DEVELOPMENT OF DEFINED SYNTHETIC DIETS FOR THE CULTURING OF ELDANA SACCHARINA (LEPIDOPTERA:PYRALIDAE).

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Abstract

Essential nutritional requirements have been identified for *Eldana saccharina* Walker. The synthetic diet was developed to provide nutrients at levels, as near as possible, to those found in undamaged and damaged (reactive) cane stalks. Sugars, fatty acids, simple phenolics, tannins and amino acid balance were found to influence survival and growth of larvae in culture. Some sugarcane varietal differences in identified biochemical traits will be presented and discussed.

Introduction

Synthetic diets for the mass rearing of laboratory insects are usually oligidic, i.e. consisting principally of crude natural products such as wheat germ or some other bulk plant material. Atkinson (1978) describes an early oligidic diet used in the culture of *Eldana saccharina* Walker which contains chickpea flour, live brewers yeast, casein and glucose as the main nutritional factors. However, if nutritional requirements or the effects of various additions and deletions are to be determined, a meridic (semi-defined) or even a holidic (fully-defined) sterile diet must be developed. Such diets should simulate the composition of the food plant. The steps taken in formulating an optimal meridic diet for eldana are described, together with the effects of several manipulations.

Preliminary diet development

Micro-organics and vitamins

In a repeat of the experiments of Atkinson (1978) the omission of ascorbic acid from the early oligidic diet led to improved survival and growth of eldana. Further manipulations revealed that this was in part due to a higher pH of the diet lacking ascorbic acid. This led to decreased effectiveness of antimicrobials, particularly sorbic acid (Funke, 1983), resulting in multiplication of the live brewers yeast. However, the concentration of ascorbic acid used by Atkinson (4 g/ℓ) was in itself found to be inhibitory to eldana survival and growth when compared with the diet lacking ascorbic acid but adjusted to the same pH. The lower concentration of 1,56 g/ℓ ascorbic acid now used in an optimal meridic diet is not inhibitory.

The omission of live brewers yeast led to reduced larval survival and development, which could be partly corrected by adding killed yeast. This suggests that multiplication of yeast cells and their maintenance in the live state ensures a better supply and preservation of certain vital nutrients. Yeast is known to contain high levels of the vitamin B complex, ribonucleic acids and lipids/fatty acids, some of which are probably of great importance in eldana nutrition. Brewers yeast was replaced in an early meridic diet by the micro-organic and fatty acid mixes given in Appendix 1.

Antimicrobials

The efficacy of antimicrobial agents in the diet is influenced markedly by pH (Funke, 1983). The anti-yeast/anti-fungal agents sorbic acid and methyl-parahydroxybenzoate (nipagin) became more effective with pH decreased to below 7. Table 1 shows that the pH of expressed juice from undamaged cane is around 5,5 while that of cane damaged by eldana can be more acidic. The concentrations of antimicrobials in a meridic diet were reduced from those considered by Singh and House (1970) to be the maximum 'safe'

Table 1

A comparison of synthetic diets with undamaged and damaged cane samples

Diet	DM % diet	Brix % diet	Fibre % diet	Sucrose % diet	pH of expressed liquid
Synthetic - undamaged Diet 0,375% N DM	30,0	16,0	14,0	13,0	5,5
Synthetic - damaged Diet 0,375% N DM	25,0	8,6	16,4 (+ 2,4)	3,0	Variable
* Cane 1.6.85	Dm % cane	Brix % cane	Fibre % cane	ERS % cane	pH of expressed juice
Undamaged	30,3	15,1	15,2	12,9	5,4 - 5,7
Damaged	25,5	7,9	17,6 (+ 2,4)	3,6	4,2 - 5,5

* Cane date from Bond (1988)

levels (see Appendix 2). These concentrations still ensured reasonable microbial stasis.

Main diet development

Bulk properties and sugars

Introduction

Analysis of undamaged and damaged stalk parts (Bond, 1988) revealed differences in bulk properties, and especially in sucrose content (Table 1). Sugars are the first and most obvious nutrients to investigate in relation to eldana diet. In addition to being nutrients, they are frequently recorded as being feeding incitants and stimulants for many lepidopteran species (Bernays and Simpson, 1982).

