Insecticidal Efficacy and Residues of Cypermethrin and Fenvalerate in Stored Wheat

by

Balbir Singh Joia

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presented to the University of Manitoba
in partial fulfillment of the
requirements for the degree of
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in
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DOCTOR OF PHILOSOPHY

Dedicated to my daughters, Aman Deep and Tina

ABSTRACT

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The effectiveness of cypermethrin and fenvalerate against the red flour beetle, Tribolium castaneum (Herbst) and the rusty grain beetle, Cryptolestes ferrugineus (Stephens) was compared with malathion in laboratory studies. A method to determine residues of cypermethrin and fenvalerate in wheat and milled fractions was developed and successfully used to determine residues of these two pyrethroids in wheat kernels, bran, middlings and flour (endosperm).

Wheat of 13.3 and 15.0% moisture content was treated with cypermethrin or fenvalerate at 8 or 12 mg kg^{-1} or with malathion at 8 mg kg^{-1}. Treated wheat was stored at 25 and -5 C for 60 weeks. Samples were removed for bioassay and residue analysis at six 12-week intervals.

Residue analysis showed that major amounts of applied pyrethroids remained on the outer surface of grain. Only a small amount (8 to 14%) penetrated to the endosperm. Thus, from 8 mg kg^{-1} of cypermethrin applied to wheat of 13.3% moisture content, residues of 7.88, 25.56, 13.00 and 0.95 mg kg^{-1} were obtained in whole grain, bran, middlings and flour, respectively, immediately after treatment. The corresponding figures for 8 mg kg^{-1} fenvalerate were 8.16, 28.24, 15.07 and 1.49 mg kg^{-1}, respectively. A similar trend was observed for 12 ppm treatments

of cypermethrin and fenvalerate. Residues of both pyrethroids degraded slowly on stored wheat, especially on wheat of 13.3% moisture content. Half-lives ($t_{1/2}$) were calculated assuming that pyrethroid disappearance follows 'pseudo first order' kinetics. The longest half-life for fenvalerate on grain was 385 weeks resulting from 8 mg kg^{-1} treatment to wheat of 13.3% moisture content and -5 C storage temperature, and the shortest was 69 weeks which resulted from 12 mg kg^{-1} treatment to wheat of 15.0% moisture content and 25 C storage temperature. Cypermethrin disappeared at a faster rate. Thus, the longest half-life for cypermethrin on grain was 169 weeks which resulted from 8 mg kg^{-1} cypermethrin applied to wheat of 13.3% moisture content and -5 C storage temperature, and the shortest was 36 weeks resulting from 8 mg kg^{-1} cypermethrin treatment to wheat of 15.0 moisture content and a storage temperature of 25 C.

Baking of bread from white and wholemeal flour resulted in a minimal reduction of residues that were initially present in the flour. White and wholemeal bread retained 84 and 79% of cypermethrin respectively, present in flour and the corresponding figures for fenvalerate were 88 and 87%, respectively. The presence of these small amounts of insecticides did not affect weight, volume, texture or taste of bread.

Bioassay studies based on mortality of adult insects exposed for one week to treated wheat, and the number of F_1 progeny produced, revealed that cypermethrin was effective against both species. When used at 8 and 12 mg kg^{-1} on wheat of 13.3 and 15.0% moisture content, stored at 25 and -5 C, cypermethrin afforded complete protection against T. castaneum throughout a storage period of 60 weeks, killing 100% of exposed adults

and allowing no emergence of F_1 progeny. Although at the 8 mg kg^{-1} level, cypermethrin did not kill 100% of exposed adults of C. ferrugineus, it successfully prevented production of F_1 progeny.

Fenvalerate failed to give effective control against C. ferrugineus, but was able to prevent F_1 progeny production of T. castaneum at both moisture contents despite the fact that initial mortalities from 8 mg kg^{-1} treatment were from 63 to 67%. In contrast, malathion at 8 mg kg^{-1} was ineffective against both the species at the 12 and 24 week period after treatment on wheat of 15.0 and 13.3%, respectively and stored at 25 C.

The results of this investigation suggest that cypermethrin is a potential grain protectant for long-term storage especially under hot and humid climatic conditions. It is under such conditions that maximum losses occur during storage of grain and other products. The insect pressure is high because of favourable conditions for rapid development and multiplication. Organophosphate insecticides and fumigants afford protection only for a short period because of quick degradation. However, further studies must be conducted using lower application rates against a variety of insect species under different storage conditions, on other products.

FOREWORD

This thesis is being prepared in the manuscript style. The first manuscript will be submitted to 'International Journal of Environmental Analytical Chemistry' and second and third to 'Pesticide Science' for publication.

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Chapter I

INTRODUCTION

Stored products are at the risk of attack by stored product insects. The estimates of losses during storage vary greatly but higher losses occur in tropical and subtropical regions than in temperate ones. Losses of up to 30% in parts of Africa, 25 to 50% in Latin America and up to 50% in Southeast Asia, have been reported (Hall, 1970). More recently, an FAO survey put an average minimum of 10% and maximum of 26% losses in developing countries (FAO, 1977).

Two of the most common and economically important stored product insects in Canada are, the rusty grain beetle, Cryptolestes ferrugineus (Stephens) and the red flour beetle, Tribolium castaneum (Herbst). These species cause considerable damage in grain storage and are a major biotic factor responsible for grain heating and spoilage (Sinha, 1971).

Treating of grain or storage structures with residual contact insecticides is the most effective means of preventing insect infestation. Insecticides used in such situations should meet certain criteria like low mammalian toxicity, high efficacy against target insects and moderate degradation rate allowing the least amount of residues but at the same time maintaining sufficient persistence for insect control. Because of its low mammalian toxicity and high efficacy against stored product insects, malathion is the most commonly used grain protectant in many parts of the world. However, continued and

repeated use of malathion has led to the development of resistant strains in a number of insect species in many countries (Champ and Dyte, 1976). This problem of resistance necessitates a search for the evaluation of alternative insecticides which can be used in grain storage. Pyrethroids are considered to have low mammalian toxicity (Elliott, 1977). During the post-Second-World-War period, pyrethrum preparations were used to protect grain from insect infestation (Rowlands, 1967). Natural and early synthetic pyrethroids were somewhat labile.

During the last 10 years several new synthetic pyrethroids with lesser lability and better efficacy have been developed Two of these insecticides, cypermethrin and fenvalerate, have proven to be highly effective against phytophagous insect pests of a number of crops (Harris et al.,1977; Hattori,1977; Thompson and White, 1977; Harris et al.,1978a; 1978b; Yoshioka, 1978). Since these insecticides have not been evaluated in detail against stored product insects, research was initiated to evaluate cypermethrin and fenvalerate as grain protectants. The objectives of the study were as follows :

1. To develop a method for the analysis of cypermethrin and fenvalerate in wheat and its milled fractions, namely bran, middlings (mostly germ) and flour (endosperm).
2. To study the insecticidal effectiveness of cypermethrin and fenvalerate against T. castaneum and C. ferrugineus.
3. To study the degradation of both insecticides on grain stored at 25 and -5 C

4. To study the distribution of cypermethrin and fenvalerate in milled fractions, and
5. To study the effect of baking on residues in flour.

Chapter II

LITERATURE REVIEW

Pyrethrum, extracted from the flower heads of Chrysanthemum cinerariaefolium (vis.), was in use as an insecticide more than a century ago in Europe and even earlier in Persia (McLaughlin, 1973). Staudinger and Ruzicka (1924) and Staudinger et al. (1924) separated and partially identified two active ingredients of pyrethrum, as pyrethrin I and pyrethrin II. LaForge and Barthal (1945, 1947) reported two additional components as cinerin I and cinerin II. Later jasmolin I and jasmolin II were reported by Godin et al. (1964, 1965, 1966). The six insecticidal constituents of pyrethrum are collectively known as "the pyrethrins" or "the natural pyrethrins" (Casida, 1973).

2.1 TOXICITY OF PYRETHROIDS

As a group of insecticides, pyrethroids are considered to be relatively less toxic to mammals than other groups of insecticides. At the same time, they the have additional advantage of being more toxic to insects as indicated in Table 1 (Elliott, 1977).

Rapid penetration and interaction at the target site in insects may be the cause of high activity against insects, whereas in mammals they are metabolized or eliminated intact before reaching the site of action (Elliott et al., 1978).

Table 1. Toxicities of Classes of Insecticides (Elliott, 1977).

Class	LD_{50} mg kg^{-1}		
	Rats	Insects	Ratio
Carbamates	45	2.8	16
Organophosphates	67	2.0	33
Organochlorines	230	2.6	91
Pyrethroids	2000	0.45	4500

2.2 DEVELOPMENT OF SYNTHETIC PYRETHROIDS

Since the discovery of the chemical structure of natural pyrethrins, there have been a number of efforts in various laboratories to synthesize pyrethroid analogues with enhanced photostability and better biological performance. Some of the early synthetic pyrethroids like allethrin (Schechter et al., 1949) and tetramethrin (Kato et al., 1964)have been used as household insecticides (Miyamoto, 1981). Insecticides with improved properties like resmethrin and bioresmethrin were developed in the late 1960's (Elliott et al.,1967). These synthetic pyrethroids have higher insecticidal activity and excellent knock-down activity, but have been used for agricultural pest control only on a very limited scale (Miyamoto, 1981). One reason for their limited use has been their ready decomposition by sunlight and air.

Significant advances in the development of stable synthetic pyrethroids were made with the discovery of permethrin (Elliott et al., 1973) and later cypermethrin was discovered by Elliott et al. (1975). The α-cyano group in cypermethrin gives greater insecticidal activity than permethrin at the cost of somewhat increased mammalian toxicity (Elliott, 1977). Deltamethrin, which is one of the stereoisomers of the

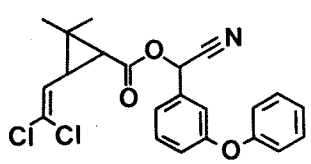
bromoanalogue of cypermethrin, is the most active pyrethroid against insects and apparently has each centre in the optimum configuration for activity (Elliott, 1977). Fenvalerate, which lacks the usual cyclopropane ring, a common feature of most of the pyrethroids, was first reported by Ohno et al. (1974). Structural similarity (Ohno et al., 1976) and mode of action (Hirano, 1979) considerations identify fenvalerate as a pyrethroid.

These newly synthesized pyrethroids, derived from 3-phenoxybenzyl alcohol and α-cyano-3-phenoxy-benzyl alcohol, have high insecticidal potency against many insect species, moderate stability in the environment and acceptable mammalian toxicity (Miyamoto, 1981).

2.3 PHYSICAL AND CHEMICAL PROPERTIES OF CYPERMETHRIN AND FENVALERATE

2.3.1 Cypermethrin

Cypermethrin is the common name of synthetic pyrethroid discovered as NRDC 149 by Elliott et al. (1975). Its International Union of Pure And Applied Chemistry (IUPAC) name is (RS)-α-cyano-3-phenoxybenzyl (IRS)-cis, trans-3(2,2-dichlorovinyl)-2,2-dimethyl cyclopropanecarboxylate. Cypermethrin has the empirical formula $C_{22}H_{19}NO_3Cl_2$ (molecular weight 416) and the following structural formula:

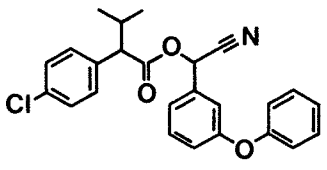


CYPERMETHRIN

Cypermethrin is considered to be relatively non-volatile (vapour pressure at 70 C is 3.8 x 10^{-8} mm Hg). Its solubility in water at 21 C is 0.01-0.2 mg/L and in organic solvents at 20 C is 103 g/L hexane, >450 g/L acetone, cyclohexane, ethanol, xylene and chloroform. It is more stable in acidic than alkaline media (Worthing, 1979). Other synonyms (alternate names) of cypermethrin are, Barricade, CCN52, Cymbush, EMC 30980, Imperator, Kafil-super, OMS 2002, PP383, Ripcord, SH1467 and WL-43467 (Worthing, 1979; Miyamoto, 1981). Technical cypermethrin and its formulated materials are of a moderate order of acute oral toxicity (rat LD_{50} 251 mg/kg in corn oil; 303 mg/kg in dimethyl sulfoxide) and a low order of acute percutaneous toxicity. The oral LD_{50} of emulsifiable concentrate against male and female rat is 242 and 542 mg/kg, respectively (Shell International Chemical Co.,1977).

2.3.2 Fenvalerate

Fenvalerate, ∝-cyano-3phenoxybenzyl 2-(4-chlorophenyl)-3-methyl butyrate, is a pyrethroid which lacks the usual cyclopropane ring, a common feature of other pyrethroids. It was first reported by Ohno et al., 1974. Fenvalerate has the empirical formula of $C_{25}H_{22}NO_3Cl$, (molecular weight 419.5) and the following structural formula:



FENVALERATE

Fenvalerate is considered to be relatively non-volatile (vapor pressure at 20 C is 2.3×10^{-7} mmHg). Solubility in most of the organic solvents is >450 g/L and in water is <1 mg/L (Worthing, 1979). Other names for fenvalerate are Belmark, S5602, SD43775, sumicidin, Pydrin and WL43775 (Worthing, 1979; Miyamoto, 1981). Fenvalerate has a moderate to low order of acute oral toxicity (rat LD_{50} 451 mg/kg in dimethyl sulphoxide; >3200 mg/kg as an aqueous suspension) and a low order of acute percutaneous toxicity. The oral LD_{50} of emulsifiable concentrate and ultra-low-volume formulation against rat is 427 and 4810 mg/kg (Shell International Chemical Co., 1977).

2.4 RESISTANCE OF STORED PRODUCT INSECTS TO MALATHION

As a grain protectant, malathion has been used widely for more than 20 years. It is an approved grain protectant in many countries of the world including Brazil, Canada, France, Italy, U.K. and the U.S.A. Unfortunately, some insects started developing resistance to malathion, soon after its introduction. Tribolium castaneum has been reported to show very widespread resistance. A strain of T. castaneum collected from peanut-pyramids in Nigeria proved to be resistant to malathion by a factor of 52 (Parkin and Foster, 1962). In the U.S.A. malathion replaced synergized pyrethrins in peanut storage in the southeastern states in 1961 and, within two years, resistant strains of T. castaneum were found in those areas (Spiers et al. 1967). Zettler (1974) reported malathion resistance in T. castaneum by a factor of 109 times in strains collected from peanuts and 38 times in those collected from rice. Recently, Zettler (1982) found T. castaneum strains from south

eastern U.S.A to be 130 times resistant to malathion. This is the highest resistance to malathion ever reported in T. castaneum in USA.

Resistance to malathion in T. castaneum has also been reported in Australia (Champ and Campbell-Brown, 1970), Egypt (Toppozada et al., 1969), India (Bhatia et al., 1971) and other countries (Champ and Dyte, 1976). In Canada, Bond (1973) noticed increased tolerance of T. castaneum to malathion and lindane, even in the strains collected from feed mills where no insecticide had been used. It is believed that T. castaneum resistance is spreading around the world through international trade (Dyte and Blackman, 1970; Champ and Dyte, 1976). A world wide survey by Champ and Dyte (1976) revealed malathion resistance in T. castaneum in 75 of the 78 countries surveyed and involved 438 of the 504 strains tested. Malathion resistance in T. confusum was detected in 27 of 33 countries surveyed and involved 78 of the 122 strains tested (Champ and Dyte, 1976).

Resistance has also been recorded in other species. In Plodia interpunctella, resistance was so high in 10 strains that a probit mortality regression line could not be established and a resistance factor of >114 was reported for all the strains (Zettler, 1982).

