Degradation of the stored grain protectant malathion as a function of drying systems and storage temperatures

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Degradation of the stored grain protectant malathion as a function of drying systems and storage temperatures

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Iowa State University, 1988
Degradation of the stored grain protectant malathion as a function of drying systems and storage temperatures

by

Wendy Kay Wintersteen

A Dissertation Submitted to the Graduate Faculty in Partial Fulfillment of the Requirements for the Degree of DOCTOR OF PHILOSOPHY Major: Entomology

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For the Graduate College

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1988
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INTRODUCTION AND LITERATURE REVIEW

Introduction

During recent years there has been a marked increase in the capacity of on-farm grain drying and storage facilities. In 1970, U.S on-farm grain storage capacity was 4.6 billion bushels. By 1983, this storage capacity had increased to more than 12 billion bushels (Anonymous 1986). In 1986, on-farm grain storage facilities in Iowa were estimated at over 2 billion bushels (Iowa Agricultural Statistics 1987). This increased capacity has been largely in response to excess production and low prices. In addition to meeting storage needs, increased storage capacity has allowed midwest corn producers to forward-price a portion of their crop, to hold grain for tax purposes, or to participate in government loan and storage programs. It is apparent that long-term farm grain storage will continue as an important component of Midwest corn production and marketing.

Approximately 50% of the corn stored for at least 1 year is held on-farm. Successful grain storage of 1 year or more requires that the farmer dry grain to moisture levels of 13% or less. Since corn must frequently be harvested at moisture levels far above this figure, artificial drying is essential. Statistics show that 68% of Iowa farmers dried grain in 1986 (Iowa Agricultural Statistics 1987).

Contemporary drying systems range from low-temperature or natural-air systems that dry grain slowly to high temperature fast drying systems. Approximately 25-30% of the corn dried on Iowa farms is dried with natural air; the rest is dried with heat. Essentially all natural dried corn
remains in the bin where it was dried. Approximately 40% of the corn
dried with heat either remains in the bin where it was dried or is placed
in final storage while still hot (Larry Van Fossen, ISU Ag. Engineering
Ext., pers. comm.).

Concurrent with increased farm storage there has been a corresponding
increase in the instance of insect infestations. Survey data collected in
1980 suggested than more than 80% of the corn stored on Midwest farms was
insect infested (Barak and Harein 1981, Storey et al. 1983). Additional
surveys show that farmers who deliver infested grain are routinely docked
in excess of $0.05/bu or were unable to market their grain (Harein et al.
1985, Weinzierl and Porter 1988). Infested grain must be treated, usually
by fumigation. Elevator managers in Illinois indicated that 10.1% of all
corn in their facilities was fumigated and that approximately 4% of the
corn they received was fumigated on-farm prior to delivery.

Grain fumigation poses a number of potential problems. Farm
fumigation constitutes a potential lethal hazard to inexperienced and ill-
equipped farmers who apply their own fumigants. Additionally, the grain
may be fumigated several times prior to reaching the end user, increasing
the chance of residue accumulation above tolerance levels. Moreover,
public concern about ethylene dibromide residues in food and the
subsequent cancellation of the compound accent the public's and the
Environmental Protection Agency's (EPA) concern over fumigant usage. In
addition, EPA has cancelled the carbon tetrachloride based fumigants,
placed user restrictions on the remaining fumigants, and required the use
of gas masks or self-contained air systems and monitoring equipment.
The only economically feasible alternative to fumigation is a preventative program that includes the application of a grain protectant, such as malathion. Malathion applications are simple, economical, and effective. Quinlan (1982) described the use of "drip-on" and dust applicators that deposit insecticide as grain is augered into the storage bin. Both applicators, if used correctly, will adequately distribute the malathion through the grain. Besides the ease of application, malathion treatments are economical, costing approximately a half-cent per bushel and are relatively effective at controlling many stored grain pests (Storey et al. 1984).

Despite its many advantages, recent surveys have shown that malathion has been applied to only 7% of the corn stored on-farm (Wintersteen and Hartzler 1987) and has been applied to only 7% of the corn and 11.6% of the wheat arriving at U.S. port terminals (Storey et al. 1982). One factor that has limited use of malathion is its instability when placed on high moisture and/or hot grain. Consequently, malathion has been recommended only for application to corn that has already been dried and cooled. This has precluded application to an estimated 40% of corn being placed into farm storage.

The breakdown of malathion as temperature increases and/or when grain moisture is above 14% moisture content (mc) has been documented by a number of workers, notably Strong and Sbur (1960). However, available data are limited to fixed temperature and moisture levels with temperatures of 50°C or lower. Grain undergoes a number of temperature and moisture fluctuations during drying and throughout storage. The
degree of change depends mainly on the type of drying and storage systems used, the changes in air temperature during storage, and the aeration practice employed.

In addition to temperature and moisture, there are other factors that influence the effectiveness of malathion applications. The presence of storage fungi may affect the degradation rate of malathion. Research has associated the presence of Aspergillus glaucus group species on stored grain with decreased levels of malathion, indicating that microbial degradation of malathion occurs on grain (Anderegg and Madison 1983). The formulation of the malathion product used may also influence effectiveness under different temperature and moisture conditions. Two formulations, a 57% emulsifiable concentrate and a 6% flour dust, are used as grain protectants. Current industry claims suggest that the flour dust formulation lasts longer, but inadequate research data are available to support that claim. Clearly a number of factors and their interactions influence the rate of malathion degradation on grain. However, current research has failed to consider all of these factors or has done so under artificial conditions, failing to reflect the actual conditions that exist in various farm drying and storage systems.

The purpose of this research was to evaluate the residual life and efficacy of two formulations of malathion under a range of drying and storage conditions both in the field and in the laboratory. This project was designed to answer the following questions: can malathion be applied before the grain is dried, can malathion be applied to hot dry grain, what effect does drying temperature have on malathion degradation during
storage, and what is the relationship, if any, between drying temperature and malathion degradation by storage fungi?

The specific objectives completed in this study are listed below.

1. An accurate, rapid and inexpensive method of extracting malathion residues in yellow dent corn was developed.

2. Effect of drying temperature and grain moisture on malathion liquid and dust formulations applied prior to drying were determined. Residual life and efficacy of these malathion applications were evaluated during 4 months of cold storage followed by 7 months of warm storage.

3. Effect of drying temperature on malathion liquid and dust formulations applied after drying to either hot or cool dry grain were determined. Residual life and efficacy of these malathion applications were evaluated during 4 months of cold storage followed by 7 months of warm storage.

4. Indigenous fungal populations on grain were evaluated and correlated with malathion residue levels present on grain dried at low temperature and high temperature.

Determining at what points malathion could be applied during the drying process and the length of time residues will remain effective under variable storage temperatures will allow farmers to utilize malathion more effectively as a grain protectant.

Literature Review

Malathion has been recognized as an effective grain protectant for many years. In 1954, Lindgren et al., completed the first studies on the
effectiveness of malathion against stored grain insects. Malathion, applied at 8 ppm to wheat, provided control of granary weevil, *Sitophilus granarius* (L.); rice weevil, *Sitophilus oryzae* (L.); and lesser grain borer, *Rhyzopertha dominica* (F.), for 6 months. LaHue (1965), showed that malathion 57% emulsifiable concentrate (EC) applied at 16.7 ppm protected wheat from insect damage for twelve months. Malathion 57% EC applied at 11 ppm provided almost the same level of control.

Strong et al. (1967) determined that the minimum effective malathion residue on wheat with 13% mc needed to control *Sitophilus* spp. and the red flour beetle, *Tribolium castaneum* (Herbst), was 1 to 3 ppm. The labeled application rate for malathion is 10 ppm and the legal tolerance for malathion residues on grain is 8 ppm (Anonymous 1958). Many of the initial studies involving malathion consisted of comparing the effectiveness of various insecticides to malathion. For example, Womack and LaHue (1959) compared the effectiveness of methoxychlor dusts to malathion EC sprayed on shelled corn. Malathion sprays were applied at 5, 10, 15 and 20 ppm; methoxychlor dusts were applied at 12.5, 25, 50, and 100 ppm. The malathion EC treatments at the dosage of 15 and 20 ppm gave excellent protection to stored shelled corn. The methoxychlor dusts were not as effective and would require an application rate of 100 ppm to provide the same protection as the malathion EC spray.

In a subsequent study LaHue (1976) applied malathion EC, pirimiphos, chlorpyrifos methyl, fenitrothion and malathion M+K (malathion-diatomaceous earth) dust to seed corn. Sixteen months after treatment malathion EC provided 88% mortality of rice weevils while pirimiphos,
chlorpyrifos methyl, and M+K dust provided 100% mortality and fenitrothion provided 86% mortality. Malathion EC and fenitrothion were less effective but still gave adequate protection.

Kramer et al. (1985) compared residual activity of fenoxycarb, Bacillus thuringiensis Berliner, and malathion on wheat during a 2 year storage period. During this time, malathion provided effective control of rice weevil and confused flour beetle, Tribolium confusum Jacquelin du Val but failed to control Indianmeal moth, Plodia interpunctella (Hubner). As expected, B. thuringiensis controlled only Indianmeal moth. Fenoxycarb was effective at controlling all the tested insect species during the 2 year storage period.

While malathion continues to be an effective grain protectant, resistance has been reported for a number of stored grain insects (Champ and Dyte 1977). A survey completed in the Midwest by Beeman et al. (1982), found that 39 of 43 strains of Indianmeal moth collected were resistant to malathion. Haliscak and Beeman (1983) collected red flour beetles, lesser grain borer, Sitophilus spp., Cryptolestes spp., and Oryzaephilus spp. from on-farm grain storage in 14 states to screen for malathion resistance. Resistance was demonstrated in red flour beetles from some specific locations. Resistance to other species was not demonstrated.

Temperature Effects

Grain temperature has been shown to dramatically affect the insecticidal activity of malathion (Teotia and Pandey 1967, O'Donnell 1980). Iordanou and Watters (1969), determined the effect of three
temperatures 26.7, 15.5 and 10.0°C, on the initial toxicity of several organochlorines and organophosphates including malathion. Five insects were used in the bioassay as measures of toxicity: red flour beetle; confused flour beetle; sawtoothed grain beetle, *Oryzaephilus surinamensis* (L.); merchant grain beetle, *Oryzaephilus mercator* (Fauvel) and rusty grain beetle, *Cryptolestes ferrugineus* (Stephens). The insects were exposed to filter papers treated with test insecticide for 24 hours. Malathion was the most effective of all the chemicals tested at all temperatures. At 26.7°C, malathion provided complete control of all test insects, with LD50 values (mg/925cm²) under 5. At 10.0°C, LD50 values ranged from 15 to over 30. As temperatures decreased, higher rates of malathion were required.

Tyler and Binns (1982) tested malathion along with seven other organophosphorus insecticides for effectiveness at three temperatures, 10, 17.5, 25°C. Red flour beetles, sawtoothed grain beetles, and the granary weevil were placed on the filter paper treated with insecticides in concentrations ranging from 10 to 5000 mg/m² for 24 hours. As temperatures were lowered the effectiveness decreased for all the insecticides. The authors speculated that the decreased effectiveness of the insecticide was probably due to the decreased activity of the insects and perhaps also due to slower penetration of the insecticide to the active site. Malathion concentrations determined by chemical residues, instead of bioassay, showed that malathion was still present on the grain at 10°C even though not biologically effective. Abdel-Kader et al. (1980), applied malathion as an emulsion of 8 ppm on wheat. The treated
grain was stored at -35, -20, -5, 5, 10, 20, 27°C for 72 weeks. At -35°C, 7.66 ppm was present at 1 week, 7.53 ppm was present at 72 weeks. At 5°C, 7.53 ppm was present at 1 week, 4.48 was present at 72 weeks. At 27°C, 6.62 ppm was present at 1 week, 0.32 ppm was present at 72 weeks.

