellonella* were far more susceptible to the venom than larvae as evidenced by a higher incidence of paralysis at low venom doses (Table 1), lower LD_{50} and LD_{99}s (Figs. 1 and 2), and suppressed rates of adult emergence (Table 1). Pupal abdominal mobility was inhibited within 30 min at 25 ± 1°C with a venom concentration of 0.1 VRE (Table 1). The ratio of individuals responding to mechanical stimulation declined with increasing venom dose (Table 1). By 24 h, all venom-injected pupae were paralyzed when injected with any dose above 0.05 VRE. In contrast, sham-injected and saline-injected pupae showed no signs of paralysis and all completed development by emerging as adults. Pupae injected with doses between 0.1–1 VREs began showing signs of melanization at the site of injection within 24 h. Within 1–2 days, venom-injected pupae displayed a darkening of the entire integument that was accompanied by a deterioration of host tissues. Pupae that failed to complete development were immediately paralyzed following venom injection and remained pupal-like in morphology until death.

Adult emergence ratio differed significantly (F = 124.838; d.f. = 8, 18; $P < 0.001$) among experimental and control groups and there was no adult emergence at doses ≥0.05 VRE. An increase in adult emergence ratio was detected with decreasing venom dose: adult emergence was 33.3, 66.6, and 86.6% at 0.02, 0.01, and 0.005 VREs (Table 1). Injection of venom had no effect on the developmental period of pupae from injection to adult emergence. The developmental periods of venom-injected groups were not significantly different from those of controls (F = 1.423; d.f. = 4, 53; $P > 0.05$).

Within 1 h post injection at 25 ± 1°C, larvae remained responsive to mechanical stimulation and showed no signs of paralysis or necrosis aside from the site of microsyringe insertion. However, by 2 h, 20% (n = 15) of the larvae injected with 1

TABLE 1. Response of *G. mellonella* Pupae to Venom Solutions of *P. turionellae*

	Response to mechanical stimulus (RMS)(%)				
	Minutes after injection				
Venom dose (VRE/5 μl/pupa)[a]	30	60	120	Adult emergence ratio (%)*	Development period (days) from injection to emergence ($\bar{x}$ ± SEM)[b,c]
1.0	33.3	13.3	6.6	0.0a	–
0.5	87.6	46.7	20.0	0.0a	–
0.1	93.0	87.6	53.3	0.0a	–
0.05	100.0	100.0	100.0	0.0a	–
0.02	100.0	100.0	100.0	33.3b	13.2 ± 1.0a (5)
0.01	100.0	100.0	100.0	66.6c	10.4 ± 0.6a (10)
0.005	100.0	100.0	100.0	86.6c	9.5 ± 0.7a (13)
Control I	100.0	100.0	100.0	100.0d	10.4 ± 0.9a (15)
Control II	100.0	100.0	100.0	100.0d	10.4 ± 0.9a (15)

[a]Every venom reservoir equivalent (VRE) of *P. turionellae* injected into host pupae weighing 90 ± 5 mg. Data are results of the three replicates using 5 host pupae per replicate.
[b]Numbers in parentheses represent the number of pupae developed to adult stage. Control I: Physiological saline; Control II: Injection only.
[c]Dashes indicate no adult emergence.
*Numbers in columns followed by the same letter are not significantly different ($P > 0.05$).

TABLE 2. Response of *G. mellonella* Larvae to Venom Solutions of *P. turionellae*

	Response to mechanical stimulus (RMS)(%)				
	Minutes after injection				
Venom dose (VRE/5 μl/larva)[a]	30	60	120	Adult emergence ratio (%)*	Development period (days) from injection to emergence ($\bar{x} \pm$ SEM)[b,c]
1.0	100.0	100.0	80.0	0.0a	–
0.5	93.0	93.0	93.0	13.3a	59.0 ± 32.0a (2)
0.1	100.0	87.6	100.0	86.6a	47.5 ± 7.2a (13)
0.05	100.0	100.0	100.0	93.3a	42.5 ± 5.9a (14)
Control I	100.0	100.0	100.0	100.0d	31.8 ± 5.1a (15)
Control II	100.0	100.0	100.0	93.3d	34.8 ± 6.8a (14)

[a]Every venom reservoir equivalent (VRE) of *P. turionellae* injected into last instars of host weighing 160 ± 5 mg. Data are results of the three replicates using 5 host larvae per replicate.
[b]Numbers in parentheses represent the number of larvae developed to adult stage. Control I: Physiological saline; Control II: Injection only.
[c]Dash indicates no adult emergence.
*Numbers in columns followed by the same letter are not significantly different ($P > 0.05$).