Malathion resistance has also been reported in Orzaephilus surinamensis, Rhyzopertha dominica and other species (Champ and Dyte, 1976). Widespread resistance of stored product insects threatens the continued use of existing organophosphate grain protectants. The phenomenon of multiple-and cross-resistance further complicates the problem. Generally, resistance of stored product insects to pyrethroids is less than to organophosphates (Carter et al., 1975; Lloyd and

Ruczkowski, 1980). Pyrethroids have the potential of becoming alternate grain protectants of the future. Bengston (1978) suggested that synthetic pyrethroids can be kept as a reserve group of insecticides to be used should resistance invalidate current materials.

2.5 USE OF PYRETHRINS AND SYNTHETIC PYRETHROIDS AGAINST STORED PRODUCT INSECTS

2.5.1 Pyrethrins

Pyrethrum has been used as an insecticide for many years. Rudolfs (1926) reported that pyrethrum mixed with vaseline gave protection against mosquitoes for up to 2 hours. The effectiveness of pyrethrins in heavy white oil against the stored product insects, P. interpunctella, and Ephestia elutella was first demonstrated by Potter (1935). Since then there have been a number of conflicting reports about the effectiveness of pyrethrum against stored product insects. Goodwin-Bailey and Holborn (1952) treated wheat with 1.3 ppm pyrethrins synergized with 27 ppm piperonyl butoxide. This treatment protected wheat for 11 months from infestation by Sitophilus granarius and O. surinamensis.

Pyrethrins (0.2%) plus piperonyl butoxide (1.0%) applied to bagged corn at 118 mL/91kg (4 oz/200 lb) provided effective control against Sitophilus zeamais and T. castaneum but was less effective against Ephestia cautella (Anonymous, 1964). Quinlan and Miller (1958) reported that synergized pyrethrins applied to the top surface of bulk corn,was not effective against P. interpunctella.

Walkden and Nelson (1959) reported that synergized pyrethrins applied to bulk wheat and corn at 1.5 to 2.5 ppm, as spray or dust formulations, were effective against a number of stored product insects. On the other hand, LaHue (1965) observed that wheat treated with 2.14 ppm pyrethrins plus 21.4 ppm piperonyl butoxide, lost its effectiveness against the rice weevil, Sitophilus orzyae after one month, and was completely ineffective against the confused flour beetle, T. confusum. The same treatments protected corn for three months against the rice weevil and confused flour beetle (LaHue, 1966).

McFarlane (1969) applied pyrethrins at 1.25 ppm synergized with piperonyl butoxide (1:5 ratio), to beans. This treatment protected beans against Acanthoscelides obtectus for 16 months. Higher concentration (2.5 ppm) of pyrethrins or synergist ratio (1:10) gave no additional advantage. Unsynergized pyrethrins were completely effective for 3 months and provided 95% control after 9 months.

Weaving (1970) recommended that 2 ppm of pyrethrins synergized with piperonyl butoxide at the ratio of 1:10 or 1:15 should be used for pulses against Callosobruchus chinensis. But Dhari et al. (1977) reported that pyrethrins alone, or with synergists, used at 2, 4 and 6 ppm failed to give satisfactory protection to gram against C. chinensis.

Pyrethrins at 3 ppm with 27 ppm piperonyl butoxide, applied to wheat and barley provided protection from infestation by T. castaneum and Rhyzopertha dominica for 17 months (Greening, 1979). Senapati and Satpathy (1972) reported that pyrethrins alone, or with synergists, used at 6 ppm failed to protect wheat for more than 2 months against S. oryzae.

Piperonyl butoxide increases the effectiveness of pyrethrin due to its synergistic action as well as enhanced stability of pyrethrins (Desmarchelier et al., 1979). Desmarchelier et al. (1979) also reported its effectiveness against R. dominica. Pyrethrins,alone applied to wheat at 2 ppm, did not provide any protection against R. dominica. But when synergized with 31 ppm piperonyl butoxide, it completely suppressed the population of R. dominica up to 26 weeks. Unsynergized pyrethrin at 4 ppm was effective up to 10 weeks. They concluded that the residual efficacy of pyrethrins was doubled by the addition of 5 to 13 ppm piperonyl butoxide.

LaHue (1965, 1966) reported that pyrethrins provide a repellent action against weevils. Laudani and Swank (1954) observed a repellent action of synergized pyrethrins, against T. castaneum at a concentraion as low as 0.37 ppm. But Chadwick (1962) reported that a low concentration (0.5-0.6 ppm) of synergized pyrethrins had no repellent action against S. oryzae. Repellent action was observed only at concentration of 3 ppm or more.

Due to its repellency, pyrethrins have been used to treat food packages to make them insect resistant. Brooke and Lomax (1969) observed that pyrethrins prevented damage by T. castaneum to food packages for 6 months. Pyrethrins offer the most suitable and safe means of achieving an insect-resistant package for packaged food (Langbridge, 1970).

Pyrethrins have been used to disinfect mills (Joubert, 1965; Evans, 1966). Watters (1969) obtained high mortality of C. ferrugineus exposed on plywood panels placed at different locations on the floor of an empty storage bin fogged with pyrethrins.

Most of the authors agree that synergized pyrethrins effectively control a number of stored product insects. In addition to this, pyrethrins also have a repellent action. This property makes pyrethrins especially suitable to protect food packages from insect infestation.

2.5.2 Synthetic Pyrethroids

Lloyd (1973) compared the toxicities of natural pyrethrins and five synthetic pyrethroids against susceptible T. castaneum and susceptible and pyrethrin-resistant S. granarius. Without the use of the synergist, piperonyl butoxide, bioresmethrin was the most toxic pyrethroid against all strains of the two species, followed by resmethrin, bioallethrin, allethrin and tetramethrin. The order of toxicity did not change by using piperonyl butoxide as a synergist, although synergism was observed with all the compounds. The highest synergism was observed against resistant S. granarius and the lowest against T. castaneum.

Ardley and Desmarchelier (1974) investigated the potential of resmethrin and bioresmethrin, as grain protectants in Australia from 1967 to 1974. Insecticide-treated grain was stored in commercial silos up to one year or more and bioassays were conducted on a number of species. After 11 months of storage, resmethrin at 2.0 ppm plus piperonyl butoxide at 20 ppm, produced 100% mortality against S. granarius and R. dominica. No F_1 generation was produced. Bioresmethrin at 0.5 ppm with 5.0 ppm piperonyl butoxide, tested 4 months after treatment, produced 100% mortality against S. granarius and R. dominica. Bioresmethrin at 4.0, 8.0 and 12.0 ppm gave 100% mortality against R. dominica after 12 months of storage. Bioresmethrin at 4.0 ppm plus 20

ppm piperonyl butoxide was considered to be the most cost/effective treatment. Both insecticides were much less effective against T. castaneum.

Bioallethrin proved to be much less effective than malathion against C. chinensis, when either insecticide-treated glass surfaces or horsebeans, cowpeas or lentils were used (El-Rafie et al, 1974).

Carter et al. (1975) tested the toxicity of natural pyrethrins and 6 synthetic pyrethroids against some stored product beetles. In topical application tests, cismethrin was the most effective compound against T. castaneum, when synergized or used alone. This was followed by bioresmethrin. Piperonyl butoxide synergized all the pyrethroids. When insects were exposed to wheat treated with insecticidal dust, synergized bioresmethrin was better than malathion against susceptible O. surinamensis, S. granarius, S. oryzae and T. castaneum. Synergized bioresmethrin proved to be the best insecticide for controlling stored product beetles except Lasioderma serricorne and Stegobium paniceum.

Bioresmethin applied to wheat at 4 or 8 ppm did not provide satisfactory control of S. oryzae, T. castaneum and E. cautella (Bengston et al., 1975). On the other hand, R. dominica was controlled only by bioresmethrin. Chlorpyrifos-methyl and pirimiphos methyl failed against this species. At 4 ppm, bioresmethrin protected bulk wheat for 25 weeks against R. dominica and prevented any F_1 progeny production. This treatment was effective against T. castaneum and S. oryzae only for 2 weeks. Bioresmethrin also proved more effective than bioallethrin and tetramethrin.

Ardley (1976) reported the effectiveness of 4 ppm bioresmethrin applied to wheat along with 20 ppm piperonyl butoxide, against three species of stored product insects. Bioassays were done after a holding period of 0.5, 1, 2, 3, 4, 7, 8, 9, 10, 11 and 12 months. Bioresmethrin-treated wheat resulted in 100% mortality of R. dominica at all intervals and 100% mortality of S. oryzae at nearly all intervals. Malathion gave a high level of control against S. oryzae, but failed against R. dominica after 10 months. Bioresmethrin was not effective against T. castaneum but produced nil or a very low number of progeny. Synergized bioresmethrin was concluded to be the best grain protectant.

In subsequent trials (Ardley and Desmarchelier, 1978), bioresmethrin did very well against S. granarius, R. dominica and S. oryzae, but failed against T. castaneum.

The effectiveness of fenvalerate against adults of C. chinensis was tested by treating green gram seeds with fenvalerate at the rate of 1:500 and 1:1000, (2000 and 1000 ppm, respectively) and exposing insects to treated seed at various intervals (Govindrajan et al., 1978). At the end of 12 months, grains treated with fenvalerate at the rate of 1:500 and 1:1000 had an infestation of 8.54 and 5.74%, respectively, compared to 100% infestation in the controls. In their discussion, the authors failed to explain the reason why the lower treatment (1:1000) resulted in a lower level of infestation. However, the massive dosages used by these authors are very unrealistic.

Ardley and Halls (1979), used laboratory and field tests to study the effectiveness of synergized phenothrin, bioresmethrin and d-phenothrin. None of the three pyrethroids showed high activity against T. castaneum.

Phenothrin proved to be more than half as active as bioresmethrin against resistant and susceptible strains of R. dominica and S. oryzae, and against a susceptible strain of S. granarius. The authors recommended a combination of 2 ppm phenothrin plus 10 ppm bioresmethrin and 12 ppm fenitrothion. This gave an excellent control for 9 months.

In a food processing plant, synergized bioresmethrin was better than permethrin or synergized pyrethrins against T. castaneum (Carter and Dodd, 1979) Permethrin and pyrethrins were better than bioresmethrin against O. surinamensis and S. granarius.

Bengston et al. (1980) did extensive field trials using synergized bioresmethrin and synergized bioresmethrin combined with chlorpyrifosmethyl and primiphos methyl. Against S. oryzae, chlorpyrifos-methyl 10 ppm plus bioresmethrin 1 ppm proved to be the best in aerated storage. Against R. dominica, bioresmethrin 4ppm plus 16 ppm piperonyl butoxide was the best treatment. They concluded that chlorpyrifos-methyl 10 ppm plus bioresmethrin 2 ppm, and primiphos-methyl 4 ppm plus bioresmethrin 2 ppm are effective grain protectants for wheat in Australia, in nonaerated storage conditions.

Taylor and Evans (1980) treated pigeon peas (Cajanus cajanus) and white haricot beans (Phaseolus vulgaris) with permethrin dust at 2.5 and 5.0 ppm, to test the effectiveness of permethrin against C. chinensis and A. obtectus. The treatment gave complete control of the insects up to 24 weeks after treatment. The treatments also prevented F_1 or F_2 generations of the insects. Applications of the insecticide to pulses already infested by bruchid pests did not significantly reduce the total number of adults produced by either species. Perhaps, this

was due to penetration of insecticides in amounts less than sufficient to kill the insects.

Bitran et al. (1980) treated corn with decamethrin alone, or synergized with piperonyl butoxide (1:4), and piperonyl butoxide synergized bioresmethrin (1:4) at the rates of 0.5 and 1.0 ppm. After 9 months of storage, decamethrin at 0.5 and 1.0 ppm produced 83.6 and 95.2% mortality, respectively, in S. zeamais. Synergized decamethrin was less effective than decamethrin alone. This observation is contrary to the usual observation on the effect of synergists on pyrethrins and a number of synthetic pyrethroids. Bioresmethrin produced 81% and higher mortality up to 30 days at 0.5 ppm level and 79% or more up to 60 days at 1 ppm.

A number of workers have reported low toxicity of pyrethroids to T. castaneum. But contrary to these, Adesuyi (1982) found that 1% permethrin dust on maize cobs prevented damage by T. castaneum. Permethrin at the rate of 2.5, 5, 10 and 15 ppm was applied to corn stored in cribs up to 8 months. A treatment of 5 ppm for the rainy season crop and 2.5 ppm for the dry season crop, greatly reduced the damage for 8 and 5 months, respectively. These treatments kept the levels of S. zeamais, T. castaneum, Cryptolestes spp. greatly reduced. The reports mentioned above show that pyrethroids gave good control of a number of stored product insect species. However, T. castaneum appears to be less susceptible to many pyrethroids, although progeny production in this species is prevented

2.6 ANALYTICAL METHODOLOGY FOR THE DETERMINATION OF CYPERMETHRIN AND FENVALERATE RESIDUES

During the last few years several methods have been developed and used for the determination of the residues of these two pyrethroids.

2.6.1 Cypermethrin

Shell International Chemical Co. (1976) reported a method for the determination of cypermethrin from grapes, apples and maize. Samples mixed with anhydrous sodium sulphate were extracted with acetone-petroleum ether (1:1). After removal of acetone through washing with water, the petroleum ether extract was subjected to Florisil column chromatography. Residues were eluted from the column with ether-petroleum ether and analyzed with gas-liquid chromatography (GLC) using electron capture detection (ECD).

Chapman and Harris (1978) described a method for the analysis of cypermethrin and three other pyrethroids from carrots, tomatoes, celery and onions. Fortified samples were extracted with acetone followed by partitioning into hexane. Several adsorbents and eluting solvents were evaluated for quantitative recoveries. Florisil column cleanup with benzene-hexane (80:20) as an eluting solvent was satisfactory. Analysis was done by ^{63}Ni-ECD-GLC.

A method for the analysis of cypermethrin and other pyrethroids from fat and brain tissue of rat, was developed by Marie et al. (1982). Extraction of residues from fat was done with hexane and from brain tissues was done with acetonitrile. Cleanup of the extracts was carried out by partitioning with acetonitrile-hexane (2:1) and silica-gel chromatography. EC-GLC was used for final analysis.

Baker and Bottomley (1982) described a method for residue analysis of 9 pyrethroids, including cypermethrin. Fortified samples of apple, pear, cabbage and potato were extracted with hexane-acetone (1:1). Acetone was removed through liquid- liquid partitioning. Concentrated hexane extracts were subjected to silica gel column chromatography and residues eluted with dichloromethane. EC-GLC and high-performance-liquid chromatography (HPLC) were used for analysis.

2.6.2 Fenvalerate

Talekar (1977) described a method for the determination of fenvalerate residues in cabbage. Chopped samples were soxhlet extracted with hexane-acetone (1:1). After removal of acetone through liquid-liquid partitioning, hexane extracts were concentrated and subjected to Florisil column cleanup using benzene-ethyl acetate as eluting solvent. Quantitative determination was done with ^{3}H-ECD-GLC, but Talekar recommended the use of a ^{63}Ni detector because of high column temperature required for analysis.

Lee et al. (1978) reported a GLC method for the analysis of fenvalerate in cabbage and lettuce. The substrates were extracted with acetonitrile followed by partitioning into petroleum ether. Concentrated petroleum ether samples were column chromatographed on a Florisil column and eluted with benzene-hexane (1:1). An additional silica gel column cleanup was required to remove a peak which eluted at about 90 minutes. Although this peak did not interfere with analysis, its removal decreased the analysis time considerably.

Residues of fenvalerate from cotton leaves were extracted with hexane (Esteen et al., 1979). The extraction was followed by a Florisil column cleanup with hexane and 5% ethyl acetate in hexane as eluting solvents.