Method

In an experiment designed to test the effects of sucrose, fructose and glucose upon feeding initiation, survival and growth of eldana larvae, a range of diets was produced which varied in bulk properties between those of undamaged (when sugar % of fresh mass was high) and damaged cane (Table 1). Unless otherwise stated five replicates of 20 larvae each were used for all subsequent experiments.

Results and discussion

Figure 1 shows the average number of survivors (in parentheses) and the average individual larval mass after three weeks on the diets.

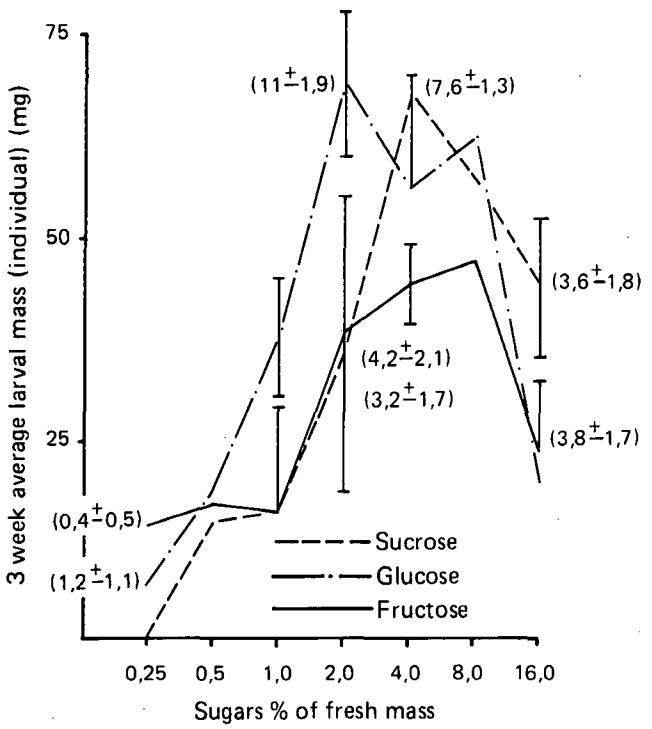

FIGURE 1 The effect of sugars on the survival and growth of *Eldana saccharina* larvae in artificial diet (0,75% NDM) (larval survival per replicate (20) in parentheses).

Fructose appeared to be the most potent initiator of feeding whilst sucrose, over the range found in cane (3–16%), did not change in activity except perhaps for a decrease in survival and growth at the highest concentration. As sugar content of the diets increased there appeared to be a carbohydrate excess, even though nitrogen was supplied at a level higher than is found in mature cane (see Table 3). Any decrease in survival and growth for the 16% sugar treatments may result from an inability of the insect gut to accommodate too osmotically active a concentration of small molecule dietary components. It appears that inadequate nitrogen nutrition limits the development of the insect in synthetic diet and in the field.

Amino acid balance

Introduction

In meridic diets the milk protein casein is one of the more commonly used nitrogen sources. Vanderzant (1958) found that a mixture of amino acids equivalent in proportion to that found in cotton protein resulted in better growth of *Pectinophora gossypiella* than did a mixture equivalent to casein. Casein was deficient in certain beneficial amino acids which consequently limited growth.

Method

For eldana a different approach was taken in determining the optimal dietary amino acid balance, in that the amino acid composition of the insect itself was determined. Whole eldana larvae were acid hydrolysed and the liberated amino acids derivatised with phenylisothiocyanate. These were separated and quantified by high performance liquid chromatography and ultraviolet detection (Anon, 1986).

Results and discussion

Columns 1 and 4 of Table 2 show the amino acid compositions of casein and whole second instar larvae. Column 2 shows the proportions of free amino acids, which when combined with casein, give a balance equivalent to that found in larvae. Added free amino acids will constitute half of the total whilst casein will contribute the remaining amino acids (see columns 1, 2 and 3).