VRE did not respond to stimulation. This loss of abdominal mobility was followed by a darkening of the integument attributed to haemolymph melanization near the site of injection. Larvae became flaccid and died within 24 h post-injection. None of these venom-injected larvae completed development to adult eclosion. At lower concentrations of wasp venom, the onset of paralysis was delayed by comparison to that evoked by 1 VRE, and few were able to pupate (Table 2). Venom doses lt;0.5 VRE did not appear to induce paralysis or alter larval development by comparison to sham-injected and untreated larvae.

The ratio of adult emergence was reduced by all doses tested, and completely stopped by injection of 1 VRE into larvae (Table 2). Adult emergence ratio significantly differed (F = 22.771; d.f. = 5, 12; $P < 0.001$) and increased with decreasing dose, being 13.3% at 0.5 VRE, increasing to 86.6 and 93.3% at 0.1 and 0.05 VREs, respectively. The developmental period of larvae from injection to adult emergence increased as the dose of venom

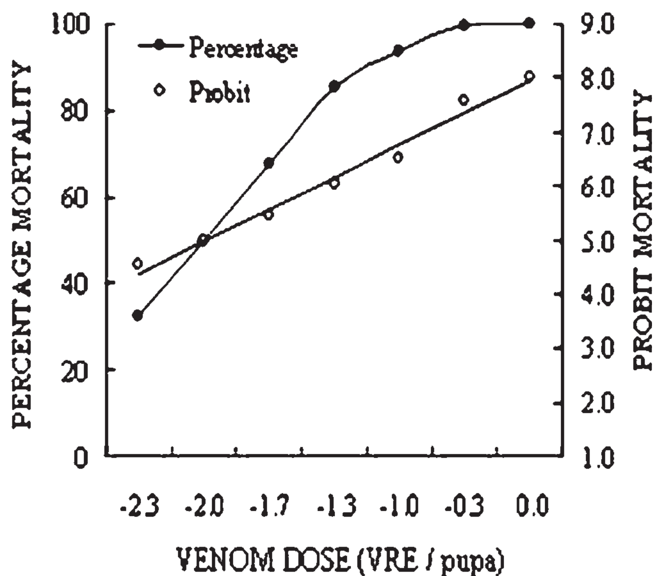

Fig. 1. Percentage and probit mortality of *G. mellonella* larvae as a function of log dosage of *P. turionellae* venom. Each point is a mean of three replicates each containing 5 larvae.

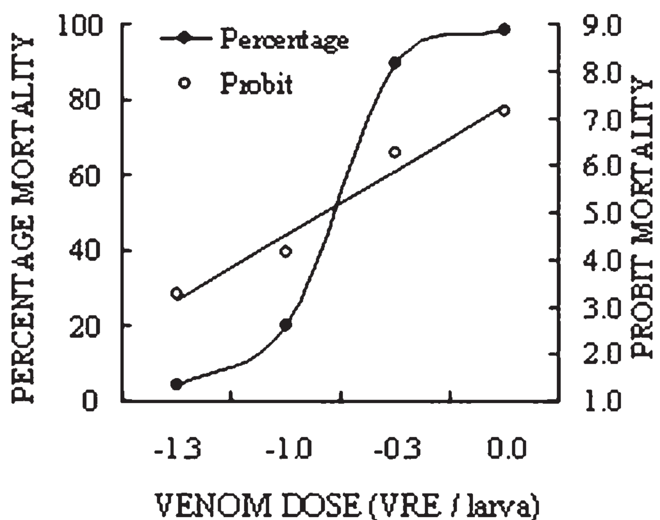

Fig. 2. Percentage and probit mortality of *G. mellonella* pupae as a function of log dosage of *P. turionellae* venom. Each point is a mean of three replicates each containing 5 pupae.

increased, but the difference among adult emergence periods was not significant (Table 2, $F = 1.283$; d.f. = 4, 53; $P > 0.05$).

