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EFFECTS OF RNA, PURINES AND PYRIMIDINES ON THE DEVELOPMENT OF *TRIBOLIUM CONFUSUM* (DUVAL)

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Abstract—Growth period and survival of *Tribolium confusum* were studied on a chemically defined diet alone or supplemented with RNA; guanine, adenine, cytosine, and uracil were also added to the basic diet either singly or in all possible combinations. The effect of supplementing a processed food (dehydrated tapioca pudding) with various levels of RNA on the growth and survival of the beetle was also studied.

Diets supplemented with RNA or a mixture of guanine + cytosine or all the four nucleotides were equally effective in stimulating growth and increasing survival of the beetle; guanine + adenine also improved the diet but not as well as the other three. In general, the purines guanine and adenine stimulated the growth and the pyrimidine cytosine acted as synergist while uracil had slight retardatory effect. The tapioca pudding, which is inadequate for supporting growth of the beetle was also improved by the addition of RNA.

INTRODUCTION

THOUGH dietary RNA promotes growth and development in many insects (HOUSE, 1965), it may have an adverse effect on some, particularly if it is not properly balanced with other dietary constituents. For instance, it inhibited adult emergence in *Phormia regina* (Meig.) (BRUST and FRAENKEL, 1955) and when the diet contained more than 0·05% RNA, it inflicted heavy larval and pharate adult mortality in *Agaria housei* Shewell (HOUSE and BARLOW, 1957). GEER (1963) demonstrated that the growth promoting effects of nucleic acids on *Drosophila melanogaster* (Meig.) depended upon the qualitative adequacy of dietary proteins. HOGAN (1972) showed that pyrimidines, purines or non-halogenated pyrimidine analogues shortened the developmental period of *Tribolium castaneum* (Herbst), whereas halogenated pyrimidines and halogenated nucleosides exerted marked toxic effects on the developing larvae. The development of *Oryzaephilus surinamensis* (L) is also retarded by certain purines and pyrimidines, though RNA promoted it (DAVIS, 1966).

In an earlier paper (PRATT *et al.*, 1972), we suggested insect pests might be controlled in processed foods by adding safe nutrient compounds which could cause nutritional imbalance for the pest but not for the consumer. The aforementioned adverse effects of RNA and certain nucleotides on the development and survival of some insects suggests that such chemicals might be added to certain processed foods for creating nutritional imbalance for insect pests. It is therefore essential to know the dietary role of each nucleotide in the development of a particular species. The present investigations were conducted to assay the effects of individual purines and pyrimidines and their mixtures on the development of *T. confusum* on a chemically defined diet. Development of the beetle was also studied on a processed food (dehydrated tapioca pudding) which was supplemented with RNA.

MATERIALS AND METHODS

Experimental diets

The basic diet was a mixture of corn starch 72·5%, amino acid mixture 20%, minerals (whole wheat ash) 3·5%, corn oil 3%, cholesterol 0·37% and vitamin mixture 0·63% (TAYLOR

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and MEDICI, 1966). All the chemicals were purchased from Nutritional Biochemicals, Cleveland, Ohio. One gram of the basic diet was blended with 0·4 mg of a particular additive, (individual nucleotides or their mixtures) in a small amount of water, freeze-dried and powdered. In preliminary experiments this concentration was found to be adequate for detecting growth and survival responses of the beetle larvae on various diets. Mixtures of nucleotides with or without D-ribose contained equal amounts of ingredients.

The common tapioca pudding contained pure granulated cane sugar (56·7 g), a large whole egg (50 g), homogenized cow's milk (473 ml) and tapioca (39·5 g). The ingredients were boiled for 15 min, cooled, freeze-dried and ground to a coarse powder. Various amounts of RNA were suspended in water, blended with known amounts of the pudding powder and then freeze-dried.