A method for the determination of fenvalerate residues in soil was developed by Williams and Brown (1979). The residues were extracted with hexane-acetone (1:1) and acetone removed through partitioning with water. Column cleanup was done on micro columns consisting of Pasteur pipettes. The residues eluted with benzene were analyzed on EC-GLC.

Hill (1981) used hexane-acetone to extract fenvalerate residues from soil. After liquid-liquid partitioning with 2% NaCl solution, concentrated hexane extracts were subjected to column chromatography using Pasteur pipettes containing deactivated acid alumina. Elution of residues was done with ether-hexane (1:9) and analysis by EC-GLC.

Greenberg (1981) described two methods to analyze fenvalerate residues. Residues from grapes, peppers and apples were extracted by blending with acetone followed by partitioning with petroleum ether. For cotton seeds, soxhlet extraction was used after blending the samples in chloroform. This was followed by partitioning with propylene carbonate and residues taken in petroleum ether. Florisil column cleanup with 1% acetone in petroleum ether as the eluting solvent, was used for both the methods. Analysis was done by GLC using a tritium ECD.

Reichel et al. (1981) used hexane soxhlet extraction to extract fenvalerate residues in grasshopper and duck tissue. Clean up was done by gel-permeation chromatography with an in-line alumina column. Analysis was done using EC-GLC and confirmation by gas-liquid chromatography- mass spectrometry (GLC-MS).

In addition to the methods described above for fenvalerate residues, other methods have been used for this pyrethroid. Chapman and Harris (1978), Marie et al. (1982) and Baker and Bottomley (1982) used the same methods for the determination of cypermethrin and fenvalerate residues.

At the time the present research was initiated, there was no published method for the determination of residues of these insecticides in cereals.

2.7 DEGRADATION OF PYRETHRINS AND SYNTHETIC PYRETHROIDS ON STORED PRODUCTS

2.7.1 Pyrethrins

Due to the non-availability of suitable analytical techniques, earlier studies on pyrethrins were done by determining the residues of piperonyl butoxide (Quinlan and Miller, 1958; LaHue, 1965; 1966). The residues of pyrethrins were estimated assuming both degrade at the same rate. However, Blinn et al., (1959) showed that this assumption is not valid. They sprayed wheat with a formulation containing 2% pyrethrins and 20% piperonyl butoxide. Treated grain was stored at 32.2 C (90 F) and residues analyzed at different intervals. Pyrethrins residues of 0.8, 0.6, 0.4 and 0.2 ppm were recovered 1, 14, 30 and 90 days after treatment, respectively. Residues of piperonyl butoxide at the respective intervals were 7.7, 6.2, 5.0 and 3.5 ppm. From these figures they calculated the half-life values of 5.8 weeks for pyrethrins and 9.9 weeks for piperonyl butoxide. Pyrethrins were considerably less persistent than assumed. However, the practice of calculating residues of pyrethrins based on the analysis of piperonyl butoxide continued for quite some time.

Quinlan and Miller (1958) applied synergized pyrethrins (pyrethrin : piperonyl butoxide 1:10) to the top surface of corn stored in bins. Pyrethrins were used at three concentrations of 0.125, 0.250 and 0.375% and at three frequencies of semi-weekly, weekly and bi-weekly. Analysis of piperonyl butoxide residues showed 75 and 90 percent reduction in residues after 3 and 6 months, respectively.

Walkden and Nelson (1959) reported that residues of pyrethrins in wheat decreased sharply after treatment, then leveled off and persisted up to 2 years. Average residues from applications of 1.5, 2.0 and 2.5 ppm decreased by about 50 percent after 2 months on wheat stored in bins. Twenty seven and 23% of residues were still present after one and two years, respectively.

LaHue (1965) applied a mixture of pyrethrins: piperonyl butoxide to provide 2.14 and 21.4 ppm treatment to wheat and determined residues of piperonyl butoxide at various intervals. The residues decreased in an erratic manner for the first few months but stabilized after six months. From a calculated dosage of 21.4 ppm, only 6.6 ppm were recovered immediately after treatment. After 1, 3, 6, 9 and 12 months, the residues on wheat were 10.7, 12.9, 8.4, 8.8 and 9.9 ppm, respectively. Pyrethrins were assumed to be present in the same proportions as in the original formulation.

On corn, LaHue (1966) found more uniform residue degradation of piperonyl butoxide. Pyrethrins and piperonyl butoxide in the ratio of 1:10 were applied to corn. From a calculated deposit of 27.52 ppm piperonyl butoxide, he recovered 12.32 ppm immediately after treatment. The residues degraded uniformly for six months and then reached a level

of stability. Amounts of 10.24, 7.72, 5.68, 5.00 and 6.12 ppm were recovered after 1, 3, 6, 9 and 12 months of storage, respectively. Pyrethrin residues were assumed to degrade in the same manner as those of piperonyl butoxide. In both studies, residues of piperonyl butoxide recovered by LaHue (1965; 1966) immediately after treatment were much less than calculated.

Desmarchelier et al. (1979) applied pyrethrins synergized with piperonyl butoxide to wheat, barley, oats and rice, and determined residues at various intervals after storing grain at different combinations of temperature and relative humidity. The loss of residues depended on temperature and equivalent relative humidity. It increased with increase in temperature or equivalent relative humidity. On average, 77% of pyrethrin residues were still present on grain 3 weeks after storage at an average temperature of 29 C and an average equivalent relative humidity of 50%.

2.7.2 Synthetic pyrethroids

Desmarchelier (1980) reported that piperonyl butoxide increases the persistence of bioresmethrin on wheat. The residues of bioresmethrin were determined on wheat 3.5, 7, 14, 21, 28 and 35 weeks after application. Treated wheat was stored at temperaure of 25 to 35 C and percent equivalent relative humidities ranging from 33 to 62. The mean half-life value at 30 C and 50% equivalent relative humidity, calculated from various storage conditions was 25 weeks.

Desmarchelier et al. (1980) studied the loss of residues of bioresmethrin and phenothrin (d-fenothrin), from treated barley and

rice. Barley treated with 7 ppm bioresmethrin and stored at 25 C and 65% equivalent relative humidity, had residues of 4.0 and 2.25 ppm after 3 and 6 months of storage, respectively. Similarly, 8 ppm phenothrin decreased to 4.6 ppm after 6 months. Under similar conditions, residues of 7 ppm bioresmethrin on husked rice decreased to 4.5 and 4.0 ppm after 3 and 6 months, respectively and those of phenothrin from 8 to 6.2 ppm after 6 months. The observed levels were close to levels predicted from a model. Both of the insecticides were persistent.

Nambu et al. (1981) studied the degradation of radio-labelled cis and trans isomers of phenothrin on wheat. Wheat was treated either with individual isomers at 4 ppm or with 4 ppm phenothrin plus 20 ppm piperonyl butoxide or 4 ppm phenothrin plus 20 ppm piperonyl butoxide plus 4 ppm fenitrothion. When applied alone, about 92 percent of cis and trans isomers of intact phenothrin were recovered from wheat stored at 15 C for 12 months. At 30 C, about 79% of cis and 87% of trans isomers were recovered after the same interval. The residual life of phenothrin increased slightly in the presence of piperonyl butoxide and fenitrothion.

Fujinami (1981) also studied the residues of phenothrin applied to wheat at 2 ppm in combination with 8 ppm piperonyl butoxide and 12 ppm fenitrothion. From a dosage level of 2 ppm, residues of 1.50, 1.50, 1.60, 0.85, 1.50, 1.60, and 1.20 ppm were recovered after 1, 2, 3, 4, 6, 7, and 9 months, respectively. His results again show a slow degradation of residues.

Noble et al. (1982) studied the degradation of permethrin, phenothrin, fenvalerate and deltamethrin on wheat of 12 and 15% moisture

content. Treated wheat was stored at 25 and 35 C and analyzed for residues after 13, 26, 39 and 52 weeks of storage. All of the four pyrethroids degraded very slowly from wheat, the rate of loss being phenothrin > deltamethrin > permethrin > fenvalerate. Half-lives under different conditions calculated by the authors are shown in table 2.

Table 2. Half-lives of four pyrethroids on wheat (Noble et al.,1982).

Pyrethroid	Temperature C	Half-lives (weeks) Moisture content(%) 12	Moisture content(%) 15
Phenothrin	25	72	54
	35	39	29
Deltamethrin	25	114	90
	35	70	35
Permethrin	25	252	149
	35	89	44
Fenvalerate	25	210	182
	35	104	74

They also calculated that fenvalerate on wheat of 12% moisture content stored at 25 C will be present at 83-87% of its original concentration after 52 weeks.

2.8 DISTRIBUTION OF RESIDUES IN MILLED FRACTIONS

To be an effective grain protectant against externally feeding insects, an insecticide applied to grain should remain on its outer surface and not penetrate into the endosperm. On the other hand, higher levels in endosperm will protect stored grain against internal feeders like Sitophilus spp.

The penetration of insecticides into grain depends upon many factors, i.e., nature of the insecticide, grain moisture content, age, variety and viability of the grain as well as formulation and method of application (Rowlands, 1967).

When insecticide-treated grain is milled, most of the residue usually remains on bran and low levels are found in endosperm. This has been reported by numerous workers involving organochlorines (Butterfield, 1949), organophosphates (Roan and Srivastava, 1965; Alnaji and Kadoum, 1979; Mensah et al., 1979) and carbamates (Rowlands, 1967). Similarly, limited studies on pyrethrins and synthetic pyrethroids have shown bran to contain maximum and endosperm minimum amounts of residues.

Strong et al. (1961) treated wheat of 10 and 13% moisture content with a mixture of pyrethrins and piperonyl butoxide (1:10) and determined residues of piperonyl butoxide after storing treated wheat for 3 months at 15.5 C (60 F) and 32.2 C (90 F). The applications were made as aqueous emulsions, wettable powder suspensions, and tetrachloroethylene solutions, to give deposits of 1.5 ppm pyrethrins and 15 ppm piperonyl butoxide. From an aqueous emulsion application, residues of piperonyl butoxide on wheat with 10% moisture content, stored at 15.5 C were 7.3 ppm in whole grain and 10.9, 4.8, 2.6 and 1.9

ppm in bran, shorts, middlings and endosperm, respectively. The corresponding levels from wheat stored at 32.2 C were 6.2, 13.0, 7.2, 9.1 and 3.3 ppm. Treatment of 13% moisture content wheat resulted in 6.1, 11.0, 2.3, 4.2 and 0.1 ppm residues in whole grain, bran, shorts, middlings and endosperm, respectively after storage of the treated wheat at 15.5 C and 6.0, 12.2, 2.5, 1.7 and 0.15 ppm, respectively after storage at 32.2 C. Generally, higher residues in endosperm were detected following the application in tetrachlorethylene solution.

Ardley and Halls (1979) reported that bioresmethrin and phenothrin applied to wheat at 8 ppm did not leave any detectable residues in bread or bran from treated wheat milled at 14 days post-treatment.

Fujinami (1981) determined phenothrin residues in milled fractions of wheat 9 months after a combined application of 2 ppm phenothrin, 8 ppm piperonyl butoxide and 12 ppm bioresmethrin. Phenothrin residues of 1.2, 4.0, 1.8, 0.3, 0.4 to 0.6 and 0.1 to 0.2 ppm, were detected in whole grain, bran, pollard, endosperm, whole meal bread and white bread, respectively.

Nambu et al. (1981) applied cis and trans isomers of phenothrin to wheat and determined the residues in milled fractions, 6 and 12 months after application. After 6 months, levels of trans-phenothrin in whole grain, bran and endosperm were 3.78, 11.4 and 0.79 ppm, respectively and after 12 months 3.13, 11.3, 0.76 ppm. The levels of cis-phenothrin in whole grain, bran and endosperm were 3.64, 9.75 and 0.77 ppm, respectively after 6 months and 3.32, 11.4 and 0.77 ppm, respectively after 12 months.

Bengston et al. (in press) obtained 0.24, 0.08, 0.13 and 0.23 ppm residues of deltamethrin, fenvalerate, permethrin and phenothrin, respectively in white flour when insecticide-treated wheat was milled 10 months after application. The corresponding levels in grain were 1.82, 0.70, 0.82 and 1.21 ppm, respectively.

2.9 EFFECT OF PROCESSING ON RESIDUES

Processing of flour into bread may further reduce insecticidal residue. Losses of up to 92% malathion (Allesandrini, 1965) and 97% fenitrothion (Bengston et al., 1974) occurred when flour was made into bread. Alnaji and Kadoum (1981) reported losses of 79 to 100% in methyl phoxim and 80-100% in malathion residues through baking of bread.

The reduction of pyrethroid residues during bread baking may not be as high as those of organophosphorus insecticides. Nambu et al. (1981) reported losses of 13 to 30% in phenothrin residues when flour containing phenothrin residues was baked into bread. Ardley and Halls (1979) could not detect any residues in bread made from wheat treated with 8 ppm phenothrin.

Desmarchelier et al. (1980) reported a reduction of about 62% in bioresmethrin residues during home malting of barley. A reduction of 88% in phenothrin (d-fenothrin) and 90% in bioresmethrin, was observed in a pilot process simulating that used in commercial production. Bengston et al. (in press) reported no loss in residues of deltamethrin, fenvalerate, permethrin and phenothrin during baking of bread from white flour.

Chapter III

MANUSCRIPTS

MANUSCRIPT I

Gas-liquid Chromatographic Determination of Cypermethrin and Fenvalerate Residues in Wheat and its Milled Fractions

A method to determine cypermethrin and fenvalerate residues in wheat and milled fractions was developed. The method involved extraction of residues with acetone-hexane, partitioning residues into hexane using aqueous sodium chloride solution. After concentrating hexane extracts, column chromatography was done on Pasteur pipette micro columns containing Florisil, using benzene as elution solvent, and electron-capture GLC was used for analysis. The limits of detection with this method were 0.02 mg kg^{-1} for cypermethrin and 0.04 mg kg^{-1} for fenvalerate. Average recoveries of 82 to 98% of cypermethrin and 80-86% of fenvalerate were otained from wheat and milled fractions fortified at 0.41 to 3.80 mg kg^{-1}. No interference was observed from co-extractives.

INTRODUCTION

Cypermethrin and fenvalerate are effective insecticides against a number of phytophagous insect pests (Harris et al., 1977; Hattori, 1977; Yoshioka, 1978; Harris et al., 1978, a,b). To date , these insecticides have been evaluated against stored product insects. only on a limited scale (Govindrajan et al., 1978; Watters et al., in press), but they are potential grain protectant candidates.

Methods to determine residues of cypermethrin and fenvalerate in vegetables (Talekar, 1977; Chapman and Harris, 1978; Lee et al., 1978; Baker and Bottomley, 1982), soil (Williams and Brown, 1979; Hill, 1981) and animal tissues (Reichel et al., 1981) have been developed. Simonaitis and Cail (1977) described a gas-liquid chromatographic method for the determination of permethrin in wheat and corn. Noble et al. (1982) and Hargreaves et al., (in press) used a high pressure liquid chromatographic method (Simpson, B.W., unpublished work) to determine residues of deltamethrin, fenvalerate, permethrin and phenothrin in stored wheat. There is no published method for the determination of cypermethrin in wheat. Before any insecticide can be tested extensively as a grain protectant, there is a need to develop residue methodology so that insecticide degradation may be studied in grain and its milled fractions, for example, bran, flour (endosperm) and germ.

A rapid and inexpensive method for the determination of cypermethrin and fenvalerate was developed. This method was used to determine residues of these two pyrethroids in stored wheat and milled fractions.