Wasp venom was relatively toxic toward both pupae and larvae of *G. mellonella* but pupae were nearly 10-fold more sensitive than larvae (Figs. 1 and 2). The LD_{50} and LD_{99} calculated for pupae were 0.01 (0.009–0.016) and 0.06 (0.057–0.080) VRE/pupa (95% CI) (slope = 3.209), respectively, whereas for larvae, the values were LD_{50} =0.2 (0.13–0.29) and LD_{99} =1.1 (0.66–3.64) VRE/larva (95% CI) (slope = 3.009).

Antimicrobial Activity

No antimicrobial activity was detected even when the highest concentration of one reservoir equivalent of wasp venom was incubated with either bacterial or fungal cultures. Therefore, the minimal inhibitory concentration (MIC) for each microorganism could not be calculated.

Cell Activity

A comparison of the cytotoxic activity of venom from *P. turionellae* was made using cells derived from *T. ni* (BTI-TN-5B1-4) and *A. aegypti* (Aag2). Both insect cell lines were susceptible to the wasp venom and the cells displayed nearly identical sensitivity in terms of LC_{50}s (Table 3). With all susceptible cells, venom caused a rounding up of cells (Fig. 3b and c), followed by swelling of the plasma (but not nuclear) membranes (Fig. 3d), and eventual death. The timing of these cell responses, however, was not identical among the cell lines. For example, nearly 50% of the BTI-TN-5B1-4 cells were swollen within 1 h after venom addition (Fig. 3b), and 85% of the cells were nonviable by 24 h post-venom incubation (Table 3). In contrast, cell swelling occurred 3–5 times more slowly in Aag2 cells and cell death exceeding 80% mortality was not achieved until 50 h post-venom incubation. For both cell types, the LT_{50} values were long with corresponding slopes (Table 3).

TABLE 3. Cytotoxicity of Venom From *P. turionellae* Toward Cultured Insect Cells*

		Susceptibility	
Cell line	Origin	LC_{50} (VRE/μl) (95% CI) slope	LT_{50} (hours) (95% CI) slope
Trichoplusia ni BTI-TN-5B1-4	Minced embryos	0.0046 (0.0031, 0.0098) 0.872	27.4 (19.1, 39.0) 4.322
Aedes aegypti Aag2	Neonate larvae	0.0065 (0.0048, 0.0125) 1.134	34.5 (18.3, 50.2) 3.875

*VRE = venom reservoir equivalent.

All of the susceptible cells displayed similar morphological changes when incubated with an LC_{99} dose of wasp venom. To further characterize morphological changes, BTI-TN-5B1-4 and Aag2 cells were continuously monitored at 27°C with a progressive scan color video camera connected to Macintosh G4 equipped with a Scion series 7 frame grabber. For both cell lines, untreated and saline-treated cells were fibroblastic-like in appearance, with numerous cytoplasmic protrusions or extensions often positioned in a bi- or multipolar arrangement. Cells in the logarithmic phase of growth formed adherent, confluent monolayers, and by the stationary phase, began to retract the cytoplasmic extensions, giving the cells a more rounded appearance. In all cell assays in this study, venom was added to cells in the logarithmic phase.

When an LC_{99} dose of venom was added to either type of insect cell, the first detectable change in cell morphology was a retraction of the cytoplasmic protrusions. The onset of this event, however, was not the same for both cell lines: Cells from the cabbage looper began to retract the cytoplasmic extensions several hours (3–5) earlier than *A. aegypti* cells. In fact, all venom-induced changes in cell morphology were always observed several hours earlier in BTI-TN-5B1-4 cells than Aag2 cells. Blebbing of the plasma membrane preceded retraction in mosquito cells but not in the caterpillar cell line. Swelling of the plasma membrane occurred 24–48 h after venom addition depending on the cell line: Typically *T. ni* cells showed signs of swelling 24 h sooner than those from *A. aegypti*. The nuclei of these cells did not swell but the nuclear material condensed (chromatin). Swelling of cells culminated with interruptions (lysis) of the

