DISCOVERY AND DEVELOPMENT OF A NEW BAIT FOR CONTROL OF PEST INSECTS

H. N. WREN

Department of Entomology, Virginia Polytechnic Institute and State University, Blacksburg, Virginia 24061-0319

Abstract—A new type of bait has been developed, aimed at control of pest insects, and tested successfully on the model insect *Blattella germanica* (L.) – the German cockroach. The active ingredient, termed a nutritional metabolism disrupter (NMD), is a non-toxic composition of the compounds oxypurinol and xanthine, and exploits a new mode of action with very effective results. The insects are not killed by an immediate toxic reaction, but die when their reserves of uric acid are not restored after mating, egg-case development, or moulting.

The composition acts by inhibiting the enzyme xanthine oxidase while simultaneously increasing its substrate. This causes a block in the purine metabolic pathway which, in cockroaches, is the synthetic pathway for uric acid. This latter compound is normally stored in the cockroach fat body cells, and is used as a resource during periods of high nitrogen demand. Whole-body uric acid levels in dead, treated insects were measured by spectrophotometric assay and were found to be extremely low, confirming this as the mechanism of action.

Laboratory colonies treated with NMD declined to total extinction within four to six weeks. About six days of feeding proved sufficient to ensure population control, and the test insects made no distinction between treated and untreated diets when given a choice. In further tests, both insecticide-resistant and susceptible cockroach strains were equally affected, indicating that there is no cross-resistance from other mechanisms. Nymphs also died when they were fed feces from treated colonies, which implies good residual effects.

The bait's inert matrix was carefully designed to enhance the effects of the active ingredient, as well as to attract the target pests for multiple feedings. Early field-trial results confirmed that the bait was effective against large populations of German cockroaches, and that it compared well with known toxic baits.

The product, named Ecologix™, is being developed in the USA, and patented world-wide, by Dominion BioSciences Inc., Blacksburg, Virginia, USA.

INTRODUCTION

It is widely acknowledged that the majority of insects are uricotelic, in that they excrete their excess nitrogen as uric acid and other uricolytic derivatives thereof (Cochran, 1975). The uric acid is synthesized, via the purine catabolic pathway, and is either excreted to the outside, or stored by the insects as a metabolic reserve.

The purine metabolic pathway is central to this process, and, as in any of the known biochemical pathways, the hydrolytic enzymes are essential to its function. One of the enzymes involved in this pathway is xanthine oxidase (E.C.1.2.3.2), which is a flavoenzyme with an iron-sulphur and molybdenum center (Coughlan, 1980). It functions late in the salvage pathway of purine catabolism from guanosine monophosphate and inosine monophosphate to uric acid. In this pathway, xanthine oxidase catalyzes the oxidation of hypoxanthine to xanthine, as well as the conversion of xanthine to uric acid (Lehninger, 1982). Functioning as xanthine dehydrogenase, the same enzyme reduces uric acid to xanthine in the uricolytic pathway of the endosymbiont bacteria in the cockroach fat body (Wren and Cochran, 1987).

An understanding of this system in cockroaches led to the discovery of a novel, non-toxic composition, termed a nutritional metabolism disrupter (NMD). This composition consists of the purine xanthine, (3,7-dihydro-1H-purine-2,6-dione), (The Merck Index, 1989), and the xanthine oxidase inhibitor, oxypurinol, (4,6-dihydroxy-pyrazolo[3,4-*d*] pyrimidine), (Massey *et al.*, 1970). Xanthine is a compound which occurs naturally in many plant and animal tissues, which constitute human foods, and oxypurinol is a metabolite of allopurinol, used as a medication in humans, to treat gout. Neither of these compounds functions alone to affect cockroaches, but as a composition they cause a virtual shut-down of nitrogen recycling in these insects.