Table 2

Percentage amino acid residues in casein and the added residues required to provide ratios equivalent to those found in 2nd instar larvae

	1	2	3	4
Amino acid	% amino acid residues in casein	added % residues as free	% amino acid residues in diet	% amino acid residues in 2nd instar larvae
Asp	7,44	+ 11,70	9,16	9,21%
Glu	17,65	+ 2,80	9,78	9,80%
Ser	5,57	+ 10,60	7,74	7,80%
Gly	3,19	+ 15,60	8,99	9,08%
His	2,27	+ 4,40	3,20	3,26%
Arg	1,93	+ 8,40	5,42	5,46%
Thre	3,69	+ 5,90	4,60	4,67%
Ala	4,29	+ 21,8	12,48	12,56%
Lys	7,05	+ 5,3	5,91	5,97%
Pro	12,72	+ —	6,09	5,32%
Tyr	3,97	+ 4,70	4,15	4,20%
Val	7,63	+ 5,70	6,34	6,45%
Met	1,99	+ 1,80	1,81	1,83%
Cyst	0,11	+ 0,35	0,22	0,21%
Isoleuc	5,59	+ 3,75	4,47	4,53%
Leuc	10,24	+ 4,50	7,05	7,13%
Phe	3,65	+ 1,60	2,51	2,53%
Total	100	109	100	100

NB: Tryptophan is destroyed during acid hydrolysis. Addition at the same level as Phenylalanine fulfilled the nutritional requirement.

Essential amino acids

Introduction

Essential amino acids are those that cannot be synthesised by the insect from other amino acids or from carbohydrates. They must be supplied by the food plant.

Method

A mixture of free amino acids, given in column 4 of Table 2, was added to a meridic diet to a total nitrogen concentration of 1,5% of dry matter (NDM). To test for essentiality, individual amino acids were omitted and replaced by aspartate. Carbohydrates were supplied in the forms of fructose and dextrin at 2 and 4,25% of fresh mass respectively.

Results and discussion

Histidine, arginine, threonine, valine, methionine, isoleucine, leucine, phenylalanine, lysine and tryptophan were found to be essential. Omission of tyrosine reduced growth, probably due to the use of phenylalanine by the insect to produce tyrosine, resulting in phenylalanine deficiency. Of interest is the reduction in growth caused by the omission of alanine, which can be produced by insects directly from pyruvic acid. Similarly, the omission of serine retarded growth.

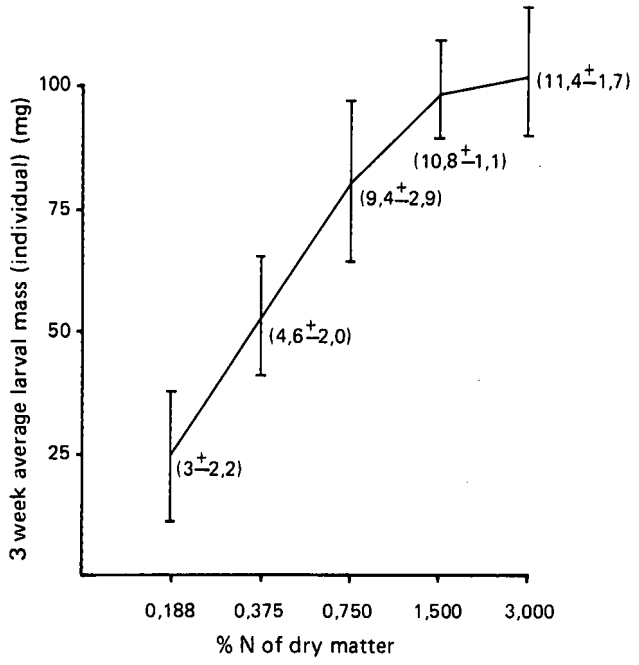

FIGURE 2 The effect of increasing nitrogen content on the survival and growth of *Eldana saccharina* larvae in artificial diet (2% fructose and 4,25% dextrin FM) (larval survival per replicate (20) in parentheses).

Nitrogen content

Introduction

Nitrogen appeared to be limiting the development of eldana in meridic diet.

Method

Nitrogen content was increased by adding additional casein and free amino acids while decreasing cellulose content. Carbohydrate was again supplied as fructose and dextrin.

