SEASONAL CHANGES IN THE ODOR VOLATILES ASSOCIATED WITH MICROFLORAL INFECTION AND ACARINE INFESTATION IN BIN-STORED WHEAT

by

DANICA TUMA

A thesis submitted to the Faculty of Graduate Studies in partial fulfillment of the requirements for the degree of MASTER OF SCIENCE

FOOD SCIENCE Department

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ABSTRACT

Hard red spring wheat, stored in five bin-duplicates at 20 and 25% moisture content for 10 months, was monitored for biotic and abiotic variables in Winnipeg, Manitoba. Major odor volatiles detected were 3-methyl-1-butanol, 3-octanone and 1-octen-3-ol. The production of these volatiles was correlated and associated with microfloral infection. Ventilation, used for cooling and drying of grain, disrupted microfloral growth patterns and production of volatiles. The highest levels of 3-methyl-1-butanol occurred in 25% moisture content wheat infected with bacteria, *Penicillium* spp. and *Fusarium* spp. In non-ventilated bins with 20% moisture content wheat, 3-methyl-1-butanol was correlated and associated with infection by *Aspergillus glaucus* gr. and bacteria. Infection by *Penicillium* spp. was correlated with 1-octen-3-ol production in control (non-ventilated) bin-stored wheat of both moisture contents. The mite volatile component, tridecane not reported previously in bin-stored grain occurred in 25% moisture content control bin-stored wheat naturally infested by *Acarus* spp. In the following years study, this volatile component was associated with three acarine species, *Acarus siro* (L.), *Aeroglyphus robustus* Banks, *Lepidoglyphus destructor* (Schrank), introduced into bins with 15% moisture content wheat. The highest surviving mite species under these storage conditions was *Aeroglyphus robustus*. An alarm pheromone, citral was detected in mite infested grain probably as a result of disturbing samples during handling.

In the laboratory, fungus-inoculated wheat produced 3-methyl-1-butanol most commonly and abundantly. Two other volatiles, 3-octanone and 1-octen-3-ol, were produced less frequently. The fungal species, isolated from bin-stored wheat in 1985-86 and tested for production of odor volatiles on wheat, included *Alternaria alternata* (Fr.) Keissler, *Aspergillus repens* (Corda) Saccardo, *A. flavus* Link ex Fries, *A. versicolor* (Vuill.) Tiraboschi, *Penicillium chrysogenum* Thom, *P. cyclopium* Westling, *Fusarium moniliforme* Sheldon, *F. semitectum* (Cooke) Sacc.. Two

bacterial species inoculated on agar are reported for the first time to produce 3-methyl-1-butanol. Two acarine species, *Acarus siro* and *Aeroglyphus robustus*, reared on wheat and fungus-inoculated wheat, produced tridecane. *Acarus siro*, a fungivorous mite with higher rates of survival and multiplication than *Aeroglyphus robustus* produced higher levels of tridecane on fungus-inoculated wheat.

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I. INTRODUCTION

Grain stored in bulk in farm bins, silos, and elevators are complex ecosystems in which many physical and biological agents interact, often diminishing the quality and quantity of stored grain (Sinha 1973). Changes within this dynamic ecosystem are primarily affected by initial moisture content, temperature, and previous storage history of the grain. Stored grain is also subjected to the deteriorative effects of one or more of biotic agents including microflora, insects, mites, and rodents (Sinha 1979). Under poor storage conditions, these can cause monetary losses to the producers through physical or quality decreases resulting in down-grading or rejection.

In North America, major qualitative and economical problems of losses to stored grain involve microfloral infection (Christensen and Kaufmann 1969). Advanced microfloral deterioration can be detected by the occurrence of grain heating, caking, sprouting, rotting, discoloration, and off-odor production. In such cases, the problem is often detected after the damage has already occurred; the grain is down-graded and farmers suffer economical losses. This deteriorated grain is often fed to livestock. Occasionally it is contaminated with harmful mycotoxins produced by fungal infection. Feeding of such contaminated grain can induce mycotoxicosis in animals and, perhaps, even enter the human food chain (Scott 1973). This makes prevention of deterioration strategically important to a grain-exporting country such as Canada, if it wishes to maintain its export markets and reputation as a producer of a high-quality grain.

Common symptoms indicating spoilage of stored-grain include: increased temperature, visual grain deterioration (discoloration, sprouting, microfloral growth), increased CO_2 and decreased O_2 concentrations in intergranular air, increase free fatty acid (FAV) levels in seeds, and loss in seed germination. None of these symptoms, however, characterize the deterioration specifically involved in

fungal infection, and insect or mite infestation. Such relatively non-specific assessment of decaying stored grain presents difficulty to storage managers when decisions must be made to carry out expensive measures to prevent further deterioration.

In Poland, Kaminski *et al.* (1972, 1973, 1974) and Stawicki *et al.* (1973), detected major odor volatiles produced by fungal species that differed from volatiles produced by dormant grain (Hougen *et al.* 1971, Maga 1978). Analyses by Polish researchers of *Aspergillus* spp., *Penicillium* spp., and other species of *Fungi Imperfecti* grown on coarse wheat meal yielded major odor volatiles such as 1-octen-3-ol, 2-octen-1-ol, 3-methyl-1-butanol, 3-octanone, and 3-octanol. Odor volatiles were also assigned a specific characteristic odor such as fungal-resinuous for 1-octen-3-ol, musty for 2-octen-1-ol, fusel oil for 3-methyl-1-butanol, fruity for 3-octanone, and citric-resinuous for 3-octanol. Dravnieks *et al.* (1973) correlated the sensory evaluation of musty and sour corn with odor-volatile profiles.

The aforesaid volatiles were also detected in airtight-stored corn at different temperatures and moisture contents by Richard-Molard *et. al.* (1976). A significant finding was the sequential increase in frequency and abundance of volatiles as storage time and microfloral infection increased. The most important odor volatile, 1-octen-3-ol, was suggested for use in early detection of microfloral spoilage. The method for collection of odor volatiles used by Richard-Molard *et. al.* (1976) and Kaminski *et al.* (1972, 1973, 1974) involved vacuum-steam extraction followed by freezing of volatiles. This common procedure made their results comparable; the major volatile in these studies was 1-octen-3-ol.

Abramson *et al.* (1980, 1983) used a different method of odor volatile collection for monitoring quality changes in wheat, barley, and oats stored in farm bins. Odor volatiles were trapped onto a porous polymer and heat-desorbed on a

cryogenically-cooled pre-column. The volatiles detected were 3-octanone, 1-octanol, 3-methyl-1-butanol, and 1-octen-3-ol. These were the same volatiles found in stored corn by Richard-Molard *et. al.* (1976), but in different relative proportions.

Tridecane was detected in large quantities from the headspaces of laboratory cultures of stored-product mites (Kuwahara *et al.* 1979, Curtis *et al.* 1981, Baker and Krantz 1984). The collection method involved the adsorption of volatiles on a porous polymer over several days using large mite population. This tedious and time-consuming method makes detection of tridecane in mite-infested grain cumbersome. The alarm pheromone, citral, was mainly detected in disturbed acarine cultures (Baker and Krantz 1984, Kuwahara *et al.* 1980).

Ventilation of stored cereals for prevention of spoilage through drying and cooling has become a common practice in Canada and elsewhere (Fraser and Muir 1981, Metzger and Muir 1983). The purging effect of the airflow could disrupt odor-volatile detection and potentially distort their association with microfloral infection of grain.

This research consisted of several field and laboratory experiments: the field study was a minor part of a larger study dealing with "Crop drying in near-ambient temperature air", undertaken by Department of Agricultural Engineering, University of Manitoba, in collaboration with Agriculture Canada Research Station, Winnipeg. Analysis of odor volatiles in grain was introduced as another possible indicator of spoilage in stored grain. The objectives of the field study were

1) to detect odor volatiles produced by microflora in wheat stored at different initial moisture contents during two crop years (1984-85 and 1985-86),

2) to determine the seasonal variation of microfloral volatiles as associated with changes in biotic and abiotic variables,

3) to determine the effect of ventilation on the development of odor volatiles, and

4) to measure seasonal fluctuations of tridecane with corresponding fluctuations of acarine populations in bin-stored wheat during one crop year (1986-87). The laboratory study was conducted under controlled conditions to establish cause-and-effect relationships between odor volatiles, and microflora or mites. The objectives of the laboratory study were

1) to determine the odor volatiles produced by fungal and bacterial species, isolated from the grain used in the 1985-86 bin study, and grown on wheat and synthetic media and

2) to determine the volatiles produced by two species of mites reared on wheat and fungus-inoculated wheat.

II. LITERATURE REVIEW

A. Cereal-odor volatiles

Odor volatiles associated with different cereals can be important in flavor composition for baked cereal products (McWilliams and Mackey 1969) or in chemotaxonomy of different cereal varieties (Hougen *et al.* 1971). Maga (1978) reviewed the literature on cereal volatiles and suggested the measurement of off-odors during storage for detection of spoilage. The identified cereal volatiles were mostly short-chained (C_1-C_8) alcohols, aldehydes, and ketones.

Hamilton-Kemp and Andersen (1984) suggested the use of plant volatiles (from whole plant tissue) to explain parasite-plant interactions (insect or pathogen). Vacuum-steam distillation of whole macerated wheat plant revealed chemicals of C_6-C_{10} carbon chains with double bonds. They suggested that these chemicals were artifacts of enzymatic cleavage of 9-10 double bonds of C_{18}-fatty acid moieties, resulting from the breaking down of plant tissue. Hamilton-Kemp and Andersen (1986) confirmed this hypothesis by comparing volatiles from fresh, frozen, cut, and uncut wheat-plant tissue. The quantity of many compounds with double bonds increased in cut and frozen (cells cut by ice crystals) plant tissues.

Dravnieks *et al.* (1973) used multivariate, stepwise discriminant and regression analyses of results from sensory panels and corn-odor profiles. They found a good correlation for musty and sour corn based on objectionable odors. Their method involved adsorption of volatiles in trap, than heat desorption, and following pre-column concentration by N_2 freezing.

B. Microfloral odor volatiles

Kaminski *et al.* (1972) analyzed volatiles of *Aspergillus flavus* Link ex Fries, grown on sterilized wheat. Odor volatiles detected on *A. flavus*-inoculated wheat were 1000 times higher than for wheat alone. Odor volatiles identified were:

3-methyl-1-butanol, 3-octanone, 3-octanol, 1-octen-3-ol, and cis 2-octen-1-ol. Of these volatiles, 1-octen-3-ol and 2-octen-1-ol were thought to be responsible for the characteristic musty-fungal odor of certain fungi. Methodology involved homogenization of wheat at -10^{0}C, vacuum distillation under N_2 at 35^{0}C, and cold trapping of volatiles at -10 to -80^{0}C. Extracts of volatiles in CH_2Cl_2 were dried and concentrated. The same method was used for collection of volatiles from *Aspergillus* spp., *Penicillium* spp., and species of *Fungi Imperfecti* (Kaminski *et al.* 1974). Most of the aforesaid odor volatiles were detected in all fungal species, but in different relative proportions. Stawicki *et al.* (1973), using the same method, analyzed odor-volatiles from different fungal and bacterial species isolated from stored wheat. The typical odor of microfloral species grown on wheat was compared with detected volatiles. Fusel oil odor was associated with 3-methyl-1-butanol, fruity with 3-octanone, citric-resinous with 3-octanol, fungal-resinuous with 1-octen-3-ol, and musty oil with 2-octen-1-ol. Bacterial cultures were characterized as acid, putrid, and musty; no odor components were identified.

Kaminski *et al.* (1973) heated coarse wheat meal at 90^{0}C for 30 min and froze headspace components at -20^{0}C. They found volatiles similar to those isolated in the vacuum-distillation method; only lesser amounts were measured and 2-octen-1-ol was mostly absent. Bacterial species, assessed for volatile fatty acids, yielded acetoine, acetone, acetic, isobutyric, and isovaleric acids as their major odor components (Kaminski *et al.* 1979).

Classification of fungal species through volatile analysis was suggested by Seifert and King (1982) and Halim *et al.* (1975). These and other aforementioned authors observed that odor production was associated with the level of sporulation in fungal cultures.

C. Stored-grain odor volatiles

Corn stored airtight at different moisture contents and temperatures was analyzed for development of volatiles (Richard-Molard *et al.* 1976). Major volatiles showing increased peak areas over time included 3-methyl-1-butanol, 1-pentanol, 3-octanone, 1-hexanol, 3-octanol, and 1-octen-3-ol. Chromatograms became more complex and peak areas increased as moisture content and temperature of the stored corn increased and promoted microfloral activity. Methodology was similar to vacuum-extraction of blended cereals by Kaminski *et al.* (1973); it was suggested that 1-octen-3-ol be used for early detection of spoilage.

Quality changes in bin-stored cereals were monitored using odor volatile analysis by Abramson *et al.* (1980). Three major volatiles, 3-octanone, 1-octanol, and to a lesser extent 3-methyl-1-butanol, were detected in damp wheat, barley, and oats stored in farm bins in Manitoba. The highest levels of volatiles occurred at seven weeks of storage . Volatiles were adsorbed in a trap and than heat desorbed onto a cryogenically-cooled pre-column. Bin-stored barley at two different moisture contents was analyzed for odor volatiles by Abramson *et al.* (1983); 3-methyl-1-butanol, 1-octen-3-ol, and 1-octanol were detected and peaked at six weeks of storage.

D. Mite odor volatiles

Tridecane was reported as an odor volatile of the headspace above cultures of acarine species including *Tyrophagus putrescentiae* (Schrank) (Kuwahara *et al.* 1979), *Rhizoglyphus robini* Claparede (Baker and Krantz 1984), and *Acarus siro* L. (Curtis *et al.* 1981). The alarm pheromone, citral, was detected in a disturbed culture of *R. robini* (Baker and Krantz 1984), *Aleuroglyphus ovatus* (Troupeau), and *Carpoglyphus lactis* (L.) (Kuwahara *et al.* 1980). These odor volatiles have never been found in bin-stored grain.

III. MATERIALS AND METHODS

A. Stored wheat ecosystem

1. Experimental design: Bins, wheat, ventilation

The experimental bins consisted of internally wax-coated cardboard-cylinders 3.66 m high and 0.61 m in diameter (Fig. 1). These bins were insulated with 0.13 m thick fiberglass and located in an unheated machine shed in Winnipeg, Manitoba. All bins were divided into 10 sampling layers, each 35 cm deep with a sampling port in the bin wall at the middle of each layer (Fig. 2). Only the top layer of grain was sampled for my analysis. A metal sampling probe was inserted radially through the port, closest to the surface, to collect samples used for biotic and abiotic analyses. Ventilated bins were equipped with uniformly-perforated floors, the air-plenum chambers were supplied with outside air by a 2.24 kW centrifugal fan (Chicago Blowers, Winnipeg, MB).

a) Bin experiment 1984-85

Number 2 Canada western hard red spring wheat (*Triticum aestivum* L., cv. Neepawa), locally harvested on 17 August 1984, was stored at 15.6% moisture content (m.c.) in a 15.8 m^3 bulk-feed tank. On 18 August, additional grain was harvested at 16.1% m.c. During the following two days the moisture content of the last batch was increased to 18±0.5% by spraying three times with rain water. The moisturized grain was placed into the test bins on 20 August. The top of ventilated bin was covered with plywood and the tops of non-ventilated control bins with plastic sheeting. None of the coverings was made airtight.

Figure 1. Layout (top view) of experimental bins;
bins used in 1984-85: 1 and 2-ventilated, 9, 10, 13 and 15-control;
bins used in 1985-86: 3 and 4-ventilated, 5 and 6-low ventilated, 7 and 8-high ventilated, 9, 10, 13 and 15-control;
bins used in 1986-87: 14-ventilated, 16-control.

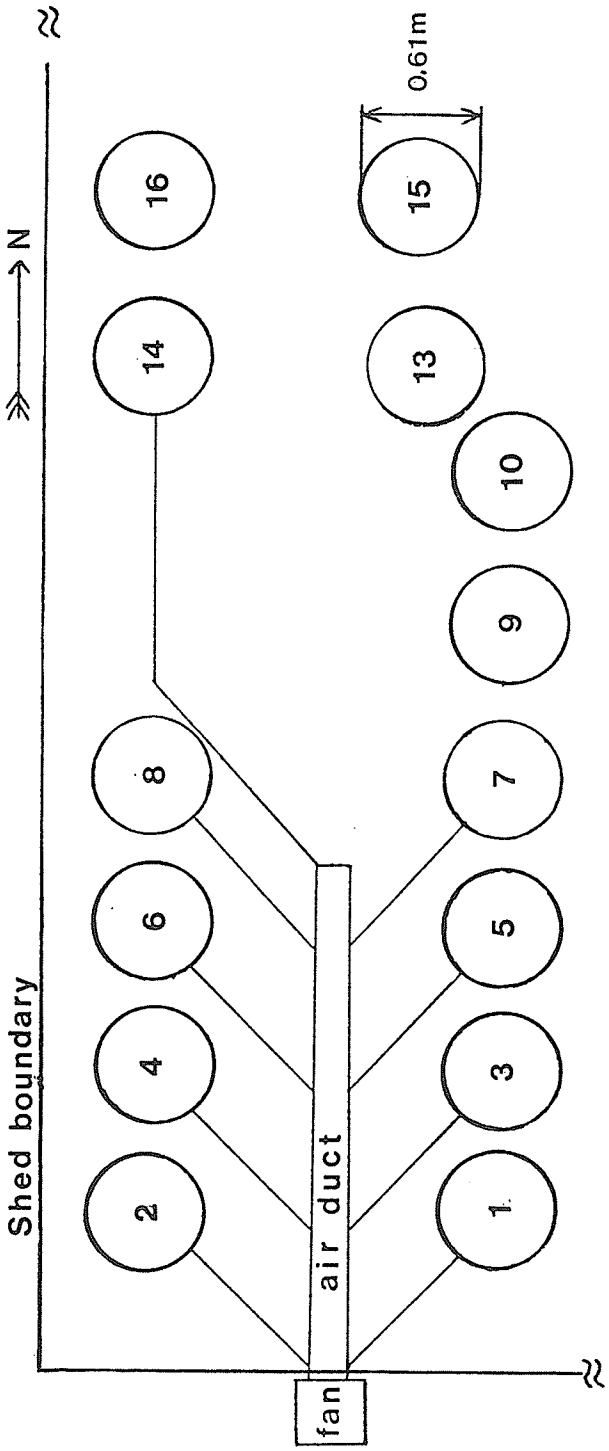
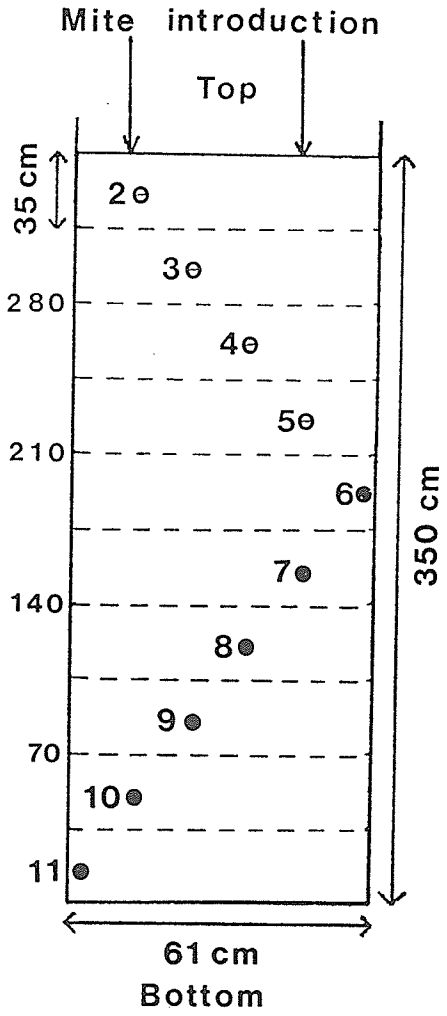

Shed boundary
N
0.61m
air duct
fan
1
2
3
4
5
6
7
8
9
10
13
14
15
16

Figure 2. Sampling locations in a vertical profile of the experimental bin; sampling ports are located spirally in the middle of each layer.


Mite introduction
Top
35 cm
2
3
280
4
5
210
6
7
140
8
9
70
10
11
350 cm
61 cm
Bottom

Ventilation with ambient air started on 20 August 1984 at a rate of 7 ($L \cdot s^{-1}$) m^{-3} in two "ventilated bins" with 18% initial m.c. and continued until 30 October 1984; the experiment was terminated on 15 July 1985. The four "control bins" (two at 15.6% and two at 18% initial m.c.) were not ventilated.

Grain samples collected during winter and summer for odor analysis from 300- and 330-cm grain heightsin each bin were taken from 2-3 cm below the surface with a scoop. Samples for biotic and abiotic analyses were taken through the bin port, using a metal probe. The samples taken at 230- and 260-cm heights are referred to as the "middle locations"; and at 300- and 330-cm heights are referred to as "surface locations", for convenience in discussion.

b) Bin experiment 1985-86

Number 2 Canada western red spring wheat (*Triticum aestivum* L., cv. Katepwa) was harvested in part and binned on 2 September 1985 at 20% moisture content (m.c.). The remainder of the grain was harvested and binned on 3 September at 25% m.c. The bin tops were covered with fine mesh and sealed with silicone. Ambient air ventilation began on 4 September at the following rates:

2 ($L \cdot s^{-1}$) m^{-3} in two ventilated bins (20% initial m.c.),

3.5 ($L \cdot s^{-1}$) m^{-3} in two low-ventilated bins (25% initial m.c.)

and

7 ($L \cdot s^{-1}$) m^{-3} in two high-ventilated bins (25% initial m.c.).