The cockroach is a good model of the essential nature of storage-excretion and recycling of uric acid, in that they rely on this metabolic resource to be able to develop and reproduce. In the cockroach fat body, *de novo* synthesis of uric acid takes place, largely through purine salvage, in the trophocytes. The uric acid pours in quantity through the cell membranes into the adjacent

Proceedings of the Second International Conference on Urban Pests.
K.B. Widey (editor). 1996

specialized urocytes (Wigglesworth, 1987; Wren, 1991), to be stored as rounded concretions for recycling (Cochran *et al.*, 1979; Cochran, 1985). This is accomplished through uricolytic digestion of the stored urates by endosymbiont bacteria sequestered in bacteriocytes (Wren and Cochran, 1987), which are the third type of cell that makes up the cockroach fat body. During reproduction, the male German cockroach passes a slurry of uric acid to the female during mating, as a paternal investment. The female, in turn, invests the developing eggs with a supply of uric acid that is used during embryogenesis (Mullins *et al.*, 1992).

Disruption of this complex nutritional cycle is highly detrimental to homeostasis of the insects, and thus, to population growth (Engebretson and Mullins, 1986; Suiter *et al.*, 1992). It is here that the NMDs function.

This technology has become the basis for development of the non-toxic Ecologix™ products. Part of this development was to ensure that the activity of the NMD is not diminished by any of the ingredients in the carriers. Further development is centered on application of the technology to other pest insects.

METHODS AND MATERIALS

Laboratory trials

The following protocol formed the basis for most of the laboratory trials. Some variations were made, in order to address specific questions of performance of the NMD.

German cockroaches (*B. germanica*) from one of the laboratory stock insecticide-susceptible strains (Virginia Polytechnic Institute (VPI), or American Cyanimid (AMCY)), were used to form experimental colonies of mixed life stages. Each colony consisted of forty-two cockroaches, made up of five each of newly-molted adult males and females, eight each of male and female nymphs at the fifth nymphal stage, and eight each of male and female nymphs at the third nymphal stage. Care was taken to select insects from the same stock colonies for each experimental block, and the insects were trapped individually in glass vials for selection and transfer. The insects were not anaesthetized or chilled, and each colony was allowed to acclimatize for 24 h prior to treatment.

In further trials, standard colonies of German cockroaches of the Hawthorne and Las Palms resistant strains were tested. The profiles of the resistance ratios of these two strains are detailed in Figure 1, and show the broad resistance among several classes of insecticides in these insects (Cochran, personal communication). The Hawthorne strain also has an aversion to glucose (Silverman and Bieman, 1993).

Colonies were housed either in covered one-gallon glass battery jars fitted with fiber-board platforms, or in lidded plastic storage containers with halves of egg-cartons for harborage. The containers were rimmed with a thin coating of petrolatum to prevent escape of the test insects.

Each test included colonies offered food treated with NMD, and untreated 'control' colonies. After initial surveys using a wide range of concentrations of the two compounds in NMD (data not shown), it was determined that 1% xanthine + 1% oxypurinol (described as 2% NMD) was optimal for the model insects, and became the norm for the trials. (Both compounds were initially purchased from Sigma Chemical Company, St. Louis, Missouri. Later, the oxypurinol was also obtained from Synthons, Inc., Blacksburg, Virginia.)

At first, the food used in all the trials was the same laboratory rat chow on which the cockroaches were reared. This was prepared by grinding the chow pellets into a fine, dry powder (RC). In the treated colonies, the NMD was incorporated into the food by grinding it finely with the RC, using a mortar and pestle. In later experiments, the food consisted of a dry, powdered bait-base (BB)(proprietary information), or a mixture of 50% BB and 50% RC (RC+BB), with all percentages by weight (w/w).

Food was packed, weighed, and offered, in stainless steel planchettes. These were placed in plastic cups to avoid loss through spillage. The formulated bait was offered in commercial plastic bait-stations which were taped or stapled shut to allow for inspection of the interiors before weighing. The filled planchettes and bait stations were weighed at the start of an experiment and weekly thereafter, and food was replenished when necessary to maintain constant availability. Clean tap-water was offered continuously in cotton-stoppered glass vials.

