DEVELOPMENT OF MASS REARING METHODS FOR *ELDANA SACCHARINA (LEPIDOPTERA: PYRALIDAE)* I: CONTROL OF FUNGAL CONTAMINATION ON THE ARTIFICIAL DIET

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Abstract

Fungi of the genus *Aspergillus* were a continuing problem on the newly developed eldana diet in multicell trays. Various control attempts are outlined. They include hygiene practises, adjustment of dietary pH, and incorporation of the fungicides benomyl, mancozeb, captab, methyl paraben and sodium propionate into the diet. Treatment of the corn cob grits used for larval inoculation was also investigated.

Introduction

Advancement in laboratory research on insects, whether for physiological, behavioural, chemical or biological control purposes, is entirely dependent on the availability of insect material produced by sound and reliable insect rearing techniques (King and Leppla[6], Singh and Moore[7]).

In 1978 an artificial diet was developed by Atkinson[1] for the rearing of *Eldana saccharina* Walker for laboratory studies and field infestations for insecticide trials. In 1981 increased emphasis was placed on the biological control programme against eldana borer, and a special unit of prefabricated buildings was designed and constructed for the laboratory rearing of parasitoids and their hosts. This increase necessitated modifications of the diet and rearing methods for eldana to ensure a reliable supply of experimental material (Conlong *et al.*[2]).

In 1987 the culture of the indigenous hymenopterous parasitoid *Goniozus natalensis* Gordh was considerably expanded, which demanded an increase in eldana larval supply. Eldana production was increased but survival rates dropped from 70% to approximately 50% (Conlong *et al.*[2]). High levels of contamination, particularly by *Aspergillus* spp appeared in the rearing jars, which often resulted in the entire contents of the jar being lost (Graham and Conlong[4]).

An alternative diet was sought that would be less susceptible to microbial contamination, would produce high levels of good quality eldana and would be acceptable to *Goniozus* for direct parasitism of the larvae in the diet.

The high moisture content of the diet developed by Atkinson[1] was unsuitable for *Goniozus* activity. The preservative sorbic acid had to be eliminated from the diet because it inhibited *Goniozus* egg hatch. A diet developed at the Experiment Station for *Sesamia calamistis* Hamps (Lepidoptera: Agrotidae) was tested but sorbic acid still exerted an inhibitory effect. Upon its exclusion the modified diet produced high quality eldana and was acceptable for *Goniozus* activity and parasitism when dispensed into multicell trays (Graham and Conlong[4]).

Sporadic outbreaks of *Aspergillus* became more frequent with increased eldana production. It also became apparent that the required standards of hygiene, microclimatic precision and efficient culture isolation were extremely difficult to implement in the existing facilities. As a result a permanent custom-designed building was erected for biological control research and development. The new facility allowed the production of insects for consistent experimental field releases of parasitoids against eldana.

Contamination of the diet with fungi may be a minor inconvenience or a serious impediment to insectary rearing. In a rearing programme, contamination can mean:

- poor quality of insects, i.e. dimunitive pupae and adults; reduced pheromone production; reduced amino and fatty-acid synthesis
- high mortality of all stages, particularly young instars
- direct pathological effects
- additional workload
- increased expense
- loss of confidence in work (King and Leppla[6], Griffith and Haskell[5], Singh and Moore[7])

Authorities on insect rearing have found that the best control is preventive control, using measures such as a clean environment, sterilised equipment and initially uncontaminated dietary ingredients. In addition microbial growth can be prevented in diets by routinely introducing chemical preservatives and antimicrobials if the diets are susceptible to contamination and spoilage (Griffith and Haskell[5], King and Leppla[6], Singh and Moore[7]).

The steps taken in developing an optimal diet for eldana production are described here.

Materials and Methods

To control *Aspergillus* contamination in the multicell trays of the newly developed eldana diet, detailed studies were conducted on the methods of inoculation, the incorporation of fungicides into the diet and the adjustment of dietary pH. Because the biological activity of antimicrobials can affect the insect as well as the target microbe, a quality control procedure was established to measure their effects on eldana growth and fecundity.

Methods of inoculation

To inoculate the multicell trays with eldana, the first instar larvae are mixed with sterile corn cob grits. The grits are sterilised in a steam autoclave at 121°C for two hours, and treated with a fungicide (Davis and Oswalt[3], Graham and Conlong[4]).

In the USA the fungicides folpet (which is not locally available) and benomyl in the powder form (0,17g/200g corn cob grits) are used for this treatment. Benomyl was tested in the eldana culture at the same concentration used in the USA, and compared with mancozeb (2g/200g corn cob grits), an inexpensive fungicide which is locally available and was already a dietary constituent.