Generally, as temperature increased, malathion residues decreased. At storage temperatures 10°C and above, the amount of residue remaining also depended on the age of the residue.

In a study that considered high temperature storage, wheat at a moisture of 10% was treated with malathion at a rate of 10 ppm. Samples were stored at temperatures ranging from 15°C to 49°C, for 12 months. Test species were granary weevil, rice weevil and confused flour beetle. For all species as the temperature increased, effectiveness of the malathion decreased. For example, at 2 weeks, 15°C, rice weevil was 100% controlled; at 2 weeks, 49°C, it was 78% controlled. At 6 months, 15°C rice weevil was 100% controlled; at 6 months 49°C, zero percent was controlled (Strong and Sbur 1960).

A comprehensive evaluation of the research reviewed here indicates that: (1) treated grain stored at temperatures below 27°C requires higher concentrations of malathion to be effective, (2) treated grain stored at low temperatures (-35°C) shows little or no malathion degradation, (3) at temperatures greater than 32°C, effective control can only be obtained for 1 month; at 15°C, effective control can be obtained for 12 months. However, temperature data are not available to answer the important questions: can malathion be applied before the grain is dried, can malathion be applied to hot dry grain, and what are the effects of storage
Grain Moisture Effects

Moisture, like temperature, directly influences the effectiveness of malathion used as a grain protectant. Watters (1959) considered the effects of three levels of grain moisture (12, 15, and 17%) on the residual toxicity of malathion formulated on low grade flour. The samples were treated at 2, 4, 8 and 16 ppm of malathion and stored for 2, 5 and 8 months. After being exposed to the treated samples for 7 days, mortality of the rusty grain beetle was recorded. As grain moisture increased, mortality decreased. Increasing the dosage compensated some for the accelerated degradation of the malathion.

Strong and Sbur (1960) evaluated the influence of moisture on malathion applied at 10 ppm to wheat. Moisture levels of 10, 12, 14, 16, 18 and 20% were tested. The samples were stored at 15°C and sampled at 1, 2, 3, 6, 9, and 12 months. The test species were granary weevil, rice weevil, and confused flour beetle. The influence of moisture on the effectiveness of malathion was shown by decreased mortalities of all the test species with increases in the moisture content. For rice weevil, at 12% moisture, 100% mortality was still achieved at 12 months. At 20% moisture, 100% mortality was not achieved after 1 month, and zero mortality was recorded at 3 months. Strong and Sbur stated, "A moisture content of 12% appears to be the maximum safe level and 14% moisture appears to be the critical level when considered in regard to the persistence of biologically effective malathion deposits."

Minett et al. (1968) showed a critical moisture level for wheat
stored at a constant temperature. Above this level the degradation rate of malathion was rapid. Malathion was applied at 10 ppm to wheat of moisture contents ranging from 8 to 15%. The samples were stored at 21°C and 32°C. Moisture readings and malathion concentrations were determined on a weekly basis for 2 months. As moisture content increased malathion concentration decreased. Minett stated "Critical moisture levels of approximately 11.8% for storage temperature of 21°C and 11.6% for a storage temperature of 32°C are clearly shown".

Fam et al. (1974) considered the influence of grain moisture on malathion applied as a protectant on wheat. Malathion was applied at 8 ppm to grain held at 9, 12, 15 and 18% moisture. As the moisture content increased rice weevil mortality decreased. Fam et al. determined that 12% was the critical moisture level, above which insect control was not possible. Additionally, as the age of the malathion residue increased the insect mortality decreased at all moisture levels.

Kadoum and LaHue (1979) considered the degradation rate of malathion on wheat and corn of different moisture contents. Four different moisture contents (10, 12, 14, 16%) were considered using procedures similar to those discussed earlier. The results of the study showed that residue loss increased as moisture content increased. After 12 months storage at 27°C, malathion residues were 34, 24, 8 and 5% of that applied for corn and 41, 21, 7 and 3% for wheat, containing 10, 12, 14, 16% moisture, respectively. This study clearly showed that malathion residues degraded similarly on wheat and corn grain at high moisture contents. Further studies by Kadoum and LaHue (1976) demonstrated that malathion residues on
high moisture sorghum grain were not degraded as rapidly as those on wheat and corn.

Quinlan et al. (1980) compared the effectiveness of pirimiphos-methyl to malathion applied to high moisture wheat (14.6% mc). As moisture content increased the residues of malathion and pirimiphos-methyl decreased.

**Moisture Content and Temperature Interactions**

Strong and Sbur (1960) considered temperature and moisture interactions as well as their individual effects. Three temperatures, 15, 32, 40°C, and three moisture contents, 10, 12, and 14%, were considered in all possible combinations. Malathion was applied at 10 ppm, 20 ppm and 40 ppm. Malathion effectiveness was evaluated throughout a 12 month storage period using the granary weevil, rice weevil and confused flour beetle. The results indicated a positive relationship between temperature and moisture. As temperature and moisture increased, malathion effectiveness decreased. Data also indicated that by increasing the dosages the negative effects of increased temperature and moisture could be avoided to some extent.

In a similar study, Kadoum and LaHue (1969) evaluated the effect of moisture and temperature on sorghum grain. Three moisture levels, 7, 12.5 and 17% were evaluated at two storage temperatures, -7°C and 12.4°C, for 6 months. Less malathion was remaining on the high moisture grain (17% mc) stored at 12.4°C.

The effects of moisture and temperature on the residual activity of malathion was further demonstrated by Watters and Mensah (1979).
Malathion was applied to wheat at two moisture levels, 16.8% and 11.7%. The treated wheat was placed in a grain bin, exposed to natural air fluctuations, and stored in the laboratory at 10, 20, 30°C. Wheat placed in the grain bin was exposed to subzero winter temperatures which slowed malathion breakdown on wheat at both moisture levels. However, as air temperatures increased in the spring, malathion residues on the high moisture wheat declined more rapidly compared to those on the low moisture wheat. The degradation rate of malathion stored in the laboratory was not affected by grain moisture content when grain temperatures remained at 10°C. However, high moisture grain stored above 10°C degraded malathion residues significantly faster than the low moisture grain.

Tyler and Green (1968) evaluated the effectiveness of fenitrothion and malathion applied to warm moist grain (barley and wheat) stored in nine on-farm bins. The grain moisture and temperature varied significantly among bins and throughout the storage period. Temperatures in some bins reached more than 50°C with grain moisture above 16%. At the end of the twelve week storage period, the dryer, cooler grain retained significantly more chemical residues than the moist warm grain.

Rowlands (1967) stated that the breakdown of malathion on grain is rapid when moisture content and grain temperatures are high due to the increased speed and occurrence of enzyme-catalyzed reactions.

**Chemical Formulation**

The malathion dust formulation commonly used today is flour-based. Most work reported in the literature considers a malathion-diatomaceous earth dust formulation (M+K). Watters (1959) evaluated malathion on flour
dust, but did not compare it to the EC formulation. LaHue and Dicke (1976) compared malathion as a water emulsion spray prepared from premium grade 57% EC and M+K dust. The treated shelled corn (11.6-13.2% mc) was stored for 12 months at 26°C. Results showed that the M+K dust was superior to malathion EC. Moreover, M+K dust degraded at a slower rate than did the EC formulation. At the end of 12 months, only 1.4 ppm remained of the malathion spray while 3.0 ppm residue was recovered from grain treated with the dust formulation. Similar results were obtained when LaHue (1978) considered the effect of high temperature, low humidity storage on the following grain protectants: diatomaceous earth impregnated with pirimiphos methyl, diatomaceous earth impregnated with malathion, pirimiphos-methyl EC, and malathion EC. The treated wheat (11.4% mc) was kept at 33°C and 40% relative humidity for 8 months. The dust formulations were more effective than the EC sprays throughout the storage period.

Biotic Factors

Biotic factors such as grain respiration or microbes associated with the grain may influence malathion degradation. Rowlands (1964) demonstrated that enzymic degradation occurred through phosphatase and carboxyesterase hydrolysis. In addition, Rowlands found that mixed-function oxidase systems resulted in the oxidation of malathion to malaoxon. Studies reviewed earlier have demonstrated the effect of increased grain temperatures and moisture content on the rate of malathion degradation (Strong and Sbur 1960). Clearly, these factors determine the speed at which enzymic degradation occurs (Rowlands 1967).
Kadoum and LaHue (1972) compared malathion degradation in sterilized sorghum kernels (no germination) to that in non-sterilized living kernels (95% germination). Their results showed that sterilized grain had 43.1% of the malathion remaining after 6 months, while the live sorghum grain had only 15.9%. Kadoum and LaHue concluded that enzymic hydrolysis was the major factor in the breakdown of malathion on the non-sterilized kernels.

Microbial degradation of malathion has also been speculated. Rowlands (1967) stated, "It is not surprising, therefore, that those fungi infecting stored cereals frequently metabolize and utilize residues of insecticides that adhere to or penetrate grains; indeed, when the storage conditions are such that enzymic activity of the grains, per se, is almost inactive, any degradation of pesticide residues ensuing is probably due to the storage fungi present." Walker and Stojanovic (1973) showed that malathion disappeared from non-sterile soil faster than it did from sterile soil. In experiments conducted by Lewis et al. (1975), Aspergillus oryzae (Ahlb.) Cohn rapidly transformed malathion into B-malathion monoacid. In another study, Anderegg and Madison (1983) showed that sterilized grain contained significantly more malathion after 6 months storage than sterilized grain inoculated with Aspergillus glaucus Link.

Fungi involved in malathion degradation are commonly found in the corn stored in the Midwest. Survey data collected in 1980 showed that storage fungi (A. glaucus) was found in 84% of the corn samples (Sauer et al. 1984). Corn samples with at least 12.5% mc, and with insects present,
averaged 54% invasion by storage fungi.

Christensen and Kaufmann (1969) stated that there were six factors that influence the development of storage fungi in grain. These factors are: (1) moisture content of the stored grain; (2) grain temperature; (3) length of storage period; (4) the degree of invasion by storage fungi; (5) amount of foreign matter present and (6) insects present. All of these factors are interacting and together influence the quality of storage grain.

Increasing moisture content results in possible fungal invasion by more than 50 fungal species, each with their own specific moisture limits (Christensen 1957). Temperature also affects the growth of fungi, with optimum growth of many species occurring around 30°C. Growth rate will be reduced below 20°C but some species of the A. glaucus group will grow at 5°C (Christensen 1957). The degree of fungal invasion will influence how long the grain can be stored, even at optimum conditions. Christensen and Kaufmann (1969) showed that grain already invaded by storage fungi and stored at 15% mc, 10°C, continued to experience fungal growth. If the grain was free of storage fungi it could be stored under those conditions for up to a year without incurring damage.

Castor (1983) stated that since storage fungi are so widespread that there is a high probability of contaminating grain during harvest and the grain drying process. The longer the seed remains moist the greater the chance for fungal invasion. Castor reported that fungi can even "colonize during the 70 to 100 hours (at 50°C) needed for drying."
Malathion Residue Extraction Procedures

Several procedures for malathion extraction from cereal grains, including corn, wheat, barley and oats, have been developed. However, these methods were typically time-consuming and expensive. Procedures developed by Storherr et al. (1964) and Kadoum (1968) used solvent systems for partitioning and chromatographic columns for cleanup. While these methods were effective, the laboratory procedures were complex and required at least two solvents for residue partitioning. Furthermore, Kadoum found cleanup columns necessary for obtaining the clean samples required by the electron capture detector.

