SCREENING INSECT PATHOGENS FOR PEST CONTROL

P.J. WIGLEY, L.A. MALONE and S. DHANA

D.S.I.R. Entomology Division, Mt Albert Research Centre, Private Bag, Auckland.

SUMMARY

A range of endemic and exotic insect viruses and protozoa have been tested for oral infectivity to grass grub (*Costelytra zealandica*), Argentine stem weevil (*Listronotus bonariensis*), porina (*Wiseana* spp.), greasy cutworm (*Agrotis ipsilon*) and the greenheaded leafroller (*Planotortrix excessana*). Assays were conducted in the laboratory using artificial diets. Grass grub, porina and Argentine stem weevil were susceptible to infection only with endemic protozoan pathogens from other pasture inhabiting species. Greasy cutworm and greenheaded leafroller were susceptible to infection by a wider range of pathogens. The implications of using such screening tests for finding and evaluating potential control agents are briefly discussed.

INTRODUCTION

Insect pathogens have long been considered to possess significant potential for the control of insect pests. The past five years have seen a substantial research effort aimed at assessing the effectiveness of pathogens mass-produced for inundative release (Kain *et al* 1982; Latch and Kain 1983; Jackson and Pearson 1985) and during this period long term ecological studies have confirmed the importance of protozoan, bacterial and viral diseases in the natural regulation of grass grub and porina population numbers (Miln 1982; Fleming *et al* 1982; Jackson 1984). However, as yet, there has been no systematic attempt to evaluate the general susceptibility of New Zealand insect pests to pathogenic microorganisms infecting other endemic or exotic insect species. Identification of new pathogenic agents would increase the range of microorganisms potentially available for biological control by introduction and natural spread, or for mass-production as microbial insecticides.

This paper reports the first results of tests of the susceptibility of five important insect pests to infection by a range of exotic and endemic microsporidia, coccidia, and cytoplasmic polyhedrosis viruses.

METHOD

The origins of inocula are summarised in Table 1 and their concentrations in Table 2.

Argentine stem weevil adults were obtained from a disease-free laboratory colony maintained on an artificial diet and ryegrass (Malone, unpublished). Grass grub larvae originated from surface-sterilised eggs laid in the laboratory by adults collected at Wanganui. Emergent larvae were fed individually on an artificial diet (Wigley and Dhana, unpublished). Greasy cutworm eggs came from a laboratory colony mass-reared on the diet of Hirai and Santa (1983). Eggs of the greenheaded leafroller were obtained from Rearing Section, Entomology Division, DSIR and larvae were fed on a wheatgerm diet (Singh 1977). Porina eggs were laid by females trapped by K. Stewart, Invermay, and larvae were grown on another artificial diet (Wigley and Dhana, unpublished).

Infectivity experiments were conducted by coating plugs of the appropriate artificial diet with about 10 microlitres of spore or viral suspension. Ten newly-hatched larvae were confined individually to these diet plugs for at least one week, before being transferred to individual plugs of fresh diet. Argentine stem weevil adults were fed individually on blades of ryegrass previously coated with inoculum. Test insects were examined weekly and grown for at least 6 weeks, at 22 °C, before survivors were killed for microscopic examination. Wet mount preparations, and, later, Giemsa-stained

Proc. 39th N.Z. Weed and Pest Control Conf.

TABLE 1: Identity and sources of insect pathogens.

Pathogen	Host	Source
PROTOZOA		
Microsporidia		
<i>Nosema costelytrae</i>	<i>Costelytra zealandica</i> (Col. Scarab.)	NZ
<i>N. carpocapsae</i>	<i>Laspeyresia pomonella</i> (Lep. Tort.)	NZ
<i>N. pyrausta</i>	<i>Ostrinia nubilalis</i> (Lep. Pyral.)	USA
<i>N. bombycis</i>	<i>Bombyx mori</i> (Lep. Bombyc.)	Japan
<i>N. whitei</i>	<i>Tribolium castaneum</i> (Col. Teneb.)	UK
<i>N. locustae</i>	<i>Melanoplus bivittatus</i> (Orthop. Acrid.)	USA
<i>Microsporidium itiiti</i>	<i>Listronotus bonariensis</i> (Col. Curcul.)	NZ
<i>Pleistophora</i> sp.*	<i>Wiseana</i> spp. (Lep. Hepial.)	NZ
<i>P. sp.*</i>	<i>L. bonariensis</i>	NZ
<i>P. sp.*</i>	<i>C. zealandica</i>	NZ
<i>P. oncoperae</i> *	<i>Oncopera alboguttata</i> (Lep. Hepial.)	Australia
<i>P. schubergi</i>	?	USA
<i>Vairimorpha plodiae</i>	<i>Plodia interpunctella</i> (Lep. Pyral.)	Czech.
<i>V. ephestiae</i>	<i>Ephestia kuehniella</i> (Lep. Pyral.)	Czech.
<i>V. heterosporum</i>	<i>Galleria mellonella</i> (Lep. Pyral.)	Czech.
<i>V. sp.</i>	<i>Galleria mellonella</i> (Lep. Pyral.)	Czech.
<i>V. sp.</i>	<i>Crambus trisectus</i> (Lep. Pyral.)	USA
<i>Vavraia</i> sp.	<i>Crambus</i> sp.	USA
<i>Vavraia</i> sp.	<i>Hyphantria cunea</i> (Lep. Arctiidae)	USA
<i>Orthosoma operophterae</i>	<i>Operophtera brumata</i> (Lep. Geometrid.)	UK
<i>Cystosporogenes operophterae</i>	<i>O. brumata</i>	UK
Coccidia		
<i>Adelina</i> sp.	<i>Heteronychus arator</i> (Col. Scarab.)	NZ
VIRUSES		
Cytoplasmic polyhedrosis viruses		
Type I	<i>B. mori</i>	UK
Type II	<i>Mamestra brassicae</i> (Lep. Noct.)	UK
Type 7 + type 12	<i>Noctua pronuba</i> (Lep. Noct.)	UK
Type ?	<i>Zizina otis labradus</i> (Lep. Lycaen.)	NZ