EXPERIMENTAL

Reagents

1. Solvents: Pesticide grade acetone, hexane and benzene -Caledon Co. Ontario (Caution- Benzene is a potential carcinogen).
2. Insecticide analytical standards: Cypermethrin, [(RS)-x-cyano-3 phenoxybenzyl (1RS)-cis,trans-3-(2,2-dichlorovinyl)-2,2-dimethyl cyclopropane carboxylate and fenvalerate [x-cyano-3-phenoxybenzyl 2-(4-chlorophenyl)-3-methyl butyrate] were supplied by Shell International Chemical Co.,Toronto, Ontario.
3. Anhydrous sodium sulphate: Reagent grade- Fisher Scientific Co.
4. Grain: Hard red spring wheat, variety Neepawa
5. Florisil: 80-100 mesh- Floridin Co., activated for 3 h at 250 C and deactivated with 8% water.
6. Glass wool: Silanized- Applied Science Laboratories Inc.

Apparatus

1. Gas-liquid chromatograph (GLC): A Varian 1800 GLC equipped with (^{3}H-ECD). The operating conditions were as follows: 0.6m x 4 mm(id) silanized glass column packed with 3% OV-210 on Gas Chrom Q, 80/100 mesh; temperature (C), inlet 250, column 200, detector 225, nitrogen carrier gas flow rate 80 mL/min.
2. Rotary grinder: GS Iona Model CG8- General Signal Appliances Ltd., Canada.
3. Extraction tubes: 50 mL round bottom stainless steel tubes (John Solomon, Freshwater Institute,Winnipeg, Manitoba) stainless steel

caps fitted with teflon O ring gaskets, stainless steel balls approximately 1.75 cm dia.

4. Shaker: Wrist action shaker- Burrell Equipment Co.
5. Mill: Ottawa micro mill No. 6012- Engineering Research Service, Agriculture Canada, Ottawa
6. Vortex -Fisher Scientific Co.
7. Disposable Pasteur pipettes,14.5 and 22.5 cm- Fisher Scientific Co.
8. Centrifuge- International Equipment Co.
9. Centrifuge tubes: 5 and 15 mL, graduated- Fisher Scientific Co.

Fortification of the Samples

Five-gram samples of ground wheat or milled fractions (3 g for bran) were placed in 50 ml stainless steel extraction tubes. The samples were fortified with insecticidal solutions of cypermethrin or fenvalerate to give three levels of fortification. The solvent was evaporated with nitrogen and samples allowed to stand for 24 h.

Extraction and liquid-liquid partitioning

One stainless steel ball (1.75 cm dia.) was put in each extraction tube. The extraction was done with 25 mL acetone-hexane (1:1) for 1 h on a wrist action shaker (Grussendorf et al., 1970). The tubes were centrifuged at 1000 rpm for 10 min. A 5 mL aliquot of this extract was pipetted into a 15 mL centrifuge tube. Six mL of 2 percent aqueous sodium chloride was added and contents agitated for about 15 sec on a

vortex mixer. After the layers had separated, the hexane layer was removed to another tube. The aqueous layer was re-extracted with 2 x 2 mL hexane. The combined hexane extract was dried over sodium sulphate and concentrated to about 1 mL with nitrogen, keeping the tube immersed in a water bath at about 40 C.

Cleanup

The extract was further purified by passing it through 500 mg of deactivated Florisil packed in a disposable Pasteur pipette (22.5 x 0.5 cm) over a plug of glass wool. The column was tapped to obtain good packing, topped with about 0.5 g sodium sulphate, and prewashed with 3 mL hexane. Then the concentrated hexane extract was transferred to the prewashed column with 1 mL of hexane and immediately eluted with 5 mL of benzene. The eluate collected in a graduated tube, adjusted to the appropriate volume and 2-8 uL injected into the EC-GLC for analysis.

RESULTS AND DISCUSSION

Under the conditions described, each of the pyrethroids eluted as a single peak. The retention times were 3.3 min for cypermethrin and 4.8 min for fenvalerate (Fig. 1). The blank extracts showed no interference peaks from wheat or milled fractions. Quantitation was done by external standards. Each of the extracts was injected twice. Excellent recoveries of cypermethrin and fenvalerate were obtained from fortified samples (Table 3). Cypermethrin recoveries ranged from 83.9 to 95.2, 91.1 to 104.0, 82.8 to 84.6 and 74.7 to 85.0 percent from fortified

grain, flour, bran and middlings (Table 3). The percent range of recoveries for fenvalerate was 77.6 to 90.5, 81.5 to 86.3, 76.2 to 82.1 and 77.2 to 86.5, respectively (Table 3). The lower limits of detection were 0.02 mg kg^{-1} for cypermethrin and 0.04 mg kg^{-1} for fenvalerate.

The method described is rapid and more economical than the method of Simonaitis and Cail (1977) for permethrin and than that used by Noble et al., (1982) and Hargreaves et al., (in press) for deltamethrin, fenvalerate, permethrin and phenothrin. Less than 40 mL of solvents are required to extract and cleanup a 5-g sample. Similarly, only 0.5 g of Florisil is needed for cleanup of each sample. The use of ball-mill extraction makes it possible to extract 12 samples in one h. Thus, the method developed is rapid and inexpensive and can be used routinely to determine residues of cypermethrin and fenvalerate (perhaps other pyrethroids as well) in wheat.

Although in some instances it may be necessary to quantify individual isomers, usually the determination of total cypermethrin and fenvalerate is feasible and within the capabilities of most laboratories. The method described here was used for the determination of residues of cypermethrin and fenvalerate in wheat and milled fractions.

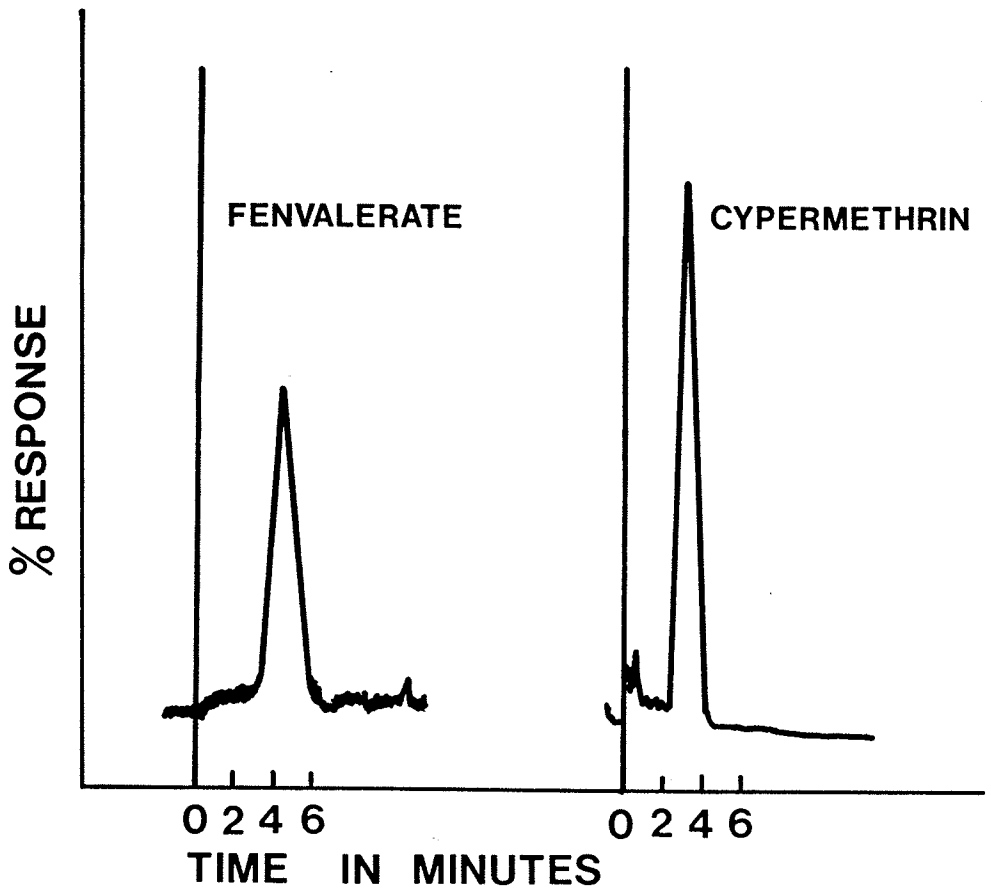


FIGURE 1. Retention times of fenvalerate and cypermethrin.

TABLE 3. Percent recovery[a] of cypermethrin and fenvalerate from wheat and its milled fractions.

Fraction	Cypermethrin		Fenvalerate	
	Fortification Level mg kg^{-1}	Percent recovery (Means±S.D.)	Fortification level mg kg^{-1}	Percent Recovery Mean±S.D.
Whole grain[b]	0.46	83.9±5.3	0.46	77.6±3.1
	1.14	95.3±1.2	0.82	90.0±4.5
	2.28	91.1±4.5	2.04	90.5±8.7
Flour	0.46	103.9±1.7	0.41	81.8±2.0
	0.91	97.5±5.3	0.82	81.5±7.2
	2.28	91.1±0.3	2.04	86.3±3.0
Middlings	0.46	74.7±1.3	0.41	82.3±1.2
	0.91	84.9±4.7	0.82	77.2±3.2
	2.28	85.0±1.7	2.04	86.5±1.6
Bran	0.76	86.4±1.5	0.68	80.3±4.2
	1.52	85.3±2.8	1.36	76.2±0.5
	3.80	82.8±1.4	3.39	82.1±3.6

a = mean of 3 replications
b = ground wheat

'Manuscript II'

Residues of Cypermethrin and Fenvalerate in Stored Wheat and Milled Fractions

Cypermethrin or fenvalerate were applied at 8 and 12 mg kg^{-1} to wheat of 13.3 and 15% moisture content. Treated wheat was stored at 25 and -5 C for 60 weeks and sampled at six 12-weekly intervals. Residues were determined in wheat and milled fractions viz. bran, middlings (mostly germ) and flour (endosperm). It was observed that the highest amounts of insecticides were present in bran and the least in endosperm. Both insecticides degraded in treated wheat at a slow rate. Half-lives of fenvalerate on grain ranged from 385 weeks on wheat of 13.3% moisture content stored at -5 C to 69 weeks on wheat of 15% moisture content stored at 25 C. Cypermethrin disappears at a faster rate than fenvalerate. Half-lives of cypermethrin on grain varied from 169 weeks on wheat of 13.3% moisture content stored at -5 C to 36 weeks on wheat of 15% moisture content stored at 25 C. Reduction of residues in flour through bread baking was low; 79 to 84% of cypermethrin and 87 to 88% of fenvalerate were present in bread made from flour (white and wholemeal) containing cypermethrin and fenvalerate residues.

1.Introduction

The development of malathion resistance in stored product insects is threatening the continued use of this commonly used grain protectant. The relatively new synthetic pyrethroids have shown their potential as alternate grain protectants in the few studies that have been conducted with these insecticides (Ardley and Desmarchelier, 1974; Bengston ,1978; Bengston et al.,1980; Desmarchelier et al.,1981; Bengston et al., in press). Before a grain protectant can be tested extensively,it is necessary to study its degradation at the residue level under varying conditions.

After an insecticide has been applied to grain, it is important from the standpoint of consumption to know its distribution in the different milled fractions. Studies on cypermethrin and fenvalerate residues in the grain storage environment are very limited. Fenvalerate, along with permethrin , phenothrin and deltamethrin have been reported to persist for very long periods on stored wheat (Noble et al., 1982). Deltamethrin applied to wheat of 12% moisture content, remained at the same level throughout a 15-month storage period at 25 C (Hargreaves et al.,in press).

The aim of this study was to measure residues of cypermethrin and fenvalerate in stored wheat and its milled fractions. Insecticide residue degradation usually increases with increased moisture content and temperature (Rowlands, 1967). Therefore, the study was conducted with wheat at two moisture contents and two storage temperatures using two levels of each of the insecticides.

2.Materials and Methods

2.1 Grain treatment and storage

Hard red spring wheat, cultivar Neepawa, was adjusted to two moisture levels, 13.3 and 15.0%. Emulsifiable concentrate formulations of cypermethrin (40%) and fenvalerate (30%), supplied by Shell International Chemical Co., Canada, were diluted with water to contain 8 and 12 mg/mL. Two-kg batches of wheat were thinly spread in a tray lined with aluminium foil. Two mL of diluted insecticide emulsions were sprayed on wheat using a Paasche airbrush sprayer at a constant pressure of 0.52 kg/cm^2. Control samples were sprayed with 2 mL distilled water. The treated lots were poured in 4.5-L glass jars and tumbled on a mechanical tumbler for 30 min to ensure uniform mixing of the insecticides and grain. Four such lots were treated for each insecticidal level. The lots from each treatment were mixed and divided into 350-g glass jars. Samples for 0 week analysis were taken immediately after treatment. The rest of the jars were stored in dark rooms maintained at 25 and -5 C for subsequent sampling at 12, 24, 36, 48, and 60 weeks after treatment. At each sampling interval, one jar was taken from each treatment.

2.2 Grinding and Milling of samples

Twenty-five g of wheat was ground in a coffee grinder (GS Iona Model CG 8) and used for determination of the residues. For milling, 100-g samples were milled on a micro mill (Ottawa micro mill No.6012, Engineering Research Service, Agriculture Canada, Ottawa) to obtain bran consisting mainly of the outer layers of grain (pericarp, seed coat and

aleurone layer), middlings (mostly germ, fine particles of bran and coarse particles of flour) and flour (mainly endosperm). Triplicate samples from ground grain and fractions were used for analyses.

2.3 Analysis of residues

Residues of cypermethrin and fenvalerate were determined using the method described earlier (Chapter III, Manuscript I). Five-gram samples were used for ground wheat, middlings, flour and bread, whereas 3 g was used for bran, in each analysis. For bread samples, 5 g of anhydrous sodium sulphate was added before extraction.

2.4 Processing of flour

In a separate experiment, cypermethrin and fenvalerate were applied to 2-kg lots of wheat. Treated wheat was stored at 25 C for 4 weeks. Samples were milled as described earlier to obtain white flour. Wholemeal flour was obtained by mixing the fractions obtained from milling. White bread and wholemeal bread was baked according to the "Remix Baking Test" (Irvine and McMullan, 1960). The formula used was as follows- flour 100 g, water variable, yeast 3.0 g, sugar 2.5 g, salt 1.0 g, malt syrup 0.3 g, ammonium dihydrogen phosphate 0.1 g, potassium bromate 1.5 mg. The procedure used was - fermentation for 165 min at 30 C, proof time 55 min at 30 C and baking for 25 min at 227 C . The residues in flour and bread were determined following the procedure described earlier (Manuscript I).

2.5 Calculation of half-lives

Half-lives of cypermethrin and fenvalerate on grain were calculated through regression analysis assuming the loss of pyrethroids follows 'pseudo first-order' kinetics, as has been shown for fenitrothion (Desmarchelier, 1978) and for deltamethrin, fenvalerate, phenothrin and permethrin (Noble et al., 1982). Thus:

$$\ln(C/C_o)=-k't$$

in which C is the concentration at time t; C_o is the concentration at time zero and k' is the pseudo first-order rate constant; k' depends upon the water activity (A_w) and is

$$k'=k\ A_w$$

in which k is the first-order rate constant;

$$t_{1/2}=-(1/k')\ln 0.5$$

and $t_{1/2}$ is the half-life at water activity A_w

3.Results

3.1 Residues of cypermethrin

Table 4 shows that the residues of cypermethrin on wheat of 13.3 and 15.0% moisture content immediately after treatment were close to the intended levels. Cypermethrin residues on wheat of 13.3% moisture content at various intervals at 25 and -5 C are shown in Table 5 and those on wheat of 15.0% moisture content Table 6. On wheat of 13.3% moisture content, the residues decreased from an initial level of 7.88 mg kg^{-1} at 0 week to 4.78 mg kg^{-1} after 60 weeks at 25 C, and to 6.25 mg

kg^{-1} at -5 C. From an initial level of 12.65 mg kg^{-1}, the residues declined to 7.43 and 9.66 mg kg^{-1} when wheat was stored at 25 and -5 C, respectively for the same interval of 60 weeks.