Minett and Belcher (1969) and Levi and Nowicki (1974) developed extraction methods that eliminated partitioning by solvent systems; this substantially reduced the time required per extraction. Additionally, their procedures eliminated the cleanup column, and relied on gas-liquid chromatographic (GLC) analysis with the less sensitive flame thermonic detector. However, these procedures were still inefficient. They required 2 hours of vigorous shaking and then prescribed soaking the grain in the solvent overnight (Minett and Belcher 1969), or ball-milling the grain in ethyl ether and hexane for 1 hour (Levi and Nowicki, 1974).

Studies by Abdel-Kader et al. (1980) and Desmarchelier et al. (1977) showed that different solvents were not equally effective in extracting aged residues. Abdel-Kader et al. (1980) modified the Levi and Nowicki (1974) method by replacing ethyl ether-hexane with methanol, resulting in increased recovery of aged residues. In a similar study by Desmarchelier
et al. (1977), recovery of aged residues was greater with methanol than with hexane. In addition, residue recovery using ethyl acetate or acetone + water was comparable to that accomplished using methanol.
REFERENCES CITED


PART I. A RAPID, ACCURATE, AND INEXPENSIVE METHOD FOR EXTRACTING MALATHION RESIDUES FROM CORN
Current procedures for malathion residue extraction from grain are typically time-consuming and expensive. In this study, a method was developed to extract malathion from corn in an accurate, rapid and inexpensive manner, using ethyl acetate as the solvent.

Yellow dent corn, dried to 12% moisture content, was ground, spiked with 10 ppm malathion and placed in separate regular pint Ball® mason jars. Fifty ml of ethyl acetate were measured into each pint jar containing the treated ground-grain and Ball® canning flats and rings were used to seal each jar. Sealed jars were shaken vigorously (275-285 oscillations/min) for 15 minutes on a Fisher-Kahn® shaker. The ethyl acetate was then decanted from each jar and filtered through 70 g of sodium sulfate held in a Whatman® no. 1 paper filter placed in a glass funnel. The filtrate was collected in a 500-ml round-bottom flask. This procedure was completed a second time using an additional 50 ml of ethyl acetate. The extract, consisting of all filtrates and rinses, was then concentrated to ca. 10 ml and then brought to a final volume of 50 ml in a volumetric flask by adding ethyl acetate.

Malathion residues in extracted samples were detected by a thermonic specific nitrogen-phosphorus detector on a Varian® 3740 gas chromatograph. This detector allowed injection of samples that contained high levels of pigments and other plant compounds.

The rapid extraction method described in this paper is 99% effective.
at removing malathion residues from ground corn. The extraction cost for this method was $32.77 per 12 samples, an amount that approximates the cost of one sample in many methods.
INTRODUCTION

Malathion has been used as an effective and inexpensive grain protectant for over 30 years. Despite its many advantages, recent surveys have shown that malathion has been applied to only 7% of the corn stored on-farm in Iowa (Wintersteen and Hartzler 1987) and that only 7% of the corn and 11.6% of the wheat arriving at U.S. port terminals have been treated (Storey et al. 1982). The recent cancellation of carbon tetrachloride based grain fumigants and additional restrictions placed on the remaining fumigants suggest that grain protectants including malathion will play a more important role in stored grain management in the future.

As malathion application becomes more common, a rapid, efficient and economical procedure to determine malathion residues on grain is needed for monitoring and efficacy determinations. Although several procedures for malathion extraction from grains, including corn, wheat, barley and oats, have been developed, these methods are typically time-consuming and expensive. Procedures developed by Storherr et al. (1964) and Kadoum (1968) used solvent systems for partitioning and chromatographic columns for cleanup. While these methods are effective, the laboratory procedures are complex and require at least two solvents for residue partitioning. Furthermore, Kadoum found cleanup columns necessary for obtaining samples of sufficient quality for analysis by gas-liquid chromatography (GLC) with an electron capture detector.

Minett and Belcher (1969) and Levi and Nowicki (1974) developed extraction methods that eliminated partitioning by solvent systems; this substantially reduced the time required per extraction. Additionally,
their procedures eliminated the cleanup column, and relied on (GLC) analysis with the less sensitive flame thermonic detector. However, these procedures were still inefficient. They required 2 hours of vigorous shaking and then prescribed soaking the grain in hexane overnight (Minett and Belcher 1969), or ball-milling the grain in ethyl ether and hexane for 1 hour (Levi and Nowicki, 1974). Studies by Abdel-Kader et al. (1980) and Desmarchelier et al. (1977) showed that different solvents were not equally effective in extracting aged residues. Abdel-Kader et al. (1980) modified the Levi and Nowicki (1974) method by replacing ethyl ether-hexane with methanol, resulting in increased recovery of aged residues. In a similar study by Desmarchelier et al. (1977), recovery of aged residues was greater with methanol than with hexane. In addition, residue recovery using ethyl acetate or acetone/water was comparable to that accomplished using methanol.

The goal of our study was to develop an accurate, rapid and inexpensive method of extracting malathion residues from yellow dent corn. The extraction method was based on procedures developed by Abou-Assaf et al. (1986) for extracting another organophosphate insecticide, isophenphos, from soil. Isophenphos residues were extracted by shaking the soil with solvent for three-45 min periods on a Fisher-Kahn shaker. Gas-liquid chromatographic analysis with a thermonic specific detector indicated that this method recovered over 90% of the isophenphos present in the soil. Modifications of this procedure included use of ethyl acetate instead of a hexane/acetone mixture and a reduction in shaking time.
MATERIALS AND METHODS

Ethyl acetate was the only solvent used in the extraction process. This solvent was selected because its moderate lipophilicity (dielectric constant at 20°C is 6.4) results in effective malathion extraction from grain while removing fewer waxes and oils than do more lipophilic solvents such as hexane. Additionally, ethyl acetate is relatively safe and nonirritating, compared to solvents used in earlier methods. For example, dermal exposure to methanol can lead to respiratory failure and death, and dermal exposure to acetone causes erythema and dryness (Windholz 1983).

Yellow dent corn, dried to 12% moisture content, was ground to a consistency of flour interspersed with larger pieces of kernal pericarp, mesh no. 8 (U.S. Standard Sieve Series). Five 20-g samples of ground corn were placed in separate regular pint Ball® mason jar and each sample was spiked with 5 ml of a 40 µg/ml malathion solution to obtain a 10 ppm residue level. Malathion solutions were prepared by diluting analytical grade malathion (98.5% purity) provided by the American Cyanamid Co. with certified grade ethyl acetate.

Fifty ml of ethyl acetate were added to each pint jar containing the treated ground-grain. Ball® canning flats and rings were used to seal each jar. Ball® brand flats were used because they did not leak over the course of a complete extraction, whereas other brands of lids permitted solvent leakage during the second of two 15-min shaking periods. Leaking flats showed a deterioration of the flexible sealing compound on their inner surfaces, which resulted in the release of unidentified contaminants to the samples.
Sealed jars were shaken vigorously (275-285 oscillations/min) for 15 minutes on a Fisher-Kahn® shaker modified to hold 12 jars horizontally. The ethyl acetate was then decanted from each jar and filtered through 70 g of anhydrous sodium sulfate held in a 9 cm diameter, Whatman® no. 1 paper filter supported by a 75 mm diameter glass funnel. The filtrate was collected in a 500 ml round-bottom flask. The sodium sulfate was then rinsed with 25 ml of ethyl acetate; the rinsate was also collected in the flask.

Fifty ml of ethyl acetate were again added to the pint jar containing the treated, ground-grain. The shaking procedure was repeated, and then the entire contents of the jar were emptied into the filtering apparatus. The jar and the lid were rinsed three times with 25 ml of ethyl acetate. The rinses were poured through the filtering apparatus and collected in the flask. The filtering apparatus and its contents were then rinsed three additional times with 25 ml of ethyl acetate. These rinses were also collected in the 500 ml flask. The extract, consisting of all filtrates and rinses, was then concentrated to ca 10 ml on a rotary evaporator. The concentrated extract was then brought to a final volume of 50 ml in a volumetric flask by adding ethyl acetate. A 7 ml subsample was placed in a 2-dram vial with a teflon lined lid. Samples were stored in the freezer at approximately -32°C prior to injection.

Malathion residues in extracted samples were detected by a thermonic specific nitrogen-phosphorus detector on a Varian® 3740 gas chromatograph. This detector allowed the injection of samples that contained high levels of pigments and other plant compounds. The column used was a 2 mm by 90
cm Pyrex® column packed with 10% DC-200, 12.50 CSTK/2% OV-225 on 80/100 mesh Chromosorb®. The column packing near the injection port was changed after approximately every 750 injections and the entire column was replaced after approximately 4000 injections. Gas-liquid chromatographic analysis was completed using the temperature and gas flow parameters of Abou-Assaf et al. (1986), where the injection port temperature was 230°C, the column temperature was 220°C, and the detector temperature was 250°C. Gas flows were maintained at 30 ml/min nitrogen, 4.5 ml/min hydrogen, and 175 ml/min air.

Three μg aliquots of the extract and standards were injected into the gas-liquid chromatography column. Malathion retention time was 4 min. Standard curves based on the log of the peak height times attenuation were used to quantify samples. Detector response was linear over the range of samples injected (ca. 0.1 to 10 ppm).
RESULTS AND DISCUSSION

The extraction method presented in this paper proved both efficient and economical. It was effective in removing more than 99% of the malathion residues from ground corn (Table 1).

This extraction method has a number of advantages over the methods previously reviewed. First, the method was simplified by using only one solvent and two extraction steps, shaking and filtering. This is a significant reduction in procedure compared to methods which may have used more than one solvent, or required solvent partitioning, soaking the grain for extended periods of time, or column cleanup. Moreover, the actual extraction was accomplished during two shaking periods of 15 min each. The extraction process for 12 samples, including shaking, filtering, rotary evaporation and glassware cleanup, was completed in 4 hours.

While this extraction method is fast and efficient, the final extract is contaminated with plant pigments and other plant compounds. The use of the thermonic specific detector allows the injection of contaminated samples since it is specific only for nitrogen and phosphorus. However, the high level of sample contamination requires that the column packing at the injection port (the initial 10 cm of the column packing) be replaced routinely.

The second advantage, low extraction cost, stems from the simplicity of the procedure that minimizes material and labor expenses. The extraction cost for this method was $32.77 per 12 samples, an amount that approximates the cost of one sample in many methods. Hourly labor ($5 per hour) accounted for $20 of the above cost with the remainder spent on
materials. The cost of GLC analysis would be similar for all methods.
Third, exposure to ethyl acetate was less irritating to laboratory
personnel than the solvents typically used for extraction. Moreover,
exposure to ethyl acetate carries less risk of severe dermal and
respiratory irritation and poisoning. Fourth, the method used relatively
inexpensive glassware, such as Ball® jars, glass filters and round bottom
flasks; other methods required the use of separatory funnels and
chromatography columns.
Table 1. Percent malathion recovery from ground corn

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<th>Replication</th>
<th>% Recovery</th>
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<tr>
<td>1</td>
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% Average Recovery 99.6
REFERENCES CITED


PART II. DEGRADATION OF MALATHION AS A FUNCTION OF GRAIN DRYING AND STORAGE SYSTEMS
ABSTRACT

Experiments were conducted to determine the effect of grain drying and grain storage temperatures on the degradation of the 6% active ingredient (AI) dust and 57% AI emulsifiable concentrate formulations of malathion. Malathion was applied to cool wet corn (CWC), 19 - 20% moisture content, prior to drying, or to either hot dry corn (HDC) or cool dry corn (CDC), dried to 12% moisture content. All treatments were dried to a final moisture content of 12% under three drying temperatures, 21, 48, 71°C. Storage temperature effects were evaluated for all treatments with corn stored at 3°C for 4 months followed by storage at 16°C for 7 months.

