DETECTION OF INSECT INFESTATIONS IN CEREALS BY MEASUREMENT OF URIC ACID[1]

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ABSTRACT

The levels of insect populations in food required to produce a measurable quantity of uric acid are discussed. The use of the uric acid content of a sample as an index of infestation or contamination was found not to be satisfactory. The test was not sufficiently sensitive. The high level of insect population generally necessary to produce a measurable quantity of uric acid (2 weeks for four adult *Tribolium confusum* per g. of food to produce a measurable amount of uric acid) seriously limits the value of the technique. The determination of the uric acid content of a sample would, however, be useful in producing evidence of a past infestation, but would be of no value in an attempt to predict the storage potential.

Many methods of determining the degree of infestation of cereal grains by insects have been devised, but none of the methods at present available is satisfactory for all purposes. The method most widely used is to count the number of insect fragments in a sample of flour or ground wheat, and a very large number of papers which describe this technique have been published and are summarized by Kurtz and Harris (1). One apparently promising technique, the measuring of uric acid produced by insect metabolism, has been described by Subrahmanyan *et al.* (2,3) and by Venkat Rao *et al.* (4,5,6,7). These workers correlated kernel damage and insect-fragment counts with the uric acid content of a sample of food. They also demonstrated and discussed the effect of varying concentrations of uric acid in flour on the bread baked from the contaminated flour. Approximately 18% of the total frass of *Tribolium confusum* is uric acid (8).

In addition to insects, the two other most prevalent contaminators of grain and, at times, flour are rodents and birds. The major nitrogenous waste product in rodents is urea, not uric acid (9). Birds do excrete uric acid in their mixed urine-feces excrements. However, bird excrement is largely removed from wheat during cleaning and milling and does not represent an important source of uric acid in flour and meal.

The present paper discusses the levels of insect population required to produce a measurable quantity of uric acid in a food, and whether or not this can be satisfactorily used as an index of contamination in

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cereals and their products, taking into account the number of insects in a given quantity of food that should be regarded as a heavy infestation.

Material and Methods

Uric acid was extracted from the ground wheat or flour by placing 200 to 2,000 mg. of the material, depending on the expected uric acid concentration, in a flask containing 100 ml. of distilled water and maintaining it at a temperature of approximately 60°C. in a water bath for 1 hr., with periodic shaking. The flask was then removed and the contents centrifuged at 2,000 r.p.m. for 10 min. To 50 ml. of the centrifugate was added 5 ml. of borate buffer (pH 9.2), 5 ml. of 10% sodium tungstate, and 5 ml. of 0.66*N* sulfuric acid. It was then diluted to 100 ml. After standing for 5 min. the solution was filtered through No. 4 Whatman paper. Ten milliliters of the filtrate was taken for the determination of uric acid by the method of Buchanan *et al.* as described by Hawk *et al.* (9). This method was preferred to that of Benedict and Franke (10) as used by other workers (2,6) because it was not affected to the same extent by substances other than uric acid (11).

To check the efficiency of this method of extracting uric acid from flour, three samples of baker's flour, which contained no added chemical improvers and had not been subjected to any bleaching treatment, were contaminated with 0.08, 0.20, and 0.32% (by wt.) of uric acid. These samples were also used to check the technique throughout this series of investigations. The recovery rate ($\nless$ 90%) was satisfactory. There was no development of color in the uninfested control flour samples at any time. Samples of fish meal, a high-protein food, were also contaminated with similar proportions of uric acid; the recovery rate was similar to that obtained with flour. No blue color developed in the uninfested control samples of fish meal.

In the first series of tests, adult beetles of *Tribolium confusum* Duv. were used on flour, with relatively high densities of insects to ensure a high level of uric acid. Six 8-g. samples of flour were infested with 8 adults and a further 6 samples with 32 adults, 2 samples of each set being tested each week for 3 weeks. Three additional samples were infested with 16 adults each and tested after 28, 35, and 43 days. A control sample of uninfested untreated flour was included with each series of tests. The samples were kept at a constant temperature of 30° ± 0.5°C. and relative humidity of 55 ± 5%.

A second series of tests used a larger beetle, *Tenebrio molitor* L. Four samples of 10 g. each were infested with three adult beetles. One sample was tested each week for 4 weeks.

In a third series of tests 10 young larvae of *Dermestes lardarius* L., less than half-grown, were used on 10 g. of fish meal. For both series the samples were kept at 25° ± 0.5°C. and 70 ± 5% r.h.