Results and discussion

Figure 2 shows that any increase in nitrogen content above 3% NDM will not lead to greatly improved growth and survival. Replacing dextrin with sucrose at 3% and glucose at 1,25% of fresh mass, in order to simulate damaged cane more closely, neither reduced nor increased larval survival and growth.

Fatty acids

Introduction

In keeping with other meridic diets for lepidopteran stem borers, the fatty acid and oil content of the diet was at first set at around 7% of nutrients (Singh, 1977). However, a content of 3,3% was found to be more suitable in terms of larval survival and growth rate when using the fatty acid mixture in Appendix A. In the plant, fatty acids are present as esters in the form of phospholipids, principally as phosphatidylcholine. Upon alkaline hydrolysis these provide saturated and unsaturated fatty acids, commonly including palmitic and stearic (saturated), and oleic, linoleic and linolenic (unsaturated) acids. The 3,3% level of fatty acids and oils, in combination with the lipogenics choline and glycerol, is equivalent to a phosphatidycholine content which would supply 0,1% P of dry matter (based on a iP/phosphatidylcholine ratio of 25). This is in excess of the P levels found in mature cane stalks (Table 3).

Table 3
Mineral composition of sugarcane

Variety	Stalk Part	% of DM					ppm			
		N	P	K	Ca	Mg	Zn	Mn	Cu	Fe
N12	Top 1/3	0,39	0,09	1,83	0,23	0,13	–	–	–	–
	Bottom 1/3	0,26	0,05	0,59	0,14	0,05	–	–	–	–
NCo376	Top 1/3	0,47	0,07	1,93	0,21	0,16	7	5	3	31
	Bottom 1/3	0,30	0,03	0,49	0,12	0,07	4	7	2	40
N14	Top 1/3	0,40	0,04	1,39	0,25	0,17	–	–	–	–
	Bottom 1/3	0,37	0,03	0,66	0,15	0,10	–	–	–	–
N11	Top 1/3	0,30	0,04	1,17	0,22	0,10	–	–	–	–
	Bottom 1/3	0,20	0,02	0,49	0,15	0,06	–	–	–	–
Average top (immature)		0,39	0,06	1,58	0,23	0,14	7	5	3	31
Average bottom (mature)		0,28	0,03	0,56	0,14	0,07	4	7	2	40

The polyunsaturated C-18 fatty acids (linoleic and linolenic) are usually found to be essential for Lepidoptera (Reinecke, 1985). Moths that eclose following dietary deficiency frequently fail to emerge, or emerge with deformed wings (David and Gardiner, 1965).

Method

The effects of various combinations of fatty acids in a meridic diet on larval growth and survival were tested. Linolenic acid, when supplied, was contained in linseed oil. β carotene (0,17 g/ℓ of water) was included with ascorbic acid as an antioxidant to protect unsaturated fatty acids against oxidative degradation. In addition propyl gallate replaced nipagin at 1 g/ℓ of water because of its combined antimicrobial and powerful antioxidative properties.

Lipids were extracted from undamaged sugarcane stalk, hydrolysed, derivatised and analysed by gas chromatography, following methods outlined by Harborne (1984). Undamaged cane fatty acids comprised approximately 35% palmitic, 10% stearic, 15% oleic, 30% linoleic and 10% linolenic by mass. Mixtures tested on eldana varied around this distribution (Table 4). Five replicates of 100 larvae each were assessed.

Results and discussion

Table 4 shows that an increasing ratio of unsaturated to saturated fatty acids improves the pupation and emergence of eldana moths. To optimise the meridic diet further, the initial fatty acid mix (column 4 of Table 4) was replaced by the mix given in column 5 of Table 4.