Insecticide	Hawthorne Strain	Las Palms Strain
Organophosphates	RR	
Diazinon	2.0	>75
Chlorpyrifos	10.8	>50
Acephate	2.0	1.2
Malathion	5.5	>50
Carbamates	RR	
Propoxur	1.7	>60
Bendiocarb	2.2	>70
Pyrethroids	RR	
Pyrethrins	>140	>140
Allethrin	>140	>140
Permethrin	0.5	3.2
Phenothrin	0.6	>120
Fenvalerate	0.9	>60
Esfenvalerate	0.8	7.0
Cyfluthrin	1.8	2.5
Cypermethrin	1.6	>80
Bio-Chemical	RR	
Avermectin	2.4	1.5

Figure 1. Resistance ratio (RR) profiles for the Hawthorne and Las Palms insecticide-resistant strains of German cockroaches, where, on a continuum of rising resistance, RR >2.0 indicates that resistance is developing, and RR >3.0 indicates that the gene frequency for resistance has increased. RR is calculated as (Test Strain LT_{50}) ÷ (Susceptible Strain LT_{50}), where LT_{50} is the time taken for an intoxicant to acheive 50% mortality in a treated population.

Replicate colonies were initiated on the same day, or on consecutive days for larger trials, with all colonies housed in the stock laboratory under the same conditions of humidity and ambient temperature (±25°C) as during rearing. A control "blank colony" (which was identical to a control colony except that no insects were introduced), was monitored for any weight changes due to loss or gain of moisture in the food. Any such changes were factored into calculations of food consumption.

To determine the duration of feeding required for the NMD to be effective, the standard protocol was followed, except that BB treated with 2% NMD was offered for durations of 3, 6, 9, 12, or 15 days. At the end of the treatment time, the NMD-treated food was removed and replaced with untreated food. The control colonies were offered either untreated or treated food continuously until the end of the trial.

A weekly census was kept of all dead insects, which were tallied by sex and stadium at the same time that the food was weighed. When required for whole-body uric acid assays, dead insects were frozen and stored at −4°C. The population of each colony was counted every three weeks unless conditions (such as rapid mortality) dictated otherwise. When all of the insects, or all of the females, were dead or moribund, the colony was determined to be non-viable and the experiment was terminated. Remaining insects in treated and untreated colonies were killed by freezing, and stored as above.

Individual consumption in milligrams (ICmg), was calculated for the first three weeks of each experiment, (prior to nymphs hatching in the controls). The percent change (Δ%) in mean population number for each colony was calculated, with the initial population (42) representing 100%. In the assays where a choice of food was offered, the ratio of treated to untreated food consumed was expressed as a percentage of the total amount of food consumed (% TOTAL). These measurements determined whether the test compositions were ingested, and whether such compositions were effective in inhibiting population growth.

Uric acid assays

Determination of whole-body uric acid content was conducted essentially according to a standard uricase assay (Cochran, 1973). Individual cockroaches, with legs and wings trimmed off, were dried for 24–48 h at 60°C., weighed, and individually ground to a fine powder, using micro-centrifuge tubes with fitted pestles.

Uric acid was extracted from the dried tissue with 0.6% aqueous lithium carbonate for three hours at 60°C, with continuous shaking. The extracts were centrifuged to remove tissue debris and aliquots were pipetted off for assay.

After mixing with uricase, the maximum absorption at 292 nm was determined spectrophotometrically. Uric acid concentrations were calculated in μg uric acid/mg dry tissue.

Field trial

A field trial was conducted, under contract, by Entomology Associates, Inc., Houston, Texas. The methods and materials used for the trial (J. B. Tucker, personal communication), are summarized here.

Three cockroach-bait treatments were randomly assigned to individual buildings in a housing complex in Houston, Texas. There were 12 apartments in each building, and all were infested with endemic populations of German cockroaches. The treatments and rates applied per apartment were; (a) 2% NMD in a formulated bait (26 stations); (b) 1.0% hydramethylnon bait (12 stations); (c) 0.528% chlorpyrifos bait (12 stations).