During the period when the fungicides were being tested as a treatment for corn cob grits, a laminar-air-flow cabinet was commissioned. Contamination of newly dispensed diet,

cooled and inoculated on the laminar-flow-cabinet, was compared with diet inoculated after surface sterilisation for one hour by ultraviolet light.

Fungicides in the diet

To reduce microbial contamination of diet in multicell trays still further, the fungicides sodium propionate (local and imported sources), mancozeb, captab, benomyl and methyl paraben were individually incorporated into the diet in a series of concentrations (g/litre of water used in the diet). With those fungicides already in the diet, the routine concentration was used as a control (1.6g methyl paraben/litre and 9g sodium propionate/litre), and test concentrations up to double routine or more were used. The concentrations for the other fungicides were arbitrarily determined. The routine diet mix (9g sodium propionate/litre, from a local source) served as a control for all the fungicides tested.

Ten litres of diet containing each of the fungicide concentrations chosen were mixed and dispensed into multicell trays using an automatic dispenser (Graham and Conlong[4]). The treatments were assessed for fungal contamination 20 days after inoculation.

Combination of fungicides in the diet

A combination of the best fungicides tested, as described in the previous section, were incorporated into the diet to achieve more successful control. Methyl paraben and sodium propionate were combined at their control concentrations, i.e. 9 and 2g/litre respectively. Results were compared with the routine sodium propionate diet and diets containing 6 and 8g methyl paraben/litre which gave good control in the initial tests.

Adjustment of dietary pH

The pH of eldana diet containing 9g sodium propionate/litre was increased from 4,6 by the addition of sodium hydroxide to a maximum of 6,2. *Aspergillus* contamination levels were monitored 20 days after inoculation.

Insect quality control

Batches of newly formed pupae were cut from their pupal cases and weighed. Results were compared with pupal weights of eldana from the routine culture (9g sodium propionate/litre) and with pupae collected from field sugarcane and from *Cyperus papyrus* L, a natural host plant.

To measure fecundity, newly emerged male and female moths were placed in lidded 250ml paper cups. Paper towelling was used as an oviposition subtrate and was changed daily, so that the numbers of eggs laid could be recorded.

Table 1

Contamination, by *Aspergillus*, of eldana diet exposed to ultraviolet light prior to inoculation, and diet cooled and inoculated on a laminar-air-flow cabinet, using mancozeb and benomyl treated corn cob grits

Fungicide in corn cob grits	Ultraviolet light			Laminar-air-flow cabinet		
	No trays inoculated	*Aspergillus* contamination		No trays inoculated	*Aspergillus* contamination	
		No trays	%		No trays	%
Mancozeb	979	231	23,6	788	62	8,0
Benomyl	313	169	54,0	331	76	23,0

Table 2

***Aspergillus* contamination of diet in multicell trays containing various individual fungicides at a range of concentrations (bold type indicates control results)**

Fungicide	Conc g/ℓ	*Aspergillus* contamination							
		No trays	No contam	%	SD	No cells	No contam	%	SD
Sodium propionate (local)	**9**	**6 401**	**1 075**	**16,8**		**204 832**	**6 072**	**3,0**	
	11	14	0	0	0	448	0	0	0
	13	15	2	13,3	0,35	480	2	0,4	1,09
	15	6	0	0	0	192	0	0	0
	17	15	5	33,3	0,49	480	20	4,2	12,76
Sodium propionate (imported)	9	14	1	7,1	0,27	448	1	0,2	0,83
	11	12	7	58,3	0,51	384	12	3,1	3,28
	13	7	4	57,1	0,53	224	5	2,2	2,37
	17	2	0	0	0	64	0	0	0
Captab	1	13	13	100,0	0	416	411	98,8	2,38
	3	14	14	100,0	0	448	448	100,0	0
	5	17	17	100,0	0	544	374	68,8	28,50
	7	15	15	100,0	0	480	223	46,5	10,75
	9	16	12	75,0	0,41	512	36	7,0	7,87
Mancozeb	1	15	15	100,0	0	480	480	100,0	0
	3	15	15	100,0	0	480	480	100,0	0
	5	16	16	100,0	0	512	512	100,0	0
	7	11	9	81,8	0,40	352	57	16,2	10,63
	9	15	15	100,0	0	480	287	59,8	27,15
Benomyl	1	13	5	38,5	0,51	416	8	1,9	1,93
	3	15	10	66,7	0,49	480	22	4,6	6,57
	5	14	6	42,9	0,51	448	9	2,0	2,65
	7	3	1	33,3	0,58	96	2	2,1	3,64
Methyl paraben	1,6	10	10	100,0	0	320	113	35,3	7,36
	2	12	10	83,8	0,39	384	65	16,9	14,99
	4	13	4	30,7	0,48	416	10	2,4	4,63
	6	11	1	9,1	0,30	352	1	0,3	0,93
	8	11	1	9,1	0,30	352	1	0,3	0,93

Results

Methods of inoculation

Mancozeb incorporated with corn cob grits was far more effective at *Aspergillus* control than benomyl (Table 1). In addition, the levels of contamination by *Aspergillus* were considerably reduced when inoculation was completed on a laminar-air-flow cabinet.