* Studies indicate that *P. oncoperae* and the three endemic *Pleistophora* isolates are one species. (Malone *et al* in prep.).

preparations were checked for the presence of protozoan spores or inclusion bodies of the cytoplasmic polyhedrosis virus. To ensure comparability, the same pathogen suspension was used for each test host and all experiments were initiated within an 8 week period.

RESULTS

There was a wide variability among the five pests in their susceptibility to infection and among the pathogens in their infectivity (Table 2).

Grass grub, porina and Argentine stem weevil were susceptible to infection only by the coccidian, *Adelina* sp. and by endemic microsporidian species found naturally in host field populations. Greasy cutworm larvae were generally more susceptible to microsporidian infection than the pasture pests, most notably to the range of *Vairimorpha* species, but were not susceptible to *Adelina* infections. The greenheaded leafroller was susceptible to most of the microsporidian pathogens tested, to *Adelina* sp. and two of the CPV isolates.

Pleistophora species were the most cross-infectious of the isolates tested. The grass grub pathogen, *Nosema costelytrae*, and the black beetle pathogen, *Adelina* sp., had an unexpectedly wide laboratory host range.

Although the assessment of infectivity rather than virulence was the primary objective of this study it was noted that the microsporidian infections of the pasture

TABLE 2: Susceptibility of various pests to infection by some insect pathogenic protozoa and viruses.

Pathogen	Concentration (spores/ml x 10 ⁶)	Susceptibility of pest species				
		GG ¹	POR	ASW	GCW	GHLR
PROTOZOA						
Microsporidia						
<i>Nosema costelytrae</i>	0.07	*	0	***	*	****
<i>N. carpocapsae</i>	0.7	0	0	0	-	****
<i>N. pyrausta</i>	1.8	0	0	0	0	****
<i>N. bombycis</i>	0.2	0	0	0	0	****
<i>N. whitei</i>	0.3	0	0	0	0	0
<i>N. locustae</i>	0.6	0	0	0	0	0
<i>Microsporidium ititii</i>	0.3	0	0	****	0	0
<i>Pleistophora</i> sp. (ex POR)	0.5	*	***	**	*	***
<i>P. sp.</i> (ex ASW)	0.2	***	****	****	-	****
<i>P. sp.</i> (ex GG)	0.4	**	***	**	****	**
<i>P. oncoperae</i>	0.5	*	**	**	-	***
<i>P. schubergi</i>	0.2	0	0	0	0	****
<i>Vairimorpha plodiae</i>	1.6	0	0	0	****	***
<i>V. ephestiae</i>	1.2	0	0	0	****	****
<i>V. sp.</i>	0.1	0	0	0	*	0
<i>V. heterosporum</i>	1.7	0	0	0	****	****
<i>V. sp.</i> (ex <i>C. trisectus</i>)	3.6	0	0	0	****	****
<i>Vavraia</i> sp (ex <i>Crambus</i>)	0.6	0	0	0	0	0
<i>Vavraia</i> sp. (ex <i>H. cunea</i>)	0.5	0	0	0	****	0
<i>Orthosoma operophterae</i>	-	0	0	0	0	0
<i>Cystosporogenes operophterae</i>	5.4	0	0	0	0	*
Coccidia						
<i>Adelina</i> sp.	-	***	*	**	0	****
VIRUSES						
Cytoplasmic polyhedrosis viruses						
Type I CPV	1.3	-	0	-	-	0
Type II CPV	2.7	-	0	-	-	***
Types 7 + 12	8.8	-	0	-	-	*
CPV (ex <i>Z. otis labradus</i>)	0.3	-	0	-	-	0

infection rate: 0=no infections, * = 25%, ** = 25% to 50%, *** = 50% to 75%, **** = >75%, -not assessed

GG¹=grass grub, POR=porina, ASW=Argentine Stem Weevil, GCW=greasy cutworm, GHLR=greenheaded leafroller

insects were generally not lethal under the conditions of this experiment. However, *Adelina* did kill most infected grass grub larvae and most infected larvae of the greenheaded leafroller. Greasy cutworm larvae were killed mostly as 3rd or 4th instar larvae by *Vairimorpha* infections, and as large larvae by the *Vavraia* species. Greenheaded leafroller larvae infected with Type II CPV were retarded in growth and died before pupation.