Prior to starting the treatments, infestation densities were assessed by setting four sticky traps in appropriate locations in each apartment kitchen, for 48h. The traps were retrieved, the trapped insects were tallied, and the average number of cockroaches per apartment was calculated from this census. This procedure was repeated at 4, 6, 9, and 12 weeks of treatment, and changes in population density were assessed.

A maximum of four of the NMD bait stations was removed from each apartment at the fourth week of treatment, and these were replaced with the same number of fresh stations. The collected bait was inspected to check consumption levels. At the end of the trial, the NMD bait stations were retrieved and returned to the laboratory for the same purpose.

Percent change (Δ%) in total population counts over time (weeks), for each of the three treated buildings, was calculated from the reported data.

RESULTS

Effects of 2% NMD

Consumption appeared lower in colonies of the AMCY strain offered BB treated with 2% NMD, than in the untreated control (Table 1), but nevertheless, the decline in population was rapid, with extinction in all replicates by the sixth week of treatment. The census of dead insects revealed that the males and nymphs started dying after the first week of treatment, and that the majority of females died immediately after the first egg-case appeared.

Table 1. Mean individual consumption (ICmg), and percent change (Δ%), in mean population numbers over time (weeks), in colonies of German cockroaches of the AMCY susceptible strain offered untreated bait-base (BB), or bait-base treated with 2% NMD.

Time (wks)	Test	BB Untreated	BB+2% NMD
3	ICmg (±SEM)	50 (±2.3)	37 (±0.6)
2	Δ%	−3%	−44%
3	Δ%	−5%	−77%
6	Δ%	+295%	−100%

(n=3, 100%=42).

Table 2a. Mean individual consumption (ICmg), and percent change (Δ%) in mean population number over time (weeks), in colonies of German cockroaches of the VPI susceptible strain offered untreated food, or food treated (w/w) with 2% NMD. The foods were 50% rat chow + 50% bait-base (RC+BB), or bait-base alone (BB).

Time (wks)	Test	Untreated	2% NMD	
		RC+BB	RC+BB	BB
3	ICmg (±SEM)	64 (±1.6)	50 (±1.3)	41 (±1.5)
3	Δ%	−10%	−14%	−64%
6	Δ%	+798%	−94%	−100%

(n=3, 100%=42).

Table 2b. Percent change (Δ%) in mean population number over time (weeks), in colonies of German cockroaches of the AMCY strain offered untreated bait-base (BB), or 2% NMD in a formulated bait (FB).

Time (wks)	Test	Untreated BB	2% NMD FB
3	Δ%	−5%	−52%
6	Δ%	+296%	−99%

(n=3, 100%=42).

Effects in different foods

Decline and extinction of the colonies was faster when they were fed 2% NMD in BB, rather than in RC+BB (Table 2a). When 2% NMD was incorporated into a formulated bait (FB) containing the same bait-base (Table 2b), the results compared closely with those in BB alone, (Table 2a).

Duration of Treatment

Treatment for at least six days was required to maintain a reduction in the population (Table 3), although there was a weak resurgence in this group. However, the populations did not return to pre-treatment levels. When treatment was maintained for longer periods, the population decline was irreversible.

Food choice

When given a choice of NMD-treated and untreated food offered together, the cockroaches consumed more of the treated food than the untreated food (Table 4). Total consumption was lower than in the untreated control, but the effects on the population were the same as when only treated food was offered (see Table 1).

Effects on resistant strains

The colonies of Hawthorne and Las Palms resistant strains declined to extinction by 6–7 weeks when fed 2% NMD in either RC+BB or BB, although the latter mixture provided faster results (Table 5). These results compare well with those of the susceptible strains.

Resistant strains – food choice

There was little distinction made between untreated and NMD-treated food, offered together, by the Hawthorne and Las Palms resistant strains (Table 6), while the populations declined to near

Table 3. Percent change (Δ%) in mean population numbers over time (weeks), in colonies of German cockroaches of the AMCY susceptible strain fed a diet of bait-base alone (BB), or bait-base combined (w/w) with 2% NMD. Duration of treatment was 3, 6, 9, 12, or 15 days, after which untreated bait-base was fed continuously.