Malathion applied before drying was significantly degraded by the drying process. The amount of degradation was dependent on drying temperature and formulation; 55, 27, and 19% of the residues from the malathion EC formulation remained on the corn when dried at 21, 48, and 71°C, respectively and 71, 30 and 37% of the residues from the malathion dust formulation remained on the corn when dried at 21, 48, and 71°C, respectively.

Degradation during 3°C storage was negligible and not affected by the variables considered. During the 16°C storage, malathion applied to CWC that was then dried at 48 or 71°C had a significantly lower rate of degradation than grain dried at 21°C. Malathion applied to HDC after drying had a significantly lower degradation rate during the 16°C storage than the malathion applied on CDC after drying.

Bioassay results using adults of the rice weevil *Sitophilus oryzae*
(L.) and the red flour beetle Tribolium castaneum (Herbst) showed that biologically active residues were present throughout the entire storage period. Rice weevils sustained 100% mortality while red flour beetle mortality declined as malathion residues decreased.

This study suggests that both malathion formulations can be applied immediately before or after drying at a wide range of temperatures and still remain efficacious. Rates should be adjusted to compensate for temperature and moisture effects when malathion is applied to CWC or HDC. In general the dust formulation was more stable than the EC formulation when exposed to high temperature and moisture.
INTRODUCTION

Malathion has proven to be an effective and economical grain protectant and has been used successfully for more than 30 years. Despite its many advantages, recent surveys have shown that malathion has been applied to only 7% of the corn stored on-farm in Iowa (Wintersteen and Hartzler 1987) and has been applied to only 7% of the corn and 11.6% of the wheat arriving at U.S. port terminals (Storey et al. 1982). One factor that has limited the use of malathion has been a lack of information concerning its application under the range of temperature and moisture conditions encountered in contemporary grain handling systems. Available data relating to these questions are limited to studies that considered malathion degradation under fixed moisture and storage conditions.

The breakdown of malathion as temperature increases and/or when grain moisture is above 14% has been documented by a number of researchers. Abdel-Kader et al. (1980), showed that as grain storage temperature increased the degradation rate increased. Strong and Sbur (1960) considered temperature and moisture interactions as well as their individual effects. Their results linked malathion degradation to increasing grain temperature and moisture. They showed that 14% moisture content (mc) wheat stored at 32°C degraded malathion more rapidly than either 12% mc stored at 32°C or 14% mc wheat stored at 15.5°C. Their data also indicated that by increasing the application rate the negative effects of increased temperature and moisture could be avoided to some extent. Rowlands (1967) stated that the rapid breakdown of malathion on
grain under high moisture or temperature conditions is due to the increased speed and occurrence of enzyme-catalyzed reactions.

Past studies have failed to consider malathion degradation under variable temperature or moisture conditions. Grain stored on-farm is frequently exposed to a wide range of temperature and moisture fluctuations throughout drying and storage. Actual temperatures and moisture levels depend on grain handling systems used, the changes in air temperature during storage, and the aeration practices employed.

The formulation of the malathion product may also influence effectiveness under different temperature and moisture conditions. Two malathion formulations, a 57% emulsifiable concentrate and a 6% flour dust are commonly used as grain protectants. Marketing interests have claimed that the flour dust formulation lasts longer, but research data are not available to support that claim.

The purpose of this research was to evaluate the residual efficacy of two formulations of malathion under a range of drying and storage conditions, both in the field and in the laboratory. The project was designed to answer the following questions: can malathion be applied before corn is dried, can malathion be applied to hot dry corn, what effect does drying temperature have on malathion degradation during both drying and storage, and are these factors affected by formulation? Determining at what points malathion could be applied during the drying process and the length of time residues will remain effective under variable storage temperatures will allow farmers to utilize malathion more effectively as a grain protectant.
MATERIALS AND METHODS

Laboratory Studies

A preliminary experiment evaluating the effect of three drying temperatures on corn treated with malathion 57% emulsifiable concentrate was completed in 1983. New crop yellow dent corn at 19% moisture content was used. The corn was divided into 25-kg lots and placed in cloth bags with plastic jackets to limit moisture fluctuations during storage. The bags of corn were held in storage at 12.5°C until malathion application. Each bag of corn was emptied into a large metal tray measuring 1.2 m by 0.9 m by 15 cm prior to treatment. Malathion 57% EC was applied as a water emulsion spray prepared with distilled water and applied with a low volume atomizer at a nominal rate of 10 ppm to cool wet corn (CWC). The corn was thoroughly mixed by hand raking during and at the end of the insecticide application. The malathion treated corn was placed in the grain dryers immediately after treatment. Samples for residue analysis were taken immediately after malathion application and immediately after drying.

Corn drying was completed in the laboratory, using four small drying bins designed by Navratil and Burris (1982). The dryers were equipped with thermostatically controlled electric elements capable of heating grain to 71°C. A centrifugal fan placed behind the heater on each bin allowed air movement at up to 196 liters/sec at 7.6 cm static pressure. Dryer airflow was adjusted and maintained at ca. 0.59 liters/sec by closing down the air intake opening. The dryers were vented to the outside to avoid increasing the room air temperature.
A randomized complete block design with four blocks was used. Each dryer bin was considered a block. Three drying temperatures (21, 48, 71°C) were the treatments. Drying temperatures were randomly applied to the drying bins.

Corn was dried at 21, 38 or 71°C for 33 ± 6.1, 12 ± 2.1, and 5 ± 0.6 hours, respectively. Dryer temperatures were monitored by placing a flask of water containing a thermometer in each dryer. Drying times were determined by sampling corn during the drying process, cooling the corn immediately and testing the corn moisture. Corn was removed from the dryer when the moisture content reached 12 ± 1%. Moisture content was determined with a Burrows Digital Moisture Computer 700 that had been calibrated using oven dried samples.

The laboratory drying experiment was repeated in 1984 and was expanded to consider the effects of malathion application after grain drying to hot corn or to cool corn, the evaluation of the EC and dust formulation of malathion, and to determine the effect of storage temperature on the degradation rate of the treated and dried corn. The procedures outlined for the 1983 study were repeated in 1984 with several modifications. A split-plot design with four blocks was used in the 1984 study. Each dryer bin was considered a block. The three drying temperatures (21, 48, 71°C) were the main-plot treatments. The split-plot treatments were application time and formulation. The initial moisture content of the corn was 20% compared with 19% in the 1983 study. The sample size was reduced from 25 kg of corn to 12.7 kg. Two formulations of malathion were applied, 57% EC or 6% dust (formulated on wheat flour).
The liquid formulation was applied as described above. The 6% dust was applied with a hand flour sifter at the rate of 10 ppm. The corn was thoroughly mixed by hand raking during and at the end of the insecticide application.

Drying times to be used during the experiment were determined by averaging the results of repeated trial runs at each temperature. The average drying times of corn dried at 21, 48 or 71°C were 24, 9, and 5 hours, respectively. These drying times resulted in corn with a final percent moisture content of 12 ± 1.5%. This change in procedure from the 1983 study standardized drying times for all treatments without significantly altering the final grain moisture content.

In the 1984 study, malathion was applied at 3 different points in the drying process: (1) on cool wet corn just prior to drying (CWC), (2) on hot dry corn immediately after drying (HDC), and (3) on cool dry corn after drying (CDC). After treatment CWC and CDC were held for an 11 month storage period in a temperature controlled storage chamber. The corn was stored at 3°C for 4 months representing the winter storage season in a grain bin. The 4 month storage period was followed by storage at 16°C for 7 months, simulating spring and summer storage conditions. Malathion residue and insecticidal activity were measured throughout the storage period. After treatment, HDC was immediately placed in an insulated container for 24 hours to prevent rapid cooling of the corn. This procedure was used to approximate the cool down period frequently experienced in farm systems. HDC was then held for an 11 month storage period, as described above.
Malathion residues were expressed as the percent malathion remaining at sampling. This percentage was obtained by dividing the residue remaining at each sampling date by the initial concentration. Percent malathion residue remaining after drying was evaluated statistically by split-plot analysis of variance. Duncan's Multiple Range Test (5 percent level) was used in evaluating and testing drying temperature and formulation differences. Degradation rate during the storage period was evaluated by regressing percent malathion remaining over time. The regressions were pooled over block, drying temperature, formulation and application. Analysis of variance was performed on the predicted slopes of these mean regressions.

Field Study

Three field trials were conducted in farm storage bins to validate the effects of drying temperature and formulation on malathion degradation applied on CWC prior to drying. Two 3000 bu. grain bins outfitted with perforated floors, propane dryers and drying fans were used to evaluate the effect of high temperature drying on the EC and dust formulations of malathion. A 9000 bu. grain bin with a perforated floor and dryer fan was used to evaluate the effect of low temperature drying on the EC formulation of malathion.

A "drip-on" applicator was used to apply the 57% EC; the 6% dust was applied with the auger dust applicator as described by Quinlan (1982). The liquid formulation was applied at the rate of 1 pint per 1000 bu.; and the 6% dust formulation was applied at the rate of 10 lbs per 1000 bu. These rates approximated the 10 ppm rates used in the laboratory study.
Grain bins were sampled immediately after malathion application, after drying and throughout the storage period. A composite sample was obtained using a 1.5-m tiered grain probe inserted into the grain surface at 8 random sites. At each sampling date, grain temperature and moisture were recorded. Temperature was determined by averaging three readings taken at three predetermined locations using a 6-foot thermometer probe. Moisture was determined using the moisture meter already discussed. Malathion residues were determined after application, after drying and at 60 and 180 days after malathion application.

**Residue Analysis**

Residue analyses for the laboratory and field studies were completed using the extraction and gas-liquid chromatographic procedures of Wintersteen et al. (In manuscript). Corn samples were ground to a consistency of flour interspersed with larger pieces of kernel pericarp, mesh no. 8 (U.S. Standard Sieve Series). Twenty-g samples of ground corn were placed in separate pint Ball® mason jars. Fifty ml of ethyl acetate were placed in each pint jar containing the treated corn and Ball® canning flats and rings were used to seal each jar. Sealed jars were shaken vigorously (275-285 oscillations/min) for 15 minutes on a Fisher-Kahn® shaker. The ethyl acetate was then decanted from each jar and filtered through 70 g of sodium sulfate held in a Whatman® no. 1 paper filter placed in a glass funnel. The filtrate was collected in a 500 ml round-bottom flask. This procedure was completed a second time using an additional 50 ml of ethyl acetate. The extract, consisting of all filtrates and rinses, was then concentrated to ca. 10 ml on a rotary
evaporator. The concentrated extract was then brought to a final volume of 50 ml by adding ethyl acetate.

Malathion concentrations were determined using a thermonic specific nitrogen-phosphorus detector with the Varian 3740 gas chromatograph equipped with a 2mm by 90 cm Pyrex column packed with 10% DC-200, 12.50 CSTK/2% OV-225 on 80/100 mesh Chromosorb. Gas-liquid chromatographic analysis was completed using the following temperature and gas flow parameters: injection port temperature was 230°C, column temperature was 220°C, and detector temperature was 250°C. Gas flows were maintained at 30 ml/min nitrogen, 4.5 ml/min hydrogen, and 175 ml/min air.