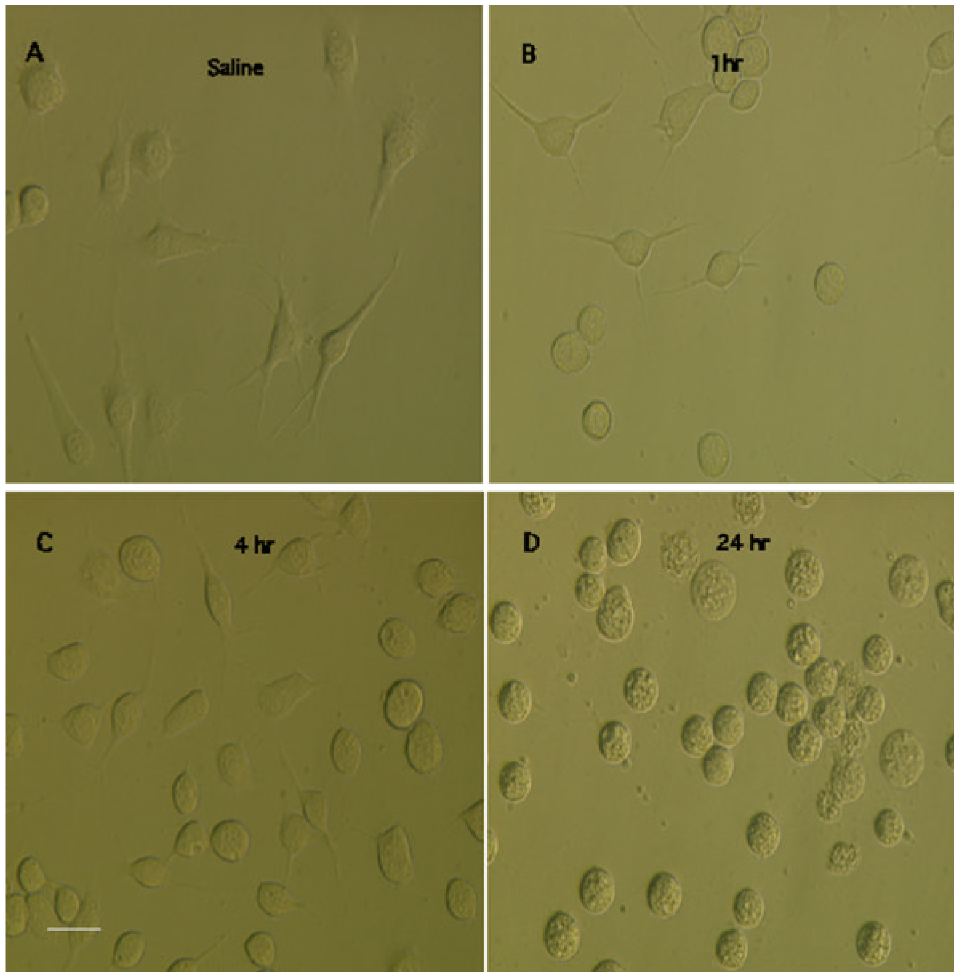

Fig. 3. Cytotoxicity of venom from *P. turionellae* toward BTI-TN-5B1-4 cells at (**B**) 1, (**C**) 4, and (**D**) 24 h post-treatment. Cells were incubated with a LC_{99} dose of crude venom at 27°C. Cell monolayers exposed to saline (**A**) served as controls (image shown is at 24 h). Photographs were made using a 40× objective connected to a 0.55× camera coupler. Scale bar = 50 µm.

plasma membrane. For both cell lines, lysis did not occur until 12–24 h after the cells became swollen.

DISCUSSION

Upon locating a suitable host, females of *P. turionellae* always inject venom prior to ovipositing a single egg into the hemocoel of pyralid hosts (Kansu and Uğur, 1984). In all cases, the host dies either due to the feeding activity of the wasp larva or due to the paralytic and/or neurotoxic effects of venom. Virtually nothing is known about the insecticidal activity or mode of action of this wasp venom. Therefore, this study was conducted to examine the ability of venom from *P. turionellae* to induce paralysis and retard development in two developmental stages (larvae and pupae) of a preferred host, *G. mellonella*. Our observations revealed that host pupae were far more sensitive to venom than larvae in terms of LD_{50}s, incidence of paralysis and mortality, and in development being retarded or halted. In fact, pupae were found to be 20-fold more susceptible than larvae to venom based on LD_{50}s and in the minimum venom dose necessary to inhibit adult emergence. This is in agreement with previous findings that suggested that though this wasp can utilize multiple life stages from at least eight families of Lepidoptera as hosts, pupae are selected in choice tests over prepupae and larvae (Kansu and Uğur, 1984).