Treatment Duration	Time (wks)	BB Control Δ%	BB + 2% NMD Δ%
3 Days	3	−2%	−8%
	6	—*	−27%
	9	+1910%	0%
6 Days	3	−2%	−10%
	6	—	−53%
	9	+1588%	−2%
9 Days	3	0%	−13%
	6	—	−75%
	9	+1452%	−90%
12 Days	3	0%	−25%
	6	—	−89%
	9	+1719%	−96%
15 Days	3	0%	−17%
	6	—	−90%
	9	+1781%	−98%
Continuous	3	0%	−2%
Treatment	6	+391%	−100%

*— = no data recorded. (n=3; 100%=42).

Table 4. Mean individual consumption (ICmg), and percent change (Δ%) in mean population number over time (weeks), in colonies of German cockroaches of the VPI strain, where untreated bait base (BB), and bait base treated (w/w) with 2% NMD were offered as a choice of food. The ratio of treated and untreated food consumed over the first three weeks is given as a percent of the total amount eaten (% TOTAL). The control colonies were fed untreated bait base.

Time (wks)	Test	Control BB	Choice of Food	
			BB	BB+2%NMD
3	ICmg (±SEM)	61 n=1	14 (±0.3)	25 (±0.6)
3	% TOTAL	100%	36%	64%
3	Δ%	−2%	−57%	
4	Δ%	−2%	−93%	
6	Δ%	+369%	−98%	

(n=3, 100%=42).

extinction by six weeks. Here, as with the susceptible strains, the rate of population decline was comparable to that achieved when no choice of diet was offered.

Residual effects

When colonies of 32 nymphs (of the same age and gender as in the standard assay) were fed on mixtures of BB and feces from treated colonies, the populations declined more rapidly than usual, to complete extinction by the fourth week of treatment (Table 7).

Table 5. Individual consumption (ICmg) and percent change (Δ%) in mean population number over time (weeks), in colonies of German cockroaches of the Hawthorne and Las Palms resistant strains offered untreated rat chow (RC), or food treated with 2% NMD that was either rat chow mixed 50% (w/w) with bait base (RC+BB), or bait base alone (BB).

Time (wks)	Test	Hawthorne			Las Palms		
		Control	2% NMD		Control	2% NMD	
		RC	RC+BB	BB	RC	RC+BB	BB
3	ICmg (±SEM)	60 (±1.1)	57 (±2.2)	47 (±1.4)	50 (±1.1)	42 (±1.7)	36 (±0.9)
3	Δ%	−2%	−17%	−58%	−4%	−16%	−39%
6	Δ%	+684%	−94%	−100%	+491%	−56%	−97%
7	Δ%	—	—	—	>+491%	−97%	−100%

— = no data. (n=3; 100%=42).

Table 6. Mean individual consumption (ICmg), consumption ratio (%TOTAL), and percent change (Δ%) in mean population numbers over time (weeks), in colonies of German cockroaches of the Hawthorne and Las Palms resistant strains, where untreated bait base (BB), and bait base treated (w/w) with 2% NMD were offered as a choice of food. The controls were offered untreated food only.

Time (wks)	Test	Hawthorne			Las Palms		
		Control	Choice of Food		Control	Choice of Food	
		BB	BB	BB+2%NMD	BB	BB	BB+2%NMD
3	ICmg (±SEM)	60 (n=1)	22 (±0.9)	23 (±1.3)	47 (n=1)	17 (±0.6)	18 (±0.6)
3	%TOTAL	100%	49%	51%	100%	49%	51%
3	Δ%	0%	−62%		0%	−48%	
4	Δ%	−2%	−94%		0%	−84%	
6	Δ%	+328%	−98%		+424%	−98%	

(n=3, 100%=42).

Table 7. Individual consumption (ICmg) and percent change (Δ%) in mean population number over time (weeks), in colonies of German cockroach nymphs of the VPI strain offered finely-ground cockroach feces (FC) from untreated control colonies mixed 1:1 (w/w) with bait-base (BB), or feces from colonies treated with 2% NMD mixed 1:1 or 1:2 (w/w) with bait-base.