On wheat of 15.0% moisture content, the decline in cypermethrin residues was faster than at 13.3% moisture content (Table 5 and 6). From an initial level of 7.46 mg kg^{-1}, the residues decreased to 2.52 mg kg^{-1} after 60 week storage at 25 C and to 4.05 mg kg^{-1} at -5 C. From an initial treatment of 11.80 mg kg^{-1}, 4.24 mg kg^{-1} cypermethrin was recovered after storage for 60 weeks at 25 and 6.27 mg kg^{-1} at -5 C.

Analysis of milled fractions of grain treated at 8 and 12 mg kg^{-1} showed maximum residues in bran followed by middlings and flour in that order (Tables 5 and 6). The residues decreased with time in all fractions, the rate being faster in 15% moisture content wheat than in 13.3% moisture content wheat samples (Appendix A and B), and also faster at 25 than at -5 C.

3.2 Residues of fenvalerate

Table 4 shows that amounts of fenvalerate present in grain immediately after treatment were in agreement with the intended dosage levels. Tables 7 and 8 show residues of fenvalerate in wheat of 13.3 and 15.0% moisture content, respectively, and its milled fractions at various post-treatment intervals. From an initial fenvalerate level of 8.16 mg kg^{-1} on wheat of 13.3% moisture content, the residues decreased to 6.10 mg kg^{-1} after 60 week at 25 C, and to 7.04 mg kg^{-1} at -5 C. The level of 12.76 mg kg^{-1} fenvalerate decreased to 9.19 and 10.68 mg kg^{-1} after storage for 60 weeks at 25 and -5 C, respectively.

The rate of decline in fenvalerate residues on 15% moisture content wheat was faster than on 13.3% moisture content wheat (Table 7 and 8). Thus, from an initial level of 8.12 mg kg^{-1}, fenvalerate residues declined to 4.30 mg kg^{-1} after 60 weeks storage at 25 C, and to 4.46 mg kg^{-1} after storage for the same length of time at -5 C. Similarly, the residues from a level of 12.81 mg kg^{-1} decreased to 6.89 and 8.37 mg kg^{-1}, after 60-weeks storage at 25 and -5 C, respectively.

The distribution of fenvalerate residues in milled fractions followed the same pattern as that in cypermethrin. The maximum amounts were in bran followed by middlings and flour in that order (Tables 7 and 8). The percent degradation of fenvalerate residues in wheat and milled fractions for both moisture contents at both temperatures and all storage periods is compared in appendix C and D. The residues decreased with time in all fractions, the rate being faster in 15% moisture content wheat than in 13.3% moisture content wheat samples and also faster at 25 than at -5 C.

3.3 Effect of processing on residues in flour

The residues of cypermethrin and fenvalerate in white and wholemeal flour and bread are presented in Table 9. There was a reduction of 15.7 and 20.6 percent in cypermethrin residues following baking of white and wholemeal bread, respectively. Fenvalerate residues decreased by 12.1 and 13.1 percent, respectively, during baking of white and wholemeal

bread. No difference was observed in bread weight, volume, texture and taste between treated and control breads.

3.4 Half-lives of cypermethrin and fenvalerate

Half-lives of cypermethrin and fenvalerate on wheat calculated from regression analysis , are presented in Table 10. The longest half-life of fenvalerate (385.1 weeks) was on wheat of 13.3% moisture content stored at -5 C and the shortest (69.3 weeks) on wheat of 15.0% moisture content stored at 25 C. In general the half-lives of cypermethrin were shorter than those of fenvalerate. The longest half-life of cypermethrin (169.1 weeks) was on wheat of 13.3% moisture content stored at -5 C and the shortest (36.3 weeks) on wheat of 15.0% moisture content stored at 25 C.

Discussion

Analyses of grain and milled fractions at various intervals showed that there was a slow decline in the residues of cypermethrin and fenvalerate. Limited studies on related pyrethroids have shown that these insecticides are highly persistent on stored grain. Hargreaves et al., (in press) reported that deltamethrin applied to wheat of 12.0% moisture content, remained at the same level throughout a 15-month storage period at 25 C.

In the present studies, the rate of reduction of fenvalerate residues was slower than that of cypermethrin. There is no reported work on the comparative degradation of these two insecticides on stored grain. However, fenvalerate was found to be the most persistent insecticide on stored wheat, amongst deltamethrin, fenvalerate, permethrin and phenothrin (Noble et al.,1982). These authors reported that fenvalerate had a half-life of 210 and 182 weeks on wheat of 12.0 and 15.0% moisture content, respectively, stored at 25 C. Bengston et al.(in press) also confirmed that fenvalerate is more persistent than deltamethrin, permethrin, and phenothrin. The longer residual life of fenvalerate as compared to cypermethrin, may be explained on the basis of their hydrolysis rate constants. In aqueous systems at pH 6, hydrolysis rate constants of 1.1×10^{-1} per day for cypermethrin and 2.33×10^{-2} for fenvalerate have been reported (Grayson, 1975). Noble et al. (1982) suggested hydrolytic mechanism to be an important pathway in the degradation of pyrethroids in stored grain environment.

A stepwise regression analysis was applied to the data in Table 10 and following general expression was obtained:-

$t_{1/2}$(weeks)=1830.7-2.43(K)-67.9(% moisture content)-911.6(rate Of hydrolysis)

where

rate of hydrolysis = 2.33×10^{-2} (fenvalerate)

1.1×10^{-1} (cypermethrin)

K = storage temperature in degrees K

% moisture content= % moisture content of wheat

$R^2 = 0.758$

when applied to the data in Table 10, this equation predicts all of the half-lives fairly well.

In the present studies, the rate of reduction in residues was faster at 25 C and 15.0% moisture content of wheat than at -5 C and 13.3% moisture moisture wheat. Temperature and moisture content influence the rate of degradation of insecticides in grain (Rowlands, 1967). Desmarchelier (1980) observed that residues of bioresmethrin and phenothrin on wheat depended on temperature and equilibrium relative humidity.

Reports on the distribution of pyrethroids in milled fractions of treated wheat are limited. However, a number of studies on organophosphorus insecticides have shown bran to contain maximum residues after milling of treated wheat. Thus, maximum residues in bran and minimum residues in flour (endosperm) were obtained from wheat treated with malathion, bromophos, iodophenphos and primiphos-methyl (Mensah et al.,1979), methyl phoxim (Alnaji and Kadoum,1979), and malathion and fenitrothion (Abdel-Kader, 1981). Bengston et al. (in press) found 0.70, 3.3 and 0.08 mg kg^{-1} fenvalerate in wheat, bran and flour (endosperm), respectively, 10 months after an application of 1 mg kg^{-1} fenvalerate to wheat. This is in agreement with the distribution of residues in milled fractions, found in the present studies.

A slow decline in residues of cypermethrin and fenvalerate observed in the present studies is similar to that observed for phenothrin,

deltamethrin, permethrin and fenvalerate (Nambu et al.,1981; Noble et al.,1982; Bengston et al., in press; Hargreaves et al., in press). However, the results differ from those of Ardley and Halls (1979) who could not detect phenothrin residues in bran or bread made about 3 weeks after an application of 8 mg kg^{-1} phenothrin to wheat.

Bengston et al.(in press) reported no loss in residues of deltamethrin, fenvalerate, phenothrin and permethrin during baking of white or wholemeal bread. The reductions of 12 to 13 percent in fenvalerate and 15 to 21 percent in cypermethrin residues, found in the present studies, may be due to a higher baking temperature and longer baking time used in the present studies. The present results agree with 13 to 30 percent reduction in phenothrin residues observed by Nambu et al. (1981). However, the reduction is much less than that observed for methyl phoxim (79 to 100%) or malathion (80 to 100%) reported by Alnaji and Kadoum (1981).

A slow degradation of these pyrethroids from stored wheat during baking might raise some concern from the consumer point of view. On the other hand, persistence of these pyrethroids on grain is a desirable property for long-term storage, especially in hot and humid climates. Under these conditions, stored product insects multiply rapidly and organophosphate insecticide degrade very quickly. Frequent repeat applications add to cost and also expose insects to low levels of rapidly degrading insecticides causing selection pressure for resistance. Fumigation is effective but only for a short period. The insects would re-infest the stored food as soon as the fumigant dissipates.

Pyrethroids would be ideal grain protectants under such conditions. Although, initial cost may be high, treatment with grain protectant is more economical in the long-term because of their greater longevity. However, further studies must be conducted involving more species, other cereal products and varying storage conditions.

TABLE 4. Mean[a] ± S.D. cypermethrin and fenvalerate residues (mg kg^{-1}) found on wheat and milled fractions immediately after treatment at 8 and 12 mg kg^{-1}.

Moisture content of wheat(%)	Insecticide	Dosage mg kg^{-1}	Residues (mg kg^{-1})			
			whole grain[b]	Bran	Middlings	Flour
13.3	cypermethrin	8	7.88±0.63	25.56±0.31	13.00±0.72	0.95±0.05
		12	12.65±1.06	39.80±0.97	21.80±0.30	1.53±0.23
15.0		8	7.46±0.10	30.66±2.13	12.09±0.24	1.01±0.04
		12	11.80±0.14	45.28±1.11	15.71±0.18	1.46±0.08
13.3	fenvalerate	8	8.16±0.29	28.24±0.62	15.07±1.20	1.49±0.06
		12	12.76±0.72	39.75±2.69	23.16±3.23	2.14±0.07
15.0		8	8.12±0.19	27.10±2.01	11.07±0.67	1.12±0.03
		12	12.81±0.62	43.61±2.07	16.03±0.52	1.64±0.14

a = mean of three replications

b = ground wheat

TABLE 5. Mean[a] ± S.D. cypermethrin residues on wheat and milled fractions after treating wheat of 13.3% moisture content at 8 and 12 mg kg^{-1} and stored at 25 and -5 C up to 60 weeks.

Intended level ($mg\ kg^{-1}$)	Storage Period (weeks)	Residues $mg\ kg^{-1}$ at two storage temperatures							
		25 C				-5 C			
		Whole Grain[b]	Bran	Middlings	Flour	Whole Grain	Bran	Middlings	Flour
8	0	7.88±0.63	25.56±0.31	13.00±0.72	0.95±0.05	7.88±0.63	25.56±0.31	13.00±0.72	0.95±0.05
	12	7.51±0.26	22.48±0.58	12.20±1.31	0.84±0.01	7.82±0.15	22.97±0.60	12.75±0.36	0.94±0.01
	24	7.49±0.15	21.26±0.35	11.15±0.51	0.78±0.04	7.59±0.25	21.84±1.60	12.49±1.17	0.91±0.01
	36	6.51±0.78	19.99±0.17	9.74±0.19	0.65±0.01	7.61±0.47	21.16±0.65	12.18±0.78	0.83±0.02
	48	5.53±0.46	18.84±1.43	8.42±1.84	0.57±0.02	6.49±0.11	20.29±0.69	11.15±0.37	0.77±0.01
	60	4.78±0.27	14.88±0.36	8.22±0.98	0.55±0.01	6.25±0.37	18.61±2.41	10.99±1.33	0.69±0.05
12	0	12.65±1.06	39.80±0.97	21.80±0.30	1.53±0.23	12.65±1.06	39.80±0.97	21.80±0.30	1.53±0.23
	12	11.87±0.23	37.00±1.92	18.84±0.34	1.42±0.02	12.42±0.17	38.35±0.88	19.87±0.49	1.53±0.03
	24	11.68±0.22	33.78±0.94	16.84±0.65	1.32±0.05	11.99±0.46	38.18±1.93	19.11±1.79	1.44±0.05
	36	9.98±0.73	31.66±1.15	16.26±0.87	1.22±0.09	11.32±0.19	34.51±1.94	18.29±1.10	1.45±0.05
	48	8.62±0.18	30.62±0.49	14.70±0.32	0.93±0.06	9.88±0.52	33.08±0.59	18.15±0.17	1.21±0.02
	60	7.43±0.26	25.23±2.30	12.47±2.16	0.89±0.04	9.66±1.25	31.68±0.33	16.63±0.29	1.21±0.01

a = mean of three replications
b = ground wheat

TABLE 6. Mean[a] ± S.D. cypermethrin residues in wheat and milled fractions after treating wheat of 15.0% moisture content at 8 and 12 mg kg^{-1} and stored at 25 and -5 C up to 60 weeks.

Intended level mg kg^{-1}	Storage Period (weeks)	Residues (mg kg^{-1}) at two storage temperatures							
		25 C				-5 C			
		Whole grain[b]	Bran	Middlings	Flour	Whole grain[b]	Bran	Middlings	Flour
8	0	7.46±0.01	30.66±2.13	12.09±0.24	1.01±0.04	7.46±0.10	30.66±2.13	12.09±0.24	1.01±0.04
	12	6.91±0.17	29.11±1.35	10.31±0.35	1.00±0.04	7.22±0.17	28.82±0.64	11.63±0.66	0.99±0.04
	24	5.45±0.34	25.93±1.09	9.59±0.31	0.72±0.04	5.91±0.21	27.16±0.92	10.29±0.58	0.94±0.11
	36	4.29±0.12	18.18±0.24	7.99±0.55	0.62±0.03	5.29±0.31	21.41±1.15	8.67±0.50	0.77±0.10
	48	3.21±0.16	16.74±1.70	7.21±0.33	0.54±0.03	4.59±0.22	19.53±1.13	8.10±0.59	0.69±0.05
	60	2.52±0.21	11.36±0.11	3.84±0.41	0.41±0.04	4.05±0.29	14.64±1.20	6.00±0.38	0.61±0.01
12	0	11.80±0.14	45.28±1.11	15.71±0.18	1.46±0.08	11.80±0.14	45.28±1.11	15.71±0.18	1.46±0.08
	12	10.89±0.19	42.79±0.88	14.69±0.45	1.30±0.06	11.39±0.35	42.42±2.28	15.22±0.86	1.43±0.13
	24	9.18±0.40	36.56±0.88	11.57±0.22	1.29±0.10	9.90±0.63	40.12±0.72	12.45±0.68	1.40±0.20
	36	7.59±0.15	25.69±0.80	11.01±0.16	0.87±0.07	8.16±0.50	33.14±0.36	13.11±0.83	1.31±0.02
	48	5.77±0.56	22.74±0.72	8.83±0.10	0.68±0.04	7.00±0.39	28.04±1.09	9.74±0.27	0.92±0.06
	60	4.24±0.15	14.97±0.54	4.99±0.79	0.68±0.01	6.27±0.19	19.42±0.56	7.58±0.41	0.71±0.03

a = mean of three replications
b = ground wheat

TABLE 7. Mean[a] ± S.D. fenvalerate residues on wheat and milled fractions after treating wheat of 13.3% moisture content at 8 and 12 mg kg^{-1} and stored at 25 and -5 C up to 60 weeks.