Ventilation was stopped after 11 weeks due to climatic changes on 25 November 1985 and was resumed on 26 March for another 14 weeks until the end of study on 9 July 1986. The four control bins (two at 20% and two at 25% initial m.c.) were not ventilated.

Grain samples for odor analysis were taken from 5 cm below the grain surface and were returned to the surface after analysis. Samples for biotic and abiotic analyses were taken through the bin port, closest to the grain surface, using a

metal probe. As the grain was shrinking, sampling changed to the lower port. Most of the samples were taken bi-weekly during late summer and early fall, and than monthly during late fall, winter and spring.

c) Bin experiment 1986-87

Grain from the bin experiment in 1985-86 was reused as follows: all unspoiled wheat, except wheat at 25% m.c. from control bins, was stored in a bulk-feed tank from 9 July 1986 until binning on 21 July 1986. Final moisture content of the wheat was 15.2%. The bin tops were covered with fine mesh and sealed with silicone. Ventilation with ambient air started on 26 August 1986 at a rate of 1 ($L \cdot s^{-1}$) m^{-3} in the ventilated bin and continued for 16 weeks until 18 December 1986. Ventilation was resumed on 12 June 1987 for another six weeks until end of experiment on 31 July 1987. The control bin was not ventilated.

Equal volumes of food media containing three species of stored-product mite pests, *Acarus siro* (L.) (Acarina; Acaridae), *Aeroglyphus robustus* Banks (Acarina; Glycyphagidae), and *Lepidoglyphus destructor* (Schr.) (Acarina, Glycyphagidae) (Hughes 1976) were introduced into the grain in control and ventilated bins. Mites were reared on yeast-wheat germ media (3:1, vol:vol) at 25±1^{0}C and 75±2% R.H. as part of the stock cultures maintained by the Agriculture Canada Research Station, Winnipeg. The estimated numbers of live mites introduced per bin location were: 443,000 for *A. siro*, 30,900 for *A. robustus*, and 97,350 for *L. destructor*. Mites were placed 0.15 m below the grain surface at two mid-way points between the sides and center of the bin (Fig. 2).

Samples for all analyses were taken through the bin port closest to the grain surface, using a metal probe. Because of grain shrinkage, the sampling port was changed to a lower port which corresponded to the grain surface. Grain used for odor analysis was returned to the grain surface. Samples for estimation of mite numbers were taken weekly, the frequency of sampling decreased to biweekly in

November and to monthly in January 1987. Samples for volatile component analysis were taken monthly until November and bimonthly thereafter.

2. Analysis of wheat samples

a) Determination and analysis of odor volatiles

For experiments in 1984-85, 355-g samples of wheat were placed in 2-L round-bottom flasks, and purged with 99.99+% N_2 (Liquid Carbonic Inc., Winnipeg, MB) at 60 mL·min^{-1} for 17 h at room temperature (23^{0}C) (Sinha *et al.* 1988). For experiments in 1985-86 and 1986-87, 500-g samples were used. The flask outlet was connected to a 75 mm x 3.5 mm I.D. glass tube packed with 200±10 mg of Johns-Manville Chromosorb 105, 60/80 mesh (Chromatographic Specialties Limited, Mississauga, ON). The adsorbent had been previously activated by purging with 99.99+% N_2 at 150^{0}C for 2 h. Volatiles were desorbed from the Chromosorb with ca. 500 µl of pesticide-grade acetone (Fisher Scientific, Winnipeg, MB) to final volume of 100 µL.

Samples were analyzed using a gas chromatograph (model 5890A, Hewlett-Packard, Avondale, PA) equipped with split/splitless injector, flame ionization detector, and computing integrator (model 3392A, Hewlett-Packard, Avondale, PA). The samples were analyzed on a Carbowax 20M fused silica capillary column (50 m x 0.31 mm I.D.). Aliquots of 1 µL were injected in the splitless mode. The injector and detector were maintained at 200 and 250^{0}C, respectively. The oven temperature program was 37^{0}C for 0.5 min, raised to 77^{0}C at a rate of 20^{0}C min^{-1}, held at 77^{0}C for 5 min, raised to 150^{0}C at a rate of 2^{0}C min^{-1}, and held at 150^{0}C for 10 min. Helium was used as carrier gas at a velocity of 45 cm·s^{-1}.

Standards of 3-methyl-1-butanol (98%), 3-octanone (99%), 1-octen-3-ol (98%), 1-octanol (99%) (Aldrich Chemical Co. Inc., Milwaukee, WIS), and 2-octanol (practical, Matheson Company Inc., Norwood, OH) were prepared in

acetone at concentrations of 40 µg mL^{-1} for each analyte. Aliquots (1-µL) were injected daily (Appendix 1). Diacetone alcohol (4-hydroxy-4-methyl-2-pentanone) was fortuitously present in the acetone used as a solvent, and served as a convenient internal standard for relative percent calculations. This analyte was identified by co-chromatography with a known standard (Aldrich Chemical Company Inc., Milwaukee, WI) and by electron-impact gas chromatography-mass spectrometry using a 5985B quadrupole system (Hewlett-Packard, Avondale, PA).

The mean relative percent of the volatile compound was calculated from two or three runs. The peak area of each compound was compared to the peak area of the diacetone alcohol internal standard for relative percent calculations.

b) Seed germination and microfloral infection assessment

The rate of seed germination and occurrence of microfloral infection were determined by the filter-paper method (Wallace and Sinha 1962); sets of 25 seeds were surface sterilized by 0.6% sodium hypochlorite and rinsed three times in sterile water. One set of 25 seeds was plated on filter paper (Whatman No.3) saturated with sterile water. Another set of 25 surface-sterilized seeds was plated on filter paper saturated with sterile 7.5% NaCl solution. This last technique is useful for determining the fungi of the *Aspergillus glaucus*, *A. versicolor*, *A. candidus* and *A. flavus* groups, and *Penicillium* spp. (Mills *et al.* 1978). Plates were stacked and incubated in a plastic bag for 7 days at 22±1^{0}C. To break seed dormancy, seeds plated for germination reading were first incubated at 10^{0}C for 7 days. The microflora that grew on the seeds were examined under a zoom stereomicroscope (Zeiss, West Germany). Final identifications of some species were made under a compound phase-contrast microscope (Nikon, Tokyo, Japan). Calculation of percentage of infection included seeds from which microflora were grown .

c) Dilution count

To dilution bottles containing 99 mL of sterilized, 0.1% peptone solution (Difco Laboratories, Detroit, MI) and 10 g of sterilized silica sand 11 g of grain was aseptically transferred . The primary dilution (1:10) was shaken at moderate speed for 30 min (Wrist Action Shaker by Burell, Pittsburgh, PA). Subsequent dilutions were prepared by pipetting 10 mL of primary solution to a new dilution bottle with 90 mL of sterilized, distilled water. Each new dilution was shaken by hand and used for further dilutions. From appropriate dilutions, 1-mL aliquots were pipetted to petri dishes and covered with an agar medium. Plates were mixed thoroughly and allowed to coagulate.

Yeast malt extract agar, consisting of 4 g yeast extract, 6 g Bacto-dextrose, 10 g malt extract, 15 g Bacto-agar in 1 L sterile water (Difco) was used for the fungal propagule count (Bothast *et al.* 1974). This medium was autoclaved at 121^{0}C for 20 min; 30 ppm of tetracyclin HCl (Onycin 1000, P.V.U. Inc., Victoriaville, CA) was blended into the medium prior to pouring to retard bacterial growth. Plates were incubated at 25^{0}C for five days and colonies counted. For further identification, plates were incubated for two more days after which percentages of fungal species developed were recorded.

Tryptone glucose yeast agar, consisting of plate count agar with 1 g of glucose added per litre of solution (Difco) was used for bacterial colony counts. After autoclaving, 4 mL of Fungizone Squibb (Flow Laboratories, McLean, VA) was blended into 1 L of medium prior to pouring to retard fungal growth. Plates were incubated at 30^{0}C for 3 days and colonies were counted. Each sample was plated in triplicate using one to two different dilutions; dilution count is expressed as the number of colonies per 1 g of grain.

d) Mite count

Mobile stages of adult and nymphal mites from grain samples were collected in the 1986-87 bin study using Burlese funnels filled with 350 mL of grain and placed under 30-W lamps (Sinha 1964). Heat from the lamps drove mobile mites from the grain into jars filled with 70% ethanol in 17 h. For the experiment in 1985-86, the extraction was done on 200 mL of grain. The numbers of mites per sample were counted using a stereomicroscope.

e) Grain moisture content and temperature measurement

Moisture content was determined on a wet-mass basis by the oven-drying at 130^0C for 19 h duplicate samples (15±1 g) from each bin (ASAE, 1983). Temperature was monitored using a Hewlett-Packard 3497A data acquisition/control unit (Hewlett-Packard, Avondale, PA) and copper-constantan thermocouples located along the centerline of the bin.

3. Statistical analysis of data from bin experiment 1985-86

To examine the relationships between observed variables, simple correlation, principal component and multiple regression analyses were carried out for a set of data collected from each storage condition. Most biotic variables monitored in natural stored-grain ecosystems usually have a J-shaped frequency distributions (Sinha 1979). The succession of populations of different species varies seasonally and annually depending on the type of interacting bio-variables involved. Monitored variables should have normal distributions so that the basic assumption for applying principal component analysis (PCA) and multiple regression analysis (MRA) to acquired data can be fulfilled (Sinha 1977). Based on the results from test of normality, variables not corresponding to requirements are transformed to achieve normality. Hypotheses on patterns of interrelationships can be generated from PCA on exploratory data in multivariable systems. The cause-and-effect

relationship can be tested in stepwise MRA by explaining the largest fraction of variation of a dependent variable with the smallest number of explaining (independent) variables (SAS Institute Inc. 1985).

Because the basic assumption for performing multivariate analysis requires measurement of all variables (p) for each sample (n) on a particular date of sampling, the number of analyzable samples had to be small; the total number of samples for all variables had to be reduced because of the low number of measurements of the odor component variable. Thus, in the 1985-86 experiment only 9-12 samples per bin were used for statistical analysis; this was achieved by matching odor analysis dates with the closest sampling date for other variables. Observations from duplicate bins were pooled to increase the sample size. This pooling of the data also helped to fulfill another basic assumption for multivariate analysis, i.e. to obtain a number of observations larger than the number of variables, which was 8-12.

a) Univariate statistics and transformations

The multivariate methods used in this study require a multivariate normal distribution and a linear relationship among the variables. To determine whether the variables had to be transformed so that normality or linearity assumption can be fulfilled, frequency distribution of all variables were examined by using simple univariate procedures on original data. Based on results from tests of normality (Shapiro-Wilk), frequency distribution plots, kurtosis, and skewness, appropriate transformations were chosen to normalize each variable (Appendix 2). Variables with more than 75% zero-observations were excluded as irrelevant for further analyses; inclusion of such variables could generate statistical errors obscuring the results (Pearce, 1965). Simple correlation matrices for all variables in all bins are given in Appendix 3-7. Interpretation of bivariate relationships are summarized in Table 3.

b) Multivariate analyses

Principal component analysis was performed on the correlation matrix of the transformed data (Appendix 2) using the SAS Princomp procedure (SAS Institute Inc. 1985); variance-covariance matrices could not be used because of different scales of variable measurements (Seal 1964). The output statistics, used in interpretation are eigenvalues, proportions of variance explained and eigenvectors (Appendices 3-7). The principal components (PC) with proportions of eigenvalues explaining >10% of variance were chosen for interpretation. In these components, a PC eigenvector cut-off point of 0.30 was applied for all variables, only components with which odor variables could be meaningfully associated (i.e. with PC loading >0.30) are included in Table 3.

Stepwise multiple regression analyses (SMRA) were done using each odor volatile as dependent variable and other biotic and abiotic variables as independent variables using SAS Stepwise procedure (SAS Institute Inc. 1985). The SMRA method explores cause-and-effect relationships based on linearity. The strength and quality of relationship between dependent and independent variables were measured by multiple correlation and tested for significance (Table 3).

B. Laboratory experiments

Two separate experiments were set up, using wheat and synthetic media artificially inoculated with single microfloral species, for analysis of odor volatiles after sevenday incubation. In another experiment, all of the treatments from the previous fungus-inoculated wheat experiment were artificially infested with a single species of mite, for analysis of mite volatiles after four week incubation.

First, an experiment was designed to determine the ability of individual fungal species grown on wheat to produce odor volatiles after seven days of incubation. Then all the wheat-fungus treatments were reused in another experiment with introduced mite species and analyzed after 4 wk for mite volatiles.

These treatments are designated as "odor analyzed" (OA) in the system description in Tables 4 and 5.

1. Media and inocula preparation

a) Wheat medium

Number 1 Canada hard red spring wheat (*Triticum aestivum* L., cv. Katepwa, 1986 crop, 15% m.c.) was moisturized to 20% by adding sterile water in a mixer for 30 min and equilibrated for 24 h at 10^0C. Round-bottom 1-L flasks were filled with 500 g of moisturized wheat, plugged with non-adsorbent cotton, and covered with aluminum foil. All flasks with moisturized wheat were autoclaved immediately at 121^0C for 20 min, then stored at -15^0C until required.

b) Synthetic media

Synthetic fungal media recommended by Onions *et al.* (1982) were: potato dextrose agar for *Fusarium* spp., Czapek agar for *Aspergillus* spp. and *Penicillium* spp., and yeast malt extract agar (Difco) for *Alternaria alternata* and *Arthrobotrys* spp. Bacterial medium was prepared by adding 1 g glucose per liter of plate count agar. For tests with each fungal species two 1-L round-bottom flasks were filled with 200 mL of medium, plugged with non-adsorbent cotton and covered with aluminum foil. The flasks were autoclaved immediately at 121^0C for 20 min.

c) Microfloral inocula

Fungal species *Alternaria alternata* (Fr.) Keissler, *Arthrobotrys* spp., *Eurotium herbariorum* (Wiggers)Link:Fr. (the perfect state of *Aspergillus repens*), *A. flavus* Link ex Fries, *A. versicolor* (Vuill.) Tiraboschi, *Penicillium cyclopium* Westling, *P. chrysogenum* Thom, *Fusarium moniliforme* Sheldon, and *Fusarium semitectum* (Cook) Sacc. were isolated on synthetic media from plated grain stored in the 1985-86 experimental study. Two bacterial species, designated as Bacterium 1 and

2,expected to be white and yellow bacteria reported by James (1955) and James *et al.* (1946), were isolated from dilution plates from 1985-86 study on plate count agar. The yellow culture obtained from an unheated grain resembled *Bacterium herbicola aureum* Duggeli (James 1955) and the white culture resembled an unnamed species of *Pseudomonas* referred as type B by James *et al.* (1946). All fungal species, excluding freshly isolated *Fusarium* spp., were stored at -15^{0}C in slant tubes for up to a year. All cultures were rejuvenated on petri dishes containing agar media for 1 wk at 25^{0}C prior to the experiment.

Spores were scraped from the culture mat that developed over 1 wk at 25^{0}C and were transferred aseptically to a tube with 5 mL of sterilized, distilled water. This suspension was mixed using a Vortex mixer (Scientific Products, McGaw Park, IL) and spore concentration determined using a hemocytometer (Bright Line, American Optical Corporation, Buffalo, NY). The final concentration was ca. 10^6 spores (cells) in 1 mL of suspension, inoculum concentration was checked by the dilution count method (Bothast *et al.* 1974).

Aliquots of 1 mL were pipetted into 1-L flasks containing media. Grain-filled flasks were swirled to mix the inocula thoroughly within the media. Flasks filled with synthetic media were inoculated with 1-mL aliquots spread over as much of the media surface as possible. Flasks were gently swirled as to not disturb the media.

A duplicate of each treatment was run and triplicates of each microfloral inoculum were plated for dilution counts. Aluminum foil from flasks was removed prior to incubation at 25^{0}C and 90% R.H. for 1 wk.

d) Mite rearing

Large populations of two acarine species, *Acarus siro* (L.) (Acarina, Acaridae) and *Aeroglyphus robustus* Banks (Acarina, Glycyphagidae) were reared on a yeast-wheat germ media (3:1, vol:vol) at 20^{0}C and 70±2% R.H. for 6 wk. A lot of

1000 adult mites was counted under the stereomicroscope and placed into each flask containing wheat using a sable-hair brush. Flasks were incubated at 25^{0}C and 90% R.H. for 4 wk.

2. Determination of odor volatiles

The determination and analyses of odor components were made by the method described earlier (A.2.a) with the following exception. Flasks with synthetic agar media were flushed over the agar surface with collection gas, rather than flushing through the media as with grain-filled flasks.

3. One-way analysis of variance

The data from tables 4 and 5 were statistically analyzed with one-way Analysis of variance (ANOVA) and Scheffe's and Student-Newman-Keuls' (SNK) multiple comparison method (SAS Institute Inc. 1985).

IV. RESULTS

A. Bin-stored wheat ecosystem

1. Preliminary study on 15.6 and 18.1% moisture content wheat - 1984-85

In wheat of 15.6% initial m.c. stored in control bins, two odor volatiles were detected during week 26-48. These volatiles and their relative percentage of occurrence were 3-methyl-1-butanol at 0.0-106.6% and 1-octen-3-ol at 0.0-14.7% (Table 1). The second odor component occurred only in the middle of bins during summer 1985. This niche had the highest fungal infection with 32 and 68% of the seeds being infected by *Aspergillus glaucus* gr.

In wheat of 18% initial m.c. stored in control bins, three odor volatiles were detected during week 13-48 (Table 1). These volatiles and their relative percentages were: 3-methyl-1-butanol at 9.2-32.5%, 3-octanone at 0.0-30.3%, and 1-octen-3-ol at 0.0-43.6%. All three volatiles occurred in the middle of bins during week 13 and 14 (in the fall), when infection levels by *A. glaucus* gr. and *Penicillium* spp. were 64 and 42%, respectively. During week 48, occurrence of all three volatiles coincided with the highest levels of infection by *A. versicolor* (94%) and *Penicillium* spp. (58%) in the middle of control bins. At the same time, seed germination decreased to 26%, and infection level by *Alternaria alternata* and *Aspergillus glaucus* gr. decreased to 22% and 26%, respectively.

In ventilated bins at 18% initial m.c., 3-methyl-1-butanol was detected only once at a 7.5% level in the middle of the bin during week 13 (Table 1). In this niche 32% of seeds were infected with the field fungus, *Alternaria alternata*. The low level of fungal activity and absence of storage fungi could have resulted from drying the grain to 12.3% m.c., thereby making it unfavorable for the growth of fungi. Thus a reduced level of production of odor volatiles and purging of volatiles by ventilation seem to account for low level of 3-methyl-1-butanol in this niche. Bacterial

Table 1. Odor volatiles and microfloral infection in bin-stored 15.6 and 18.1% initial moisture content wheat in 1984-85

Storage week-season	Sample location-height(cm)[a]	Moisture content (% w.b.)	Temp. (^{0}C)	Odor volatiles[b] (%)			Seed germination (%)	Microflora[b,c] (%)					
				3-methyl-1-butanol	1-octen-3-ol	3-octanone		Alt	Ag	Aver	Af	Pen	Bac
15.6% initial moisture content wheat - control bins													
26-Winter	surface-330	15.0	-6	20.4			84	4					16
45-Summer	surface-330	13.8	16	7.7			88	40	8				
45-Summer	surface-330	14.8	17	24.6			92	40	12				
47-Summer	surface-330	13.4	23	12.7			88	40	8				
47-Summer	surface-330	14.4	24	27.6			92	40	12				
48-Summer	middle-260	15.2	21	106.6	12.1		76	16	32				32
48-Summer	middle-260	15.7	22	65.2	14.7		80	24	68				8
18.1% initial moisture content wheat - control bins													
13-Fall	middle-190	17.9	-1	32.5	14.6	21.1	80	80	64			44	
14-Fall	middle-175	18.0	-4	15.6	8.3	7.5	56	70	64	4		40	
26-Winter	surface-330	17.1	-6	11.0			84	56	52				
26-Winter	surface-330	16.5	-6	29.9			84	44	52	4			
45-Summer	surface-330	16.9	16	9.2	13.5		68	76	64	4	4	24	8
45-Summer	surface-330	15.7	18	9.7	7.4		92	64	52				
47-Summer	surface-330	16.5	26	17.8			52	72	68			12	24
47-Summer	surface-330	16.0	26	13.1			80	48	32	20	8	48	12
48-Summer	middle-230	18.2	26	21.4	33.4	19.0	16	16	28	92	24	60	40
48-Summer	middle-230	18.0	26	24.5	43.6	30.3	36	28	24	96	4	56	16

Table 1. (continued)

Storage week-season	Sample location-height(cm)[a]	Moisture content (% w.b.)	Temp. (^{0}C)	Odor volatiles[b] (%)			Seed germination (%)	Microflora[b,c] (%)					
				3-methyl-1-butanol	1-octen-3-ol	3-octanone		Alt	Ag	Aver	Af	Pen	Bac
18.1% initial moisture content wheat - ventilated bins[d]													
13-Fall	middle-190	12.3	-2	7.5			92	32					
14-Fall	middle-190	12.2	-5				92	32					
26-Winter	surface-300	12.8	-7				84	32					
26-Winter	surface-300	13.1	-7				76	32					64
46-Summer	surface-300	12.2	20				72	56					4
46-Summer	surface-300	12.3	20				96	12					60

[a]grain height from bin bottom

[b]blanks indicate zero values

[c]Alt-*Alternaria alternata*; Ag-*Aspergillus glaucus* gr.; Aver-*Aspergillus versicolor*; Af-*Aspergillus flavus*; Pen-*Penicillium* spp.; Bac-bacteria

[d]airflow rate:
ventilated bins - 7 ($L \cdot s^{-1}$) m^{-3}

infection did not seem to coincide with odor production in the control or in the ventilated bins.

a) Abiotic variables

Grain moisture content in paired control bins with 15.6% initial m.c. wheat decreased to 13.4-14.4% in surface locations in the summer 1985 after week 39 (Table 1). In the middle of these bins, during the summer, grain moisture content was 15.2-15.7%. Grain moisture content in control bins with 18% initial m.c. wheat decreased to 15.7-16.5% in surface locations in the summer. In the middle of these bins, grain retained its initial moisture content of 17.9-18.2%. Grain in ventilated bins with 18% initial m.c. was dried to 12.3% m.c. Drying occurred in the middle of bins in the fall 1984 and in surface layer in the summer 1985. Temperature of grain in control bins and in aerated bin followed climatic changes (Table 1).

b) Biotic variables

In control bins with 15.6% initial m.c. wheat only 3-methyl-1-butanol, ranging from 7.7 to 27.6%, was detected in surface samples (grain height-330 cm) (Table 1). The highest level of this volatile component, 65.2-106.6%, occurred in the middle of control bins (grain height-260 cm) in July 1985 . This niche also recorded 12.1-14.7% of another volatile component, 1-octen-3-ol.