Experimental insects

Cultures of *T. confusum* were maintained on whole wheat flour. In order to obtain experimental larvae which had fed only on the basic diets (pudding or the chemically defined) from the time of hatching to the start of experiments a few hours later, adults were allowed to oviposit directly on the basic diets. First instar larvae, 1–15 hr after hatching were then removed from the diet and transferred to various experimental diets. One hundred larvae (20/replicate) were used in each experiment. The experimental larvae were maintained at 30°C and 55% r.h.

Observation on the mortality and larval–pupal or pupal–adult ecdysis were recorded daily. The dead larvae or pupae were always removed to avoid their being consumed by the living larvae. No cannibalism was noticed. The data on the growth of larvae and pharate adults were subjected to the analysis of variance followed by Duncan's multiple range test. Differences in the data on pupation and adult emergence were analysed by χ^2 test.

RESULTS

Nucleotides in basic diet

The effects of supplementing the basic diet with individual purines and pyrimidines or with mixtures on the duration of larval stages and on the percentage of pupation are shown in Table 1. The basic diet itself was not quite suitable for larval growth as only 49% pupated. The larvae which eventually died were lighter in weight and looked weaker. They appeared to have died just before ecdysis and were not consumed by those who moulted successfully. When the nucleotides were added individually, the earliest pupation and the largest number of pupae were obtained on diets containing 0·4 or 0·8 mg of guanine/gm of the diet (Table 1a). The presence of adenine also promoted growth but had no effect on the survival of the larvae. Diets with cytosine or uracil showed no difference from the basic control diet.

Supplementing diets with mixtures of two nucleotides (Table 1b) enabled more individuals to reach pupation than on the control diet or those containing adenine, or cytosine or uracil. However, when guanine was one of the components of the nucleotide mixture, the larval periods were shorter and the pupation was higher than on the other diets. Thus the most suitable diet was the one containing guanine + cytosine followed by those with guanine + adenine, guanine + uracil and adenine + cytosine; results for these four diets were significantly different from each other also.

Addition of mixtures of three nucleotides to the basic diet shortened the larval period and increased the incidence of pupation as compared with the basic control diet; however, these diets were never as good as those with guanine + cytosine, guanine + adenine or guanine + uracil (Table 1b, c).

The duration of the pupal stage and the number of adults obtained when the larvae were reared on diets supplemented with various nucleotides or their mixtures are presented in Table 2. No significant mortality was observed during the pupal stage on

TABLE 1. GROWTH AND SURVIVAL OF *T. confusum* LARVAE ON A CHEMICALLY DEFINED DIET SUPPLEMENTED WITH PURINES AND PYRIMIDINES

Additives[1]	Larval period (days)	% pupation	Additives	Larval period (days)	% pupation
		A. Single nucleotides			
None	42.12 ± 0.55 a,e	49*	A	39.53 ± 0.15 c,f	54*
G	33.60 ± 0.47 b	68	C	43.80 ± 0.47 a	50*
G (0.8 mg/g)	34.39 ± 0.41 b	71	U	44.78 ± 0.63 a	53*
		B. Mixtures of two nucleotides			
G + C	28.52 ± 0.44,d,	71	A + C	37.24 ± 0.41 c,g	63
G + A	33.89 ± 0.50,b,	71	A + U	41.49 ± 0.46 e,f	65
G + U	37.52 ± 0.38 c,g	76	C + U	40.55 ± 0.60 e,f,h	65
		C. Mixtures of three nucleotides			
G + A + C	36.39 ± 0.37,g	68	G + C + U	38.91 ± 0.43 c,h	66
G + A + U	39.64 ± 0.40,c,f	56	A + C + U	40.61 ± 0.21 e,f,h	68

L 1, added at 0.4 mg/g of diet. G, guanine ; A, adenine ; C, cytosine ; U, uracil . Figures followed by the same letter are not significantly different from each other at 1 % level. * , not significantly different from control.

any diet; however, the developmental periods on some diets were significantly different from the control and from each other.