Insect Bioassay

Bioassays were conducted for each sampling date in the 1984 study to measure the insecticidal activity of each treatment. Procedures followed were similar to those used by Strong and Sbur (1960). Adults of red flour beetles, Tribolium castaneum (Herbst) and rice weevils, Sitophilus oryzae (L.) were used as the test species. Colonies, established from stock obtained from the USDA/ARS Grain Laboratory, Manhattan, Kansas, were maintained in our laboratory.

The bioassays were completed using 1 quart of grain divided into 4 replicates, each placed in half-pint containers. Twenty 7 to 14 day old adults of both species were added to each container. Bioassay containers were held at ca. 24°C and at a relative humidity of 60%. After 14 days, all the adults were removed from the grain and mortality was determined. Individuals were considered dead when no movement was observed after
prodding with a small paint brush. Bioassay controls, using untreated corn, were run simultaneously. Insect mortality in the controls ranged from 0 to 7%.
RESULTS

Malathion application on CWC, before drying significantly reduced the malathion residue remaining on the corn. In 1983, corn dried at 21, 38, and 71°C, had 53, 47, and 25% malathion remaining, respectively (Figure 1). Analysis of variance showed a significant drying temperature effect (F = 6.6; df = 2,4; P = 0.05). Duncan’s Multiple Range Test showed that corn dried at 71°C lost significantly more malathion during drying than corn dried at 21°C (P = 0.05).

The analysis of variance procedure performed on the 1984 study indicated a significant F value for drying temperature (F = 30.61; df = 2,6; P = 0.0007). Results from the 1983 and 1984 studies were similar for the EC formulation at the low and high drying temperatures (Figure 1). However, the levels of malathion remaining after drying at 38°C in the 1983 study and 48°C in the 1984 study were different; 47% of the malathion remained on the corn in the 1983 study while only 27% of the malathion remained on the corn in the 1984 study. Duncan’s Multiple Range Test values showed that in the 1984 study, corn dried at 71°C or 48°C resulted in significantly less malathion remaining on the corn than when it was dried at 21°C (P = 0.05). This lack of agreement between the two studies for the middle drying temperature was apparently due to the 10°C temperature difference in the two studies.

The analysis of variance procedure performed on the 1984 study indicated a significant F-value for formulation (F = 7.73; df = 1,9; P = 0.02). At all drying temperatures, corn treated with the dust formulation had significantly greater levels of malathion remaining than did the EC
formulation. Despite this significant difference, the decay curves during storage for the EC and dust formulations were not significantly different. Consequently, formulation data were pooled for statistical analysis of degradation rates during storage.

All treatments were sampled on 0, 15, 30, and 60 days during 3°C storage, at the end of 3°C storage (120 days), and three times during the 16°C storage, at 240, 270, 330 days. Results of the analysis of variance performed on sampling date 15 showed a temperature by application interaction (F = 10.1; df = 4,40; P = 0.0001). Cool wet corn dried at 21°C retained more malathion than CWC dried at 48°C or 71°C. There was no difference in the percent malathion remaining for application to HDC compared with application to CDC (Figure 2). Malathion residues were also significantly affected by application time (F = 358.28; df = 2,40; P = .0001). Duncan's Multiple Range Test values showed that corn treated before drying (CWC) had significantly less malathion remaining than HDC or CDC treated after drying (P = 0.05).

To avoid complications because of drying temperature effects, analyses of the residue degradation during storage were conducted separately for malathion application before and after drying. In addition, the rates of degradation during the 3°C storage period and the 16°C storage period were observed to be different, so analysis of variance was completed separately for the two storage periods.

The degradation rates of malathion applied to CWC before drying are presented in Figure 3A. Results of the analysis of variance on the rates of degradation during 3°C storage showed a lack of significance by
temperature or formulation. The mean slopes for the 16°C storage period were also subjected to analysis of variance which indicated that degradation rates during 16°C storage were significantly different for corn dried at different drying temperatures \( (F = 5.84; \text{df} = 2,6; \text{P} = 0.04) \). Duncan's Multiple Range Test values showed that corn dried at 21°C had a significantly faster rate of malathion degradation than corn dried at 48°C and 71°C (Figure 3B) \( (P < 0.05) \).

An evaluation of degradation rates of malathion applied to HDC and CDC showed similar results to those just discussed for CWC at 3°C. Analysis of variance was completed on the mean slopes of drying temperature, formulation and application time. Formulation was the only variable that affected the percent malathion present at the beginning of the 3°C storage period with significantly more dust than EC remaining \( (F = 7.13; \text{df} = 1,24; \text{P} = 0.01) \). These data suggest that regardless of whether malathion was placed on HDC or CDC, malathion residues were not significantly different. None of the variables considered influenced the rate of degradation during the 3°C storage period (Figure 4A).

During storage at 16°C, the degradation rate was significantly affected by application time. Malathion applied on CDC degraded at a significantly higher rate \( (F = 11.95; \text{df} = 1,24; \text{P} = 0.002) \) than malathion applied on HDC (Figure 4B).

Although the temperature at which corn was dried significantly effects malathion residues, adequate residues remained on the grain to provide insect control 11 months after application during the 1984 laboratory study. Because malathion degradation was calculated as a
percentage of the initial concentration and not as actual ppm, bioassay results are not correlated to percent malathion remaining. Bioassay results indicated that susceptible rice weevil (RW) cultures were 100% controlled by residues of less than 5% of that applied (ca. 0.5 ppm). Because RW's high level of susceptibility to malathion, 100% mortality was recorded for all treatments, at all temperatures, on all sampling dates. Red flour beetle (RFB) cultures were more difficult to control, requiring at least 20% residue remaining (ca. 2 ppm) to obtain 90% control. Consequently, RFB mortality decreased over time as the ppm malathion remaining decreased, but overall the malathion treatments provided effective control during the storage period. Table 1 shows the level of control obtained at sampling date 270.

Results of the on-farm grain drying studies were similar to the results of the laboratory studies (Table 2). Cool wet corn treated with 57% EC and dried at 21°C in a 9000 bu grain bin retained 53% of the applied malathion. Corn treated and dried under similar conditions in the laboratory retained 53% and 55% of the malathion applied in the 1983 and 1984 studies, respectively.

In comparison to 21°C drying, high temperature drying severely reduced residues applied on CWC. Cool wet corn treated with 57% EC or 6% dust and dried at 71°C in 3000 bu grain bins retained 13% and 14% of the applied malathion for EC and dust formulations, respectively. The effect of high temperature grain drying was more severe in on-farm bins than in laboratory drying bins. Cool wet corn treated with 57% EC prior to drying at 71°C retained 19% of the applied malathion in the 1984 laboratory study
and 25% of the applied malathion in the 1983 laboratory study. Cool wet corn treated with 6% dust before drying at 71°C retained 37% of the applied malathion. This difference in results between the field and laboratory studies is partially explained by the fact that the corn dried on-farm was dried to 10% mc as compared with 12% mc in the laboratory study. In addition, the time required to cool corn in the farm bin was greater than the 24 hour cool down period allowed in the lab study. Corn dried in a 3000 bu. bin is exposed to higher temperatures for a longer period of time.

In the field study, the dust and EC formulations applied to CWC before 71°C drying had almost the same percentage of malathion remaining after drying. However, a significant formulation difference was shown in the 1984 laboratory study. Laboratory results showed that the malathion dust formulation withstood the moisture and temperature effects during drying significantly better than did the EC formulation. The similarity of the dust and EC residues in the field study may be due to variation in drying systems and/or the location of the grain bins.

An examination of the degradation rate of malathion during on-farm grain storage showed results similar to those found in the laboratory study. Malathion applied to CWC dried at 48°C or 71°C showed little or no degradation during the storage period. Malathion applied to CWC dried at 21°C degraded more rapidly during the first 60 day but showed only slight degradation in the following 4 months.
DISCUSSION

The 1983 and 1984 laboratory results showed that drying CWC at 71°C removed significantly more malathion than drying it at 21°C. In addition, the 1984 laboratory study indicated that the 48°C drying temperature removed significantly more malathion than corn dried at 21°C. The higher drying temperatures resulted in rapid malathion volatilization from the corn during a short, 5 or 9 hour drying period. Moreover, at the start of the drying period, the malathion was exposed to the high moisture conditions of the corn, which may have allowed malathion hydrolysis to occur (Rowlands 1967).

Low temperature (21°C) drying of CWC required a longer drying period of 24 hours. While the malathion was not subjected to high temperatures it was exposed to high moisture corn for a much longer time period, providing greater opportunity for hydrolysis to occur.

Results of the 1984 laboratory study showed that the dust formulation withstood volatilization and hydrolysis during drying better than the EC formulation. This difference is presumed to be real even though it was not apparent in the less precise and unreplicated field study.

Malathion residues remaining after application to HDC were not significantly different than residues remaining after application to CDC. However, the malathion degradation that resulted from the application to HDC may be more severe in farm bins, if corn is allowed to cool more slowly.

The 3°C storage period slowed malathion degradation in all treatments and none of the variables considered (application time, formulation or
drying temperature) effected the rate of degradation during 3°C storage. These results are in agreement with other studies which have shown that malathion degradation slows considerably under cold storage conditions (Abdel-Kader et al. 1980).

Storage at 16°C resulted in significant differences in the degradation rate for malathion. Analysis of variance performed on the mean slopes showed that drying temperature significantly effected the degradation rate for applications on CWC made before drying. Malathion applied to CWC dried at 21°C degraded at a significantly higher rate than treated CWC dried at 48°C or 71°C. Analysis of variance on the mean slopes for malathion applications after drying showed that during 16°C storage, malathion applied on CDC degraded faster than malathion applied to HDC.

Treatments with significantly steeper degradation rates were those treatments with the initial highest concentration present at the start of the 16°C storage period. Previously published research data have shown that the rate of malathion degradation is a function of its initial concentration (Abdel-Kader et al. 1980; LaHue and Dicke 1976), and results in a negative exponential decay curve. To fully explain the degradation rates, as depicted in Figures 3 and 4, decay curves were generated by regressing percent malathion remaining against sampling dates over the 16°C storage period. Malathion applications on CWC before drying at 48°C or 71°C, or applications to HDC show a flat line with a very low $r^2$ value (Table 3). Because of the significant volatilization of malathion by heat (Figure 1), the residue remaining represents the flat final portion of the
degradation curve. On CDC, the malathion level was not reduced by volatilization. Here, sufficient amounts of malathion remained to allow rapid degradation, as shown by a strong initial response during the 16°C storage period (120 to 330 days). This rapid, linear decay represents the initial portion of the exponential decay curve. Significant differences in degradation rates are due to differences in concentration remaining after drying. None of the variables (drying temperature, application time, formulation) evaluated significantly affected the rate of degradation during the 16°C storage period.

Results from the field study validated the use of the small laboratory drying bins to simulate the effect of grain drying bins used on-farm. The effect of grain drying on treated CWC was similar in both studies, although the drying effect was more severe for both formulations in the field study. Overall, the similar results between the laboratory and field studies show that the drying bins used in this study approximated the effect of grain drying systems used on-farm.

The bioassay data confirmed that biologically active malathion residues were present throughout the storage period. Complete control of the RW was achieved at all sampling dates, while RFB control was reduced as malathion residues decreased.

The results of this study suggest that when either formulation of malathion is applied immediately before or after the drying process and at a wide range of drying temperatures, effective insect control is maintained during an eleven month storage period. The data indicate that the 6% malathion dust survives the impact of initial temperature and
moisture effects significantly better than the 57% EC formulation.