In control bins with 18% initial m.c. wheat, 3-methyl-1-butanol ranging from 9.2 to 29.9%, and 1-octen-3-ol ranging from 7.4 to 13.5%, were detected in surface samples (grain height-330 cm). All three odor volatiles were more common (measure of frequency) and abundant (measure of amount) in the middle of bins (grain height-175-230 cm) in November 1984 and July 1985, than anywhere else. In ventilated bins 3-methyl-1-butanol was detected only once at 7.5% level in the middle of bin (grain height-190 cm) in November 1984.

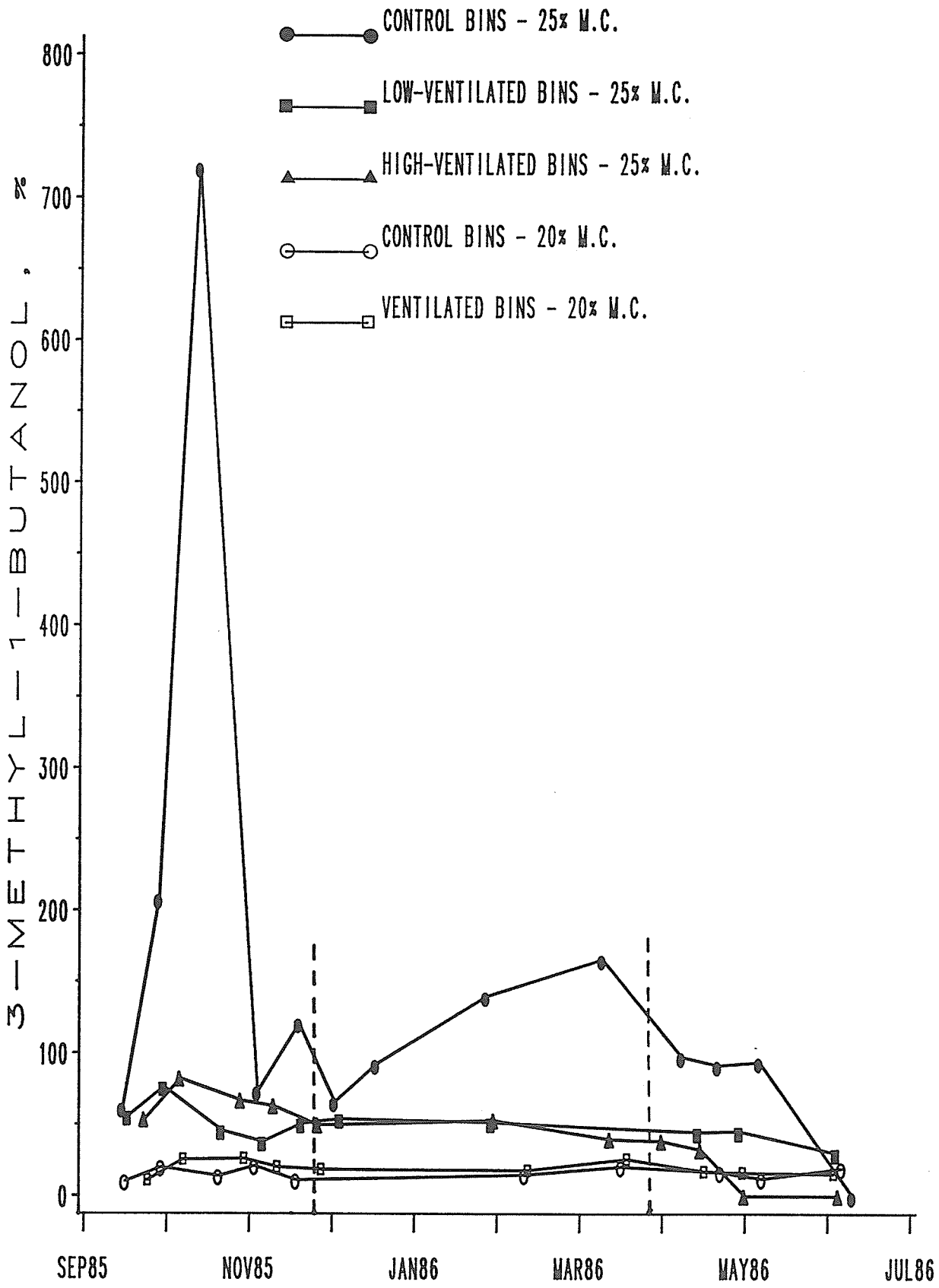
In control bins with 15.6% initial m.c. seed germination ranged from 76 to 90% during week 26-48. Active microflora consisted of the field fungus *Alternaria alternata*, storage fungi of *Aspergillus glaucus* gr., and bacteria (Table 1). In control bins with 18% initial m.c. seed germination ranged from 16 to 92% during week 13-48. The lowest germination, 16-36%, was found in the middle of bins (grain height-230 cm) in July 1985. Active microflora consisted of *Alternaria alternata*, and storage fungi of *Aspergillus glaucus* gr., *A. versicolor*, *A. flavus*, *Penicillium* spp., and bacteria (Table 1). The highest infection level of *A. versicolor* was found in the middle of bins in summer 1985. In ventilated bins, seed germination ranged from 76 to 92% during week 13-46. Active microflora consisted of *Alternaria alternata*, and bacteria.

2. Study on 20 and 25% moisture content wheat - 1985-86

The three odor volatiles, i.e. 3-methyl-1-butanol, 3-octanone, and 1-octen-3-ol, were detected during the 1985-86 experiment. These volatiles are the same as those ones detected during 1984-85 experiment. The unexpected volatile tridecane, which is known to be produced by mites, was detected only in control bins with 25% initial m.c.; grain was unintentionally infested with mites.

The most common volatile 3-methyl-1-butanol was detected in all bins regardless of initial moisture content and levels of ventilation (Fig. 3). The lowest overall levels of 3-methyl-1-butanol, 10-20%, occurred in bins with 20% initial m.c. wheat. The highest overall levels were recorded in bins with 25% initial m.c. wheat. In the control bins, 3-methyl-1-butanol occurred at its highest average (av.) level of 720% in October 1985. In all ventilated bins with 25% initial m.c. wheat, the levels of 3-methyl-1-butanol were always higher (150% av.) than in any of those of the four bins with 20% initial m.c. wheat (20% av.) (Fig. 3). No odor volatiles were detected in high-ventilated bins during April-June 1986. Absence of volatiles in post-winter

Figure 3. The effect of initial moisture content and ventilation on 3-methyl-1-butanol levels in bin-stored wheat in 1985-86; (ventilation periods are designated with broken line).


CONTROL BINS - 25% M.C.
LOW-VENTILATED BINS - 25% M.C.
HIGH-VENTILATED BINS - 25% M.C.
CONTROL BINS - 20% M.C.
VENTILATED BINS - 20% M.C.
3-METHYL-1-BUTANOL, %
800
700
600
500
400
300
200
100
0
SEP85
NOV85
JAN86
MAR86
MAY86
JUL86

samples is possible because low levels of volatiles generated by slow-growing microflora could be easily carried away with the resumption of ventilation in late March 1986.

Two other volatiles, 3-octanone and 1-octen-3-ol, occurred sporadically in bins with 20% initial m.c. and in control bins with 25% initial m.c. wheat. In control bins with 25% m.c. 3-octanone level was considerably higher than that recorded in bins with 20% m.c. during September 1985 to June 1986 (Fig. 4). The third volatile, 1-octen-3-ol, was most common and abundant in bins with 20% m.c.; the level of this component increased at the end of study in June 1986 (Fig. 5).

a) 20% initial moisture content wheat

In bins with 20% initial m.c. wheat, 3-methyl-1-butanol was the most common and stable volatile compared with the other two volatiles which occurred less frequently, although they increased at the end of the experiment in June 1986 (Figs. 6c, 8c, 10c, 12c). This increase coincided with high levels of infection by *Aspergillus glaucus* gr. and *Penicillium* spp. in control bins only (Figs. 6b and 8b). Ventilation seems to have contributed to lower levels of infection by post-harvest fungi in ventilated bins as compared to those levels in control bins. The fungal dilution count was at lower level of 10^3 per 1 g of grain in ventilated bins as compared with 10^4 per 1 g of grain in control bins.

(1) Abiotic variables

Grain moisture content in control bins (bin 13 and 15) did not change appreciably until the last few months of the experiment. During May-June 1986 moisture content dropped to 16% in bin 15 and to 19% in bin 13 (Figs. 7a and 9a). In ventilated bins (bin 3 and 4) moisture content dropped to 19% in the first week and remained unchanged for the following 8 mo., until May 1986. During May-June 1986 moisture content further dropped to 17% (Figs. 11a and 13a).

Figure 4. The effect of initial moisture content and ventilation on 3-octanone level in bin-stored wheat in 1985-86; (ventilation periods are designated with broken line).

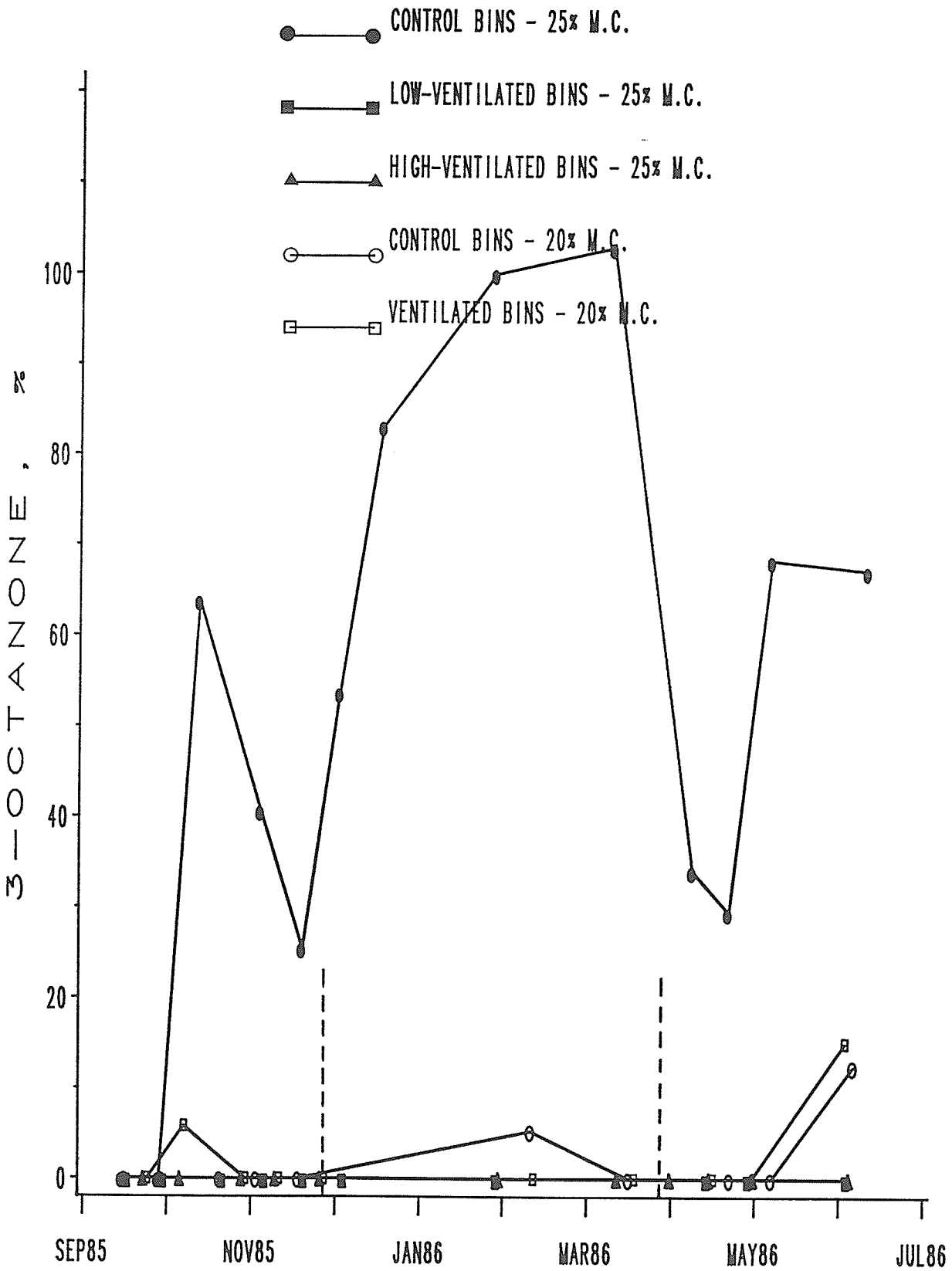

CONTROL BINS - 25% M.C.
LOW-VENTILATED BINS - 25% M.C.
HIGH-VENTILATED BINS - 25% M.C.
CONTROL BINS - 20% M.C.
VENTILATED BINS - 20% M.C.
3-OCTANONE, %
100
80
60
40
20
0
SEP85
NOV85
JAN86
MAR86
MAY86
JUL86

Figure 5. The effect of initial moisture content and ventilation on 1-octen-3-ol level in bin-stored wheat in 1985-86; (ventilation periods are designated with broken line).

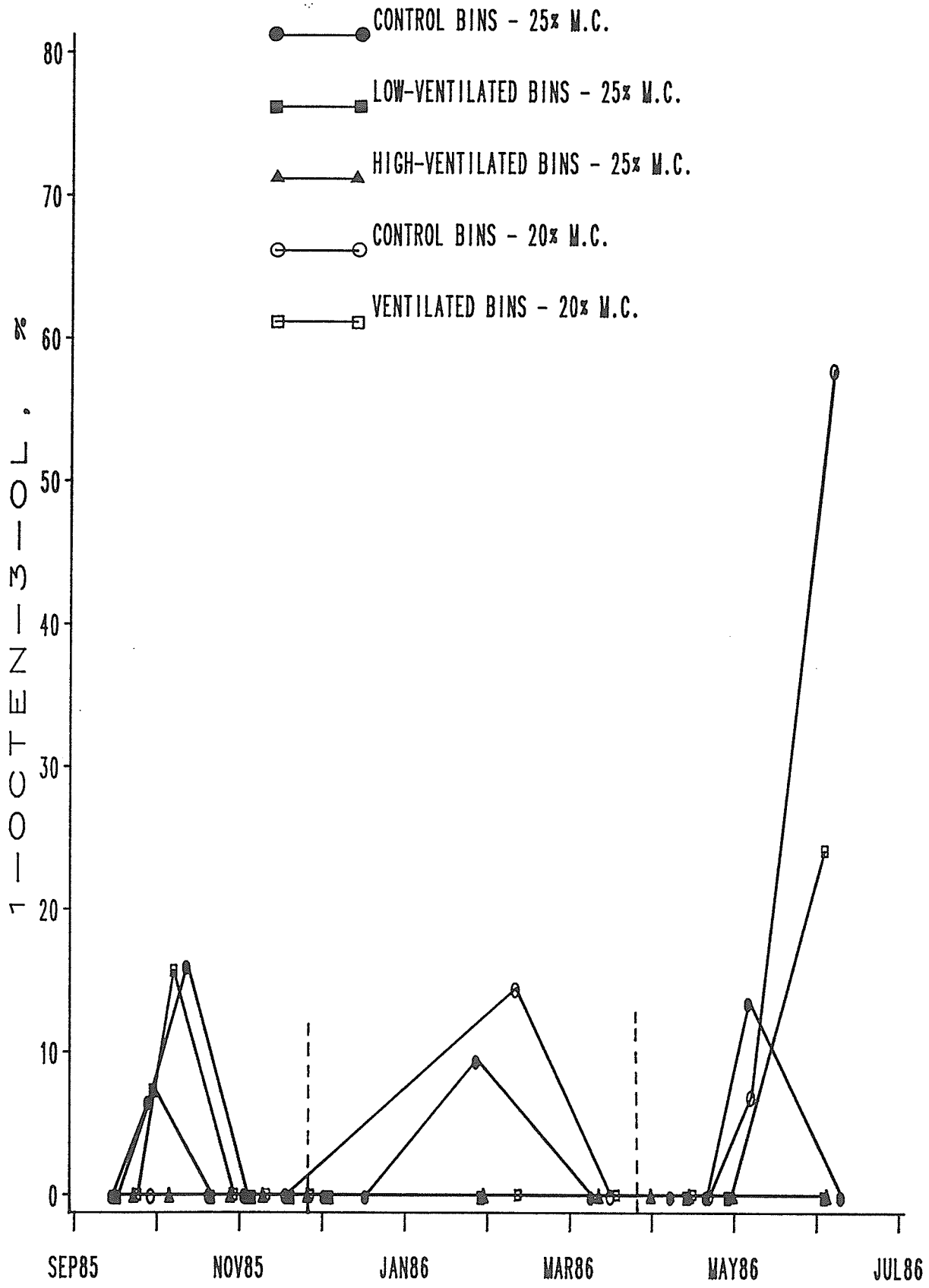
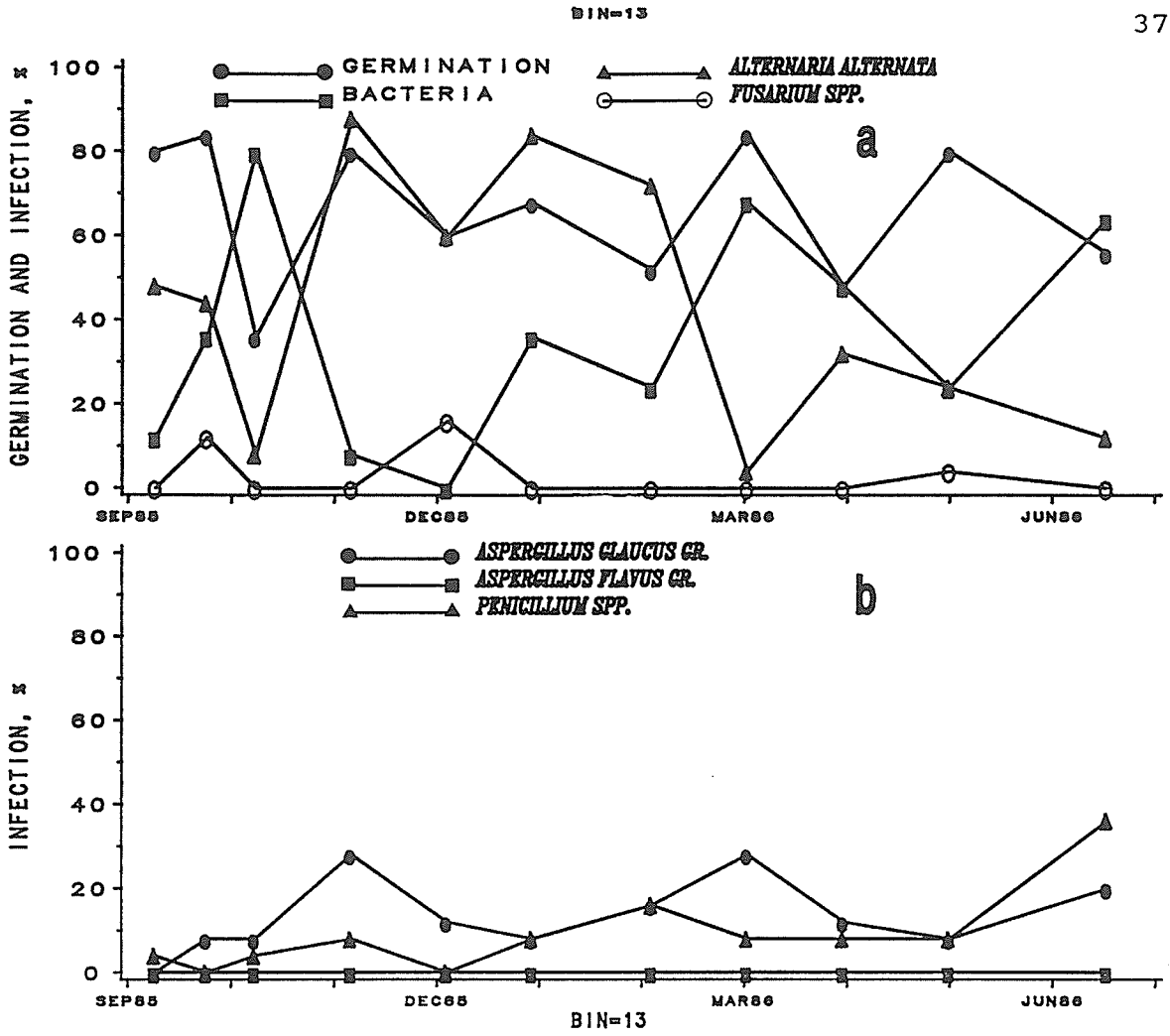

CONTROL BINS - 25% M.C.
LOW-VENTILATED BINS - 25% M.C.
HIGH-VENTILATED BINS - 25% M.C.
CONTROL BINS - 20% M.C.
VENTILATED BINS - 20% M.C.
1-OCTEN-3-OL, %
80
70
60
50
40
30
20
10
0
SEP85
NOV85
JAN86
MAR86
MAY86
JUL86

Figure 6. Microfloral infection, germination and odor volatiles of wheat stored at 20% moisture content in control bin 13 in 1985-86.