Table 3 shows that addition fof D-ribose along with guanine + cytosine to the basic diet reduced the combined growth stimulatory effect of the two nucleotides. The best results in terms of shortest growth period and maximum number of pupae and adults were obtained on diets containing guanine + cytosine or guanine + cytosine + adenine + uracil or RNA.

RNA in pudding

Table 4 shows the larval period and the overall survival of *T. confusum* on the dehydrated tapioca pudding to which various amounts of RNA were added. On the control pudding diet, larval development was slow and mortality was very high; most of the surviving larvae pupated within 11 wk. Pupal and adult mortality was also very high, the adults dying during emergence or soon afterwards. At the end of the experimental

TABLE 2. DURATION OF PUPAL STAGE AND ADULT EMERGENCE OF *T. confusum* REARED ON A CHEMICALLY DEFINED DIET SUPPLEMENTED WITH PURINES AND PYRIMIDINES

Additives	Pupal period (days)	% adult	Additives	Pupal period (days)	% adult
		A. Single Nucleotides			
None	5.80 ± 0.15 a,g	49 *	A	5.70 ± 0.10 a	54 *
G	6.10 ± 0.46 b,g	66	C	7.20 ± 0.18 c	53 *
G (0.8 mg/g)	6.06 ± 0.08 b,g	64	U	6.23 ± 0.14 b	50 *
		B. Mixtures of two nucleotides			
G + C	6.18 ± 0.09 b,	71	A + C	5.62 ± 0.09 a	62
G + A	5.02 ± 0.06 d	70	A + U	6.42 ± 0.20 e	62
G + U	6.06 ± 0.06 b,g	71	C + U	7.80 ± 0.19 c	55 *
		C. Mixtures of three nucleotides			
G + A + C	6.42 ± 0.14 e	67	G + C + U	8.29 ± 0.15 f	64
G + A + U	6.09 ± 0.09 b,g	54	A + C + U	6.16 ± 0.12 b,g	64

Legends as in table 1.

TABLE 3. DEVELOPMENT AND SURVIVAL OF *T. confusum* ON CHEMICALLY DEFINED DIET SUPPLEMENTED WITH NUCLEOTIDES OR RNA

Additives	Larval period (days)	% pupation	Pupal period (days)	% adult
None	42.12 ± 0.55 a	49	5.80 ± 0.15 d	49
G + C	28.52 ± 0.44 b	71	6.18 ± 0.09 e	71
G + C + D – ribose	31.52 ± 0.55 c	80	5.70 ± 0.05 d	75
G + C + A + U	29.82 ± 0.36 bc	82	6.18 ± 0.11 e	76
RNA (5mg/g)	29.46 ± 0.37 bc	73	6.20 ± 0.17 e	71

Legends as in table 1. % pupation and adult emergence on experimental diets is significantly different from control diet at 1% level.

period, only 35% of the initial number of larvae had survived—28% as adults, the rest as pupae. Addition of 1 or 5% of RNA significantly improved larval growth, though more pupae and adults were obtained at lower levels of RNA.

DISCUSSION

Our results show that like most other insect species (HOUSE, 1965), *T. confusum* also requires RNA for optimal growth and survival. The purine guanine and to a lesser extent adenine stimulated growth in the beetle; the pyrimidine cytosine enhanced the action of the purines while uracil retarded it.

In general purines appear to stimulate growth in other species also. For instance, though guanine and its derivatives were ineffective on *D. melanogaster* (HINTON *et al.*, 1951; GEER, 1963), adenylic acid shortened the growth period: however, these results varied in different strains of the fruit fly (GEER, 1963). Earlier SANG (1957) had found that guanylic acid did in fact promote the growth of the fruit fly though not as effectively as adenylic acid. The present results are in agreement also with HOGAN'S (1972) data on the growth stimulatory effects of guanine and adenine on an allied species of the flour beetle *T. castaneum*. Only in the case of *O. surinamensis* did guanine have growth retardatory effect while adenine was ineffective (DAVIS, 1966).