The results agree with previously published research, showing that
the rate of degradation is a function of temperature, grain moisture and
initial concentration. Within the relatively narrow parameters of this
study, the effect of moisture was modified by temperature. Both
temperature and moisture effected the concentration of malathion remaining
after drying. However, it is not known how these factors interact given
the wider range of temperature, moisture and initial concentration
combinations that are expected to exist in on-farm storage conditions.

The results suggest that actual application rates on CWC and HDC
should be adjusted to compensate for the effects of temperature and
moisture. While precise rates cannot be determined for the multitude of
conditions that might exist when grain is treated, approximate rates can
be suggested. When malathion is applied to CWC before drying at ca. 21°C,
the rate of application should be increased from 10 to 15 ppm. When
malathion is applied on CWC before drying at moderate to high
temperatures, ca. 48 to 71°C, the application rate should be increased to
20 ppm. When malathion is applied to HDC, after drying at moderate to
high temperatures, the application rate should be increased to 20 ppm.
Figure 1. Percent malathion residues remaining from dust and emulsifiable concentrate (EC) formulations applied prior to drying in the 1983 and 1984 laboratory studies.
Figure 2. Percent malathion remaining 15 days after application on CWC (cool wet corn), HDC (hot dry corn), or CDC (cool dry corn)
Figure 3A. Effect of drying temperatures on the predicted malathion degradation on corn treated prior to drying.

Figure 3B. Effect of drying temperatures on the predicted rate of malathion degradation during 16°C storage for corn treated prior to drying.
Figure 4A. Predicted malathion degradation during storage on hot (HDC) or cool (CDC) grain treated after drying.

Figure 4B. Predicted malathion degradation rate during 16°C storage on hot dry corn (HDC) versus cool dry corn (CDC).
Table 1. Percent red flour beetle mortality at 270 days post application

<table>
<thead>
<tr>
<th>Application</th>
<th>Formulation</th>
<th>Drying Temperature</th>
<th>21°C</th>
<th>48°C</th>
<th>71°C</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWC</td>
<td>Dust</td>
<td></td>
<td>94</td>
<td>91</td>
<td>86</td>
</tr>
<tr>
<td>HDC</td>
<td>Dust</td>
<td></td>
<td>95</td>
<td>97</td>
<td>97</td>
</tr>
<tr>
<td>CDC</td>
<td>Dust</td>
<td></td>
<td>94</td>
<td>93</td>
<td>82</td>
</tr>
<tr>
<td>CWC</td>
<td>EC</td>
<td></td>
<td>78</td>
<td>93</td>
<td>50</td>
</tr>
<tr>
<td>HDC</td>
<td>EC</td>
<td></td>
<td>99</td>
<td>94</td>
<td>97</td>
</tr>
<tr>
<td>CDC</td>
<td>EC</td>
<td></td>
<td>83</td>
<td>86</td>
<td>80</td>
</tr>
</tbody>
</table>

*aApplication: CWC is application to cool wet corn before drying; HDC is application to hot dry corn after drying; CDC is application to cool dry corn after drying.

*bFormulation: Dust is 6% malathion dust; EC is 57% malathion emulsifiable concentrate.

Table 2. Percent malathion remaining on grain after drying in on-farm grain bins

<table>
<thead>
<tr>
<th>Bin No.</th>
<th>Bushels</th>
<th>Drying Temp. °C</th>
<th>Form.</th>
<th>Moisture Content</th>
<th>% Residue Remaining</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Initial</td>
<td>Final</td>
</tr>
<tr>
<td>1</td>
<td>3000</td>
<td>71</td>
<td>EC</td>
<td>23%</td>
<td>10%</td>
</tr>
<tr>
<td>2</td>
<td>3000</td>
<td>71</td>
<td>Dust</td>
<td>21%</td>
<td>10%</td>
</tr>
<tr>
<td>3</td>
<td>9000</td>
<td>21</td>
<td>EC</td>
<td>23%</td>
<td>12%</td>
</tr>
</tbody>
</table>

*aFormulation: Dust is 6% malathion dust; EC is 57% malathion emulsifiable concentrate.
Table 3. Slope, intercept and $r^2$ of actual ppm regressed over residues remaining during 16°C storage

<table>
<thead>
<tr>
<th>Appl.</th>
<th>Formulation</th>
<th>Drying Temp °C</th>
<th>Slope</th>
<th>Intercept</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CWC</td>
<td>Dust</td>
<td>22</td>
<td>-0.0007</td>
<td>5.26</td>
<td>0.66</td>
</tr>
<tr>
<td>HDC</td>
<td></td>
<td></td>
<td>-0.0248</td>
<td>10.58</td>
<td>0.93</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td></td>
<td>-0.0134</td>
<td>7.17</td>
<td>0.64</td>
</tr>
<tr>
<td>CWC</td>
<td>EC</td>
<td></td>
<td>-0.0008</td>
<td>1.67</td>
<td>0.63</td>
</tr>
<tr>
<td>HDC</td>
<td></td>
<td></td>
<td>-0.0053</td>
<td>3.61</td>
<td>0.35</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td></td>
<td>-0.0075</td>
<td>3.66</td>
<td>0.66</td>
</tr>
<tr>
<td>CWC</td>
<td>Dust</td>
<td>48</td>
<td>-0.0021</td>
<td>2.41</td>
<td>0.21</td>
</tr>
<tr>
<td>HDC</td>
<td></td>
<td></td>
<td>-0.0182</td>
<td>9.36</td>
<td>0.63</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td></td>
<td>-0.0135</td>
<td>7.32</td>
<td>0.57</td>
</tr>
<tr>
<td>CWC</td>
<td>EC</td>
<td></td>
<td>0.0003</td>
<td>1.37</td>
<td>0.002</td>
</tr>
<tr>
<td>HDC</td>
<td></td>
<td></td>
<td>-0.0046</td>
<td>4.09</td>
<td>0.02</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td></td>
<td>-0.0107</td>
<td>5.58</td>
<td>0.20</td>
</tr>
<tr>
<td>CWC</td>
<td>Dust</td>
<td>71</td>
<td>-0.0021</td>
<td>2.12</td>
<td>0.22</td>
</tr>
<tr>
<td>HDC</td>
<td></td>
<td></td>
<td>-0.0093</td>
<td>6.78</td>
<td>0.36</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td></td>
<td>-0.0113</td>
<td>6.90</td>
<td>0.11</td>
</tr>
<tr>
<td>CWC</td>
<td>EC</td>
<td></td>
<td>-0.0001</td>
<td>0.83</td>
<td>0.002</td>
</tr>
<tr>
<td>HDC</td>
<td></td>
<td></td>
<td>-0.0077</td>
<td>6.46</td>
<td>0.11</td>
</tr>
<tr>
<td>CDC</td>
<td></td>
<td></td>
<td>-0.00519</td>
<td>3.97</td>
<td>0.06</td>
</tr>
</tbody>
</table>

^Application: CWC is application to cool wet corn before drying; HDC is application to hot dry corn after drying; CDC is application to cool dry corn after drying.

^Low $r^2$ value due to the flatness of the line.
REFERENCES CITED


Wintersteen, W., L. L. Karr, S. P. Bradbury and J. R. Coats. A rapid and inexpensive method for extracting malathion residues from corn. In manuscript.
PART III. EFFECT OF INDIGENOUS PENICILLIUM SPP. ON MALATHION DEGRADATION UNDER VARIABLE DRYING AND STORAGE TEMPERATURES
ABSTRACT

The influence of indigenous storage fungi on the degradation rate of malathion under typical on-farm drying and storage conditions was examined. Corn at 25% moisture content (mc) was either autoclaved (AC) to eliminate fungal populations or left non-autoclaved (NAC), dried to a final moisture content of 12% at either 21°C or 71°C, cooled and treated with 57% emulsifiable concentrate (EC) malathion. The rate of malathion degradation and the growth of fungal populations were evaluated during 4 months of storage at 3°C followed by 10 months of storage at 16°C. *Penicillium* spp. (PEN) were the most common storage fungi present prior to and after autoclaving and during storage. The occurrence of other storage fungi was too sporadic and variable to allow statistical comparison. At the end of 3°C storage, both AC and NAC dried at 71°C had significantly lower PEN populations than corn dried at 21°C. When storage temperature was increased to 16°C, increased fungal growth eliminated the drying temperature effect. The number of PEN colonies present on AC vs NAC varied significantly. At the end of both the 3°C and 16°C storage, significantly higher levels of PEN were present on the NAC. The greatest increase in PEN colonies during storage was shown on the NAC. The lack of significant differences among malathion decay curves suggest that PEN did not degrade malathion.

The degradation rate of malathion during the 14 month storage period was not affected significantly by drying temperature, sterilization technique (AC vs NAC), or by PEN population. Furthermore, there were no
statistically significant interactions among PEN populations, drying temperatures and sterilization techniques on the rate of malathion degradation.
INTRODUCTION

Insect infestation and fungal invasion presents a widespread and common problem in grain storage. Survey data collected in 1980 suggested that more than 80% of the corn stored on Midwest farms was insect infested (Barak and Harein 1981, Storey et al. 1983). In addition, survey data collected and analyzed by Sauer et al. (1984) documented the presence of the storage fungus Aspergillus glaucus (Link) in 84% of the corn samples. The rate of fungal invasion was related to grain moisture; samples with less than 11% moisture content (mc) had 14% of the kernels invaded, while samples above 13% mc had 44% of the kernels invaded. A strong interaction between the presence of storage fungi and grain insects was shown; insect infested corn samples with at least 12.5% moisture content averaged 54% invasion by storage fungi.

Malathion has been used successfully as a grain protectant for over 30 years; however, past studies have indicated that fungal populations may degrade the malathion applied to grain. Rowlands (1967) stated that storage fungi could metabolize insecticide residues on grain and that when enzymic activity of grains is low, malathion degradation could be primarily due to fungal populations present. Walker and Stojanovic (1973) showed that malathion disappeared from non-sterile soil faster than it did from sterile soil. In experiments conducted by Lewis et al. (1975), Aspergillus oryzae (Ahlb.) Cohn rapidly transformed malathion into B-malathion monoacid. In another study, Anderegg and Madison (1983) showed that sterilized grain contained significantly more malathion after 6 months storage than sterilized grain inoculated with A. glaucus.
Christensen and Kaufmann (1969) stated that there were six factors that influence the development of storage fungi in grain: grain moisture content, grain temperature, length of storage period, the degree of invasion by storage fungi, amount of foreign matter present, and insects present. All of these factors interact to influence the quality of storage grain. Yellow dent corn at 13.2% mc can be invaded by some species of *Aspergillus*. Increasing moisture content results in possible fungal invasion by more than 50 fungal species, each with their own specific moisture limits. Temperature also affects the growth of fungi with optimum growth of many species occurring around 30°C. Growth rate will be reduced below 20°C but some species of the *A. glaucus* group will grow at 5°C (Christensen 1957). The degree of fungal invasion will influence how long the grain can be stored, even at optimum conditions. Christensen and Kaufman (1969) showed that grain already invaded by storage fungi and stored at 15% mc, 10°C, continued to experience fungal growth. If the grain was free of storage fungi it could be stored under those conditions for up to a year without incurring damage.

Castor (1983) stated that since storage fungi are so widespread, there is a high probability of contaminating grain during harvest and the grain drying process. The longer the seed remains moist the greater the chance for fungal invasion. He reported that fungi can even "colonize during the 70 to 100 hours (at 50°C) needed for drying."