Table 4

Effect of various fatty acid mixes on the pupation and emergence of *Eldana saccharina* moths

Fatty acid	% composition by mass				
	1	2	3*	4**	5
Palmitic C16.0	50	35	35	25	15
Stearic C18.0	50	30	10	10	10
Oleic C18.1	0	15	15	15	15
Linoleic C18.2	0	20	30	30	30
Linolenic C18.3	0	0	10	20	30
Ratio unsaturated / saturated	–	0,54	1,22	1,86	3
% pupating	21 ± 8,4	40 ± 10,8	42,2 ± 10,8	63 ± 13,5	80,4 ± 8,2
% emerging	7,4 ± 4,1	25,8 ± 6,8	29,4 ± 9,7	55,8 ± 8,3	73,6 ± 6,6
% of emergents that were deformed	43	36	18	10	2

* Balance of fatty acids found in mature undamaged NCo376 stalk
** Initial fatty acid mix in Appendix 1

Phenolics

Introduction

During the development of the meridic diet, pure cellulose was at one stage replaced by trash powder which had been washed and leached by the neutral detergent method of Hartley (1987). Free phenolic acids were found to be present in resultant eldana frass. These were primarily ferulic and coumaric acids. In monocotyledonous plants, these acids are esterified to cell walls in large quantities where they protect the polysaccharide from enzymic attack by animals, pathogens and leaf litter microbes (Fry, 1979). They can be released by mild alkaline hydrolysis. This probably occurs in the alkaline mid-guts of lepidopteran larvae. Berenbaum (1980) lists many lepidopteran species and their mid-gut pHs. Some phenolic acids have been found to stimulate growth of insects in artificial diets, notably the 3,4 dihydroxyphenols (Kato, 1978 and Bernays and Woodhead, 1982).

Method

Several dihydroxyphenolics and ferulic acid, a monohydroxyphenol, were tested at various concentrations ranging up to 3 000 ppm in meridic diets containing 3 and 10% sucrose. The 3% sucrose treatments contained 7% dextrin.

Results and discussion

At 194 ppm (1 mM) ferulic acid appeared to increase larval survival and individual larval mass (Figure 3). However, at higher concentrations it had a detrimental effect, especially in the 3% sucrose treatment. With high ferulic acid concentrations 10% sucrose may offset this determent with stimulation. Similar activity was recorded for each of the dihydroxyphenolics (data not shown).

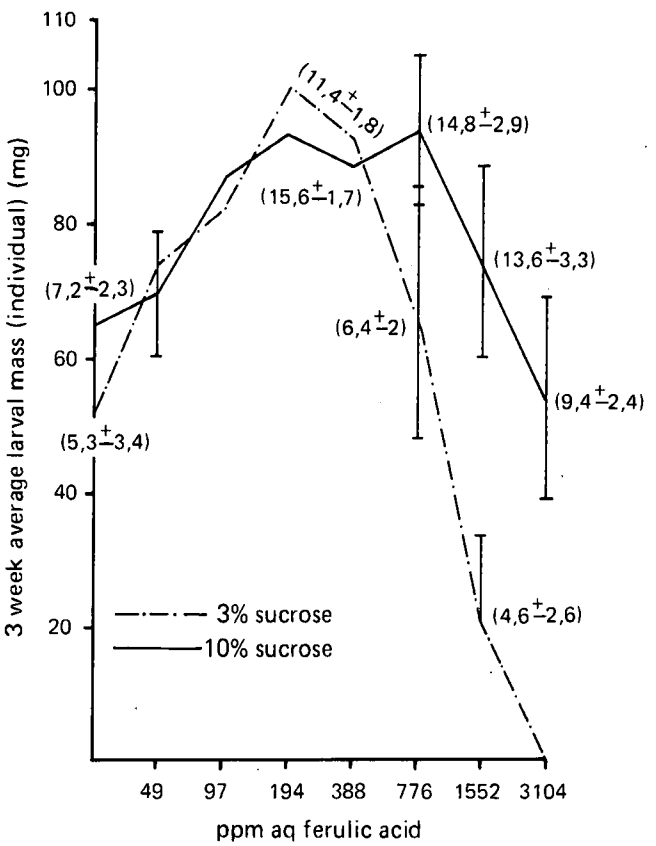

FIGURE 3 The effect of increasing ferulic acid concentration on the survival and growth of *Eldana saccharina* larvae at two sucrose levels in artificial diet (0,375% NDM) (larval survival per replicate (20) in parentheses).