In nature, larvae are not typically selected as hosts by *P. turionellae*. However, this study has demonstrated that multiple life stages of *G. mellonella* are susceptible, albeit not equally, to wasp venom. This is consistent with several investigations (Rivers et al., 1993; Coudron and Brandt, 1996; Gupta

and Ferkovich, 1998; Parkinson and Weaver 1999; Coudron et al., 2000) that have shown venom from many other parasitoid species is effective toward factitious host stages as well as natural hosts. For example, the larval ectoparasitoid *Euplectrus comstockii* Howard (Hymenoptera: Eulophidae) normally parasitizes fourth instars of its host, *T. ni*, and has also been shown to be capable of arresting development in last larval instars (fifth) and pupal stages (Coudron and Brandt, 1996). Likewise, venom from the ectoparasitic wasp *Nasonia vitripennis* (Walker) (Hymenoptera: Pteromalidae) is equally toxic to larvae, pupae, pharate adults, and imagoes of its preferred flesh fly host, *Sarcophaga bullata* Parker (Diptera: Sarcophagidae) (Rivers et al., 1993). However, parasitoid females only attack fly puparia in nature. Considering effective biological control can only be achieved by controlling the foraging developmental stage of the host species, it is essential to establish the susceptibility of larvae of *G. mellonella* to wasp venom, as this study has done, before attempting to construct recombinant bio-selective insecticides toward pyralids infesting honeycomb.

The venom from *P. turionellae* was found to be neurotropic toward larvae and pupae. The rather sudden inhibition of pupal abdominal mobility may suggest that venom targets neuromuscular junctions (Zdarek et al., 1987), or that central motor programs have been disrupted (Rivers et al., 2004). The fact that multiple host stages were sensitive to wasp venom, and that paralysis was induced argues for the presence of either lytic components or modulators of ion channels, or at least venom constituents that are likely non-selective (Schmidt, 1982). These observations are consistent with the activity of several compounds identified in the venom of *P. hypochondriaca* (Retzius) (Hymenoptera: Ichneumonidae), in particular pimplin, a paralytic peptide that elicits rapid flaccid paralysis in multiple stages of insects from at least 3 orders (Parkinson and Weaver, 1999; Parkinson et al., 2001, 2002a). cDNAs encoding a venom serine protease, a reprolysin-type protease, and trehalase- and laccase-type enzymes have also been recently characterized from venom of this wasp and may contribute to host paralysis (Parkinson et al., 2002b,c, 2003). Two enzymes causing cell or tissue lysis, phospholipase, and hyaluronidase have been determined in *Eupelmus orientalis* (Crawford) (Hymenoptera: Eupelmidae). Though specific enzymes have not been characterized in crude venom from *P. turionellae*. Uçkan et al. (2004) have identified melittin, apamin, and noradrenaline, components that are known to induce paralysis and indiscriminate cell lysis (Schmidt, 1982; Piek and Spanjier, 1986), and i.r. spectral analyses suggest an abundance of enzymes in crude venom. Supporting the non-specific action of the venom are our observations from in vitro venom assays: Cultured cells from a caterpillar (*T. ni*) and mosquito (*A. aegypti*) displayed nearly identical LD_{50}s values. Additionally, both types of cells required several hours for the onset of cell swelling and death, and at least 48 h was needed before cell lysis was evident. These features are consistent with the presence of paralytic peptides in the wasp venom, and parallel the action of venoms from social Hymenoptera (Piek and Spanjier, 1986). However, the delayed cytotoxic effects observed in this study are not in agreement with the very rapid lytic action of melittin, nor is the venom activity consistent with the cytotoxic action described for venom of *P. hypochondriaca* (Parkinson and Weaver, 1999). Further studies are needed to uncover the mode of action and target tissues of this wasp venom. A determination whether venom is directly modulating specific ion channels or perhaps disrupting calcium homeostasis, to eventually lead to a gradual loss of membrane integrity several hours to days later, is currently being investigated.