BIN=13
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
GERMINATION AND INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS CLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
INFECTION, %
BIN=13

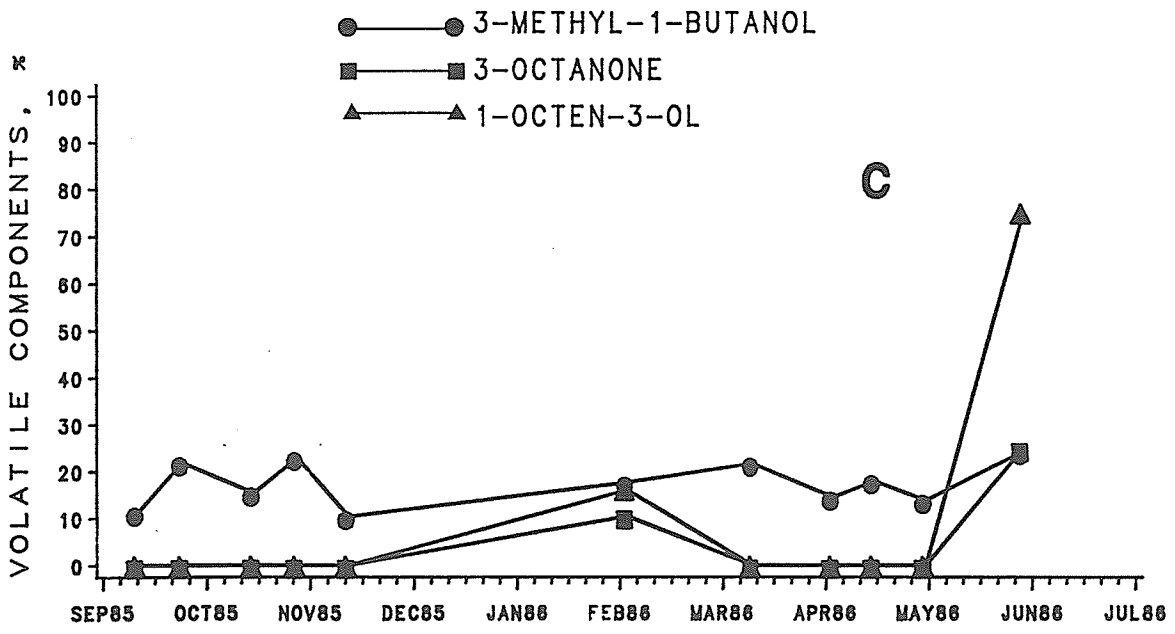
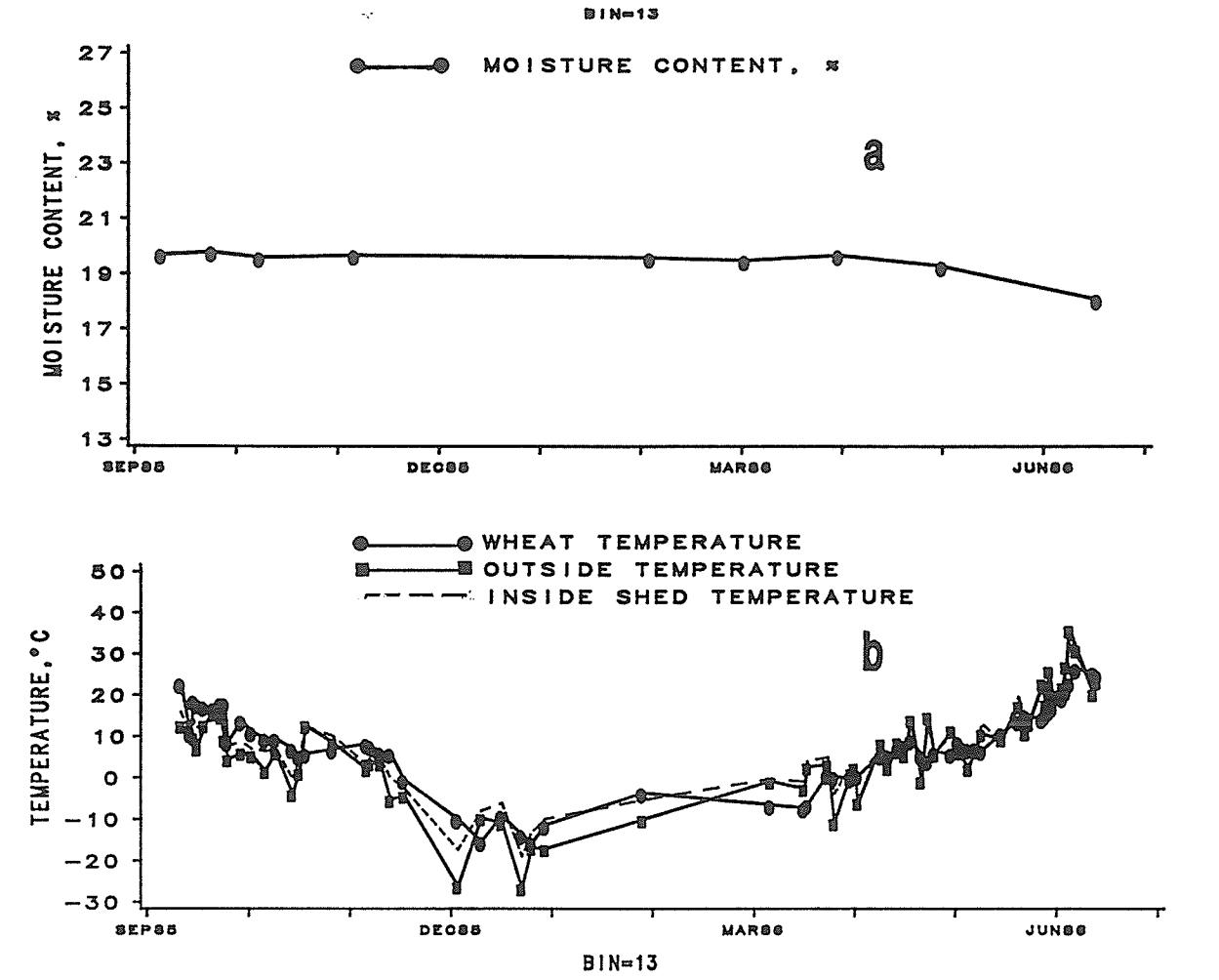

3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
100
90
80
70
60
50
40
30
20
10
0
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 7. Moisture content, temperature and dilution plating of wheat stored at 20% moisture content in control bin 13 in 1985-86.


BIN=13
MOISTURE CONTENT, %
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
a
SEP85
DEC85
MAR86
JUN86
WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
b
SEP85
DEC85
MAR86
JUN86
BIN=13

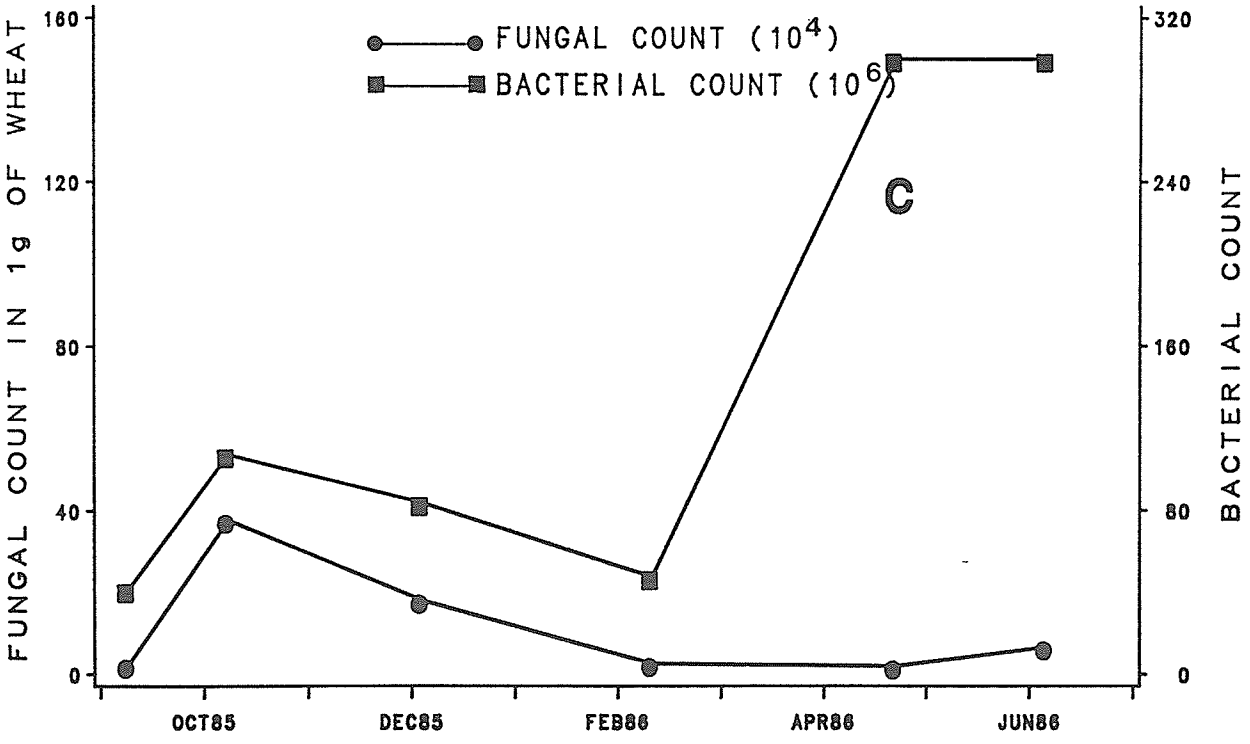
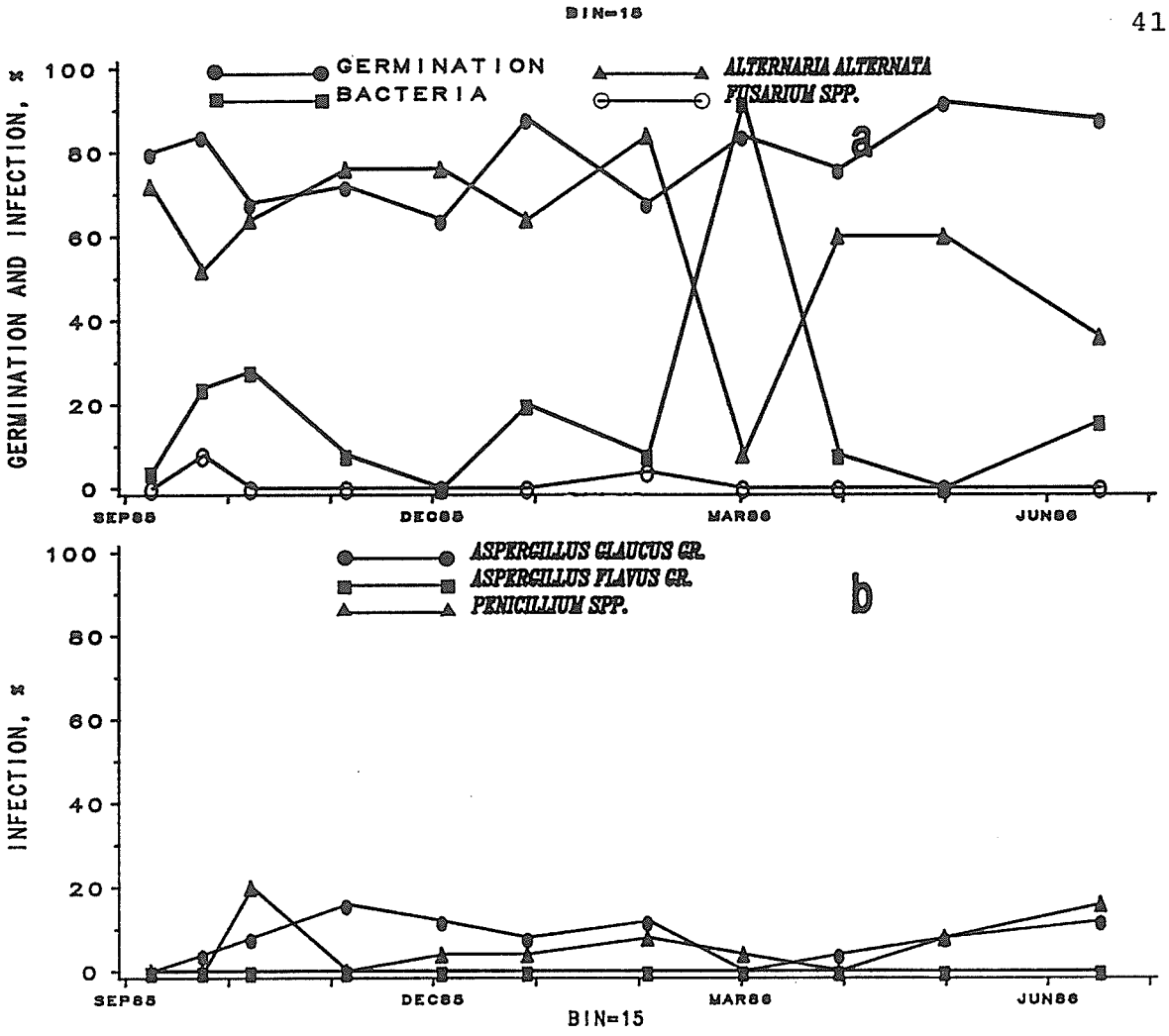

FUNGAL COUNT (10^4)
BACTERIAL COUNT (10^6)
FUNGAL COUNT IN 1g OF WHEAT
160
120
80
40
0
BACTERIAL COUNT
320
240
160
80
0
c
OCT85
DEC85
FEB86
APR86
JUN86

Figure 8. Microfloral infection, germination and odor volatiles of wheat stored at 20% moisture content in control bin 15 in 1985-86.


BIN=15
GERMINATION AND INFECTION, %
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
INFECTION, %
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
BIN=15

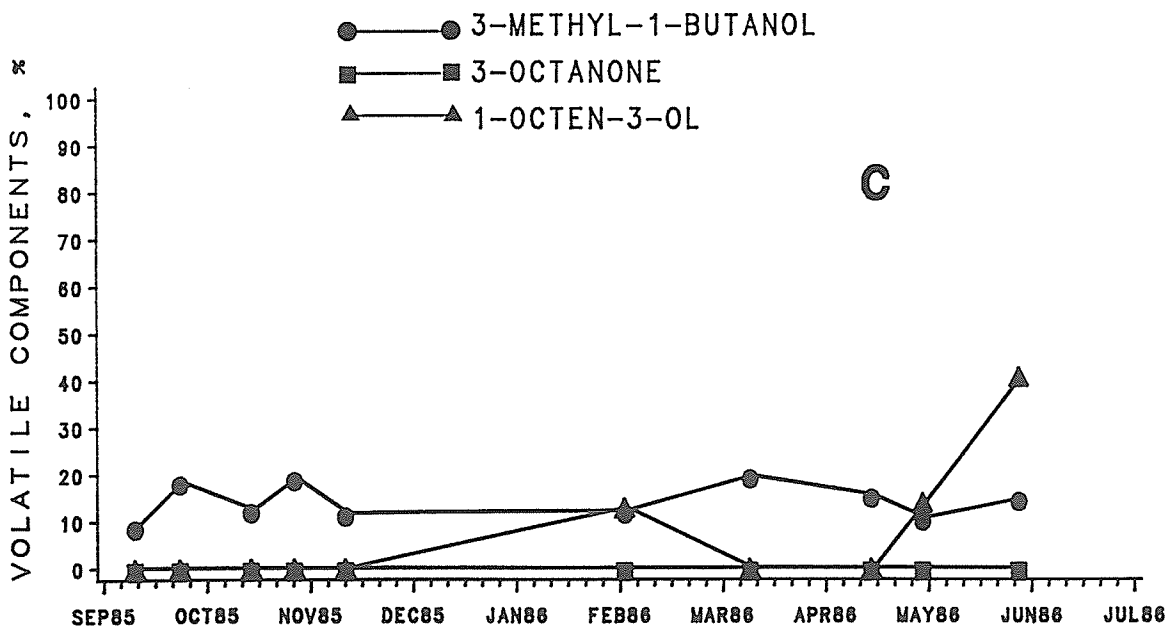
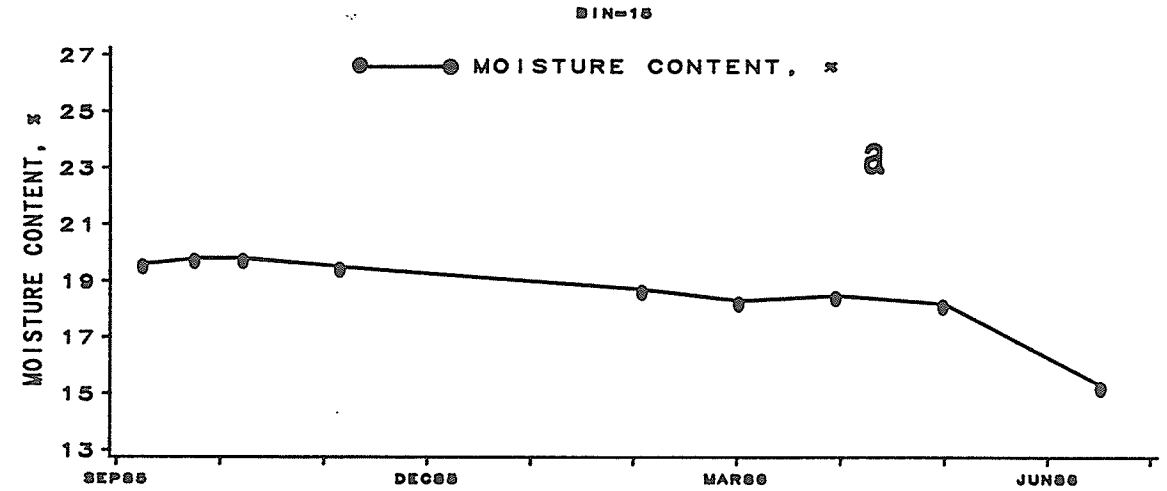
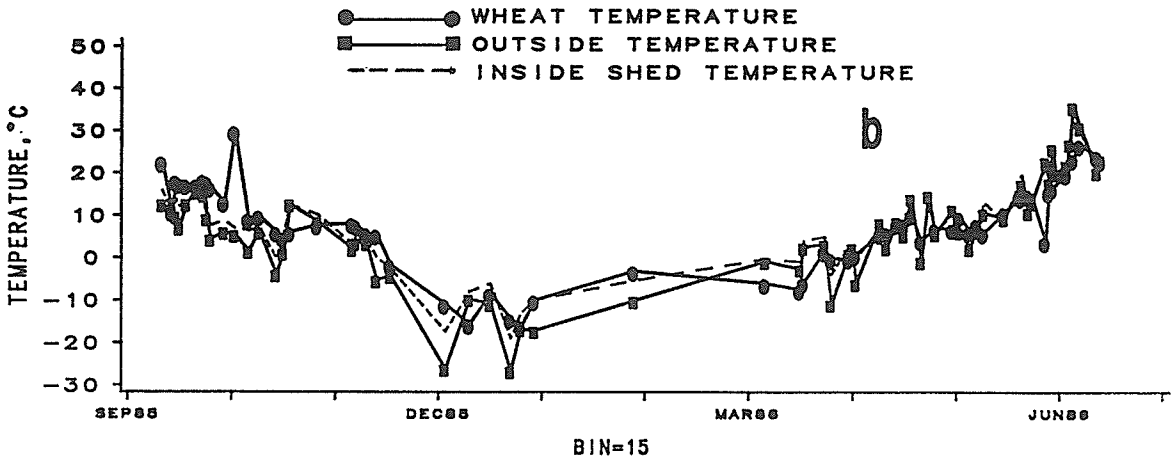

3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
100
90
80
70
60
50
40
30
20
10
0
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 9. Moisture content, temperature and dilution plating of wheat stored at 20% moisture content in control bin 15 in 1985-86.