Effect of ferulic acid on protein digestion

Method

At the end of the previous experiment larvae were recovered and placed onto fresh medium containing 0,75% NDM. Nipagin stock was omitted. After three days frass was collected from the different sucrose and ferulic acid treatments. This was extracted for protein, which would consist mostly of casein, using 1/30th strength pH 8 detergent solution based on that of Hartley (1987). Protein was assayed by the method of Bradford (1976).

Results and discussion

At 0,75% NDM there is 2,584 g of protein to 55,2 g of cellulose and agar. The latter two components would be expected to appear entirely in the frass. If it is assumed that all other dietary components are absorbed and that no protein is digested, then the maximum result for a protein assay

on frass would be 45 mg/g of dry mass. Figure 4 shows that ferulic acid affected protein digestion at higher concentrations, especially if the absorption of other nutrients is similarly affected (not tested).

There were no differences within ferulic treatments for the different sucrose concentrations, indicating that increased survival and growth at 10% sucrose with high ferulic acid levels was probably due to increased feeding.

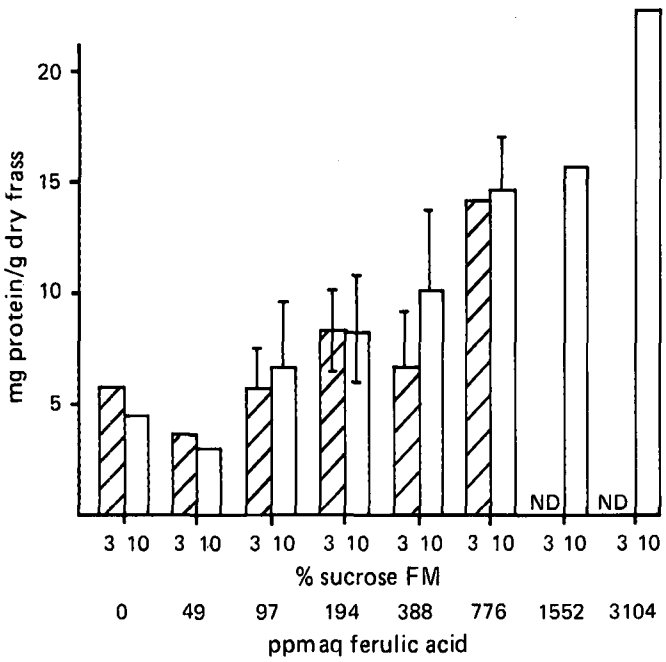

FIGURE 4 The effect of ferulic acid on the amount of dietary protein remaining in the frass of *Eldana saccharina* larvae fed on synthetic diet (0,75% NDM) at two sucrose levels (ND = insufficient frass for one determination).

Tannins

Introduction

It is thought that the generally high gut pHs of lepidopteran larvae allow them to feed on plants rich in tannins (Berenbaum, 1980), which bind to proteins at lower pH values (Goldstein and Swain, 1965), so reducing the level of protein digestion (Feeny, 1969). Monocotyledonous plants contain tannins known as proanthocyanidins or condensed tannins. These can be detected following acid hydrolysis. Measurement of absorbance at a wavelength characteristic of the coloured anthocyanidin breakdown product allows them to be tentatively quantified (Bate-Smith, 1975). In sugarcane the breakdown product is luteolinidin.

Method

The method of hydrolysis used by Bate-Smith had to be modified to avoid interference by coloured cellulose hydrolylsis products (unpublished data). Several varieties were analysed. Tannins were extracted from internodes of the tanniniferous variety N8 using the method of Feeny (1968). These were complexed with the casein of 0,375% NDM meridic diets at pH 5,7 and 4,2. Lactic acid was used to acidify. The fresh mass of the tannin diet was the same as the fresh mass of the extracted cane.

Results and discussion

Figure 5 shows relative tannin contents measured by the acid hydrolysis method for several varieties with their documented relative eldana susceptibilities. There appeared to be a loose relationship between resistance and tannin content. Figure 6 shows the effect of tannins and dietary pH on eldana survival and growth. Tannins reduced survival and growth rate. The effect seemed more pronounced at lower dietary pH.