Time (wks)	Test	FC:BB	FC:BB	FC:BB
		Untreated	1:1	1:2
3	ICmg (±SEM)	65 (±4.0)	40.5 (±3.0)	36 (±1.0)
3	Δ%	−5%	−97%	−90%
4	Δ%	−5%	−100%	−100%

(n=2, 100%=32).

Table 8. Mean whole-body uric acid concentrations in (μg/mg of dry tissue weight (±SEM), after five weeks of treatment, in German cockroaches offered untreated rat chow (RC), or rat chow treated with 2% NMD (w/w).

Time (wks)	Adult Males		Adult Females		Nymphs	
	RC	RC+2%NMD	RC	RC+2%NMD	RC	RC+2%NMD
5	2.42 (±0.12) n=5	0.32 (±0.06) n=17	2.63 (±0.14) n=3	0.31 (±0.04) n=8	1.95 (±0.36) n=4	0.32 (±0.18) n=2

Table 9. Percent change (Δ%) in total number of insects trapped over time (weeks), in endemic German cockroach populations in each of three buildings treated individually with baits containing either 1% hydramethylnon (HYD), or 2% NMD, or 0.528% chlorpyrifos (CHL), respectively. The (Δ%) is calculated from the averaged totals of the number of insects found in four sticky traps per apartment for each treated building, counted pre-treatment (0 wks), and at 4, 6, 9 and 12 weeks of treatment.

Time (wks)	Test	Treatment		
		HYD	NMD	CHL
0	Initial Count	1067	1371	1506
4	Δ%	−53%	−19%	−30%
6	Δ%	−69%	−42%	−52%
9	Δ%	−74%	−57%	−53%
12	Δ%	−70%	−64%	−42%

Uric acid assays

The whole-body uric acid levels in insects treated with 2% NMD were determined and calculated as described, and the results are shown in Table 8. It is apparent that the treated insects had only 'background' levels of uric acid remaining in their tissues after five weeks of treatment.

Field trial

The 2% NMD bait achieved a steady reduction in cockroach densities in a randomly-assigned apartment building comprising 12 apartments. The results were compared with two toxic cockroach baits applied for 'standard' comparison (Table 9), and achieved a comparable rate of control. With both of the toxic treatments, there was a pattern of population resurgence late in the trial, which did not occur with the NMD treatment.

DISCUSSION

The development of Ecologix™ stemmed from basic research which explored the physiology of nitrogen metabolism in cockroaches. In this case, the basic research illuminated a potentially vulnerable physiological target for disruption of insect homeostasis. A search followed for compounds and compositions that might produce the sought-after effects, with the overriding consideration that these should be non-toxic in the environment. Extensive testing of potential candidate compositions followed, resulting in the selection of the most promising of these for further development.

This differs from the usual "chemistry first" scenario in pesticide development, where numbers of synthesized molecules are screened for biological activity and insecticidal potential. The research that follows seeks to understand the mode of action of a selected molecule.

Another difference in the development of this new product is that, with the target-site physiology in mind, potential bait ingredients were carefully screened to avoid interfering with the activity of

the NMD. With these factors resolved, experimental trials then flowed from questions generated from application and marketing standpoints, bringing the product closer to a commercial reality.

Questions arose concerning the necessity for multiple feedings on the NMD as a possible hindrance to continued effectiveness, and concerning the time taken for effects to become obvious. These were broached in relation to fast-acting toxic bait products, in which a lethal dose is usually ingested in a few feedings.

However, the new bait proved to be both a nutritional and gustatory attractant, which draws cockroaches to continue feeding on it, regardless of what other food is available to them. Also, the first field trial demonstrated that the new bait achieved population reductions that were within the range of those achieved with standard toxic products, in the face of high-density infestations of German cockroaches.

When compared with the hormonal insect growth regulators (IGRs), the NMD bait also shows to advantage, in that it stops reproduction, and also causes death, by exhaustion of their nutritional reserves, of both adults and nymphs. The IGRs generally affect only the nymphs of the target insects as they molt.