In a fourth series, larvae of two species, *Tribolium confusum* and *Tenebrio molitor,* were used. Larvae of *T. confusum* about 1 week old were collected into groups containing different numbers of larvae and weighing about 25, 50, 100, and 250 mg. respectively. Four batches of each group were obtained and each batch was placed on about 10 g. of whole-wheat flour which had been ground fine enough to pass through a wire-mesh sieve (60 s.w.g.). The samples were kept at 25° ± 0.5°C. and 70 ± 5% r.h. Batches were examined after 7, 14, 21, and 28 days respectively, when the insects were removed and weighed. Exuviae were also removed and the uric acid content of the flour was determined.

Larvae of *T. molitor* about 1 week old were treated similarly. They were collected into eight weight groups of about 3, 6, 12.5, 25, 50, 75, 100, and 250 mg. Four batches of each weight were obtained and tested after 7, 21, 35, and 49 days respectively.

Results and Discussion

The results of tests with adult *T. confusum* (Table I) show that the increase in uric acid content of the flour was associated with an increase in weight of eggs and larvae produced. It took 32 adult beetles 14 days to produce a measurable amount of uric acid. Since the experimental error is approximately 0.02%, values below this may be regarded as zero (i.e., 20 mg. per 100 g. flour).

No trace of uric acid was found in flour in which *T. molitor* had been feeding for up to 28 days, which indicates that results of uric acid

TABLE I

AMOUNT OF URIC ACID AND INCREASE IN WEIGHT OF EGGS AND LARVAE PRODUCED BY DIFFERENT POPULATION DENSITIES OF ADULT INSECTS (*T. confusum*) ON UNTREATED FLOUR IN DIFFERENT PERIODS

PERIOD	8 ADULTS[a]		16 ADULTS[a]		32 ADULTS[a]	
	Uric Acid	Increase in Weight	Uric Acid	Increase in Weight	Uric Acid	Increase in Weight
days	*% by wt.*	*mg.*	*% by wt.*	*mg.*	*% by wt.*	*mg.*
7	0.02	9.5	...	...	0	9.3
14	0.02	23.1	...	...	0.05	16.4
21	0.02	50.0	...	...	0.09	50.0
28	..	..	0.11	127.1	..	..
35	..	..	0.20	...	..	..
43	..	..	0.27	98.1	..	..

[a] Per 8 g. flour.

determinations would not be useful in detecting infestations of this beetle.

Table II summarizes the results obtained with *D. lardarius* on fish meal using half-grown larvae (13–15 days old approx.). The uric acid

TABLE II

RESULTS OBTAINED WITH TEN HALF-GROWN LARVAE OF *D. lardarius* ON 10 GRAMS OF FISH MEAL[a]

	Uric Acid	Uric Acid Produced per Larva per Day	Weight of Ten Larvae at End of Experiments
days	*% by wt.*	*% by wt.*	*mg.*
7	0.47	0.007	....
14	1.13	0.008	....
21	1.04	0.005	369.0
28	1.00	0.004	331.6

[a] Weights of larvae after 7 and 14 days not recorded.

reached a peak in 14 days and then did not increase further. This, however, could be because the larvae had then reached their full size and were no longer feeding, although no pupae were formed.

In the series of tests using larvae of *T. confusum* the percentage of uric acid in the flour increased with time and with population density (Table III). The greatest increase in weight of insects was usually recorded at 14 days, by which time some of the larvae had pupated. After this the weight decreased; by 21 days all the larvae had pupated and there were some adults, and by 28 days almost all were adults. Figure 1 shows the relationship between the gain in insect weight and the uric acid content of the infested flour for each storage period. It is evident from this graph and Table III that the weekly rate of uric acid production was irregular, the largest increase being in the second week when large larvae were present, and the smallest

TABLE III

AMOUNT OF URIC ACID, AND INCREASE IN WEIGHT, PRODUCED BY DIFFERENT POPULATION DENSITIES OF LARVAE OF *T. confusum* ON 10 GRAMS OF UNTREATED FLOUR FOR DIFFERENT PERIODS

Period	Initial Weight of Larvae							
	25 mg.		50 mg.		100 mg.		250 mg.	
	Uric Acid	Increase in Wt.	Uric Acid	Increase in Wt.	Uric Acid	Increase in Wt.	Uric Acid	Increase in Wt.
days	*% by wt.*	*mg.*	*% by wt.*	*mg.*	*% by wt.*	*mg.*	*% by wt.*	*mg.*
7	0.06	79	0.12	116	0.18	311	0.25	530
14	0.14	146	0.11	186	0.45	468	0.65	827
21	0.11	119	0.23	205	0.44	426	0.76	754
28	0.16	99	0.25	169	0.54	277	0.94	606