BIN=15
MOISTURE CONTENT, %
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
a
SEP85
DEC85
MAR86
JUN86


WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
b
SEP85
DEC85
MAR86
JUN86

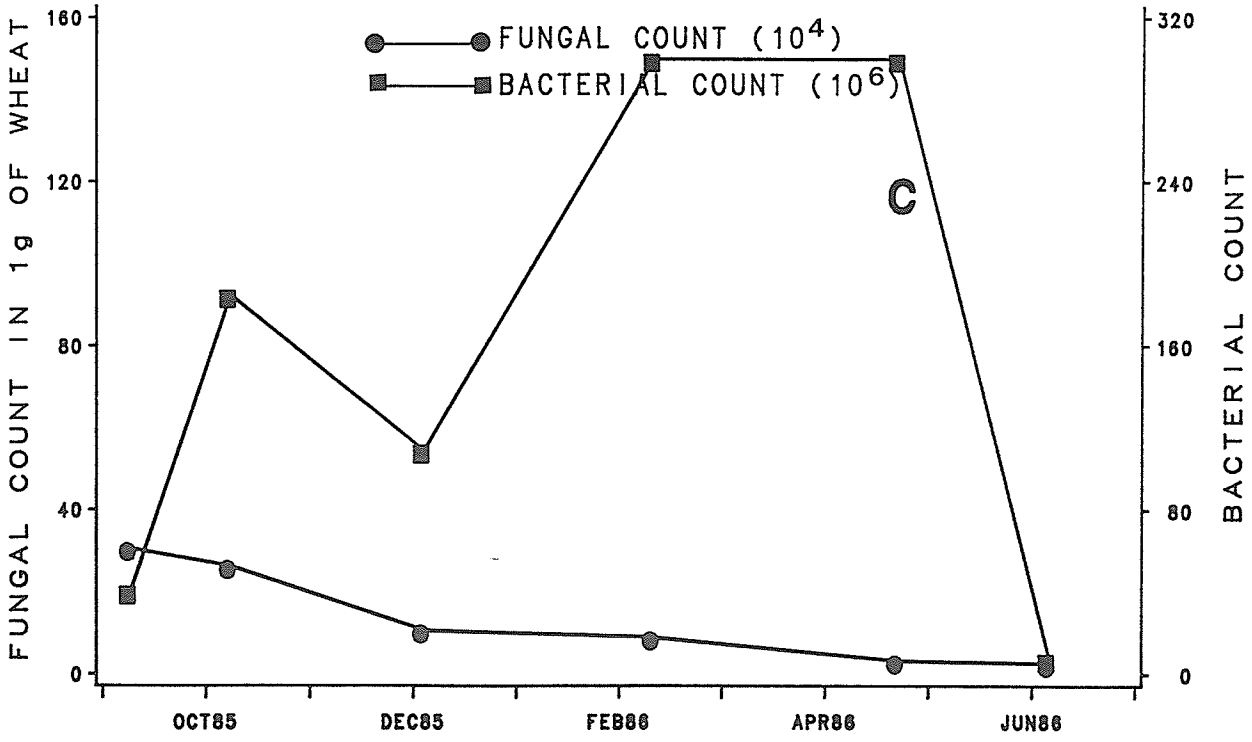
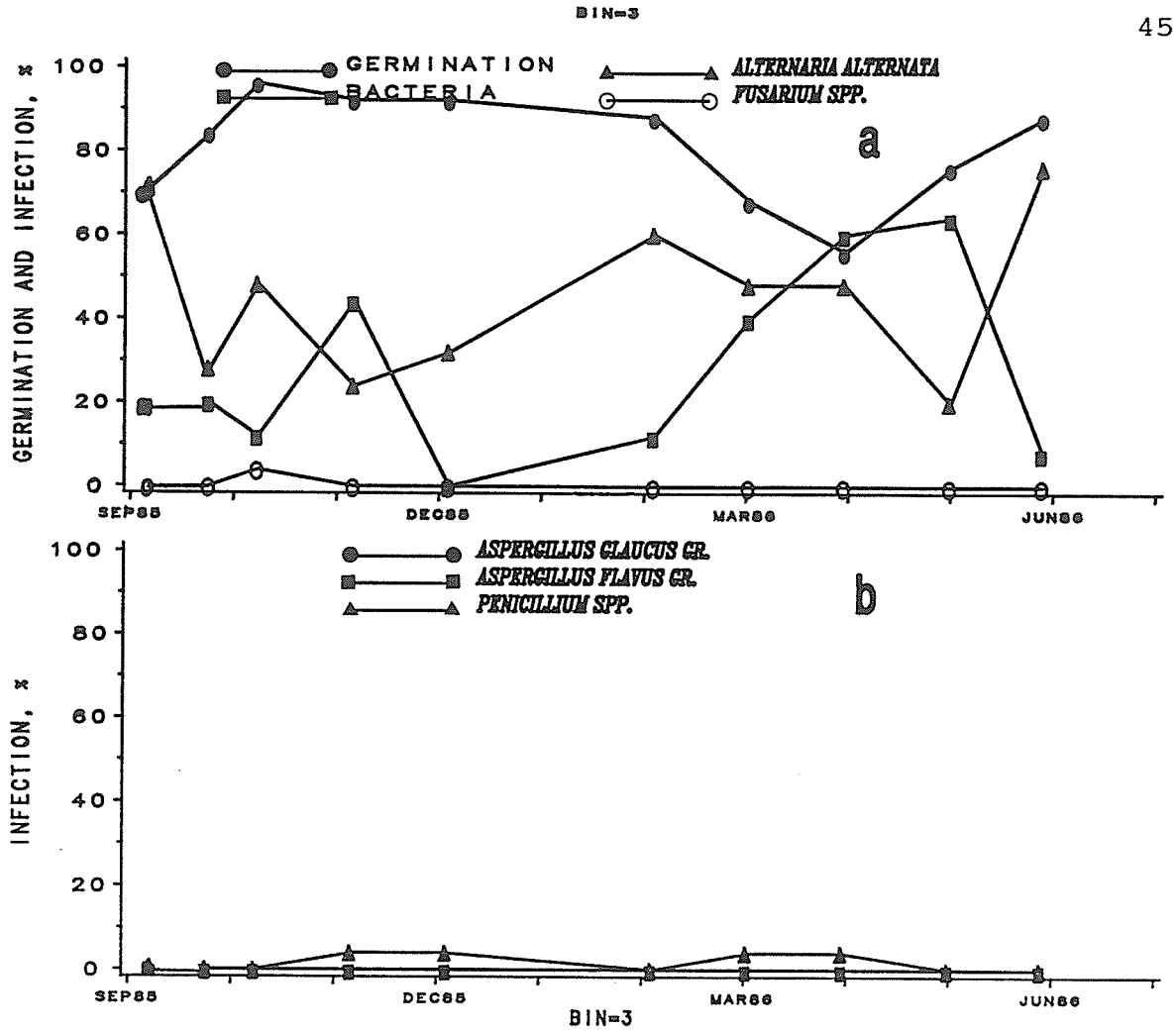

BIN=15
FUNGAL COUNT (10⁴)
BACTERIAL COUNT (10⁶)
FUNGAL COUNT IN 1g OF WHEAT
BACTERIAL COUNT
160
120
80
40
0
320
240
160
80
0
c
OCT85
DEC85
FEB86
APR86
JUN86

Figure 10. Microfloral infection, germination and odor volatiles of wheat stored at 20% moisture content in ventilated bin 3 in 1985-86; (ventilation periods are designated with broken line).


BIN=3
GERMINATION AND INFECTION, %
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
INFECTION, %
BIN=3

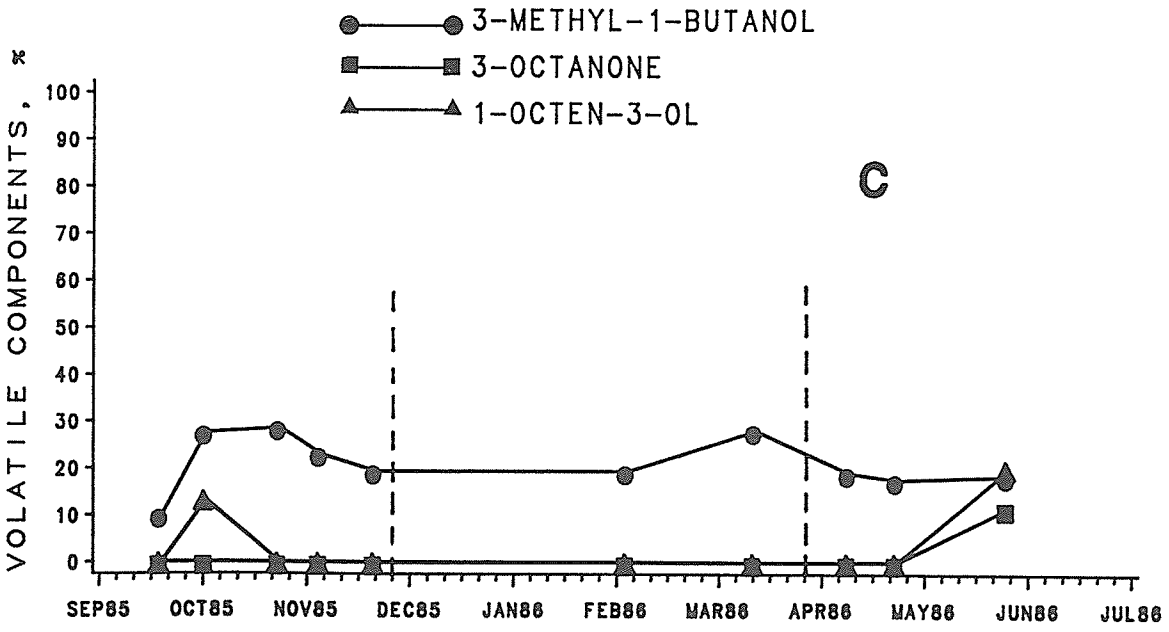

3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
100
90
80
70
60
50
40
30
20
10
0
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 11. Moisture content, temperature and dilution plating of wheat stored at 20% moisture content in ventilated bin 3 in 1985-86.

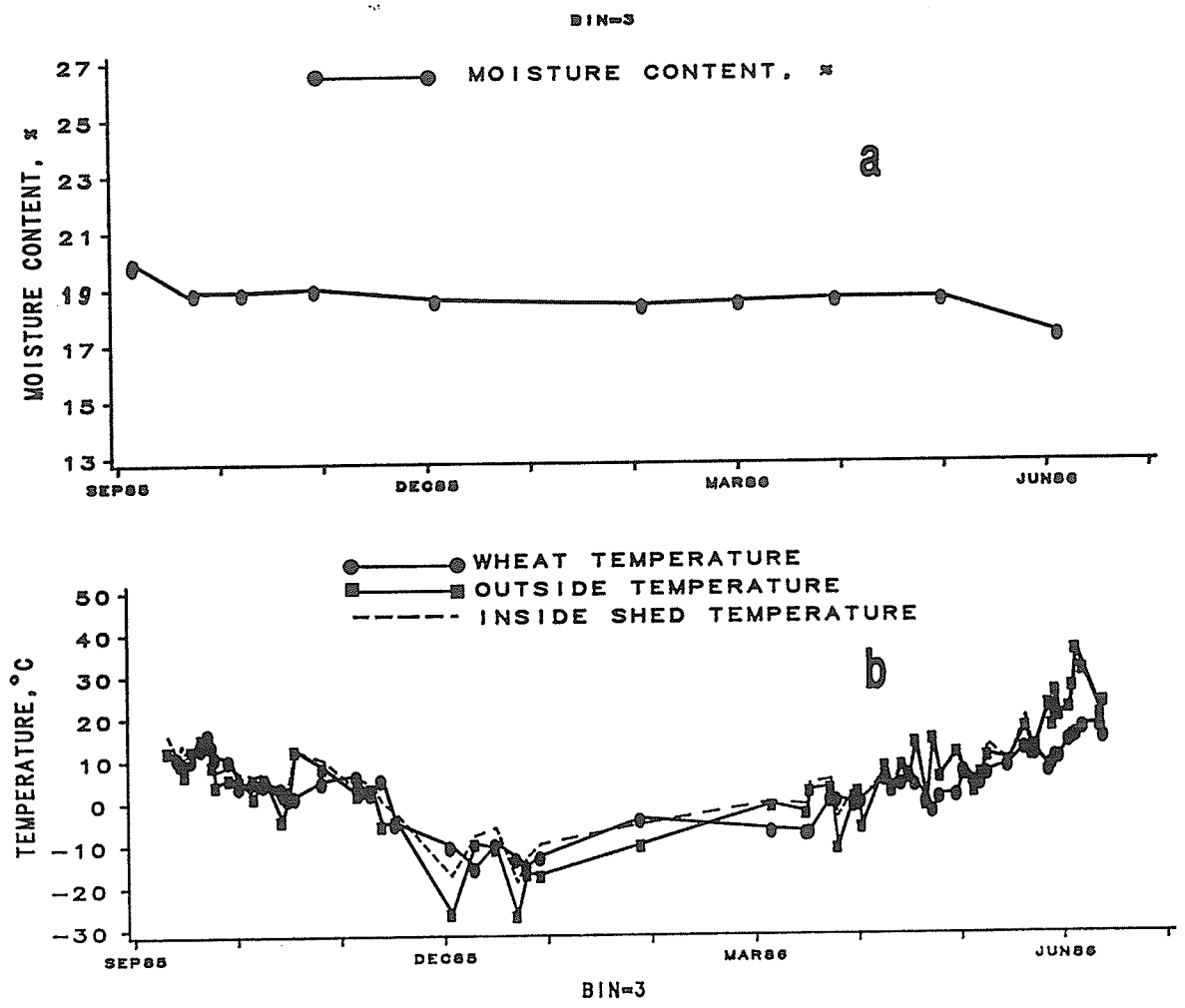
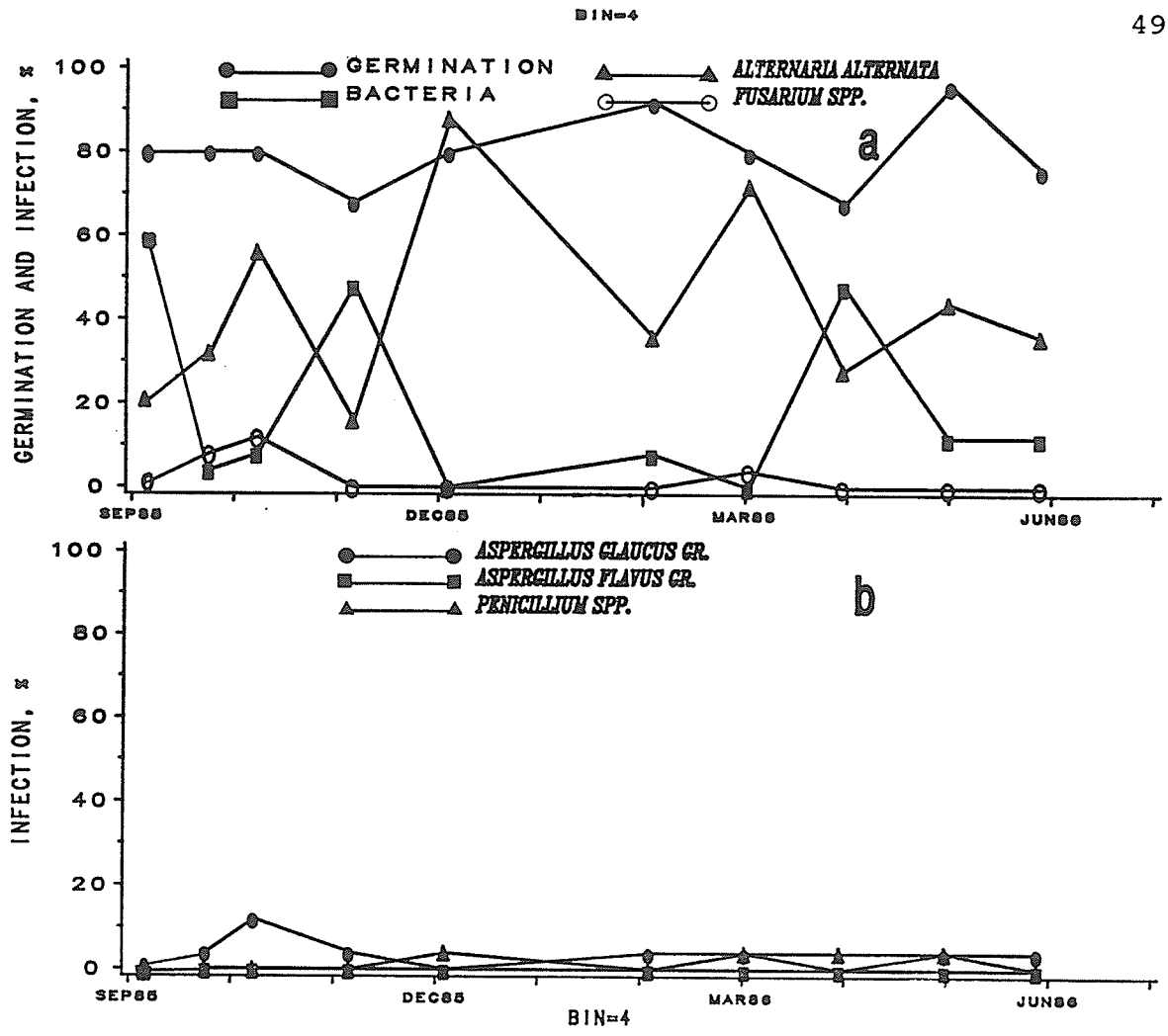

BIN=3
MOISTURE CONTENT, %
a
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
SEP85
DEC85
MAR86
JUN86
WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
b
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
SEP85
DEC85
MAR86
JUN86
BIN=3
FUNGAL COUNT (10^3)
BACTERIAL COUNT (10^6)
c
FUNGAL COUNT IN 1g OF WHEAT
120
90
60
30
0
BACTERIAL COUNT
320
240
160
80
0
OCT85
DEC85
FEB86
APR86
JUN86

Figure 12. Microfloral infection, germination and odor volatiles of wheat stored at 20% moisture content in ventilated bin 4 in 1985-86; (ventilation periods are designated with broken line).