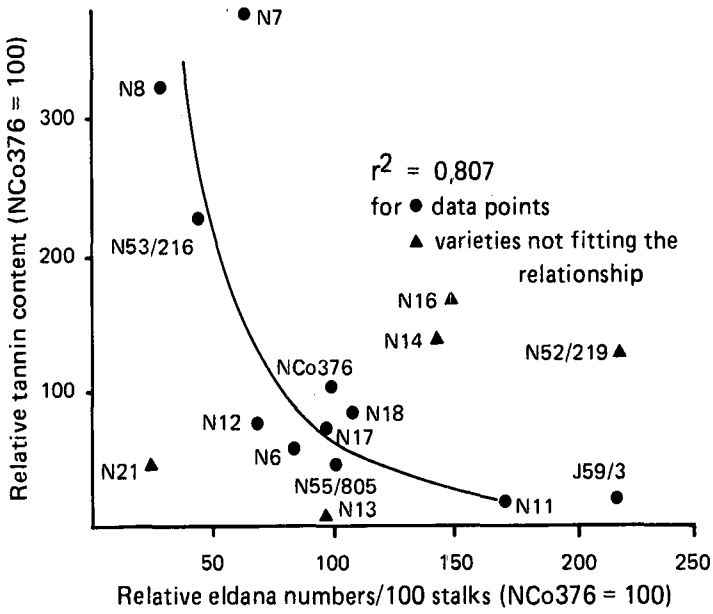

FIGURE 5 Relationship between relative tannin content (internodes) and relative eldana numbers for several varieties.

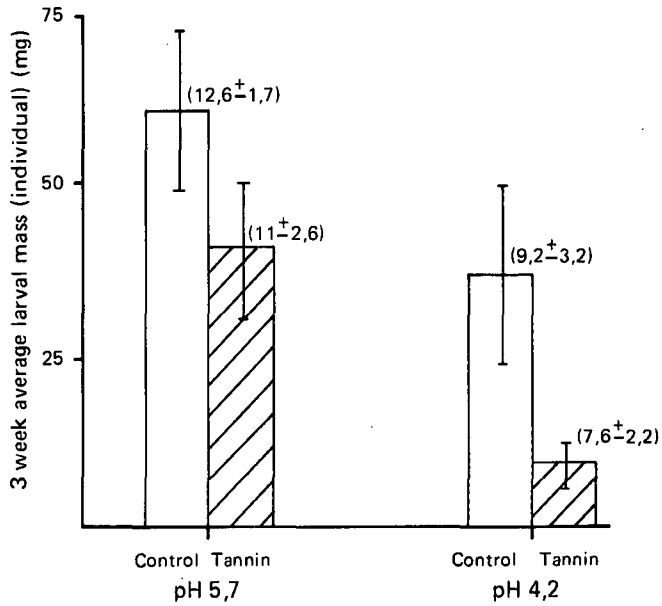

FIGURE 6 The effect of tannin and dietary pH on the survival and growth of *Eldana saccharina* larvae in artificial diet (0,375% NDM, 3% sucrose, 2% fructose, 1,25% glucose) (larval survival per replicate (20) in parentheses).

General discussion

Unlike synthetic diets, intact plants are capable of reaction to damage. In the case of sugarcane, the food that the insect finally consumes can have little in common with undamaged cane (Table 1). Atkinson and Nuss (1989) found that water stress and excessive nitrogen fertilisation increased stored amino acid levels in the stalk. These were predominantly

the amides asparagine and glutamine. Preliminary stalk analyses of the variety N11 which had been damaged by eldana, have revealed that the amides almost totally disappear. Other amino acids increase in level and the amino acid balance alters in such a way that might favour eldana (unpublished data). Amino acids which may favour eldana if they increased greatly in proportion are the essentials, plus alanine, serine and tyrosine.

Fibre in cane consists of all the water insoluble solids, these being cellulose/lignin (non-nutritional for eldana) and lipids (nutritional). Since there is an increase in fibre during damage (Table 1), it is possible that phospholipids increase in quantity. This will probably be dependent upon an increase in P content. Similarly the fatty acid balance of undamaged cane does not appear to be the best for eldana. Any increase in the ratio of unsaturated: saturated in combination with an increase in total quantity during damage should favour eldana (Table 4) provided an adequate antioxidative capacity is present. Changes in lipids following damage to cane await investigation.