Fungicides in the diet

Aspergillus contamination was completely inhibited by the local source of sodium propionate at concentrations of 11 and 15g/litre, and 17g/litre of the imported brand (Table 2). Good control was also obtained with 9g imported sodium propionate/litre. The level of *Aspergillus* contamination of diet treated with methyl paraben decreased with increase in concentration, good control being achieved with concentrations of 6g/litre and greater.

Even though it appeared that numerous multicell trays were contaminated with *Aspergillus* in certain of the sodium propionate and methyl paraben treatments, the percentage of contaminated cells in the trays was never greater than 5%.

High levels of tray and cell contamination were obtained with all concentrations of captab and mancozeb. Benomyl gave better control than either captab or mancozeb, and cell contamination was less.

Combination of fungicides in the diet

A combination of sodium propionate and methyl paraben proved to be more effective in controlling *Aspergillus* than either of the fungicides used individually (Table 3).

All fungicide testing was completed in the old prefabricated Biological Control Unit. Table 4 shows a comparison of the extent of *Aspergillus* contamination in the last few diet mixes in the old facility, with mixes completed from October 1988 to November 1989 in the new facility. The sodium propionate/methyl paraben diet was used.

Adjustment of dietary pH

The degree of contamination increased with an increase in alkalinity, but *Aspergillus* was inhibited at a pH of 5,0 (Table 5).

Before altering the pH of the routine diet, the modified sodium propionate/methyl paraben diet was tested for pH, and *Aspergillus* contamination levels were monitored (Table 6). An average pH of 5,0 produced tolerable levels of *Aspergillus* in the modified eldana diet.

Quality control

There were no significant differences between weights of pupae which had developed in diets containing different fungicide treatments and those of pupae collected from sugarcane and papyrus (Table 7).

Table 4

***Aspergillus* contamination of diet containing a 9g/litre sodium propionate and 2g/litre methyl paraben combination in the old and new insect rearing facilities**

Site of diet preparation	No trays inoculated	No cells	*Aspergillus* contamination			
			No trays	%	No cells	%
Old facility	285	9 120	138	48,4	1 628	17,9
New facility	46 206	1 478 592	360	0,8	1 465	0,1

Table 5

***Aspergillus* contamination of diet containing 9g/litre sodium propionate after adjustment of pH using sodium hydroxide (bold type indicates control results)**

NaOH conc g/ℓ	Approx pH of diet	No trays	No cells	*Aspergillus* contamination					
				No trays	%	SD	No trays	%	SD
0	**4,6**	**256**	**8 192**	**28**	**10,9**		**111**	**1,4**	
2,5	5,0	11	352	0	0	0	0	0	0
5,0	5,4	10	320	2	20,0	0,42	2	0,6	1,31
10,0	6,2	11	352	10	90,9	0,30	31	8,8	7,39

Table 6

Testing of pH and *Aspergillus* contamination level in diet containing 9g sodium propionate/litre and 2g methyl paraben/litre

No trays inoculated	No cells	Ave pH	SD	*Aspergillus* contamination			
				No trays	%	No cells	%
5 140	164 480	5,0	0,1632	53	1,0	132	0,1

Table 7

The average pupal weights of eldana developing in diet containing different fungicides and sodium hydroxide to adjust pH levels and of field collected pupae

Pupal source	Female pupae		Male pupae	
	Weight (mg)	SD	Weight (mg)	SD
Sodium propionate (local) control	147	18	97	14
Sodium propionate (local)	153	27	81	19
Sodium propionate (imported)	154	19	75	28
Captab	162	0	90	15
Mancozeb	141	39	88	12
Benomyl	162	26	105	13
Methyl paraben	147	32	96	11
Sodium propionate methyl paraben	191	30	113	16
Sodium hydroxide	144	27	89	16
Field sugarcane	126	26	83	29
Field papyrus	155	36	90	16

Table 3

Contamination of diet by *Aspergillus* in multicell trays containing a sodium propionate/methyl paraben combination, and methyl paraben and sodium propionate separately

Fungicide	Conc g/ℓ	No trays	No cells	*Aspergillus* contamination			
				No trays	%	No cells	%
Sodium propionate	9	7 699	246 368	1 324	17,2	7 214	2,9
Methyl paraben	6	827	26 464	373	45,1	7 941	30,0
Methyl paraben	8	816	26 112	154	18,9	705	2,7
Sodium propionate	9)	719	23 008	23	3,2	98	0,4
Methylparaben	2)						

There were no significant differences in female fecundity of eldana reared in diets receiving different fungicide treatments (Table 8). The addition of sodium hydroxide to the diet for pH adjustment also did not affect fecundity.