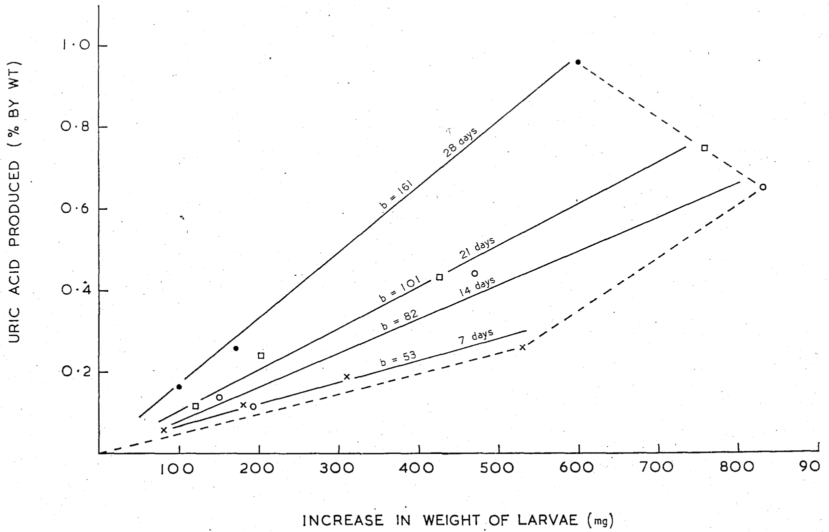

Fig. 1. Relation between gain in insect (*T. confusum*) weight and uric acid content of the infested flour for each storage period; b = regression coefficient.

in the third week when pupae were predominant. However, examination of the regression lines relating uric acid content to gain in weight shows that the greatest increase in the size of this regression coefficient (b) occurred during the first and fourth weeks, not the second, and least in the third. This is because the uric acid content continued to increase even when the insect weight was decreasing. The uric acid content at a particular time cannot, therefore, be simply related to the weight of insects present at that time. The uric acid is produced by the insects throughout the period of infestation, during which time they increase in weight and lose weight in turn.

Growing larvae of *Tribolium confusum* increase in weight exponentially. Therefore the best average for the growing period that can be obtained from an initial and a final weight is their geometric mean. The total weight of uric acid-producing tissue in these experiments can be obtained for this growing period by multiplying the geometric mean by the period of growth. After reaching maximum weight and ceasing to feed, the larvae lose weight approximately linearly, although there are greater losses accompanying the casting of skins at pupation and at the adult molt. For this postfeeding period the arithmetic mean is a fair measure of average weight. The total weight of uric acid-producing tissue for the postgrowth stages can be obtained by multiplying the arithmetic mean by the requisite period of time. Figure 2,

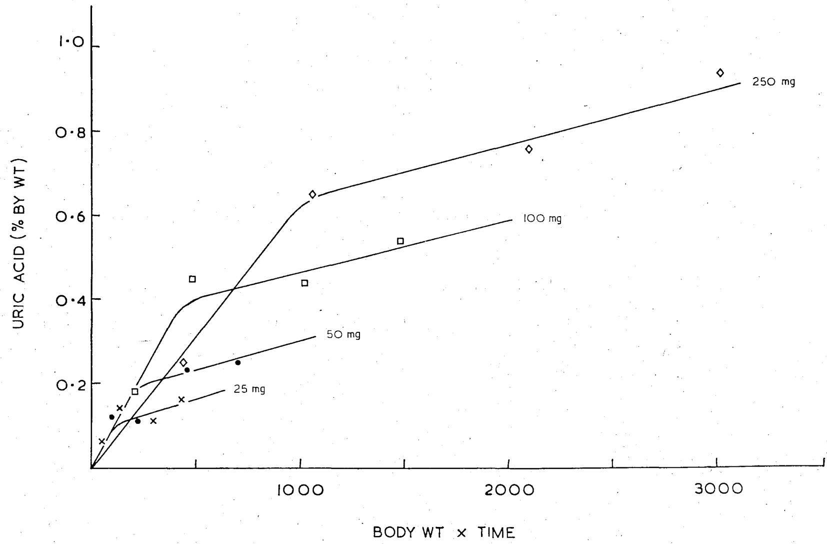

Fig. 2. Relation between uric acid production and total weight of living tissue, and time, for four different population levels of larvae of *T. confusum.*