Galliard (1978) states that fleshy roots, rhizomes and other storage organs on maceration can exhibit extensive lipoxygenase activity. This enzyme destroys polyunsaturated fatty acids and produces toxic hydroperoxides. These can adversely affect enzyme function and membranes (Matsushita, 1975).

Schuckle and Murdock (1983) have proposed that rapid and extensive lipoxygenase activity during insect feeding might be a resistance factor. Essential fatty acids are destroyed and toxic hydroperoxides are produced. Any involvement of lipoxygenase in the sugarcane-eldana interaction remains to be investigated.

Bernays and Woodhead (1982) found that label from isotopic dihydroxyphenols appeared in the cuticle of the tree locust. They suggested this would spare phenylalanine and tyrosine, the normal precursors of cuticle tanning quinones leading to increased growth rates. Similar growth stimulations for dihydroxyphenolics occur with eldana, though ferulic acid has been found to act as a stimulant as well. However, accumulation of excess phenolics in wounds at the expense of sugars, should lead to decreased eldana survival and growth (Figure 3).

While still esterified to cell walls, ferulic and coumaric acids cannot be expected to act as stimulants or deterrents. Any effect, such as that on protein digestion (Figure 4), would occur after alkaline hydrolysis in the gut. A high gut pH may be the consequence of feeding on a tanniniferous plant and it may be variable depending upon tannin content. Release of esterified phenolic acids is likely to increase with pH.

A combination of high tannin and high levels of esterified ferulic and coumaric acids might be advantageous to the plant, given the effectiveness of tannin in artificial diet (Figure 6) and an apparent relationship with field resistance (Figure 5).

Conclusions

- It may be possible to select in the breeding programme for tannin-containing genotypes by the simple method of acid hydrolysis. Similarly an assay for esterified ferulic and coumaric acids is easy to perform.
- Pre-formed resistance measures such as tannins should complement reactive resistance which may take the forms of:
  1. rapid accumulation of free phenolics and decrease in sugars leading to feeding deterrency,
  2. limited accumulation of essential and other growth promoting amino acids,
  3. limited accumulation of essential fatty acids and/or high lipoxygenase activity following maceration.

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APPENDIX 1

MICRO-ORGANIC/VITAMIN MIXTURE

Micro-organic	Mass	µg/g dwt
Adenine phosphate	4,660 g	1 864
P-aminobenzoic acid	0,020 g	8
Cyanocobalamin	0,050 g	20
Carnitine	0,050 g	20
Nicotinic acid	2,500 g	1 000
Ca pantothenate	0,250 g	100
Riboflavin	0,125 g	50
Aneurine HCl	0,500 g	200
Pyridoxine HCl	0,080 g	32
Folic Acid	0,013 g	5
D-biotin	0,010 g	4
Yeast RNA	5,000 g	2 000
Ascorbic acid	11,412 g	4 697
Total	25 g	

Use 3,3 g per litre of water in the diet

Oil/fatty acid mixture		% acid composition of mixture	
Oil/acid	Mass (g)	Acid	%
Linseed oil	40	Linolenic acid	20
Linoleic acid	24	Linoleic acid	30
Oleic acid	9	Oleic acid	15
Stearic acid	6	Stearic acid	10
Palmitic acid	21	Palmitic acid	25
TOTAL	100 g	TOTAL	100

Use 5,9 g per litre of water in the diet

APPENDIX 2

Concentration of antimicrobials/litre of water in diet

Benomyl	0,28 g/ℓ (0,57 g/ℓ Benlate)	anti-fungal/yeast
Nipagin	1 g/ℓ	
Sorbic	1,25 g/ℓ	
Formaldehyde	3,33 mℓ/ℓ of a 40% solution	anti-bacterial
Streptomycin	0,33 g/ℓ	

NB: Nipagin and formaldehyde are added as Nipagin Stock. Benomyl, streptomycin and sorbic acid are added individually.

Nipagin stock

Nipagin	45 g
Formaldehyde	150 mℓ of a 40% solution
Ethanol to	600 mℓ

Use 13,3 mℓ of Nipagin stock per litre of water in the diet.