BIN=4
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
GERMINATION AND INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
INFECTION, %
BIN=4

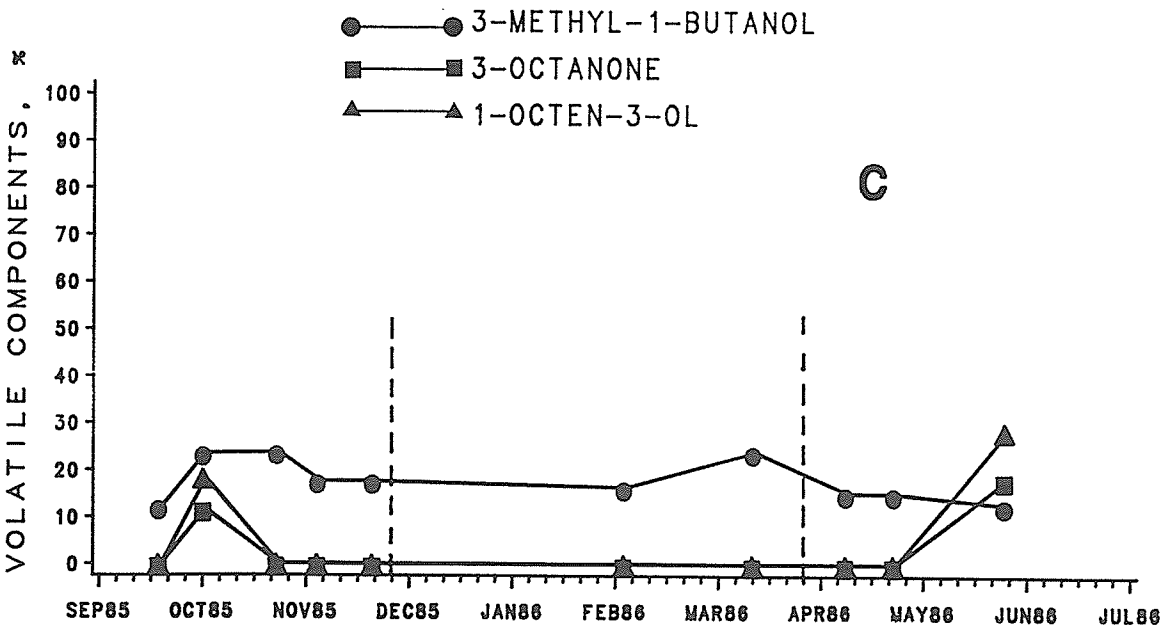
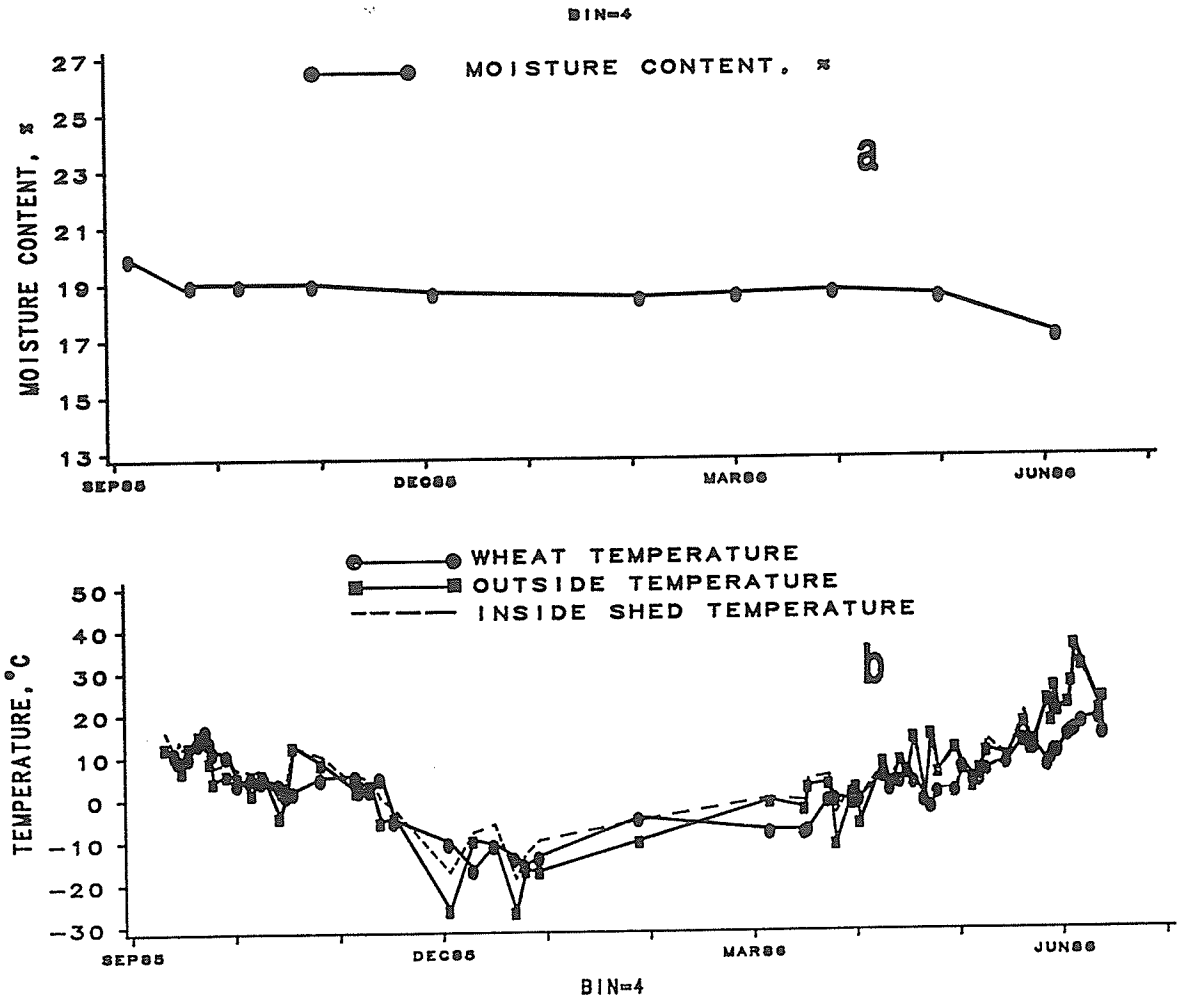
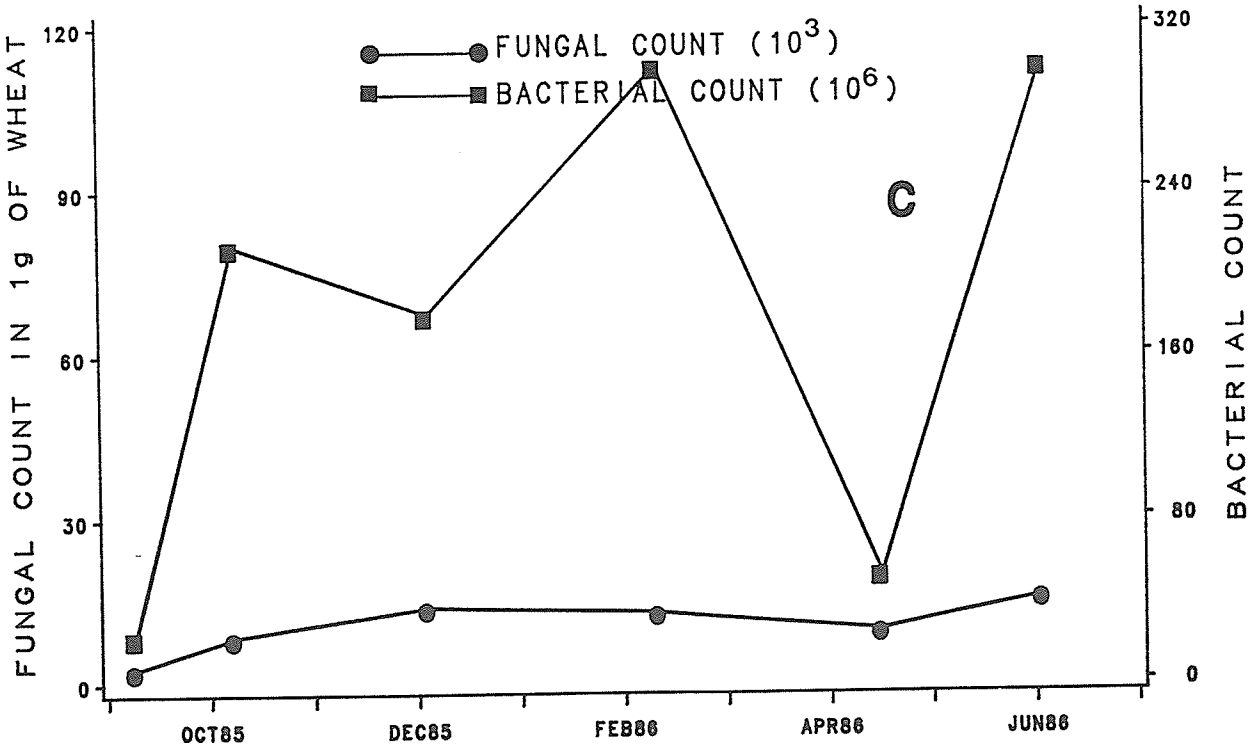

3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
100
90
80
70
60
50
40
30
20
10
0
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 13. Moisture content, temperature and dilution plating of wheat stored at 20% moisture content in ventilated bin 4 in 1985-86.


BIN=4
MOISTURE CONTENT, %
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
SEP85
DEC85
MAR86
JUN86
a
WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
SEP85
DEC85
MAR86
JUN86
b
BIN=4


FUNGAL COUNT (10^3)
BACTERIAL COUNT (10^6)
FUNGAL COUNT IN 1g OF WHEAT
120
90
60
30
0
320
240
160
80
0
BACTERIAL COUNT
OCT85
DEC85
FEB86
APR86
JUN86
c

Grain temperature in control bins stayed 5-10^{0}C higher than the inside shed temperature during the first 10 wk until November 1985 (Figs. 7b and 9b). During December, the grain temperature followed the inside shed temperature, dropping to its lowest of -15^{0}C. In the spring and summer months grain temperature approximated the inside shed temperature (15-30^{0}C) but without fluctuations.

Grain temperature in ventilated bins closely corresponded to the outside temperature during the first 10 wk until November 1985 (Figs. 11b and 13b). During December grain temperature followed the inside shed temperature dropping to its lowest of -15^{0}C. In the spring and summer months grain temperature remained a few degrees below, and having less fluctuations than the outside temperature.

(2) Biotic variables

Seed germination in control bins fluctuated between 40 and 90% (Figs. 6a and 8a). Microfloral infection consisted of the field fungi, *Alternaria alternata* ranging from 5 to 90%, and *Fusarium* spp. ranging from 0 to 15%. Bacterial infection fluctuated reciprocally to *Alternaria alternata* infection, ranging from 0 to 90%, as a possible result of species competition. The infection by storage fungi of the *Aspergillus glaucus* gr. ranged from 0 to 35% and *Penicillium* spp. ranged from 0 to 30% (Figs. 6b and 8b). Fungal dilution counts ranged from $2x10^4$ to $38x10^4$ per 1 g of grain during the first 4 mo., and later leveled off around $7x10^4$ per 1 g of grain during March-June 1986 (Figs. 7c and 9c). Bacterial dilution counts fluctuated between $5x10^6$ and $300x10^6$ per 1 g of grain; the highest counts occurred during April-June 1986 in bin 13 and during February-April in bin 15.

In ventilated bins seed germination fluctuated between 60 and 95% (Figs. 10a and 12a). Microfloral infection consisted of *Alternaria alternata* ranging from 20 to 90% and *Fusarium* spp. ranging from 0 to 10%. Bacterial infection fluctuated reciprocally to *Alternaria alternata* infection, ranging from 0 to 70%. The infection

by *Aspergillus glaucus* gr. ranged from 0 to 10% and by *Penicillium* spp. ranged from 0 to 15% (Figs. 10b and 12b). Fungal dilution counts remained unchanged at ca. $9x10^3$ per 1 g of grain, while bacterial dilution counts fluctuated between $4x10^6$ and $320x10^6$ per 1 g of grain (Figs. 11c and 13c).

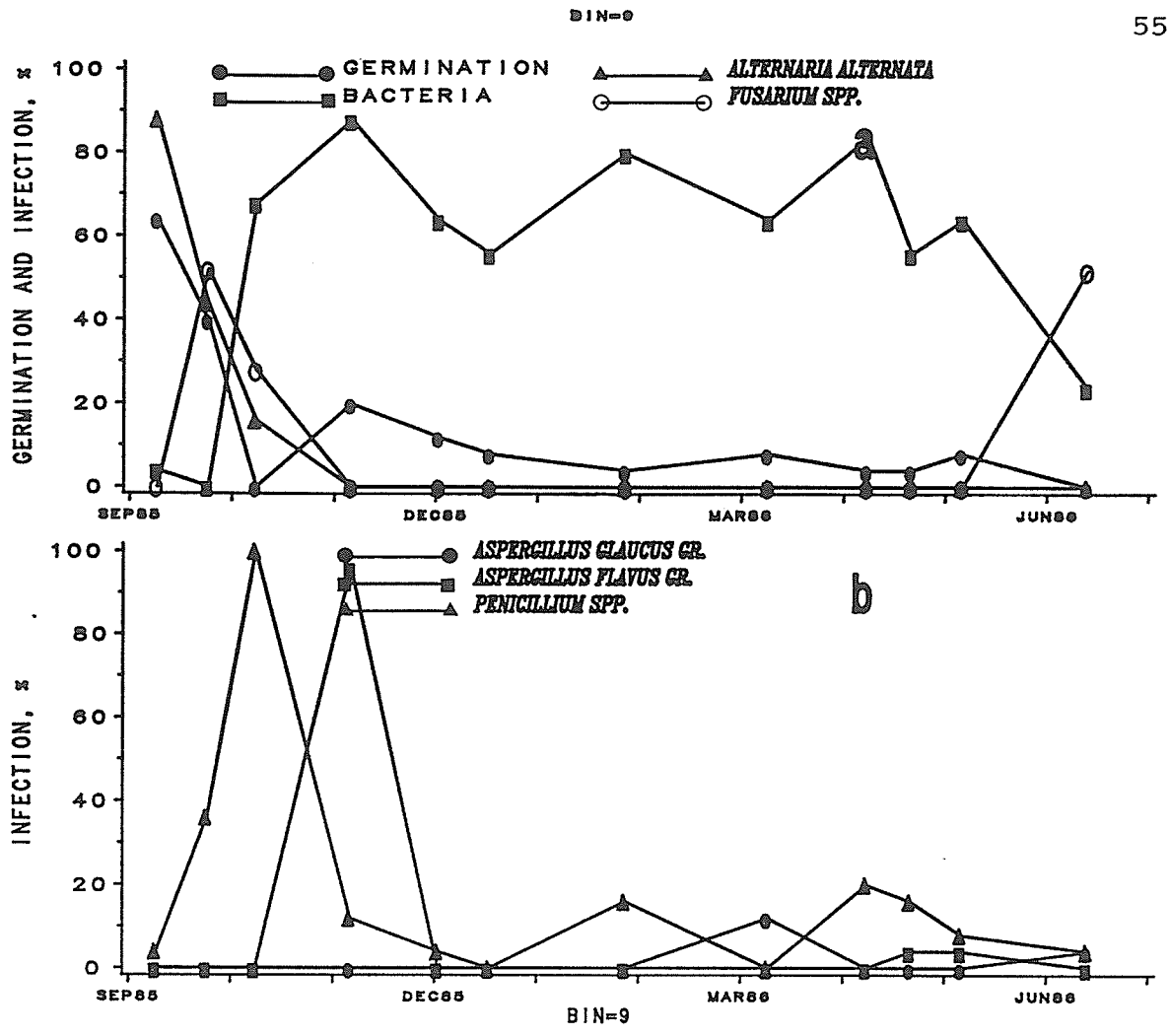
In control bins three odor volatiles were detected at different levels (Figs. 6c and 8c). The most common volatile 3-methyl-1-butanol ranged from 9 to 24%. The most abundant volatile, 1-octen-3-ol, occurred only twice, once in February at a 13% level in both bins, and again in June at a 40% level in bin 15 and at a 70% level in bin 13. The third component 3-octanone was detected only once in bin 13 at a 10% level.

In ventilated bins these three volatiles were also detected (Figs. 10c and 12c). The most common and abundant volatile 3-methyl-1-butanol ranged from 10 to 29%. The other two volatiles, 3-octanone and 1-octen-3-ol, occurred at low levels (12-30%) once in October 1985 and on the last day of the experiment in June 1985.

b) 25% initial moisture content wheat

In control bins with 25% initial m.c. wheat, 3-methyl-1-butanol was the most common and abundant volatile. The highest levels of 920% in bin 9 and 530% in bin 10 were recorded in October 1985 (Figs. 14c and 16c). At this time grain temperature reached 40^0C which was 20^0C above the outside temperature. Increased levels of volatiles coincided with increased infection levels by *Fusarium* spp. and *Penicillium* spp. at 52 and 100%, respectively, in bin 9, and at 68 and 75%, respectively, in bin 10 (Figs. 14a,b and 16a,b). During September-October, seed germination and *Alternaria alternata* infection decreased sharply from 65 to 0% and 88% to 15%, respectively, in bin 9 (Fig. 14a); from 48 to 8% and 80% to 0%, respectively, in bin 10 (Figs. 16a).

Figure 14. Microfloral infection, germination and odor volatiles of wheat stored at 25% moisture content in control bin 9 in 1985-86.


BIN=0
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
GERMINATION AND INFECTION, %
100
80
60
40
20
0
a
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
INFECTION, %
b
BIN=9

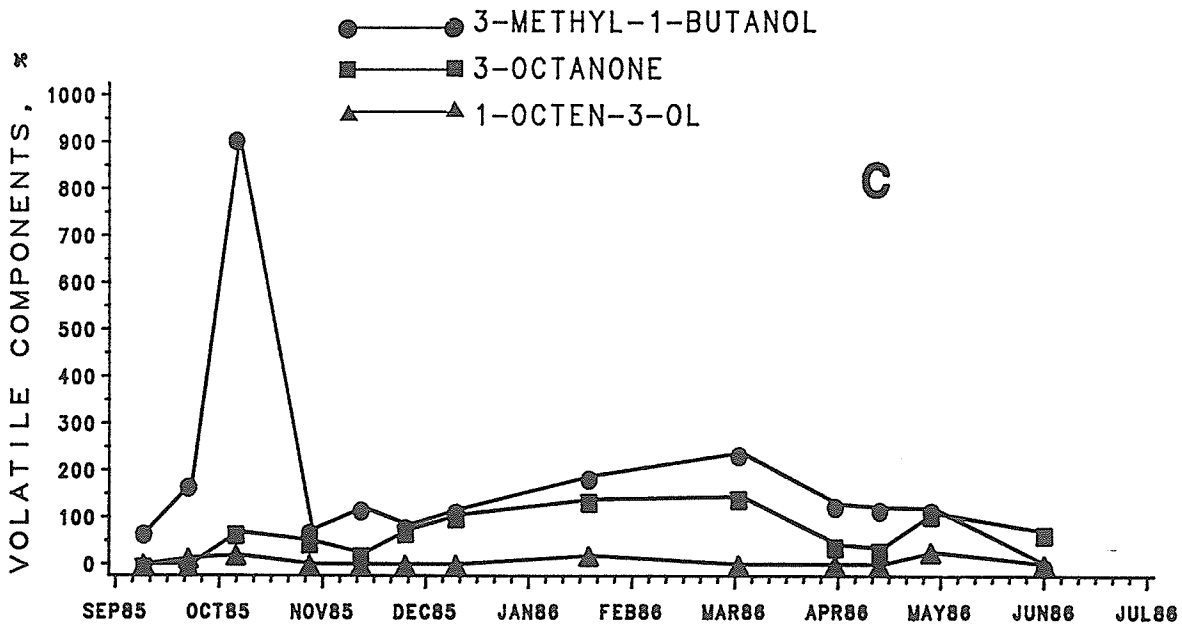
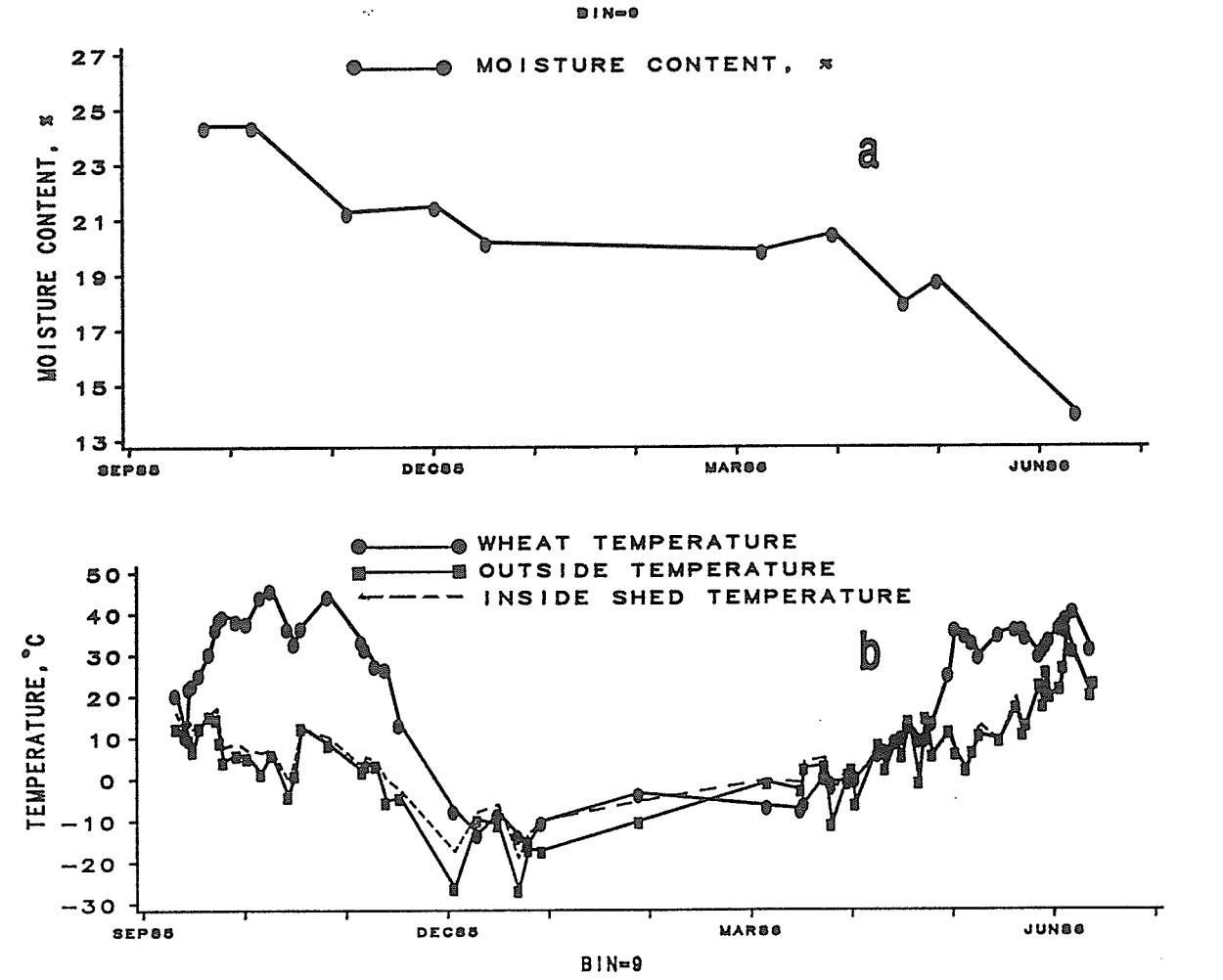

3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
VOLATILE COMPONENTS, %
1000
900
800
700
600
500
400
300
200
100
0
c
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 15. Moisture content, temperature and dilution plating of wheat stored at 25% moisture content in control bin 9 in 1985-86.