Intended level (mg kg^{-1})	Storage Period (weeks)	Residues (mg kg^{-1}) at two storage temperatures							
		25 C				-5 C			
		Whole grain[b]	Bran	Middlings	Flour	Whole grain	Bran	Middlings	Flour
8	0	8.16±0.29	28.24±0.62	15.07±1.20	1.49±0.06	8.16±0.29	28.24±0.62	25.07±1.20	1.49±0.06
	12	7.51±0.18	24.52±1.80	13.68±1.16	1.30±0.07	7.76±0.22	26.14±1.22	14.95±1.74	1.43±0.10
	24	7.58±0.15	24.40±0.30	13.17±1.03	1.27±0.14	8.11±0.43	26.02±1.19	14.61±0.40	1.41±0.10
	36	7.32±0.36	24.11±0.44	13.02±0.94	1.22±0.06	7.94±0.18	25.61±1.55	14.15±1.29	1.43±0.06
	48	6.93±0.37	23.68±0.32	12.81±1.61	1.16±0.06	7.81±0.11	25.57±0.67	14.03±0.28	1.38±0.08
	60	6.10±0.12	22.69±2.13	11.15±2.59	1.08±0.14	7.04±0.36	25.08±0.56	12.36±1.37	1.35±0.03
12	0	12.76±0.72	39.75±2.69	23.16±3.23	2.14±0.07	12.76±0.72	39.75±2.69	23.16±3.23	2.14±0.07
	12	11.93±0.27	36.70±5.28	21.03±0.07	1.98±0.03	12.23±0.10	37.83±0.74	23.04±2.65	2.13±0.05
	24	11.72±0.66	35.86±2.49	20.84±1.82	1.92±0.08	11.44±0.61	37.80±2.11	22.95±0.42	2.13±0.28
	36	11.38±0.53	35.62±3.30	20.55±0.98	1.85±0.04	11.88±1.09	37.45±4.79	22.06±0.37	2.01±0.21
	48	10.70±0.09	33.94±0.18	19.61±1.51	1.73±0.06	11.39±0.23	36.76±0.90	21.78±0.43	1.98±0.15
	60	9.19±0.32	31.88±2.93	17.60±3.95	1.67±0.10	10.68±0.58	34.14±1.67	20.76±1.12	2.00±0.03

a = mean of three replications

b = ground wheat

TABLE 8. Mean[a] ± S.D. fenvalerate residues in wheat and milled fractions after treating wheat of 15.0% moisture content at 8 and 12 mg kg^{-1} stored at 25 and -5 C up to 60 weeks.

Intended level (mg kg^{-1})	Storage Period (weeks)	Residues (mg kg^{-1}) at two storage temperatures							
		25 C				-5 C			
		Whole grain[b]	Bran	Middlings	Flour	Whole grain	Bran	Middlings	Flour
8	0	8.12±0.19	27.10±2.01	11.07±0.67	1.12±0.03	8.12±0.19	27.10±2.01	11.07±0.67	1.12±0.03
	12	7.29±0.33	24.58±1.29	9.58±0.45	1.00±0.03	7.94±0.10	26.12±2.03	11.00±1.13	1.10±0.02
	24	6.91±0.25	22.49±1.97	8.71±1.21	0.89±0.03	7.81±0.07	25.37±3.36	10.48±1.44	1.07±0.02
	36	6.39±0.16	21.43±0.77	8.54±1.27	0.85±0.02	7.53±0.19	24.37±0.39	9.68±0.94	1.01±0.05
	48	5.47±0.10	19.09±0.80	7.79±0.32	0.81±0.04	6.22±0.06	22.53±0.02	8.88±1.14	0.92±0.09
	60	4.30±0.06	14.99±0.18	6.17±0.07	0.76±0.02	4.46±0.09	18.55±0.72	7.25±0.82	0.87±0.04
12	0	12.81±0.62	43.61±2.07	16.03±0.52	1.64±0.14	12.81±0.62	43.61±2.07	16.03±0.52	1.64±0.14
	12	11.30±0.51	39.15±1.31	14.10±0.54	1.38±0.13	12.75±0.45	39.90±2.79	15.74±3.25	1.58±0.11
	24	10.99±0.29	36.84±1.29	13.52±1.31	1.33±0.01	12.15±0.48	39.92±5.24	13.52±1.60	1.58±0.14
	36	10.67±0.44	35.30±0.60	11.56±0.93	1.29±0.05	12.37±0.34	38.29±1.23	13.69±2.56	1.55±0.04
	48	7.90±0.17	30.47±0.44	9.52±0.54	1.09±0.04	9.93±0.33	35.96±0.92	12.47±0.04	1.42±0.09
	60	6.89±0.19	28.18±0.81	7.71±0.26	1.06±0.04	8.37±0.52	33.20±2.99	10.93±0.15	1.40±0.11

a = mean of three replications

b = ground wheat

TABLE 9. Mean[a] residue levels in white and wholemeal flour and bread (expressed on a moisture-free basis).

Insecticides	Residue (mg kg^{-1})			
	white flour	white bread	wholemeal flour	wholemeal bread
Cypermethrin	0.51	0.43 (84.3)[b]	3.50	2.78 (79.4)
Fenvalerate	0.66	0.58 (87.9)	3.96	3.44 (86.9)

a = mean of three replications

b = percent residues remaining after baking.

TABLE 10. Pseudo first-order rate constants and half-lives of cypermethrin on treated wheat of 13.3 or 15.0% moisture content stored at 25 or -5 C for 60 weeks.

	Storage conditions					
Insecticides	Treatment level ($mg\ kg^{-1}$)	Temperature (C)	Moisture Content (%)	Pseudo first-order rate constants $10^3 k^-$ ($week^{-1}$)	Half-life $t_{1/2}$ (weeks)	95% confidence limits on $t_{1/2}$
Fenvalerate	8	-5	13.3	1.8	385.1	231-1155
	12	-5	13.3	2.5	277.3	182-578
	8	25	13.3	4.1	169.1	133-231
	12	25	13.3	4.7	147.5	115-204
	8	-5	15.0	8.9	77.9	59-114
	12	-5	15.0	6.8	101.9	77-157
	8	25	15.0	9.7	71.5	61-87
	12	25	15.0	10.0	69.3	57-88
Cypermethrin	8	-5	13.3	4.1	169.1	124-267
	12	-5	13.3	5.0	138.6	105-204
	8	25	13.3	8.5	81.5	65-108
	12	25	13.3	9.0	77.0	65-94
	8	-5	15.0	10.8	64.2	57-74
	12	-5	15.0	11.5	60.3	53-69
	8	25	15.0	19.1	36.3	33-41
	12	25	15.0	17.2	40.3	36-46

'Manuscript III'

Efficacy of Cypermethrin, Fenvalerate and Malathion against the Red Flour Beetle, Tribolium castaneum (Herbst) and the Rusty Grain Beetle, Cryptolestes ferrugineus (Stephens)

The effectiveness of cypermethrin and fenvalerate applied at 8 and 12 mg kg^{-1} to wheat of 13.3 and 15.0% moisture contents, was evaluated against Tribolium castaneum and Cryptolestes ferrugineus and compared with malathion at 8 mg kg^{-1}. Treated wheat was stored at 25 and -5 C and sampled at six 12-weekly intervals during a 60-week storage period. Bioassays on adult mortality and F_1 progeny revealed cypermethrin to be effective against both species at all storage conditions and intervals. Fenvalerate was ineffective against C. ferrugineus. It caused 63.3 to 66.7% initial mortality of T. castaneum at 8 mg kg^{-1} and 90 to 100% at 12 mg kg^{-1} but prevented progeny production at all intervals. At a storage temperature of 25 C, malathion became ineffective at 12 weeks on wheat of 15% moisture content and 24 weeks on wheat of 13.3% moisture content. However, on malathion-treated wheat stored at -5 C, 100% mortality of both species was maintained during the entire storage period of 60 weeks.

1. Introduction

Widespread resistance of stored product insects to malathion (Champ and Dyte, 1976) has necessitated the evaluation of alternate grain protectants. Some of the recent synthetic pyrethroids have shown promise against stored product insects. Bioresmethrin is effective against Sitophilus granarius, Sitophilus oryzae and Rhyzopertha dominica (Ardley and Desmarchelier, 1978; Bengston et al., 1980). Permethrin provides good control of Callosobruchus chinensis, Acanthoscelides obtectus (Taylor and Evans, 1980) and Tribolium castaneum (Adesuyi, 1982). Recently, Bengston et al. (in press) reported that fenvalerate, permethrin and phenothrin are promising for control of R. dominica. There is not much published work on the effectiveness of cypermethrin and fenvalerate against T. castaneum and C. ferrugineus. The present study describes results of laboratory experiments conducted to determine the grain protecant potential of cypermethrin and fenvalerate against these two economically important pests in the Canadian grain industry.

2.Material and Methods

2.1 Insecticidal treatment and storage of wheat

Insecticidal treatment and storage of wheat was described earlier (Chapter III, Manuscript II). However, in addition, wheat was treated with malathion at 8 mg kg^{-1} and stored and sampled at six 12-weekly intervals in the same manner as the wheat treated with pyrethroids.

2.2 Test insects

The test insects used for bioassays were adults of the red flour beetle, T. castaneum and the rusty grain beetle, C. ferrugineus. The insects for stock cultures were obtained from standard laboratory cultures maintained at the Agriculture Canada Research Station, Winnipeg. The cultures were reared in temperature-controlled cabinets at 30 C using white flour plus 5 percent Brewer's yeast as food medium for T. castaneum and wheat kernels plus 5 percent wheat germ for C. ferrugineus. Open trays filled with water were placed in cabinets to maintain humidity.

2.3 Bioassays

Bioassays were conducted at various sampling intervals using three replicates, each containing 20 grams of wheat for each species. Samples were taken in 90 g glass jars and 1 g of wheat germ was added to each. Twenty, four to six-week old adults (unsexed) of either species were added to each jar. The jars were covered with cheesecloth secured with rubber bands and placed in incubators maintained at 30 C and 65-70% relative humidity. After one week, mortality of the insects was recorded. Wheat was returned to the jars which were stored at the same conditions to allow the development of F_1 adults. The number of live F_1 adults was recorded after a 5-week incubation period.

2.4.Statistical analysis

Mortality data was statistically analyzed following arcsin percentage transformation.

3.Results

3.1 Mortality of adults

Percent mortality of T. castaneum and C. ferrugineus exposed for one week to wheat at various intervals is presented in Tables 11 to 14. Statistical analysis of data showed that all the insecticidal treatments provided significantly higher ($P<0.05$) mortalities than the control. Mortalities in fenvalerate- and cypermethrin-treated wheat were significantly higher ($P<0.05$) than malathion. Cypermethrin caused significantly higher ($P<0.05$) mortalities than fenvalerate. The susceptibility of T. castanuem was significantly higher than that of C. ferrugineus to both pyrethroids. Cypermethrin at 8 and 12 mg kg^{-1} caused 100 percent or nearly 100 percent mortality of T. castaneum at both temperatures, both moisture contents and all intervals (Tables 11 and 12). These were significantly higher ($P<0.05$) than those caused by fenvalerate. Fenvalerate was able to produce 100% initial kill in T. castaneum only at 12 mg kg^{-1} on wheat of 13.3% msoiture content. However, the decline in percent mortality on wheat stored at 25 C, as the time progressed was less rapid on wheat treated with fenvalerate than with malathion. Malathion-treated wheat of 13.3 and 15.0% moisture, stored at 25 C produced 78.7 and 1.7 percent mortality, respectively, at the 12-week interval. The effect of moisture content was significantly ($P<0.05$) different on malathion compared with cypermethrin and fenvalerate but between cypermethrin and fenvalerate the difference was not significant ($P>0.05$). Malathion-treated wheat stored at -5 C, continued to kill 100 percent T. castaneum at both moisture contents for the entire storage period of 60 weeks.

Against C. ferrugineus, 8 mg kg^{-1} cypermethrin produced high but not 100% mortalities (Table 13 and 14). At 0 week, this treatment caused 91.7 mortality on wheat of 13.3% moisture content and 81.7 percent on that of 15% moisture content. After 60 weeks of storage at 25 C, the mortality on wheat with 13.3% moisture content was 53.3 percent and on 15% moisture content wheat was 31.7 percent. At a storage temperature of -5 C, mortality on 13.3% moisture content wheat was 68.3 percent and on 15% moisture content wheat was 46.7 percent. Cypermethrin at 12 mg kg^{-1} caused 100 percent initial mortality on wheat of 13.3% moisture content and 95 percent on wheat of 15% moisture content at 25 C. Percent mortalities decreased with time. At the 60-week interval, mortalities of C. ferrugineus on wheat of 13.3% moisture content ranged from 80.0 to 86.7 percent at 25 C and -5 C, respectively and on wheat of 15% moisture content from 55.0 to 68.3 percent, respectively. Mortalities induced by cypermethrin were significantly higher ($P<0.05$) than those by fenvalerate. Fenvalerate caused very low mortality even at the 12 mg kg^{-1} treatment. Malathion at 8 mg kg^{-1} caused 100 percent initial mortality on wheat of both moisture contents at both storage temperatures. That level was maintained throughout the storage period on wheat stored at -5 C. However, at 25 C and 15% moisture content only 3.3% mortality was recorded at the 12-week interval and 0% at the 24-week interval. Wheat of 13.3% moisture content induced 80.0 percent at the 12-week and 8.3 percent mortality at the 24-week interval.

3.2 F_1 progeny

The number of live F_1 adults that emerged is presented in Tables 15 to 17. Cypermethrin and fenvalerate prevented production of progeny of T. castaneum at the two moisture contents and storage temperatures and at all intervals (Tables 15 and 16). In the fenvalerate treatments a few live F_1 adults were recorded at some intervals. At a storage temperature of 25 C, live adults started emerging after 12 weeks from malathion-treated wheat of 15% moisture content and after 36 weeks on wheat with 13.3% moisture content stored at 25 C.

F_1 progeny of C. ferrugineus from 13.3% moisture content wheat could not be recorded because there were few or no F_1 adults produced in untreated wheat. On wheat of 15% moisture content, cypermethrin prevented progeny production at both storage temperatures and all intervals (Table 17). Fenvalerate did not prevent production of F_1 adults of C. ferrugineus, but the numbers were low compared to control. Adults started emerging from malathion-treated wheat after 12 weeks of storage at 25 C, but no adults emerged at any interval from treated wheat stored at -5 C.

4.Discussion

Some studies reported in the literature indicate that pyrethroids are less effective against T. castaneum than against R. dominica, S. granarius and S. oryzae (Bengston et al., 1975; Ardley, 1976: Ardley and Desmarchelier, 1978). But, Adesuyi (1982) found that permethrin was highly effective against T. castaneum. Apparently, effectiveness of pyrethroids varies from species to species and from one pyrethroid to another.

In the present studies, cypermethrin was found to be highly effective against T. castaneum and C. ferrugineus. Cypermethrin-treated wheat at both moisture contents and storage temperatures caused complete mortality of T. castaneum at every interval up to 60 weeks of storage. In addition, it prevented F_1 progeny production in both species. The level of control achieved with fenvalerate was much less than with cypermethrin. Similar observations were made by Watters et al. (in press) who observed cypermethrin and permethrin to be more effective than fenvalerate against T. castaneum. Although fenvalerate was not completely effective against T. castaneum, it successfully prevented F_1 progeny production at all storage conditions and intervals. Ardley (1976) showed that bioresmethrin caused only low mortalities in T. castaneum, but it suppressed F_1 progeny production. In grain storage, an effective grain protectant should be able to prevent progeny production as well as kill insects. Fenvalerate was more effective in preventing progeny production than in causing adult mortality in T. castaneum. The absence of progeny may have been due to the mortality of eggs or early instar larvae or to the suppression of oviposition . However, this insecticide failed against C. ferrugineus by both criteria.

Malathion lost its effectiveness rapidly against both species in terms of mortality and prevention of progeny production at a storage temperature of 25 C. Malathion is known to lose its effectiveness on grain exceeding moisture content of 13.5% and temperature above 15.6 C (Watters, 1959; Strong and Sbur, 1960). Therefore, it is not surprising that malathion became ineffective during the 12-week interval on wheat

of 15.0% moisture content stored at 25 C. Mensah and Watters (1979) observed that malathion at 12 mg kg^{-1} on wheat of 12 and 16% moisture content was effective against /T. castaneum for 24 and 12 weeks, respectively. This observation agrees with the observation in this study, namely, that the period of protection decreases with an increase in moisture content. Abdel-Kader (1981) reported that malathion treated wheat (12.5% moisture content) stored at -5 C for 72 weeks, contained 74% of the malathion initially applied. The extremely slow degradation at low temperatures may explain why, in the present study malathion-treated wheat stored at -5 C continued to induce 100 percent mortality of both species for 60 weeks.