in which the uric acid production is plotted against the total weight of living tissue calculated in these ways, shows that the rate of uric acid production increases sharply during the period of rapid growth but levels off during the postgrowth stage. This supports the view that larvae produce uric acid at a greater rate than adults. This statement must, however, be made with caution, since pupae are present from about the 14th day and they presumably produce no uric acid at all. The present investigation also shows the effect of increased population density, by a reduction in uric acid production per mg. per day. Farn and Smith (12), using *Tribolium castaneum,* also found that the amount of uric acid excreted per insect per day depends on the life stage, population pressure, and adequacy of nutrition. It is difficult to extrapolate from laboratory life history studies to commercial storage.

Larvae of *Tenebrio molitor* grow more slowly than those of *Tribolium confusum* and are therefore likely to produce uric acid at a much slower rate. Comparing the results given in Table IV with those for *T. confusum* in Table III, for comparable periods, it will be seen that *T. molitor* produces only about half the amount of uric acid per mg. per day. Whereas 100 mg. of *T. confusum* gain nearly 500 mg. in weight in 14 days, 100 mg. of *T. molitor* require 35 days, i.e., nearly two and one-half times as long, to make this gain. Both species produce about 0.5% of uric acid in this time. The experiments with *T. molitor*

TABLE IV

AMOUNT OF URIC ACID, AND INCREASE IN WEIGHT, PRODUCED BY DIFFERENT POPULATION DENSITIES OF LARVAE OF *T. molitor* ON 10 GRAMS OF UNTREATED FLOUR FOR DIFFERENT PERIODS

PERIOD	INITIAL WEIGHT OF LARVAE							
	3 mg.		6 mg.		12.5 mg.		25 mg.	
	Uric Acid	Increase in Wt.	Uric Acid	Increase in Wt.	Uric Acid	Increase in Wt.	Uric Acid	Increase in Wt.
days	*% by wt.*	*mg.*	*% by wt.*	*mg.*	*% by wt.*	*mg.*	*% by wt.*	*mg*
7	0	1.2	0	6.2	0	9.9	0.04	17
21	0.03	3.5	0.04	24	0.03	47	0.06	64
35	0.03	15	0.06	66	0.08	102	0.11	124
49	0.06	43	0.08	88	0.15	141	0.25	271
	Wt. 50 mg.		Wt. 75 mg.		Wt. 100 mg.		Wt. 250 mg.	
7	0.05	34	0.05	50	0.07	70	0.13	163
21	0.12	143	0.17	201	0.28	295	0.62	644
35	0.29	343	0.36	490	0.51	506	1.12	1,103
49	0.48	454	0.70	744	0.82	889	.2.44	1,667

were continued for nearly twice as long as those with *T. confusum* and eventually yielded about three times the weight of insects but only twice the amount of uric acid.

In the present work with *T. confusum*, it required 2 weeks for four adults per g. of food to produce a measurable amount of uric acid. This is roughly comparable to one insect in 2 g. of food for 16 weeks. In experiments with *Sitophilus granarius* to determine the influence of low density on population establishment, Surtees (13) showed that one male and one virgin female in a 20-kg. bag of English wheat, at 25°C. and 14% moisture content, yielded a total of 6,500 progeny in 4 months, i.e., a density of one insect to approximately 3 g. of wheat. This is slightly less than that mentioned earlier for minimum detectable production of uric acid. In 4 to 8 weeks one unmated pair in 20 kg. of wheat was sufficient to initiate a substantial population.

From Surtees' results it is evident that, under tropical conditions, a population of one adult to 1 cwt. of grain could lead to a heavy infestation. The amount of uric acid produced would not be detectable until the insects themselves were obvious and abundant. The method, therefore, is not sufficiently sensitive to predict the storage potential of grain with the uric acid content as an index of infestation.

The results of low infestations, even for long periods, would not be detected by this method. A high uric acid figure from a sample apparently free from insects, however, would be evidence of a heavy infestation which had been removed and would therefore not be detectable by fragment counts.

Conclusions

Since very low levels of insect population density could result in heavy infestation in favorable environments, the measurement of uric acid by the usual methods is not sufficiently sensitive to enable a prediction to be made as to the storage potential of the sample.

In tropical conditions where the climate makes high infestations more likely to develop, the uric acid content of a sample might be used as an index of population density, but it would be most useful in producing evidence of a past infestation.

The high level of insect population generally necessary to produce measurable uric acid seriously limits the value of the technique.

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