BIN=0
MOISTURE CONTENT, %
a
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
SEP85
DEC85
MAR86
JUN86
WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
b
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
SEP85
DEC85
MAR86
JUN86
BIN=9

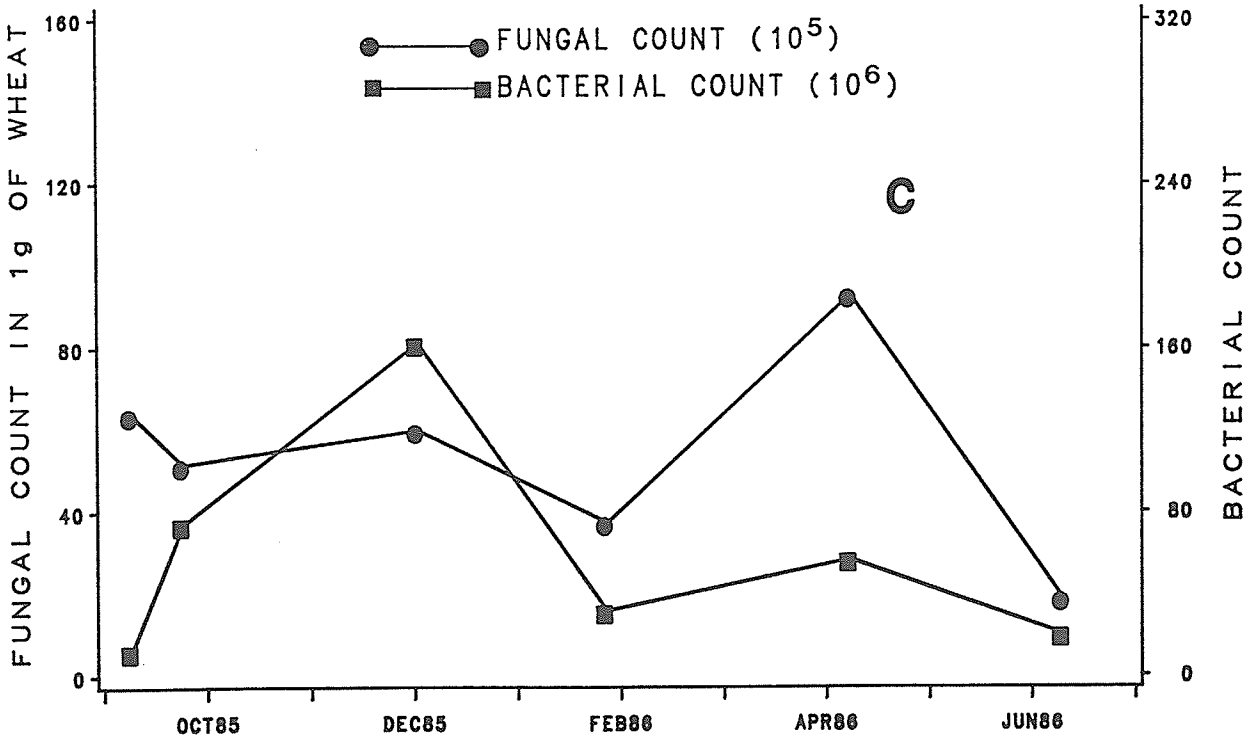
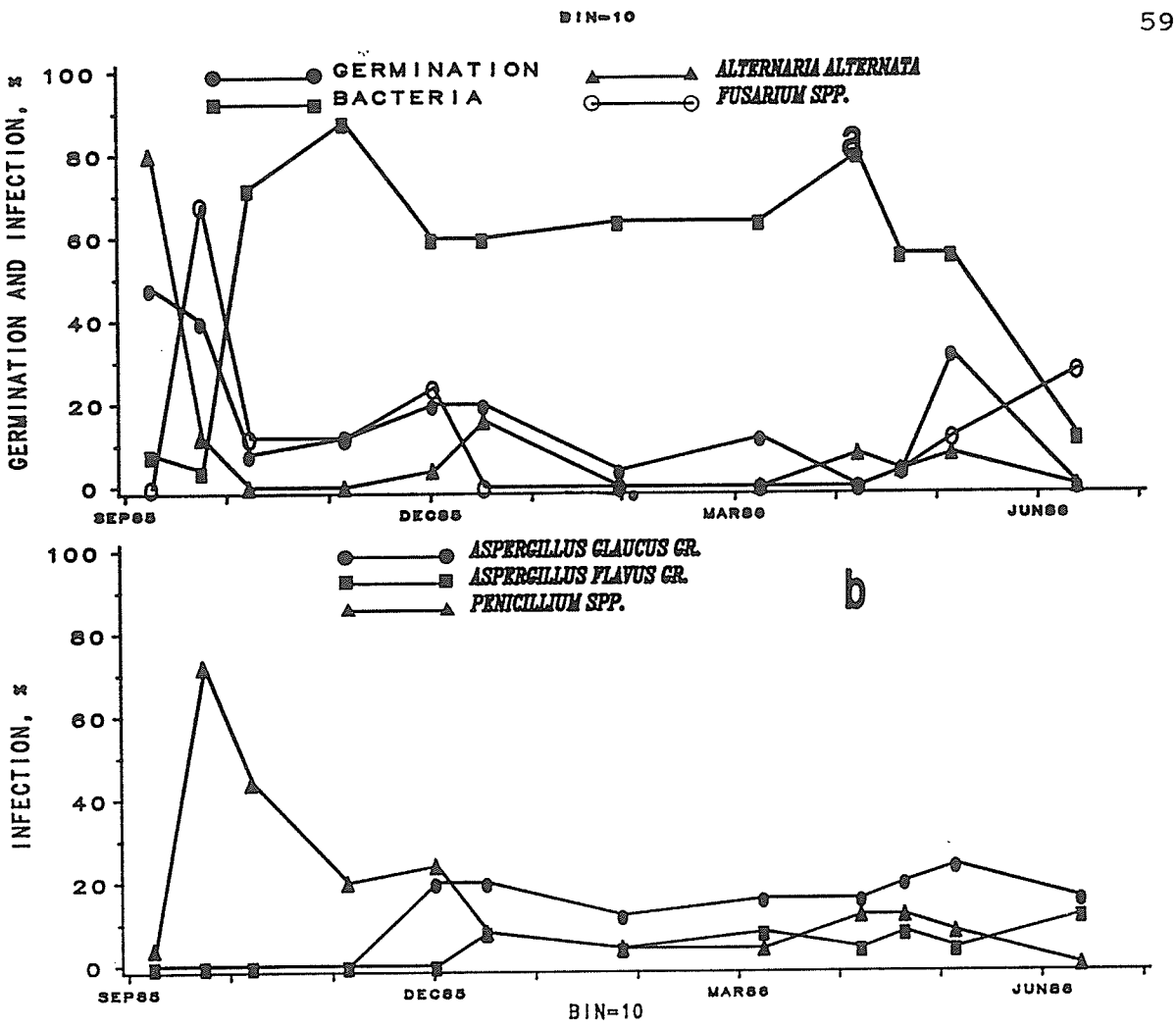
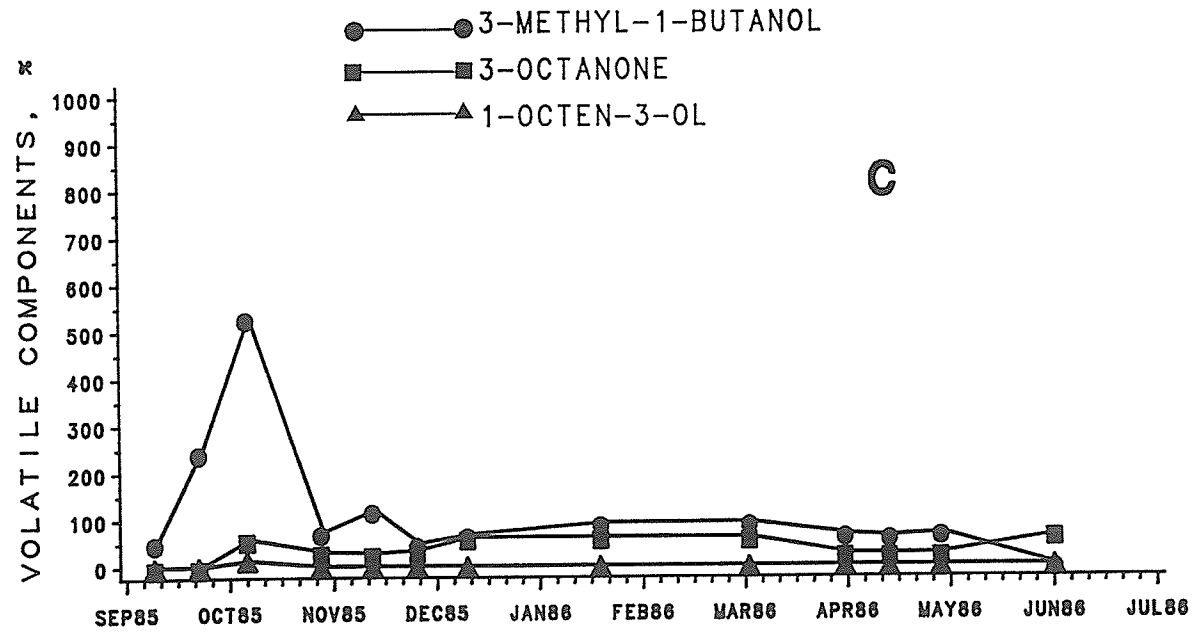

FUNGAL COUNT (10^5)
BACTERIAL COUNT (10^6)
c
FUNGAL COUNT IN 1g OF WHEAT
160
120
80
40
0
BACTERIAL COUNT
320
240
160
80
0
OCT85
DEC85
FEB86
APR86
JUN86

Figure 16. Microfloral infection, germination and odor volatiles of wheat stored at 25% moisture content in control bin 10 in 1985-86.

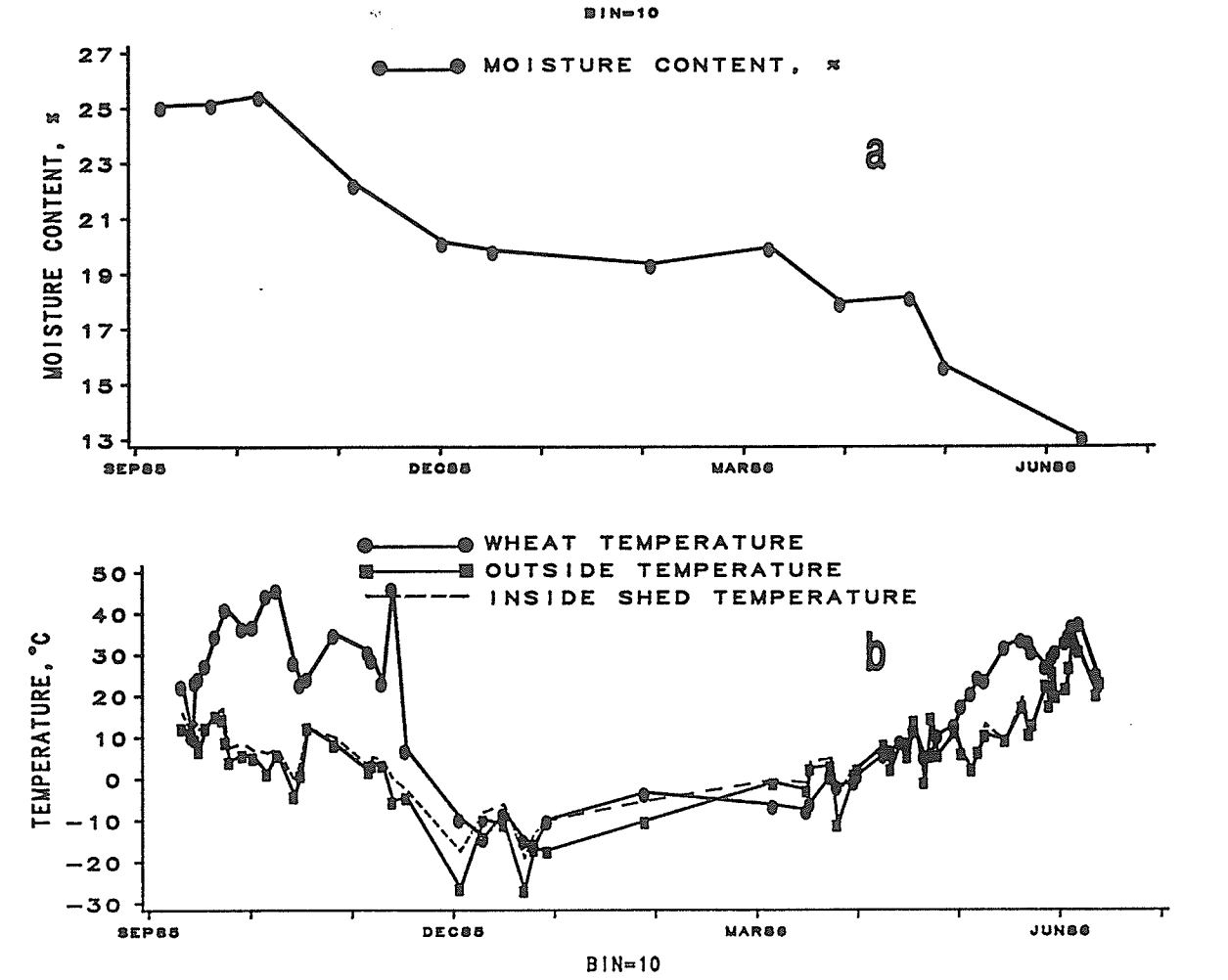

BIN=10
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
GERMINATION AND INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
BIN=10


3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
1000
900
800
700
600
500
400
300
200
100
0
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Bacterial infection in both control bins increased sharply from an initial <10% level to 70% level as the fungal infection declined during the first 2 mo. The high bacterial infection levels declined only in June 1986, coinciding with an increase in *Fusarium* spp. infection to 30% in bin 10 and to 50% in bin 9, possibly resulting from moisture decrease. In control bin 10, *Aspergillus glaucus* gr. infection was higher than that of *Penicillium* spp. and *A. flavus* during the winter and summer (Fig. 16b). Fungal and bacterial dilution counts were 10^5 and 10^6 per 1 g of grain, respectively. Moisture content decreased gradually to 13% in bin 10 and to 14% in bin 9, possibly as a result of rise in grain temperature facilitating moisture transfer to outside air.

In low-ventilated bins, 3-methyl-1-butanol increased from a 52 to 91% level in September 1985, coinciding with increasing infection by *Fusarium* spp. to 80% in bin 5 (Fig. 18a,c). A gradual increase in the level of *Penicillium* spp. infection seems to have kept 3-methyl-1-butanol at around the 50% level during November-June 1986, despite fall and spring ventilation and low winter temperatures of -10^0C. During spring the low-airflow ventilation kept grain temperatures a few degrees cooler than daily ambient temperatures (Figs. 19b and 21b). Seed germination gradually decreased to 45% coinciding with an increase of *Penicillium* spp. infection to ca. 40% in June 1986 (Figs. 18a,b and 20a,b). Bacterial dilution counts were 10^6 per 1 g of grain, while fungal dilution counts leveled off in December 1985 at ca. $40x10^4$ per 1 g of grain.

Figure 17. Moisture content, temperature and dilution plating of wheat stored at 25% moisture content in control bin 10 in 1985-86.


BIN=10
MOISTURE CONTENT, %
a
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
SEP85
DEC85
MAR86
JUN86
WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
b
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
SEP85
DEC85
MAR86
JUN86
BIN=10

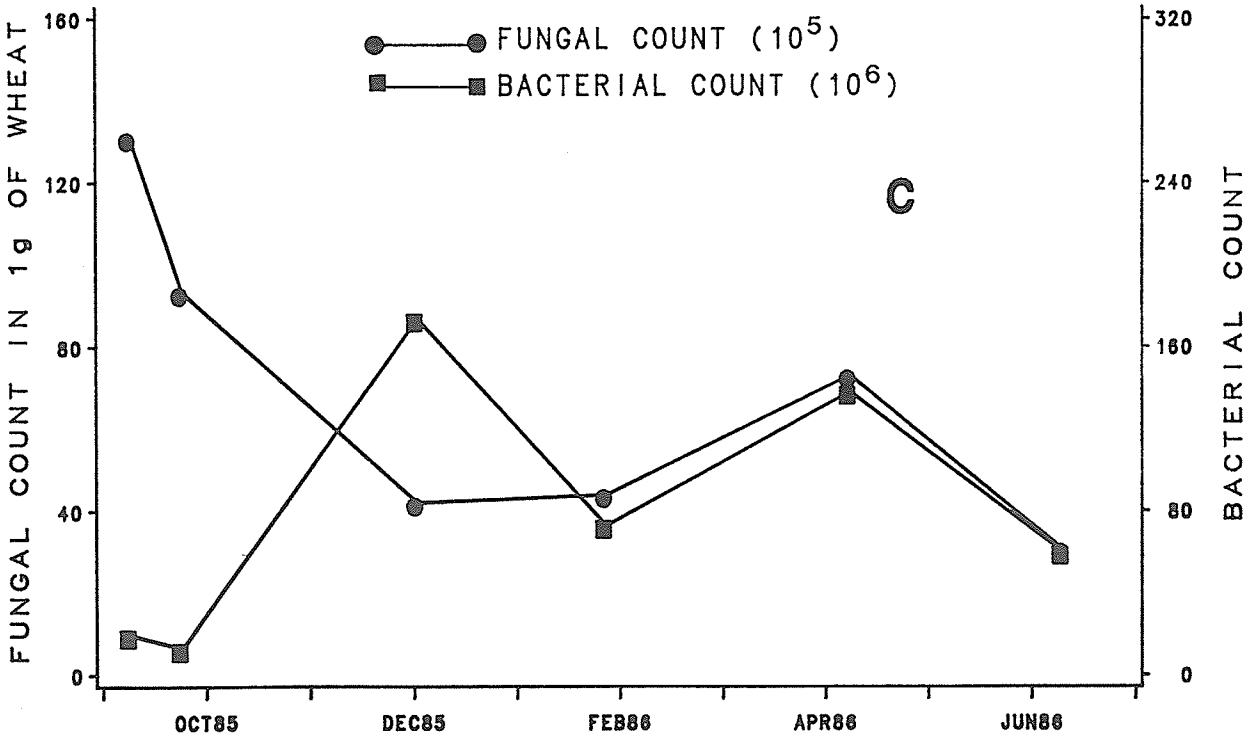
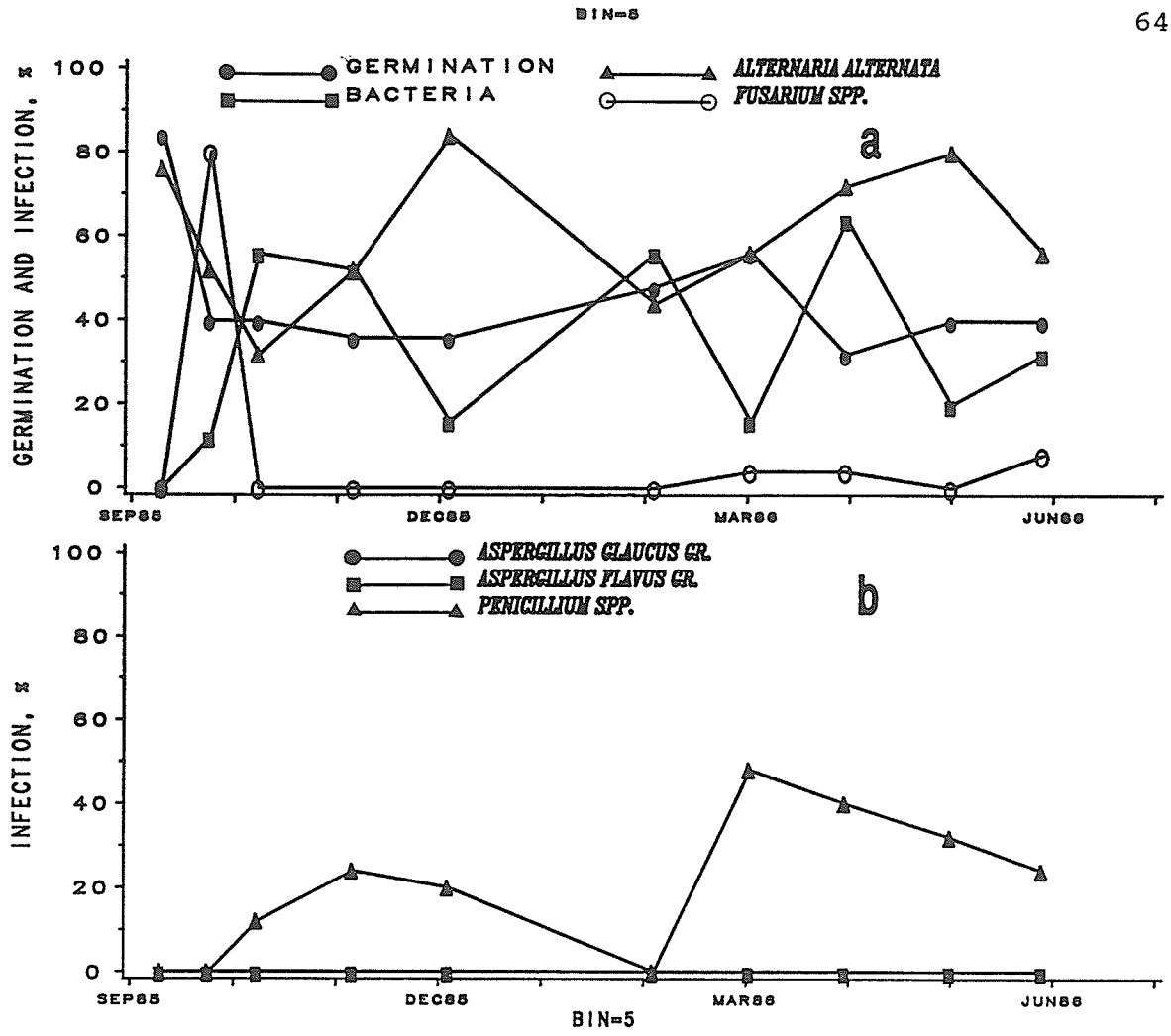

FUNGAL COUNT (10^5)
BACTERIAL COUNT (10^6)
c
FUNGAL COUNT IN 1g OF WHEAT
160
120
80
40
0
BACTERIAL COUNT
320
240
160
80
0
OCT85
DEC85
FEB86
APR86
JUN86

Figure 18. Microfloral infection, germination and odor volatiles of wheat stored at 25% moisture content in low-ventilated bin 5 in 1985-86; (ventilation periods are designated with broken line).