The results reported here suggest that cypermethrin may be used as a long-term grain protectant particularly in hot and humid climates. Under such conditions, the insect pressure on stored products is high because of favourable conditions for rapid development and multiplication. Organophosphate insecticides degrade very quickly when temperature and moisture contents are high. Fumigants dissipate quickly and provide protection only for a short period.

In light of these facts, cypermethrin may prove a valuable grain protectant. However, further studies must be conducted involving more species of stored product insects, other cereals and varying storage conditions. Studies should also be conducted to investigate the effect of synergists on the effectiveness and lower application rates should be evaluated. It would be of interest to evaluate cypermethrin against malathion resistant strains.

TABLE 11. Mean[a] percent mortality of T.castaneum exposed for one week to treated wheat of 13.3% moisture content stored at 25 and -5 C up to 60 weeks.

Treatments	mg insecticide kg^{-1}	Storage temperature											
		25 C						-5 C					
		Storage period (weeks)											
		0	12	24	36	48	60	0	12	24	36	48	60
Control	0	1.7	0	0	5.0	0	0	1.7	1.7	1.7	0	0	1.7
Fenvalerate	8	66.7	71.7	61.7	43.3	57.7	53.3	66.7	83.3	73.3	60.0	68.3	58.3
Fenvalerate	12	100	86.9	83.3	71.7	78.3	73.3	100	93.3	83.3	83.3	88.3	81.7
Cypermethrin	8	100	100	100	98.3	100	100	100	100	100	100	100	100
Cypermethrin	12	100	100	100	100	100	100	100	100	100	100	100	100
Malathion	8	100	78.7	30.0	8.3	0	0	100	100	100	100	100	100

a = based on three replications

TABLE 12. Mean[a] percent mortality of T. castaneum exposed for one week to treated wheat of 15.0% moisture content stored at 25 and -5 C up to 60 weeks.

Treatments mg insecticide kg^{-1}		Storage temperature											
		25 C						-5 C					
		Storage period (weeks)											
		0	12	24	36	48	60	0	12	24	36	48	60
Control	0	0	0	0	0	0	1.7	0	1.7	0	0	0	0
Fenvalerate	8	63.3	68.3	61.7	56.7	46.7	38.3	63.3	78.3	81.7	60.0	50.0	43.3
Fenvalerate	12	90.0	83.3	81.7	76.7	70.0	61.7	90.0	91.7	95.0	83.3	78.3	71.7
Cypermethrin	8	100	100	100	96.7	100	96.7	100	100	100	100	100	100
Cypermethrin	12	100	100	100	100	100	100	100	100	100	100	100	100
Malathion	8	100	1.7	0	0	0	0	100	100	100	100	100	100

a = based on three replications

TABLE 13. Mean[a] percent mortality of C. ferrugineus exposed for one week to treated wheat of 13.3% moisture content stored at 25 and -5 C up to 60 weeks.

Treatments	Storage temperature											
	25 C						-5 C					
mg insecticide kg^{-1}	Storage period (weeks)											
	0	12	24	36	48	60	0	12	24	36	48	60
Control 0	0	1.7	0	3.4	0	6.7	0	1.7	3.3	3.3	1.7	1.7
Fenvalerate 8	6.7	6.7	1.7	5.0	3.3	5.1	6.7	11.7	15.0	10.2	3.3	3.4
Fenvelerate 12	18.3	20.0	6.7	6.7	8.3	6.7	18.3	33.3	23.3	18.3	10.0	16.7
Cypermethrin 8	91.7	88.3	73.3	63.3	56.7	53.3	91.7	91.7	80.0	75.0	75.0	68.3
Cypermethrin 12	100	95.0	91.7	86.7	86.7	80.0	100	100	96.7	88.3	91.7	86.7
Malathion 8	100	80.0	8.3	0	0	0	100	100	100	100	100	100

a = based on three replications

TABLE 14. Mean[a] percent mortality of C. ferrugineus exposed for one week to treated wheat of 15.0% moisture content stored at 25 and -5 C up to 60 weeks.

Treatments	Storage temperature											
	25 C						-5 C					
mg insecticide kg^{-1}	Storage period (weeks)											
	0	12	24	36	48	60	0	12	24	36	48	60
Control 0	0	1.7	1.7	3.3	1.7	1.7	0	0	1.7	1.7	1.7	0
Fenvalerate 8	3.3	6.7	6.7	3.3	3.3	1.7	3.3	11.7	10.0	6.7	3.3	0
Fenvalerate 12	23.3	26.7	15.0	8.3	5.0	1.7	23.3	25.0	31.7	8.3	1.7	1.7
Cypermethrin 8	81.7	86.7	78.3	45.0	40.0	31.7	81.7	93.3	83.3	61.7	60.0	46.7
Cypermethrin 12	95.0	88.3	93.3	66.7	63.3	55.0	95.0	95 0	100	83.3	88.3	68.3
Malathion 8	100	3.3	0	3.3	0	0	100	100	100	100	100	100

a - based on three replications

TABLE 15. Mean[a] number of F_1 progeny from 20 *T. castaneum* adults exposed for one week to treated wheat of 13.3% moisture content stored at 25 and -5 C up to 60 weeks.

Treatments	Storage temperature											
	25 C						-5 C					
mg insecticide kg^{-1}	Storage period (weeks)											
	0	12	24	36	48	60	0	12	24	36	48	60
Control 0	50.7	77.3	91.3	77.0	68.3	83.0	50.7	71.3	85.0	87.7	72.3	74.0
Fenvalerate 8	0.7	0	0	0	1.0	2.7	0.7	0	0	0	0	0.3
Fenvalerate 12	0	0	0	0	0.3	0	0	0	0	0	0	0
Cypermethrin 8	0	0	0	0	0	0	0	0	0	0	0	0
Cypermethrin 12	0	0	0	0	0	0	0	0	0	0	0	0
Malathion 8	0	0	0	5.7	22.0	52.3	0	0	0	0	0	0

a = based on three replications

TABLE 16. Mean[a] number of F_1 progeny from 20 T. castaneum adults exposed for one week to treated wheat of 15.0% moisture content stored at 25 and -5 C up to 60 weeks.

Treatments	Storage temperatures											
	25 C						-5 C					
mg insecticide kg^{-1}	Storage period (weeks)											
	0	12	24	36	48	60	0	12	24	36	48	60
Control 0	104.7	112.0	66.3	80.3	84.0	81.0	104.7	117.7	83.7	82.7	85.0	69.7
Fenvalerate 8	0.3	0.3	0	0	0	0	0.3	0	0	0	0	1.0
Fenvalerate 12	0	0.7	0	0	0	0	0	0	0	0	0	0
Cypermethrin 8	0	0	0	0	0	0	0	0	0	0	0	0
Cypermethrin 12	0	0	0	0	0	0	0	0	0	0	0	0
Malathion 8	0	3.7	57.7	60.7	67.3	68.7	0	0	0	0	0	0

a = based on three replications

TABLE 17. Mean[a] number of F_1 progeny from 20 C. Ferrugineus adults exposed for one week to treated wheat of 15.0% moisture content stored at 25 and -5 C up to 60 weeks.

Treatments	Storage temperatures											
	25 C						-5 C					
mg insecticide kg^{-1}	Storage period (weeks)											
	0	12	24	36	48	60	0	12	24	36	48	60
Control 0	33.0	37.3	18.3	36.7	29.3	18.0	33.0	41.0	27.7	41.3	25.3	25.3
Fenvalerate 8	7.3	1.3	7.3	11.3	9.3	12.3	7.3	3.0	4.7	8.0	15.3	8.0
Fenvalerate 12	2.3	4.0	1.0	8.0	10.7	5.3	2.3	1.3	0	7.0	5.3	6.3
Cypermethrin 8	0	0	0	0	0	0	0	0	0	0	0	0
Cypermethrin 12	0	0	0	0	0	0	0	0	0	0	0	0
Malathion 8	0	4.0	16.3	35.7	36.0	28.7	0	0	0	0	0	0

a - based on three replications

Chapter IV

GENERAL DISCUSSION

The development of resistance to malathion in stored product insects is causing concern about the protection of stored products from attack by insects. This concern has prompted research to evaluate alternate grain protectants. Recent work with synthetic pyrethroids especially in Australia, has shown several members of this group of insecticides to be promising grain protectants (Ardley and Desmarchelier, 1974, 1978; Bengston, 1978 ; Bengston et al., 1980; Desmarchelier et al., 1981). Studies regarding the evaluation of new grain protectants are not limited to their bioefficacy only but involve their degradation, penetration and metabolism in grain and its milled fractions.

Present studies on the effectiveness and residues of cypermethrin and fenvalerate have shown these insecticides to be persistent on wheat of 13.3 and 15.0% moisture content stored at 25 and -5 C.

The micro analytical method developed for residue determination of cypermethrin and fenvalerate in wheat and its milled fractions proved satisfactory. Apart from giving excellent recoveries without substrate interference, the method is rapid and inexpensive because only small volumes of solvents are required. Micro columns were used in pyrethroid residue determination in soil (Williams and Brown, 1979; Hill et al., 1982) and alfalfa (Hill, 1982). Other methods used for residue determination of pyrethroids on stored grain (Simonaitis and Cail, 1977;

Noble et al., 1982; Hargreaves et al., in press) require large volumes of solvents.

In the present studies, it was observed that on wheat of 13.3% moisture content, 58.7 to 60.7 percent of the initially applied amount of cypermethrin was still present in grain after 60 weeks of storage at 25 C. The amount present after storage at -5 C for a similar period ranged from 76.4 to 79.3 percent of the initial deposits. On wheat of 15.0% moisture content, 33.8 to 35.9 percent of the initial deposits remained in grain after a storage period of 60 weeks at 25 C, and 54.3 to 56.1 percent after storage at -5 C for the same period.

Fenvalerate degraded at a slower rate than cypermethrin. Fenvalerate-treated wheat of 13.3% moisture content stored at 25 C for 60 weeks contained 72.0 to 74.6 percent of the initial amount and that stored at -5 C contained 83.7 to 86.2 percent. On wheat of 15% moisture content, 53.0 to 53.8 percent of the initial deposits remained in grain after a period of 60 weeks at 25 C and 54.9 to 64.6 percent at -5 C. The differences between fenvalerate residues at both temperatures are lower than those of cypermethrin. These lower differences of fenvalerate residues between two temperatures, may be due to higher stability of fenvalerate than cypermethrin. The observations agree in general with results obtained for other related pyrethroids. For example, Hargreaves et al. (in press) observed that wheat of 12.0% moisture content treated with deltamethrin and stored at 25 C, contained same level of this insecticide over a 15-month storage period. Nambu et al. (1981) reported that phenothrin applied to wheat degraded only by 13 to 21 percent after a 12-month storage at 30 C.

Half-lives calculated for fenvalerate on grain ranged from 385.1 weeks on wheat of 13.3% moisture content stored at -5 C to 69.3 weeks on wheat of 15.0% moisture content stored at 25 C. Compared to fenvalerate, cypermethrin had shorter half-lives under similar storage conditions. Noble et al. (1982) observed fenvalerate to be most persistent among deltamethrin, fenvalerate permethrin and phenothrin. The longer residual life of fenvalerate than of cypermethrin may be explained on the basis of their hydrolysis rate constants. In an aqueous system at pH6, Grayson (1975) measured hydrolysis rate constants of 1.1×10^{-1} per day for cypermethrin and 2.33×10^{-2} per day for fenvalerate. Noble et al., (1982) suggested hydrolytic mechanism to be an important pathway in the degradation of pyrethroids in stored grain environment. Therefore, it is clear that cypermethrin degrades at a faster rate than fenvalerate.

The major amounts of pyrethroids applied to wheat remained on the outer layers of grain. About 8 to 10 percent of cypermethrin and 10 to 14 percent of fenvalerate penetrated the endosperm and 60 to 70 percent of the insecticides remained in bran fractions. Bengston et al. (in press) observed that 8.5 percent of fenvalerate and 14 percent of phenothrin penetrated the endosperm of treated wheat. The small differences between the two studies may be due to different variety, formulation or method of application. Nambu et al. (1981) remarked that most of the phenothrin applied to wheat remains on the seed coat.

The fact that these insecticides remain mostly on the surface is beneficial in terms of externally feeding insects like Tribolium castaneum. Hargreaves et al. (in press) suggested that insects like

Sitophilus oryzae may lay eggs in the endosperm of grain treated with deltamethrin allowing development within grain.

Some workers have observed that there is little or no reduction in pyrethroid residue during bread baking. Nambu et al. (1981) observed only slight reduction of phenothrin through this process. Bengston et al. (in press) noted no reduction in residues of deltamethrin, fenvalerate, permethrin and phenothrin. In the present study, losses of 16 to 21 percent in cypermethrin and 12 to 13 percent in fenvalerate residues during baking were observed. The differences in the present study from that by Bengston et al. (in press) may be due to the higher baking temperature and longer baking time used in the present study. Little or no reduction in pyrethroid residues during baking of bread might raise some concern from a consumer point of view; however, if the initial application rate to whole grain is low then insignificant amounts of residue can be expected in endosperm. Generally, residues in endosperm represent about 10 percent of those present in whole grain. Lower application rates and long storage period will contribute to lower amounts of residues in white flour, wholemeal flour as well as bran.

Cypermethrin was highly effective against both species even on wheat of 15.0% moisture content stored at 25 C for 60 weeks. It not only caused complete mortality of T. castaneum and moderately high mortality of C. ferrugineus, but also successfully prevented F_1 progeny production in both species. The failure of F_1 progeny to appear would naturally prevent any damage until residue levels decline to the point that they no longer provide protection against outside infestation. The combined residue and mortality clearly show that after 60 weeks storage of

treated wheat of 15% moisture content, the level of cypermethrin from 8 mg kg^{-1} treatment was only 2.52 mg kg^{-1}. Even at that level this insecticide proved highly effective. Thus, it is possible that cypermethrin at 2.5 mg kg^{-1} or even lower concentrations may give protection against T. castaneum and perhaps other species for a long time. It may be possible to further reduce the application rates by using a synergist such as piperonyl butoxide.

On the other hand, fenvalerate does not appear to have great potential especially against C. ferrugineus. In addition, it was less effective than cypermethrin against T. castaneum. Watters et al. (in press) reported fenvalerate to be less effective than cypermethrin and permethrin against T. castaneum.

It was observed in the present studies that malathion at 8 mg kg^{-1} applied to wheat of 15% moisture content and stored at 25 C became ineffective against both species during a 12-week interval. Watters (1959) and Strong and Sbur (1961) reported that malathion loses its toxicity very rapidly above the critical level of 14% moisture content. This is because of enzymic hydrolysis and decarboxylation of organic phosphorothionates which are more pronounced in grain above 14% moisture content (Rowlands, 1967).

The fact that degradation of these pyrethroids is slow even at 25 C and 15% moisture content makes them very suitable grain protectants for hot and humid climates. Due to the rapid degradation of organophosphorus insecticides under such conditions, it may become necessary to apply these insecticides repeatedly. Apart from additional costs, such situations also expose insects to non-lethal levels thus

enhancing the chances of a build-up of resistance. Fumigation is effective but only for a short time. As soon as the fumigant disappears the product is once again vulnerable to insects. The initial costs of applying pyrethroids may be high, but a single application of an effective pyrethroid such as cypermethrin may last for a long time and thus prove more economical than repeated applications of organophosphorus insecticides. Cypermethrin may prove to be a very useful grain protectant under conditions where grain or other stored products must be stored for long periods and where large numbers of insects occur because of favourable conditions for development and reproduction.