Pyrimidines alone appear to have no role in the growth of insects, but enhance the stimulatory effects of purines. For instance, cytosine had no effect on *T. confusum* (Table 1), *T. castaneum* (HOGAN, 1972) or *O. surinamensis* (DAVIS, 1966), but increased uniformity in growth-time and survival in *D. melanogaster* (GEER, 1963). Conversely, uracil retarded growth in *T. confusam* (Table 1) *Cochliomya hominivorax* (Coquerel) (GINGRICH, 1964) and *O. surinamensis* (DAVIS, 1966) but had a stimulatory effect on the growth of *T.*

TABLE 4. DEVELOPMENT AND SURVIVAL OF *T. confusum* ON DEHYDRATED TAPIOCA PUDDING, SUPPLEMENTED WITH VARIOUS AMOUNTS OF RNA

% added RNA	Larval Period Mean ± S.E. (weeks)	% Pupae[1] Obtained	% Surviving[1] Pupae	% Surviving[1] Adults
0	8.4 ± 1.0	49	7	28
0.25	7.7 ± 1.1	58	20	30
0.5	8.1 ± 1.8	57	20	28
1.0	7.6 ± 1.0	60	15	35*
5.0	6.5 ± 0.5*	49	7	42*

[1] at the end of 11 week experimental period.

* significantly different from control at 1% level.

castaneum (HOGAN, 1972). It may be pointed out that protein in Hogan's diet was qualitatively different from others and this can modify the dietary role of nucleotides (GEER, 1963).

Mixtures of purines and pyrimidines increased survival, and in most cases, reduced the growth period of *T. confusum* (Table 1); these results are similar to those on *O. surinamensis* (DAVIS, 1966). The effects of the purines guanine and adenine on the flour beetle were enhanced by the pyrimidine cytosine and reduced by uracil; similarly, the effectiveness of guanine on *O. surinamensis* was increased greatly by cytosine but only slightly by uracil (DAVIS, 1966). In *D. melanogaster*, however, guanine was found to improve the action of another purine adenine (VILLEE and BISSEL, 1948).

In the present experiment, a mixture of guanine + cytosine + adenine was not as effective as guanine + cytosine or even guanine + adenine; this may be due to nutritional imbalance between purines and pyrimidines. For, when all the four nucleotides were present in the diet, the growth and survival of *T. confusum* (Table 1) or *O. surinamensis* (DAVIS, 1966) were just as good as on RNA-supplemented diet. Similarly, a mixture of guanylic acid + cytidylic acid + uridylic acid retarded growth of *D. melanogaster*; however, addition of proper proportions of adenylic acid to the mixture could successfully replace RNA in the diet (GEER, 1963). The importance of proper purine-pyrimidine balance in the diet of *D. immigrans* was also demonstrated by ROYES and ROBERTSON (1964). Only in the diet of *C. hominivorax* could a mixture of only three nucleotides replace RNA effectively (GINGRICH, 1964).

The dietary effect of nucleotides on the pupae period in the flour beetle showed no relationship with larval development or survival; such a confusing pattern was also observed by DAVIS (1966) on *O. surinamensis*. Most of the observed differences in the developmental period of pharate adults on different diets may have no or little biological significance.

The growth promoting effect of RNA on *T. confusum* is further confirmed by the improvement of the imbalanced tapioca pudding (Table 4) which was rich in fat and carbohydrates but poor in vitamins, particularly niacin. Probably RNA helped food utilization by enabling the synthesis of vitamins. CHARBONNEAU and LEMONDE (1960) reported the presence of an unidentified growth factor in brewer's yeast which, according to HOGAN (1972) may have pyrimidine or pyrimidine-like structure.

On the basis of the present results, it seems unlikely that the purines or pyrimidines can be used for creating imbalance in processed foods to protect against insect infestation. However, it is possible that halogenated and non-halogenated analogues of purines and pyrimidines which are known to have adverse effects on metamorphosis and fertility of many insects (MUTH, 1962; PLUS, 1962; HOGAN, 1968) might be used for the nutritional control of pests of processed foods.

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