DISCUSSION

The two pathogens most infectious to the three pasture pests were *Pleistophora* sp. and *Adelina*, but of these only *Adelina* holds promise as a biological control agent. *Pleistophora* sp. is already widely distributed in porina and grass grub populations and has been found sporadically in a wide range of other insect species. Its effects on an infected host are primarily sublethal. The potential of *Adelina* for biological control of black beetles has already been recognised by King *et al* (1985) who demonstrated the feasibility of transmitting *Adelina* using inoculum from crushed, infected cadavers and

suggested its spread into uninfected black beetle populations. The discovery that *Adelina* will infect other pasture pests in the laboratory and may be mass-produced in an easily-reared lepidopteran host considerably enlarges the biological control potential of this pathogen. Experiments are being conducted to assess the susceptibility of other pest insects and laboratory hosts to this protozoan. The greasy cutworm and the greenheaded leafroller were more susceptible to infection and these tests have identified a number of insect pathogens that may have some future use in biological or integrated control programmes for these pests.

A notable feature of the results was the general lack of susceptibility of the three pasture pests to the small random array of insect pathogenic microsporidia tested. The microsporidian inocula used in these experiments originated from three insect orders and ten insect families. It was expected that if all five hosts were equally susceptible to infection the broad pattern of infections would have been similar between hosts. This did not occur. The basis of this comparative lack of susceptibility is being investigated to determine whether it was a consequence of the laboratory infection procedure or whether it represents a more generalised resistance to microsporidian infection.

During these initial trials no systematic attempt was made to obtain pathogenic microorganisms from closely related endemic or exotic host species, or from species associated with specific habitats. The cross-infectivity exhibited by some pathogens of the pasture pests, however, demonstrates the desirability of acquiring such isolates, particularly for the less susceptible pests. A programme of obtaining pathogens from overseas laboratories is in progress.

These results have demonstrated that insect pathogens from a wide range of hosts and geographic areas may have potential for the biological control of pests which they would never normally encounter. Screening trials are an efficient method of discovering new virulent agents for insect pests and, perhaps in the long term, of providing information on the comparative resistance to infection of insects representative of particular habitats and taxonomic groups.

ACKNOWLEDGEMENTS

We would like to thank our colleagues from N.Z. and overseas who kindly provided us with many of the pathogen isolates. Sharyn Westlake provided excellent technical assistance.

REFERENCES

- Fleming, S., Archibald, R., Stewart, K. and Kalmakoff, J., 1982. Natural regulation of porina spp. by disease. *Proc. 3rd Aust. Conf. Grassland Invert. Ecol.*: 283-290.
- Hirai, K. and Santa, H., 1983. Comparative physio-ecological studies on the armyworms *Pseudeletia seperata* Walker and *Leucania loreyi* Duponchel (Lepidoptera: Noctuidae). *Bull. Chugoku Nat. Ag. Exp. Sta. Ser. E.*: 55-101.
- Jackson, T.A., 1984. Honey disease, an indicator of population decline in grass grub. *Proc. 37th N.Z. Weed and Pest Control Conf.*: 113-116.
- Jackson, T.A. and Pearson, J.F., 1985. Honey disease (*Serratia* spp.) for the biological control of grass grub (*Costelytra zealandica*). *Proc. of the 4th Aust. Conf. Grassld. Invert. Ecol.*: 376.
- Kain, W.M., Bedding, R.A. and Van der Mespel, G.J., 1982. Preliminary evaluations of parasitic nematodes for grass grub (*Costelytra zealandica* (White)) control in Central Hawke's Bay of New Zealand. *N.Z. J. Exp. Agric.* 10: 447-450.
- King, P.D., Meekings, J.S. and Phillip, K.C., 1985. A protozoan pathogen of black beetle. *Proc. 4th Aust. Conf. Grassld Invert. Ecol.*: 201-209.
- Latch, G.C.M. and Kain, W.M., 1983. Control of porina caterpillar (*Wiseana* spp.) in pasture by the fungus *Metarhizium anisopliae*. *N.Z. J. Exp. Agric.* 11: 351-354.
- Miln, A.J., 1982. The role of disease in the population dynamics of grass grub, (*Costelytra zealandica* (White)). *Proc. 3rd Aust. Conf. Grassld. Invert. Ecol.*: 147-154.
- Singh, P.S., 1977. Artificial diets for insects, mites, and spiders. Plenum, New York. p. 31.