Further studies must be conducted using lower application rates of cypermethrin and fenvalerate and combinations with synergists. It is also, necessary to study the effectiveness and residues of synthetic pyrethroids at different storage temperatures and moisture contents. The susceptibility of other insect species to pyrethroid treated wheat and other stored products, for example, rice, maize, barley, oats and millet should also be studied.

Chapter V

SUMMARY AND CONCLUSIONS

Cypermethrin and fenvalerate at 8 and 12 mg kg^{-1} applied to wheat of 13.3 and 15.0% disappeared extremely slowly during a period of 60 weeks storage at 25 and -5 C. The rate of loss of fenvalerate from treated wheat was slower than that of cypermethrin at both temperatures and moisture contents. Higher storage temperature and moisture content of wheat increased the rate of loss of both pyrethroids. Analysis of milled fractions of pyrethroid-treated wheat showed that maximum amounts of residues were present in bran followed by middlings and flour (endosperm). The longest calculated half-life of fenvalerate (385 weeks) was observed on wheat of 13.3% moisture content stored at -5 C and shortest (69 weeks) on wheat of 15% moisture content stored at 25 C. The longest half-life for cypermethrin (169 weeks) was observed on 13.3% moisture content wheat stored at -5 C and shortest (36 weeks) on wheat of 15.0% moisture content stored at 25 C. The residues of cypermethrin and fenvalerate present in white and wholemeal flour degraded only by about 12 to 21% during the bread baking process.

Bioassay studies showed cypermethrin was effective against Tribolium castaneum and Cryptolestes ferrugineus both in causing mortality of exposed adults and prevention of F_1 progeny production. It provided a high level of control throughout the 60-week storage period even on 15% moisture content wheat stored at 25 C. Fenvalerate successfully

prevented F_1 progeny production of T.castaneum, despite the fact that mortality of adults was less than that caused by cypermethrin. Fenvalerate had very little effect against C. ferrugineus.

From the present study, it is concluded that rate of loss of cypermethrin and fenvalerate from stored wheat is slow. The rate increases with an increase in temperature and moisture content of wheat. Cypermethrin disappears at a relatively faster rate than fenvalerate. Small amounts of residues are found in flour (endosperm) when treated wheat is processed, but the levels in wholemeal flour are high. The maximum amount is found in bran. Bread baking does not reduce the residues in white or wholemeal flour to any appreciable extent.

Cypermethrin is an effective grain protectant and may be quite useful for long-term storage in hot and humid climates where many kinds of stored product insects occur in large numbers because of favourable environmental conditions for development and reproduction.

Chapter VI

CONTRIBUTION TO KNOWLEDGE

The studies reported in this manuscript clearly show the value of cypermethrin as a long-term grain protectant. This insecticide proved to be highly effective against Tribolium castaneum and Cryptolestes ferrugineus and may be equally effective against other stored product insect species. In the light of present investigations, the following recommendations for future studies can be suggested.

1. Degradation of cypermethrin and fenvalerate at other storage temperatures should be studied.
2. Other stored products such as corn, rice, barley, millets, oats and peanuts, etc., should be included in further studies.
3. Cypermethrin should be evaluated against a variety of stored product insect species and, also, at lower application rates.
4. The effects of synergists such as piperonyl butoxide on the effectiveness of these pyrethroids should be investigated.
5. These insecticides should also be evaluated against organophosphate resistant strains of stored product insects.
6. As pyrethroids are known to have a negative temperature coefficient the susceptibility of insects at different temperatures should be investigated.

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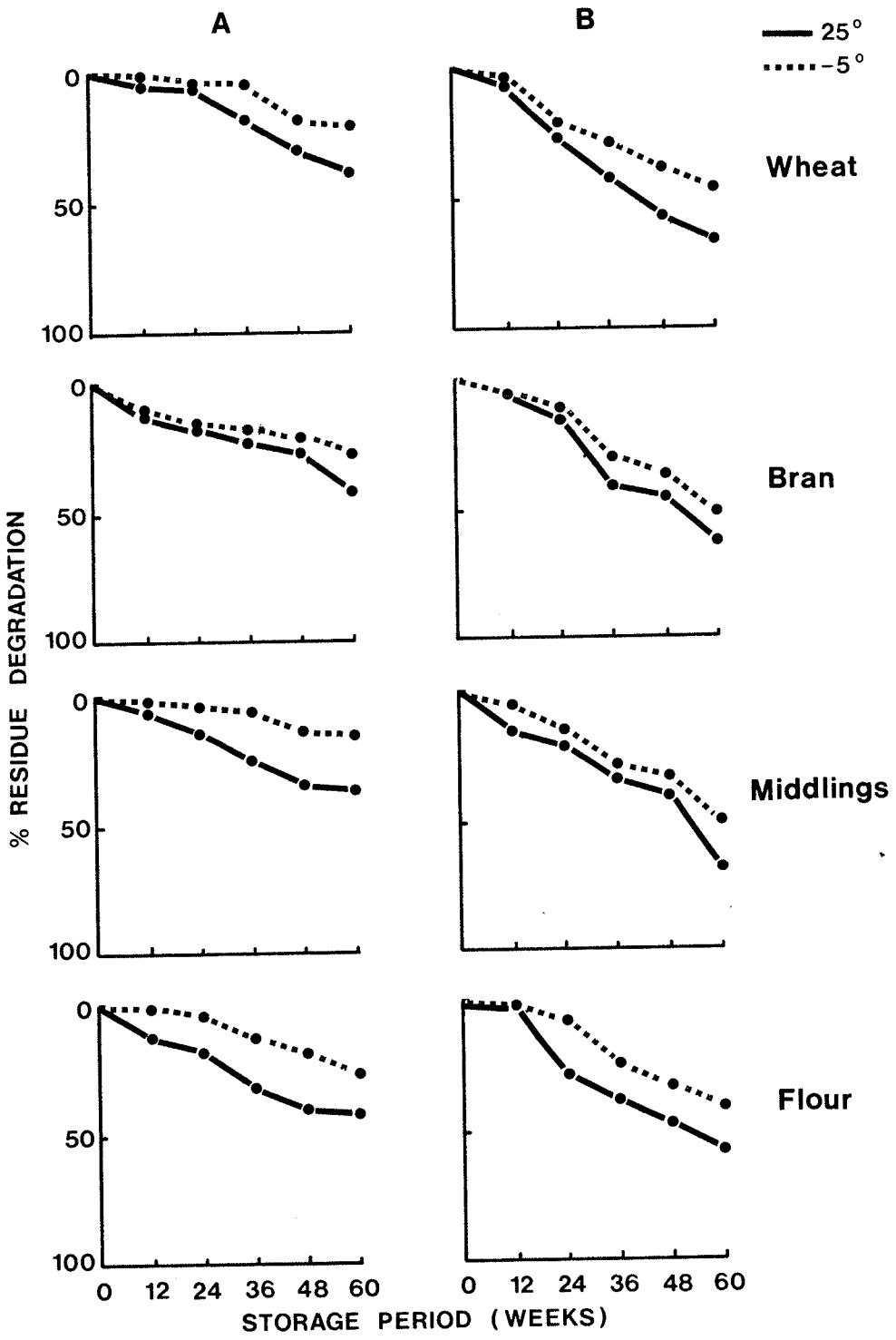
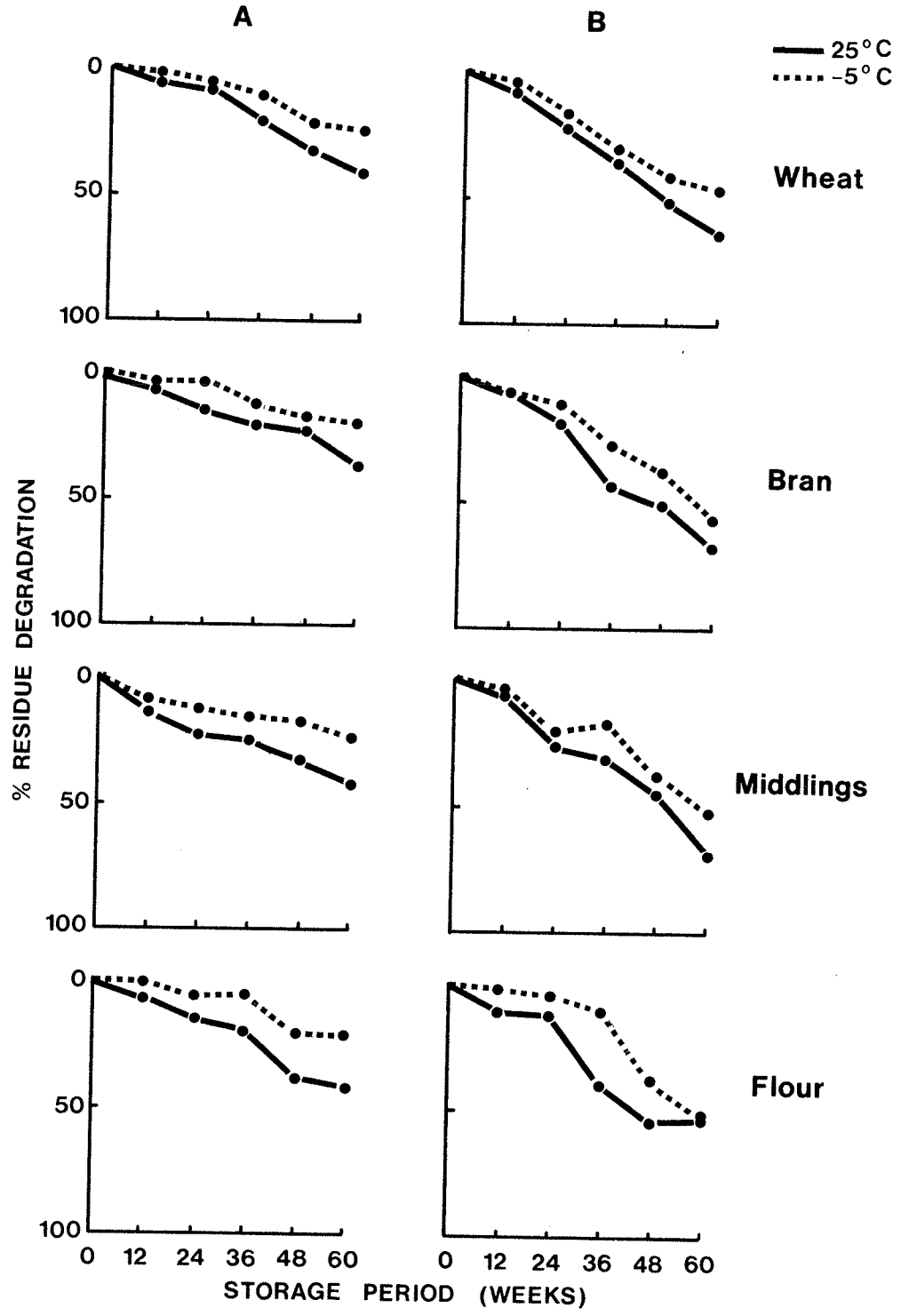
Appendix A

Percent residue degradation of cypermethrin (8 mg kg^{-1}) in wheat and milled fractions during 60-week storage of wheat of 13.3(A) and 15.0%(B) moisture content.



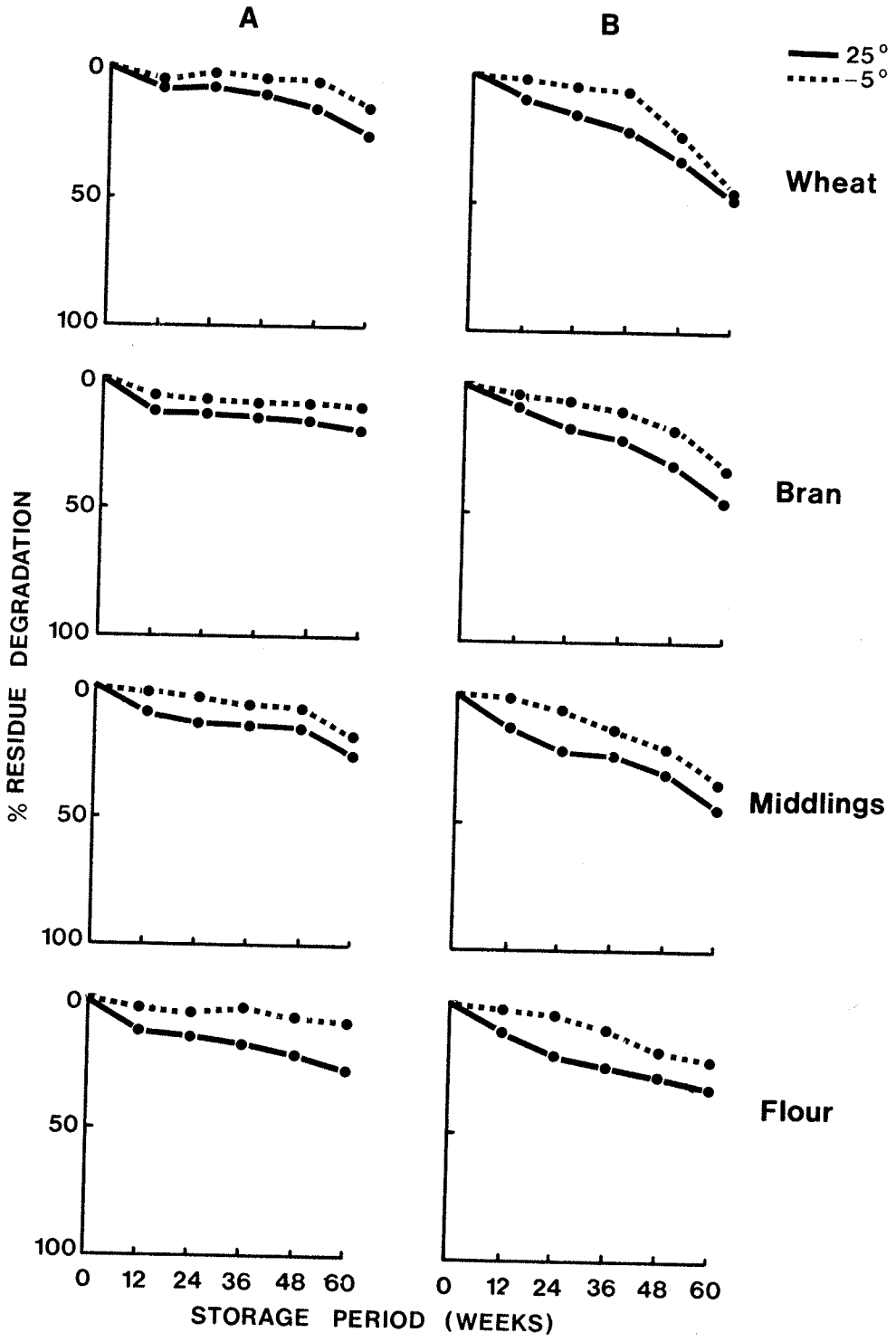
Appendix B

Percent residue degradation of cypermethrin (12 mg kg^{-1}) in wheat and milled fractions during 60-week storage of wheat of 13.3(A) and 15.0%(B) moisture content.



Appendix C

Percent residue degradation of fenvalerate (8 mg kg^{-1}) in wheat and milled fractions during 60-week storage of wheat of 13.3(A) and 15.0%(B) moisture content.



Appendix D

Percent residue degradation of fenvalerate (12 mg kg^{-1}) in wheat and milled fractions during 60-week storage of wheat of 13.3(A) and 15.0%(B) moisture content.