Ecologix™ utilizes a new mode of action, and, as was demonstrated with highly resistant strains, there is no cross-resistance from any known mechanisms of resistance. Thus, the NMD bait is also seen as a useful addition to cockroach integrated pest management (IPM) programs, as an aid in preventing the rise of insecticide resistance, and as a tool where control is thwarted due to established resistance in the pest population.

Development is continuing, with the goal of applying NMD compositions to other pest insects in the laboratory and in the field. This is being accomplished by collaboration with scientists who have a full understanding of the physiology of other insect-pest species.

ACKNOWLEDGEMENTS

I gratefully acknowledge Dominion BioSciences Inc., the Virginia Centre for Innovative Technology, and the Virginia Pesticide Control Board for providing financial support for different parts of this research. My thanks also go to Miss Sandra Walker for her enthusiastic technical assistance at VPI&SU, and to Drs. Coby Schal and Robert Kopanic at North Carolina State University, for their continuing cooperation.

REFERENCES

Cochran, D. G. (1973). Comparative analysis of excreta from twenty cockroach species. *Comp. Biochem. Physiol.*, 46A: 409–419

Cochran, D. G. (1975). Excretion in insects. In: *Insect Biochemistry and Function.* (Eds. D. J. Candy & B. A. Kilby). Chapman and Hall, London. pp. 177–281.

Cochran, D. G. (1985). Nitrogen excretion in cockroaches. *Ann. Rev. Entomol.*, 30: 29–49.

Cochran, D. G., Mullins, D. E., and Mullins, K. J. (1979). Cytological changes in the fat body of the American cockroach in relation to dietary nitrogen level. *Ann. Entomol. Soc. Amer.*, 72: 197–205.

Coughlan, M. P. (1980). *Molybdenum and Molybdenum-Containing Enzymes.* Pergamon Press, New York. pp. 121–185.

Engebretson, J. A. and Mullins, D. E. (1986). Effects of a purine inhibitor, allopurinol, on urate metabolism in the German cockroach, *Blattella germanica* (L.) (Dictyoptera: Blattellidae). *Comp. Biochem. Physiol.* 83B: 93–97.

Lehninger, A. L. (1982). *Principles of Biochemistry.* Worth Publishers, Inc,. New York. pp. 634–635.

Massey, V., Komai, H., Palmer, G., and Elion, G. B. (1970). On the mechanism of inactivation of xanthine oxidase by allopurinol and other pyrazolo[3,4–*d*]pyrimidines. *J. Biol. Chem.*, 245: 2837–2844.

Merck Index (1989). Merck & Co., Inc., Rahway, New Jersey.

Mullins, D. E., Keil, C. B., and White R. H. (1992). Maternal and paternal nitrogen investment in *Blattella germanica* (L.)(Dictyoptera; Blattellidae). *J. exp. Biol.*, 162: 55–72

Silverman, J., and Bieman, D. N. (1993). Glucose aversion in the German cockroach, *Blattella germanica. J. Insect Physiol.*, 39: 925–933

Suiter, D. R., Koehler, P. G., and Patterson, R. S. (1992). Dietary effects of allopurinol and sulfinpyrazone on development, survival, and reproduction of German cockroaches (Dictyoptera: Blattellidae). *J. Econ. Entomol.*, 85: 117–122.

Wigglesworth, V. B. (1987). Histological studies of uric acid in some insects. I. Storage in the fat body of *Periplaneta americana* and the action of the symbiotic bacteria. *Tissue and Cell,* 19: 83–91.

Wren, H. N. (1991). TEM evidence of urocytes in the cockroach fat body. *Tissue and Cell,* 23: 291–292.

Wren, H. N. and Cochran, D. G. (1987). Xanthine dehydrogenase activity in the cockroach endosymbiont *Blattabacterium cuenoti* (Mercier 1906) Hollande and Favre 1931, and in the cockroach fat body. *Comp. Biochem. Physiol.*, 88B: 1023–1026.