Venoms from parasitic Hymenoptera that are paralytic in action are more typically associated with ectoparasitic species (Piek and Spanjier, 1986; Quicke, 1997). This association is due to the ectoparasitic wasps using mobile host stages like lepidopteran larvae. In such scenarios, a mobile host poses a threat to adult females during oviposition as well as to developing parasitoid larvae (Quicke, 1997). A paralytic action seems unnecessary for the venoms from parasitoids that utilize an egg or pupae as hosts since these stages are already non-mo-

bile. Taylor (1937) has reported that *Pleurotropis passei* (Hymenoptera: Eulophidae) does apparently paralyze beetle pupae. In the case of this beetle host and several other Coleoptera and Lepidoptera, the pupal stage is characterized by limited mobility, and therefore paralysis may facilitate oviposition. However, if this were so, venom from *P. turionellae* would be predicted to induce a temporary paralysis, and not a permanent cessation of host development as observed in this study (Quicke, 1997).

An alternative explanation for the presence of paralytic venom may be associated with the host's immune system. Many endoparasitic species that use larvae as hosts have specific venom components acting to neutralize the host's immune and endocrine systems, thereby causing a delay in growth and development (Tanaka and Vinson, 1991; Parkinson and Weaver 1999; Richards and Parkinson, 2000), because premature death of a larval host would be disadvantageous to a parasitoid developing inside the host (Vinson and Iwantsch, 1980). In contrast, pupal parasitoids may not need venom components facilitating a delay in host development since the pupa cannot feed and represents a closed nutritional container for the parasitoid progeny (Vinson and Barbosa, 1987). In addition, Parkinson and Weaver (1999) reported that host pupae that had been killed by freezing and then offered as hosts yielded as many *P. hypochondriaca* offspring as nonfrozen host pupae. They concluded that pupae need not be alive at the time of oviposition for successful parasitism to occur and suggested that early killing of the pupal host may represent an effective strategy for controlling the host's immune responses to parasitism (Parkinson and Weaver, 1999). Consequently, the paralytic activity of venom from *P. turionellae* may be a rapid means to suppress host cellular and/or humoral immune responses to facilitate parasitoid development. The observation that darkening of the host integument and haemolymph attributed to melanization was only evident when pharmacological doses (≥0.5 VRE/host) of venom was injected into pupae or larvae of *G. mellonella* supports this view.

Several investigators have shown that low molecular weight peptides such as melittin, mastoparan, and anoplin found in the venom of the honeybee, *Apis mellifera* (Subbalakshmi et al., 1999), vespids (Ming-Liang et al., 2000), and a solitary wasp (Konno et al., 2001) display antibacterial activity. Similarly, venom from the closely related endoparasitoid *P. hypochondriaca* also shows anti-microbial activity toward *E. coli* grown on plates and in liquid cultures (Dani et al., 2003). When venom from *P. turionellae* was tested for similar activity, no microbial death was achieved with gram-negative or -positive bacteria, or with fungal cultures. The amount of venom protein used, however, was far less than in the above-mentioned assays. It is, therefore, entirely plausible that the lack of antimicrobial activity in this study may have been solely due to insufficient concentration of venom (proteins) tested. Anti-microbial activity has been predicted for endoparasitoid venoms as a necessary requirement when the host is immunocompromised (Parkinson and Weaver, 1999). This would seem especially critical for *P. turionellae* since it has been shown that female accessory glands release various compounds such as mucoproteins, lipoproteins, hyaluronic acid, lecithin, and cholesterol esters that evoke cytolysis of host haemocytes and prevent parasitoid eggs from being encapsulated (Kılınçer, 1975; Osman, 1978). Correspondingly, larvae of *P. turionellae* produce anal secretions that possess antibacterial and antifungal activity (Führer and Willers, 1986). Thus, protection from microbial infection for parasitoid larvae developing in immunosuppressed hosts appears to be derived from larval secretions and not from wasp venom.

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