Because of the high incidence of storage fungi in corn stored on-farm and the evidence that storage fungi can metabolize malathion, this
study was designed to determine the influence of indigenous fungal populations on the degradation rate of malathion under typical on-farm drying and storage conditions.
MATERIALS AND METHODS

New crop yellow dent corn at ca. 25% mc was stored for 4 months at 12°C. A composite sample was removed from the corn to identify and quantify the indigenous fungal populations. The corn was weighed and divided into 3.2 kg lots which were placed in cloth bags with plastic jackets to prevent moisture fluctuations during storage. The bags of corn were held in storage at 12°C until grain drying.

Before drying each experimental unit of corn was either autoclaved (AC) or non-autoclaved (NAC). Autoclaving was used to eliminate fungal populations. Corn was autoclaved for 20 minutes (18 pounds chamber pressure; 20 pounds jacket pressure, temperature 118°C). Autoclaved corn and NAC were dried at 21°C or 71°C for 24 and 5 hours, respectively. These drying times resulted in corn with a final moisture content of 12 ± 1.7%.

Grain drying was completed in the laboratory, using four small drying bins designed by Navratil and Burris (1982). The dryers were equipped with thermostatically controlled electric elements capable of heating grain to 71°C. A centrifugal fan placed behind the heater on each bin allowed air movement at up to 196 liters/sec at 7.6 cm static pressure. Dryer airflow was adjusted and maintained at ca. 0.59 liters/sec by closing down the air intake opening. The dryers were vented to the outside to avoid increasing the room air temperature.

A split-plot design with four blocks was used. Each drier bin was considered a block. Two drying temperatures, 21°C and 71°C, were the main plot treatments. Drying temperature was randomly assigned to the drying
bins. The split-plot treatment was sterilization status (AC vs NAC).

After drying, malathion 57% emulsifiable concentration (EC) was prepared as an emulsion spray with distilled water and applied to cool dry corn with a low volume atomizer at the rate of 10 ppm. Each bag of corn was emptied into a large metal tray measuring 1.2 m by 0.9 m by 15 cm and the malathion was applied. The grain was thoroughly mixed by hand raking during and at the end of the insecticide application. Samples for zero-time residue analysis were taken immediately after malathion application.

After treatment, the corn was returned to individual cloth bags with plastic jackets. All treated corn was held during a 14 month storage period in a temperature controlled storage chamber. The corn was stored at 3°C for 4 months representing the winter storage season in a grain bin. The 4 month storage period was followed by storage at 16°C for 10 months, simulating spring and summer storage conditions. Malathion residue levels were measured at 1, 30, 120, 390, and 420 days post application. Fungal populations were determined at 0, 120 and 420 days post application.

Malathion residues were expressed as the percent malathion remaining at sampling. This percentage was obtained by dividing the residue remaining at each sampling date by the initial deposit. Degradation rates during the storage period were evaluated by regressing percent malathion over time. The regressions were pooled over block, drying temperature and sterilization technique. Analysis of variance was performed on the predicted slopes of these mean regressions. Fungal populations present at each sampling date were evaluated by split-plot analysis of variance.
Residue Analysis

Residue analysis was completed using the extraction and gas-liquid chromatographic procedures of Wintersteen et al. (In manuscript). Corn samples were ground to a consistency of flour interspersed with larger pieces of kernel pericarp, mesh no. 8 (U.S. Standard Sieve Series). Twenty g samples of ground corn were placed in separate pint Ball® mason jars. Fifty ml of ethyl acetate was placed in each pint jar containing the treated grain and Ball® canning flats and rings were used to seal each jar. Sealed jars were shaken vigorously (275-285 oscillations/min) for 15 minutes on a Fisher-Kahn® shaker. The ethyl acetate was then decanted from each jar and filtered through 70 g of sodium sulfate held in a Whatman® no. 1 paper filter placed in a glass funnel. The filtrate was collected in a 500 ml round-bottom flask. This procedure was completed a second time using an additional 50 ml of ethyl acetate. The extract, consisting of all filtrates and rinses, was then concentrated to ca 10 ml on a rotary evaporator. The concentrated extract was then brought to a final volume of 50 ml in a volumetric flask by adding ethyl acetate.

Malathion concentrations were determined using a theromic specific nitrogen-phosphorus detector with the Varian® 3740 gas chromatograph equipped with a 2 mm by 90 cm Pyrex® column packed with 10% DC-200, 12.50 CSTK/2% OV-225 on 80/100 mesh Chromosorb®. Gas-liquid chromatographic analysis was completed using the following temperature and gas flow parameters: injection port temperature was 230°C, column temperature was 220°C, and detector temperature was 250°C. Gas flows were maintained at 30 ml/min nitrogen, 4.5 ml/min hydrogen, and 175 ml/min air.
Fungal Population Determinations

Corn was plated on 4 types of agar media to ensure the detection of a wide range of storage fungi. The agar mediums used were Tomato juice--salt agar (T6), Acid potato dextrose agar, PCNB agar, and Water agar (Christensen and Meronuck 1974). Five disposable petri dishes containing each agar were prepared for each treatment replication at each sampling date. Before plating, kernels were surface sterilized in a 2.0% aqueous sodium hypochlorite solution for 2 minutes and then rinsed in sterile water for 30 seconds. Kernel plating was completed under a sterile hood using rigid aseptic procedures. Twenty kernels were placed on each plate. Agar plates were incubated at 25°C for 1 week and the type and number of fungal colonies per 100 kernels were counted. All fungi developing on plates were identified to genus using the standards of Barnett and Hunter (1987) and the number of colonies of each genus counted and recorded.
RESULTS

The analysis of variance performed on the rate of degradation during the 14 month storage period showed a lack of significance for drying temperature or sterilization (Figure 1).

Comparison of the results of plating kernels from composite corn samples taken before and after autoclaving are shown in Table 1. *Penicillium* spp. (PEN) were the primary storage fungi present before and after corn was autoclaved. Statistical analysis on fungal populations present during storage were completed only for PEN. The occurrence of other storage fungi was too sporadic and variable to allow statistical comparison. The T6 media was evaluated because it is selective for PEN and provided relatively high and consistent numbers of PEN.

Analysis of variance completed on PEN colony counts at the end of the 3°C storage period (120 days post treatment), showed a significant interaction between temperature and sterilization ($F = 28.79; df = 1,5; P = 0.003$). Non-autoclaved corn dried at 21°C had the highest numbers of penicillium colonies present. There was also a significant difference between fungal populations on AC and NAC ($F = 40.79; df = 1,3; P = 0.001$) and between drying temperatures ($F = 419.29; df = 1; P = 0.0003$). Autoclaved corn had significantly lower PEN populations compared with NAC and corn dried at 71°C had significantly lower PEN populations compared with corn dried at 21°C.

Analysis of variance performed on PEN colony counts at the end of the 16°C storage period showed similar results. The significant difference between fungal populations on AC and NAC was still present ($F = 28.50; df$
However the drying temperature effect and the temperature by sterilization interaction were no longer significant. Analysis of variance performed across sampling dates showed a significant interaction between sterilization technique and sampling date (F = 9.5; df = 1,5; P = 0.01). The increase in PEN colonies during warm storage was significantly higher on NAC compared with AC. A significant sampling date effect (F = 11.68; df =1,12; P = 0.006) was also shown. Corn at day 420 had higher numbers of penicillium colonies present compared with corn at day 120.
DISCUSSION

The results of this study show that under environmental conditions unfavorable for fungal growth (12% mc, 3°C storage temperature), or under more favorable conditions (12% mc, 16°C storage temperature), indigenous PEN populations did not affect the degradation rate of malathion. The significant differences in PEN populations on AC and NAC at the end of 3°C and 16°C storage, and the fact that no differences were found in the degradation rates of AC and NAC, confirm that PEN populations did not influence malathion's decay rate.

The effect of drying temperature on PEN numbers was significant both at 0 and after 120 days storage at 3°C. Corn dried at 71°C had fewer PEN colonies present compared with corn dried at 21°C. These results suggest that drying at high temperatures reduces the numbers of PEN colonies present. However, when the storage temperature was increased to 16°C, the increased fungal growth eliminated the drying temperature effect.

Compared with colonies present at the end of 3°C storage, PEN colonies present at the end of the 16°C storage period had increased on all the treatments except AC dried at 71°C. This suggests that the increase was due to invasion and growth by the PEN under the elevated temperature conditions, and that airborne contamination was not a significant factor. The greatest increase in PEN colonies during 16°C storage was shown on the NAC. If PEN had been metabolizing malathion as an energy source, the decay curve for the NAC treated with malathion would have been steeper than the curves for AC, treated corn. However there were no significant differences in the decay rate of malathion treated AC...
and NAC.

Under the prescribed conditions of this study, the degradation rate of malathion was not significantly affected by PEN populations during either the 3°C or 16°C storage period. Furthermore, there were no statistically significant interactions among PEN populations, drying temperatures and sterilization techniques on the rate of malathion degradation. These data contrast with those of previous workers who dealt primarily with *A. glaucus*, and demonstrated its positive effect on malathion degradation.
Figure 1. Predicted malathion degradation during storage on autoclaved corn (AC) and non-autoclaved corn (NAC)
Table 1. Storage fungi present when autoclaved and non-autoclaved corn are exposed to various media

<table>
<thead>
<tr>
<th>Media</th>
<th>Non-Autoclaved</th>
<th>Autoclaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>T6</td>
<td>122</td>
<td>36</td>
</tr>
<tr>
<td>APDA</td>
<td>148</td>
<td>0</td>
</tr>
<tr>
<td>PCNB</td>
<td>116</td>
<td>0</td>
</tr>
<tr>
<td>WA</td>
<td>48</td>
<td>0</td>
</tr>
</tbody>
</table>

^Colonies present per 100 kernels: Pen. equals Penicillium spp.; Asp. equals Aspergillus spp.; Fus. equals Fusarium spp.

^Agar Media Types: T6 is tomato juice-salt agar; APDA is acid potato dextrose agar; PCNB is pentachloronitrobenzene agar; WA is water agar.
Table 2. Mean *Penicillium* spp. colonies\(^a\) present during storage

<table>
<thead>
<tr>
<th>Days Post Application</th>
<th>Dryer Temp °C</th>
<th>Sterilization(^b) NAC</th>
<th>AC</th>
</tr>
</thead>
<tbody>
<tr>
<td>120</td>
<td>21</td>
<td>58</td>
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<tr>
<td>120</td>
<td>71</td>
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<tr>
<td>420</td>
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</tr>
<tr>
<td>420</td>
<td>71</td>
<td>70.3</td>
<td>10.3</td>
</tr>
</tbody>
</table>

\(^a\)Colonies present per 100 kernels plated on tomato juice-salt agar.

\(^b\)Sterilization: NAC is non-autoclaved corn and AC is autoclaved corn.
REFERENCES CITED


Wintersteen, W., L. L. Karr, S. P. Bradbury, and J. R. Coats. A rapid and inexpensive method for extracting malathion residues from corn. In manuscript.
SUMMARY

Malathion has been used as an effective and inexpensive grain protectant for over 30 years. Despite its many advantages, recent surveys have shown that malathion has been applied to only 7% of the corn stored on-farm. One factor that has limited the use of malathion has been a lack of information concerning its application under the range of temperature and moisture conditions encountered in contemporary grain handling systems. Available data relating to these questions are limited to studies that considered malathion degradation under fixed moisture and storage conditions. Grain stored on-farm is frequently exposed to a wide range of temperature and moisture fluctuations throughout drying and storage.