Table 8

Female fecundity of eldana developing in diets containing different fungicides and their range of concentrations, and pH levels (bold type indicates control results)

Ingredient	Conc g/ℓ	Ave no eggs per female	SD
Sodium propionate (local)	**9**	**113,2**	**59,9**
	15	126,7	81,4
	18	168,9	106,3
Sodium propionate (imported)	9	116,0	62,9
	11	98,9	75,3
	13	117,4	74,5
Benomyl	5	95,0	43,3
	7	86,1	35,7
Mancozeb	7	130,3	63,3
Methyl paraben	2	118,7	80,1
	4	68,8	43,7
	6	53,5	9,5
	8	108,4	58,1
Sodium hydroxide	2,5	98,0	77,8
	5	73,1	79,0

Discussion

The use of medicated corn cob grits in conjunction with laminar-air-flow cabinets greatly reduces contact of the exposed diet surface with contaminants associated with first instar larvae and unfiltered air. In most insect rearing facilities extensive use is made of sanitation and air filtration or laminar-air-flow cabinets to reduce contamination levels (Singh and Moore[7]). However a further reduction in contamination levels was needed, as *Aspergillus* was still a major contaminant.

The next step was treatment of the diet with fungicides. Imported sodium propionate completely inhibited *Aspergillus* growth only at high concentrations, while local sodium propionate was more effective at lower concentrations in the diet (Table 3). In addition, imported sodium propionate was costly and supply did not meet demand. Because of the better *Aspergillus* control and lower cost, the local source of sodium propionate was chosen for use.

Methyl paraben showed increased control of contamination with an increase in concentration, and gave low levels of cell contamination, although complete inhibition was not attained (Table 2). When tested on a large scale (Table 3), *Aspergillus* levels increased markedly in comparison with the original tests for 6 and 8g methyl paraben/litre (Table 2), though the latter concentration was considerably more effective than the former. Comparable results were obtained with 9g sodium propionate/litre (Table 3). Although these two fungicides contributed to a further improvement in *Aspergillus* control, contamination levels were still unacceptable.

A combination of the lower concentrations of sodium propionate (9g/litre) and methyl paraben (2g/litre) gave lower tray and cell contamination levels than did either of these fungicides used alone (Table 3). This fungicide combination was thus incorporated into the routine mix prior to the move into the new rearing facility.

At the Otis Development Centre, Massachusetts, elimination of antimicrobials from the gypsy moth diet allowed contamination at pH's 4-8. Antimicrobials could not inhibit microbial development at a dietary pH greater than 6,5 and larval growth was retarded at pH 7 or higher, regardless of the diet used. Dietary pH's of 4,5-6,5 were satisfactory for rearing the insect (Davis and Oswalt[3]).

Adjustment of dietary pH was found to be unnecessary as the modified diet containing sodium propionate and methyl paraben was within the optimal pH range. The higher pH's increased the levels of contamination and this was confirmed by Davis and Oswalt[3], who reported that contamination was minimal at the acid end of the pH range. Dietary pH is also known to affect the activity of fungicides; and the best pH for the parabens is 3-9, and for propionic acid 3-5.

Increased rates of production in the old rearing facility caused problems: lack of adequate space, hygiene, isolation of cultures and microclimatic control. Suppression of *Aspergillus* could not be maintained by fungicide treatments and sterile inoculation procedures alone. With the advent of a custom-built insectary contamination was reduced and maintained below 1% (Table 4).

Although none of the fungicides, over the range of concentrations tested, had any adverse effect on eldana growth and fecundity, efforts were made to use minimal amounts of these antimicrobial compounds in the diet. This would ensure minimal detrimental effects by the compounds on the reared insects (King and Leppla[6]).

Conclusions

Aspergillus contamination of the eldana diet was minimised by:

- The incorporation of a fungicide combination of 9g sodium propionate and 2g methyl paraben/litre into the diet
- the inoculation of the newly dispensed diet with larvae in autoclaved corn cob grits containing 0,17g mancozeb in 200g corn cob grits
- the inoculation process being done on a laminar-air-flow cabinet fitted with a High Efficiency Particulate Air (HEPA) filter
- strict maintenance of cleanliness procedures in a facility specifically designed for insect production.

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