The objectives of this study were: (1) develop an accurate, rapid and inexpensive method of extracting malathion residues in yellow dent corn, (2) determine the effect of drying temperature and grain moisture on malathion liquid and dust formulations applied prior to drying and evaluate residual life and efficacy of these applications during 4 months of 3°C followed by 7 months storage at 16°C, (3) determine the effect of drying temperature on malathion liquid and dust formulations applied after drying to either hot or cool dry grain and evaluate residual life and efficacy of these applications during 4 months of 3°C followed by 7 months storage at 16°C, and (4) evaluate indigenous fungal populations on corn and correlate fungal populations to malathion degradation.

An accurate method of extracting malathion from corn was developed. Residue extraction was achieved by placing the treated ground corn and the
solvent, ethyl acetate in a pint Ball® mason jar sealed with Ball® canning flats and rings. Sealed jars were shaken vigorously (275-285 oscillations/min) for 15 minutes on a Fisher-Kahn® shaker. The ethyl acetate was decanted from each jar and filtered. This procedure was completed a second time using additional ethyl acetate. The extract, consisting of all filtrates and rinses, was concentrated and then brought to a final volume using ethyl acetate. Malathion residues in extracted samples were detected by a thermonic specific nitrogen-phosphorus detector on a Varian® 3740 gas chromatograph, and showed a 95% rate of recovery.

Malathion applied to cool wet corn before drying was significantly degraded by the drying process. The greatest degradation occurred when corn was dried at 48°C and 71°C. The higher drying temperatures resulted in rapid malathion volatilization. Laboratory results showed that the dust formulation withstood the temperature and moisture stresses of grain drying better than the EC formulation.

Grain treated after drying retained significantly more malathion than grain treated before drying. Malathion residues remaining after application to hot dry corn were not significantly different than residues remaining after application to cool dry corn. However, the malathion degradation that results from application to hot dry corn may be more severe under field conditions.

The 3°C storage period slowed malathion degradation for all treatments and none of the variables considered (application time, formulation, or drying temperature) affected the rate of degradation during this storage.
Increased degradation occurred during the 16°C storage period, but the degradation rate was not influenced by any of the aforementioned variables considered. However, the decay curve was influenced by the initial malathion concentration present at the start of the 16°C storage; high initial concentrations showed a rapid linear decay, while low initial concentrations showed a flat decay.

Results from the field study validated the use of the small laboratory drying bins to simulate the effect of grain drying bins used on-farm. The effect of grain drying on malathion applied to cool wet corn prior to drying was similar in both studies, although the drying effect was more severe for both formulations in the field study.

The bioassay data confirmed that biologically active malathion residues were present throughout the storage period. Complete control of the rice weevil was achieved at all sampling dates, while red flour beetle control was reduced as malathion residues decreased.

These results show that both malathion formulations can be applied immediately before or after drying at a wide range of temperatures and still remain efficacious. Rates should be adjusted to compensate for temperature and moisture effects when malathion is applied on cool wet corn prior to drying or on hot dry corn after drying.

The effect of indigenous *Penicillium* spp. on the degradation rate of malathion was examined. Malathion degradation and fungal growth were evaluated during 4 months storage at 3°C followed by 10 months storage at 16°C. Under these conditions, the degradation rate of malathion was not
significantly affected by \textit{Penicillium} spp. and there were no significant interactions among fungal populations, drying temperatures and sterilization on the rate of malathion degradation.
ACKNOWLEDGMENTS

Sincere appreciation is expressed to David Foster, my major professor, for guidance, support, and friendship throughout my graduate program. I am also grateful to Drs. Carl Bern, Joe Burris, Joel Coats, and Bill Showers for serving on my graduate committee and providing input on the dissertation. Special appreciation is expressed to Dr. Burris and Dr. Coats for providing the necessary laboratory equipment, space, and expertise needed for this project. I am also indebted to Dr. Steve Bradbury, Laura Karr, Leon Higley, and Phyllis Higley for their assistance on this project and their friendship and support. I also thank Dr. Laura Sweets for her invaluable assistance and support she provided on the storage fungus study and for her friendship she extended to me as a fellow cooperative extension service professional.

Most importantly, I would like to thank my husband, Robert Waggoner, for the encouragement and support he provided me throughout my graduate program. Without his friendship and love, I would not have been able to complete this degree.
APPENDIX
Table 1. Percent malathion remaining at 2, 15, 30, and 60 days after application to cool wet corn (CWC), hot dry corn (HDC) or to cool dry corn (CDC) and dried at 21, 48, or 71°C (Part II, 1984 laboratory study).

<table>
<thead>
<tr>
<th>Drying Temp. °C</th>
<th>Form.</th>
<th>Application Time</th>
<th>Trt</th>
<th>2</th>
<th>15</th>
<th>30</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>21</td>
<td>Dust</td>
<td>CWC</td>
<td>1</td>
<td>71.3 ± 10.5</td>
<td>63.4 ± 8.9</td>
<td>63.2 ± 6.5</td>
<td>63.3 ± 5.2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDC</td>
<td>2</td>
<td>91.2 ± 2.9</td>
<td>80.2 ± 16.9</td>
<td>74.3 ± 11.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDC</td>
<td>3</td>
<td>104.3 ± 6.1</td>
<td>92.5 ± 19.2</td>
<td>79.8 ± 15.0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>CWC</td>
<td>4</td>
<td>55.0 ± 15.4</td>
<td>48.6 ± 16.2</td>
<td>46.9 ± 16.8</td>
<td>42.1 ± 13.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDC</td>
<td>5</td>
<td>96.6 ± 3.7</td>
<td>94.2 ± 7.2</td>
<td>91.9 ± 10.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDC</td>
<td>6</td>
<td>91.9 ± 6.1</td>
<td>88.7 ± 12.9</td>
<td>87.8 ± 11.9</td>
<td></td>
</tr>
<tr>
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<td>Dust</td>
<td>CWC</td>
<td>1</td>
<td>30.4 ± 3.8</td>
<td>21.1 ± 2.8</td>
<td>24.3 ± 2.4</td>
<td>20.6 ± 1.4</td>
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<td></td>
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<td>95.9 ± 9.1</td>
<td>91.4 ± 6.7</td>
<td>79.4 ± 3.5</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDC</td>
<td>3</td>
<td>101.9 ± 4.6</td>
<td>95.1 ± 23.0</td>
<td>96.5 ± 14.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>CWC</td>
<td>4</td>
<td>26.8 ± 9.9</td>
<td>29.1 ± 8.7</td>
<td>32.4 ± 5.1</td>
<td>20.0 ± 4.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDC</td>
<td>5</td>
<td>92.6 ± 2.5</td>
<td>92.7 ± 19.9</td>
<td>65.2 ± 17.2</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDC</td>
<td>6</td>
<td>89.1 ± 15.3</td>
<td>76.2 ± 14.4</td>
<td>82.5 ± 24.8</td>
<td></td>
</tr>
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<td>Dust</td>
<td>CWC</td>
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<td>37.2 ± 11.0</td>
<td>34.9 ± 1.8</td>
<td>29.8 ± 6.5</td>
<td>30.5 ± 6.6</td>
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<tr>
<td></td>
<td></td>
<td>HDC</td>
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<td>61.9 ± 3.4</td>
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<td></td>
<td>CDC</td>
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<td>98.7 ± 6.4</td>
<td>86.9 ± 18.4</td>
<td>85.7 ± 12.4</td>
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<tr>
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<td>CWC</td>
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<td>20.6 ± 8.2</td>
<td>23.3 ± 3.9</td>
<td>17.2 ± 6.0</td>
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<tr>
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<td>HDC</td>
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<td>77.6 ± 6.9</td>
<td>72.7 ± 11.8</td>
<td>79.1 ± 9.9</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>CDC</td>
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<td>89.1 ± 12.9</td>
<td>70.6 ± 19.7</td>
<td>71.4 ± 11.3</td>
<td></td>
</tr>
</tbody>
</table>

*Formulation: Dust = 6% malathion dust and EC = 57% malathion emulsifiable concentrate.
Table 2. Percent malathion remaining at 120, 240, 270, and 330 days after application to cool wet corn (CWC), hot dry corn (HDC) or to cool dry corn (CDC) and dried at 21, 48, or 71°C (Part II, 1984 laboratory study)

<table>
<thead>
<tr>
<th>Drying Temp. °C</th>
<th>Form.</th>
<th>Application Form.</th>
<th>Time</th>
<th>% Malathion Remaining</th>
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</thead>
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<td>120</td>
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<td>Dust</td>
<td>CWC</td>
<td>1</td>
<td>66.5 ± 2.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDC</td>
<td>2</td>
<td>78.3 ± 10.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDC</td>
<td>3</td>
<td>84.4 ± 17.7</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>CWC</td>
<td>4</td>
<td>41.7 ± 15.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>HDC</td>
<td>5</td>
<td>80.9 ± 11.6</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDC</td>
<td>6</td>
<td>90.9 ± 18.8</td>
</tr>
<tr>
<td>48</td>
<td>Dust</td>
<td>CWC</td>
<td>1</td>
<td>20.7 ± 7.8</td>
</tr>
<tr>
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<td></td>
<td>HDC</td>
<td>2</td>
<td>75.8 ± 6.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CDC</td>
<td>3</td>
<td>93.2 ± 16.9</td>
</tr>
<tr>
<td></td>
<td>EC</td>
<td>CWC</td>
<td>4</td>
<td>19.4 ± 6.7</td>
</tr>
<tr>
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<td></td>
<td>HDC</td>
<td>5</td>
<td>75.8 ± 19.6</td>
</tr>
<tr>
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<td></td>
<td>CDC</td>
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<td>86.6 ± 25.8</td>
</tr>
<tr>
<td>71</td>
<td>Dust</td>
<td>CWC</td>
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<td>33.7 ± 3.9</td>
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<td>67.3 ± 6.1</td>
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<td>22.5 ± 4.1</td>
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<td></td>
<td></td>
<td>CDC</td>
<td>6</td>
<td>74.2 ± 3.2</td>
</tr>
</tbody>
</table>

*Formulation: Dust = 6% malathion dust and EC = 57% malathion emulsifiable concentrate*
Table 3. Moisture Content and temperature of grain stored on-farm sampled 1, 60, and 180 days after malathion application (Part II, Field Study)

<table>
<thead>
<tr>
<th>Bin No.</th>
<th>Sampling Date</th>
<th>Moisture Content</th>
<th>Temperature °C</th>
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<td>1</td>
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<td>10.0</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>60</td>
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<tr>
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<td>180</td>
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<td>4</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>10.0</td>
<td>10</td>
</tr>
<tr>
<td></td>
<td>60</td>
<td>9.4</td>
<td>1</td>
</tr>
<tr>
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<td>180</td>
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<td>7</td>
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<tr>
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<td>4</td>
<td>21.0</td>
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<tr>
<td></td>
<td>60</td>
<td>11.3</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>11.7</td>
<td>6</td>
</tr>
</tbody>
</table>
Table 4. Percent malathion remaining at 30, 120, 390, and 420 days after application to autoclaved corn (AC) and non-autoclaved corn (NAC) dried at 21 or 71°C (Part III)

<table>
<thead>
<tr>
<th>Drying Temp. °C</th>
<th>Sterilization Technique</th>
<th>% Malathion Remaining Days after Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
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</tr>
<tr>
<td>21</td>
<td>AC</td>
<td>84.2 ± 10.6</td>
</tr>
<tr>
<td>21</td>
<td>NAC</td>
<td>82.1 ± 13.7</td>
</tr>
<tr>
<td>71</td>
<td>AC</td>
<td>70.0 ± 19.9</td>
</tr>
<tr>
<td>71</td>
<td>NAC</td>
<td>74.5 ± 9.0</td>
</tr>
</tbody>
</table>