BIN=5
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
GERMINATION AND INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
BIN=5

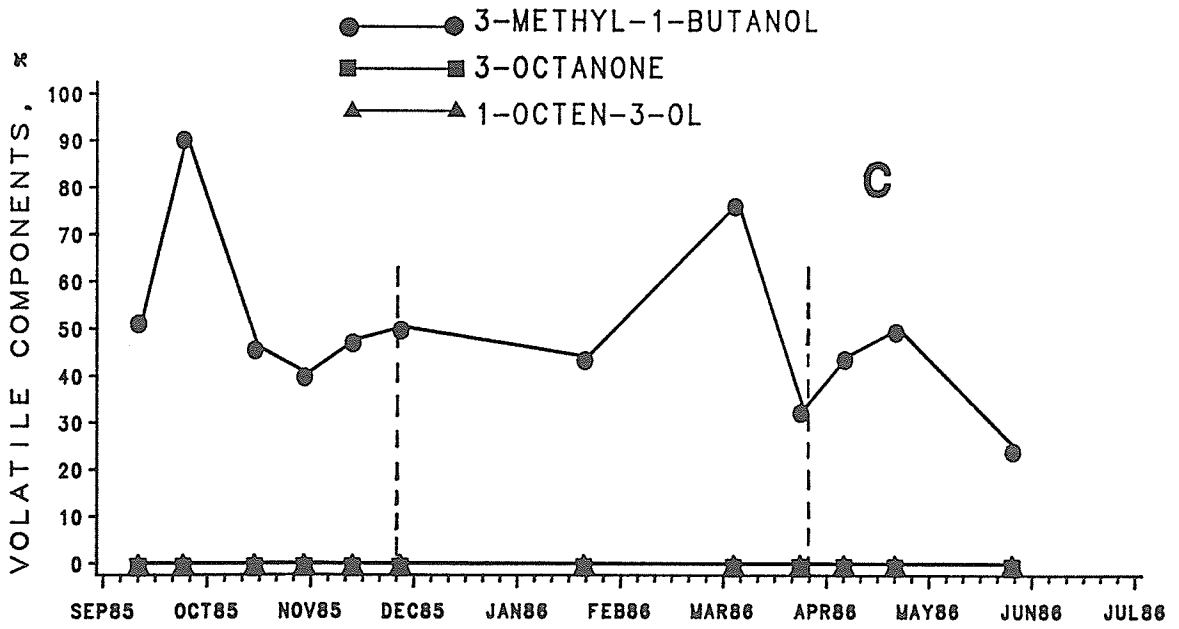
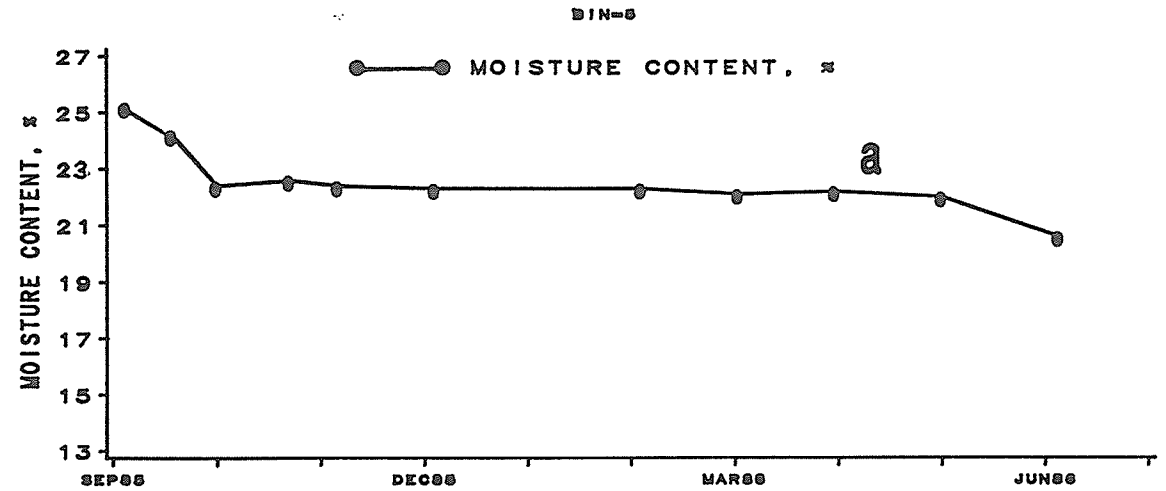
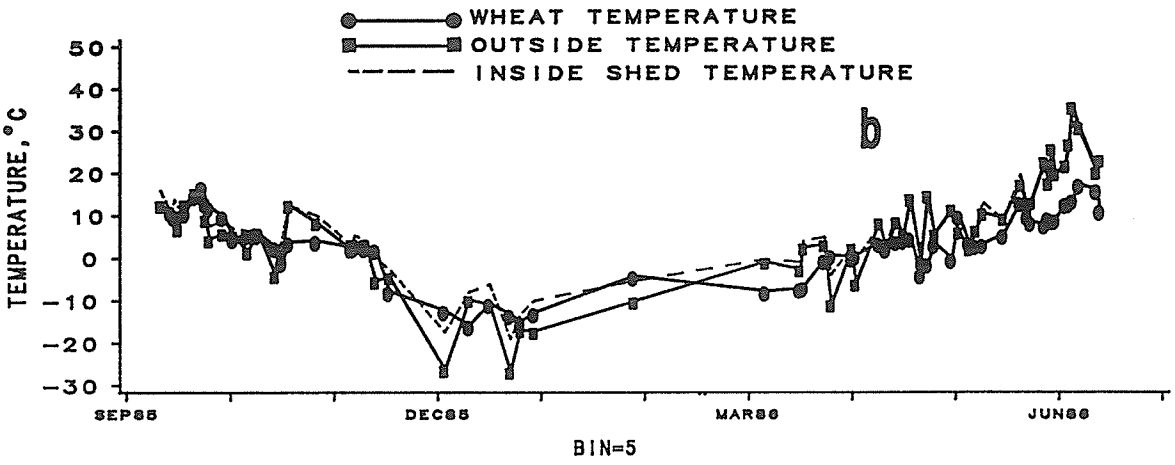

3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
100
90
80
70
60
50
40
30
20
10
0
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 19. Moisture content, temperature and dilution plating of wheat stored at 25% moisture content in low-ventilated bin 5 in 1985-86.


BIN=5
MOISTURE CONTENT, %
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
a
SEP85
DEC85
MAR86
JUN86


WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
b
SEP85
DEC85
MAR86
JUN86

BIN=5

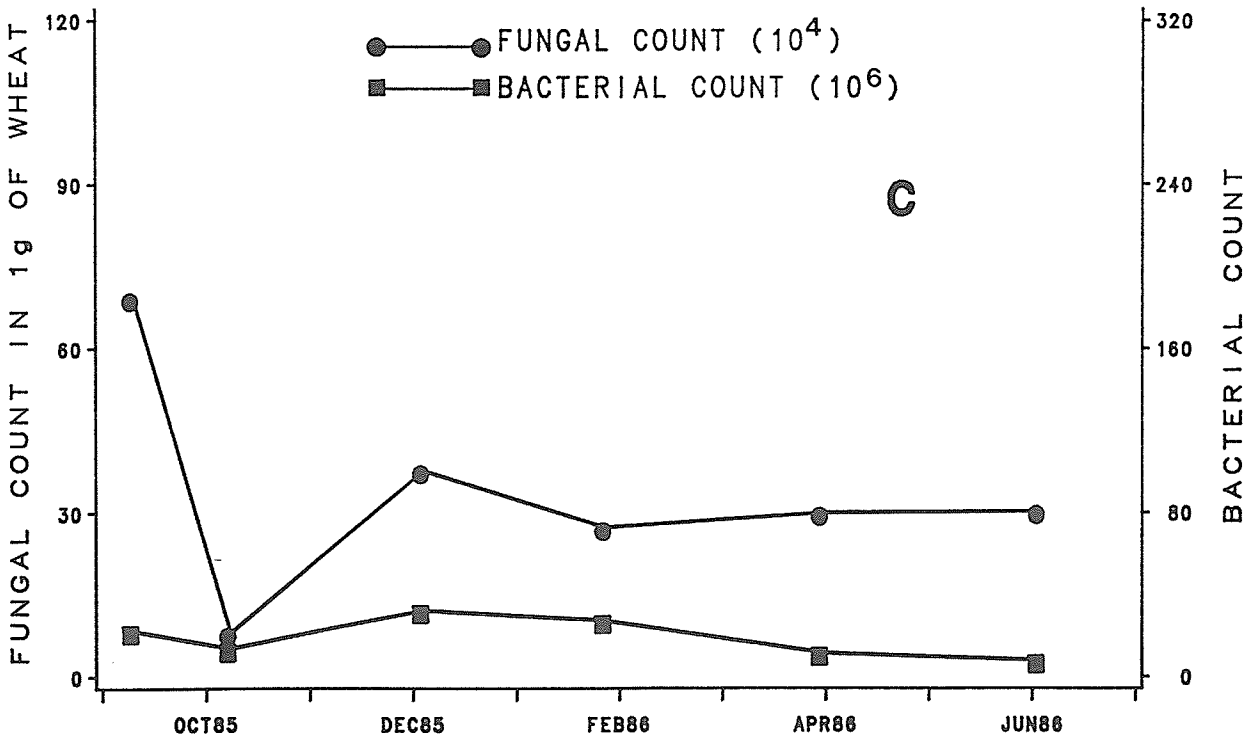
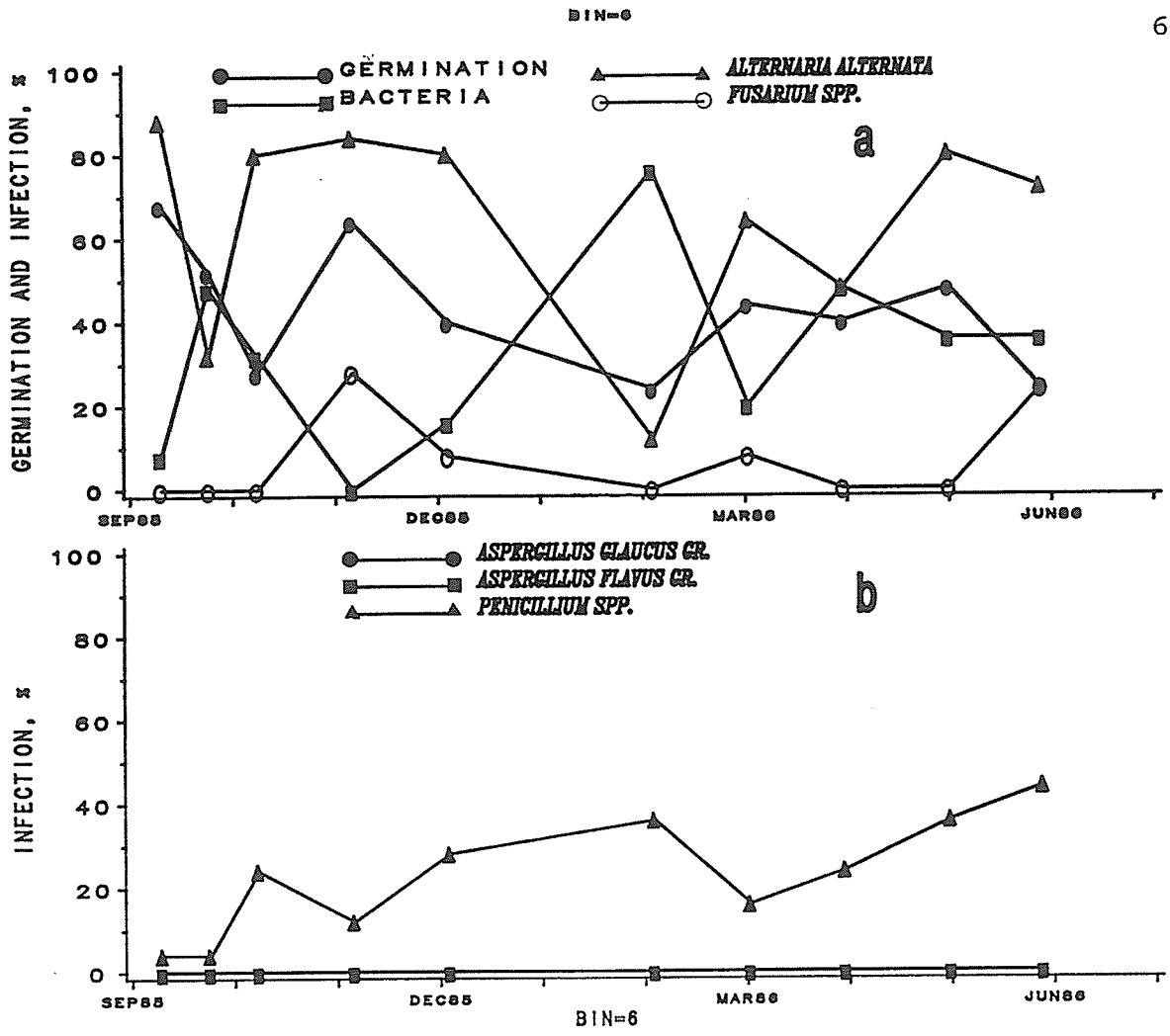

FUNGAL COUNT (10^4)
BACTERIAL COUNT (10^6)
FUNGAL COUNT IN 1g OF WHEAT
BACTERIAL COUNT
120
90
60
30
0
320
240
160
80
0
c
OCT85
DEC85
FEB86
APR86
JUN86

Figure 20. Microfloral infection, germination and odor volatiles of wheat stored at 25% moisture content in low-ventilated bin 6 in 1985-86; (ventilation periods are designated with broken line).


BIN=6
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
GERMINATION AND INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
BIN=6

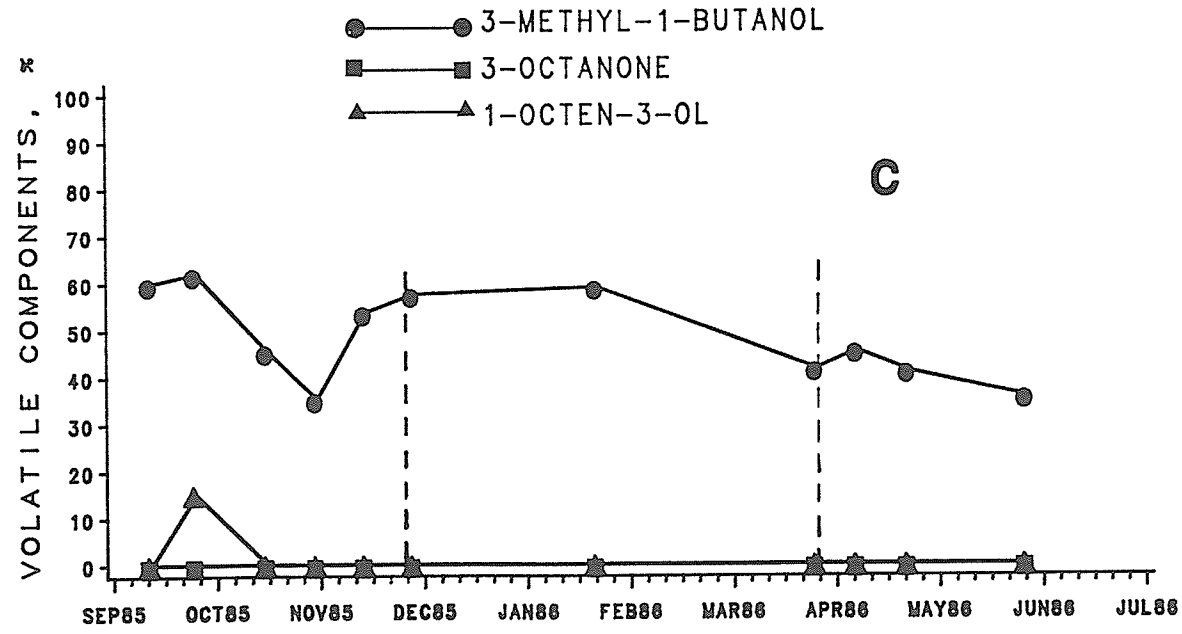
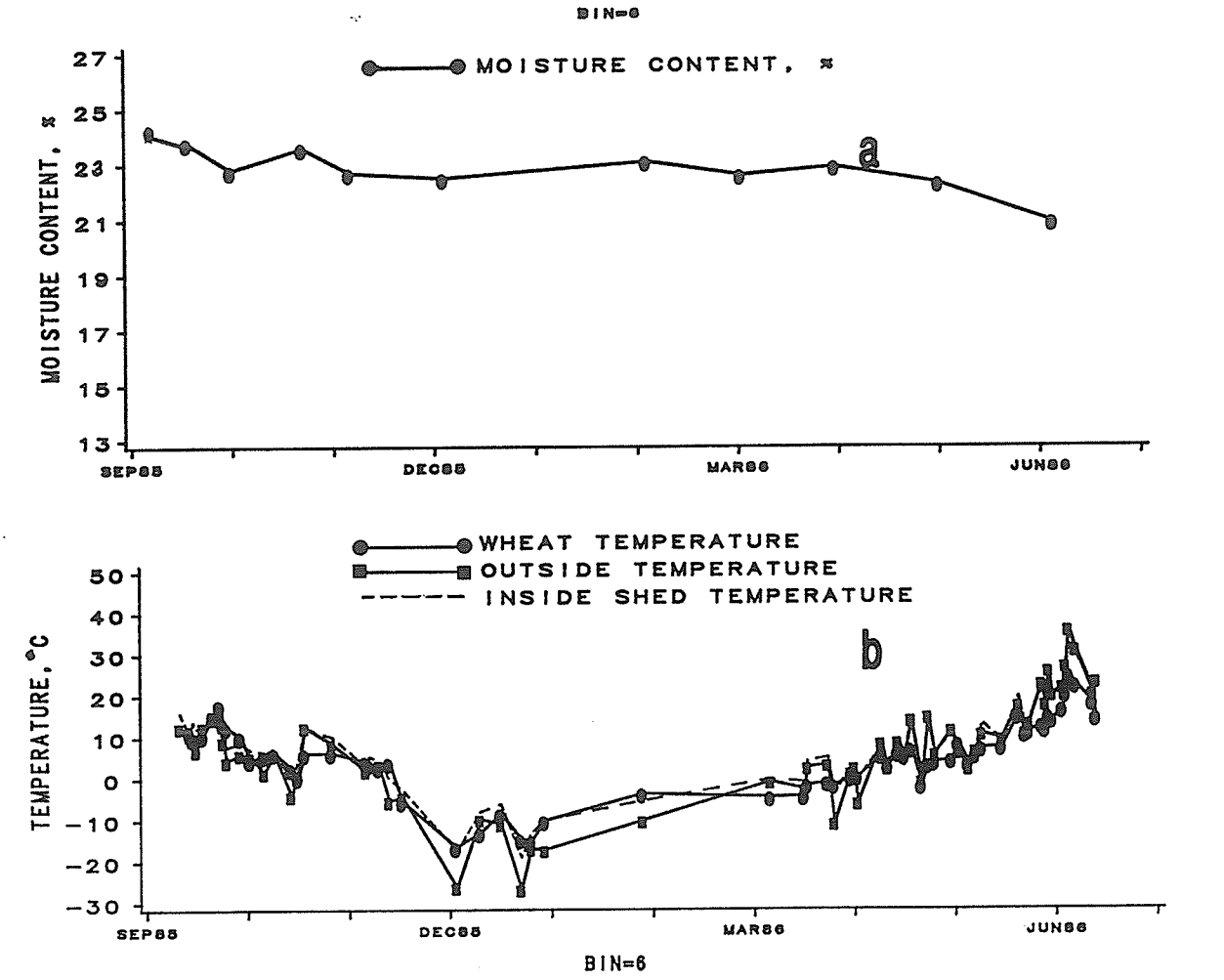
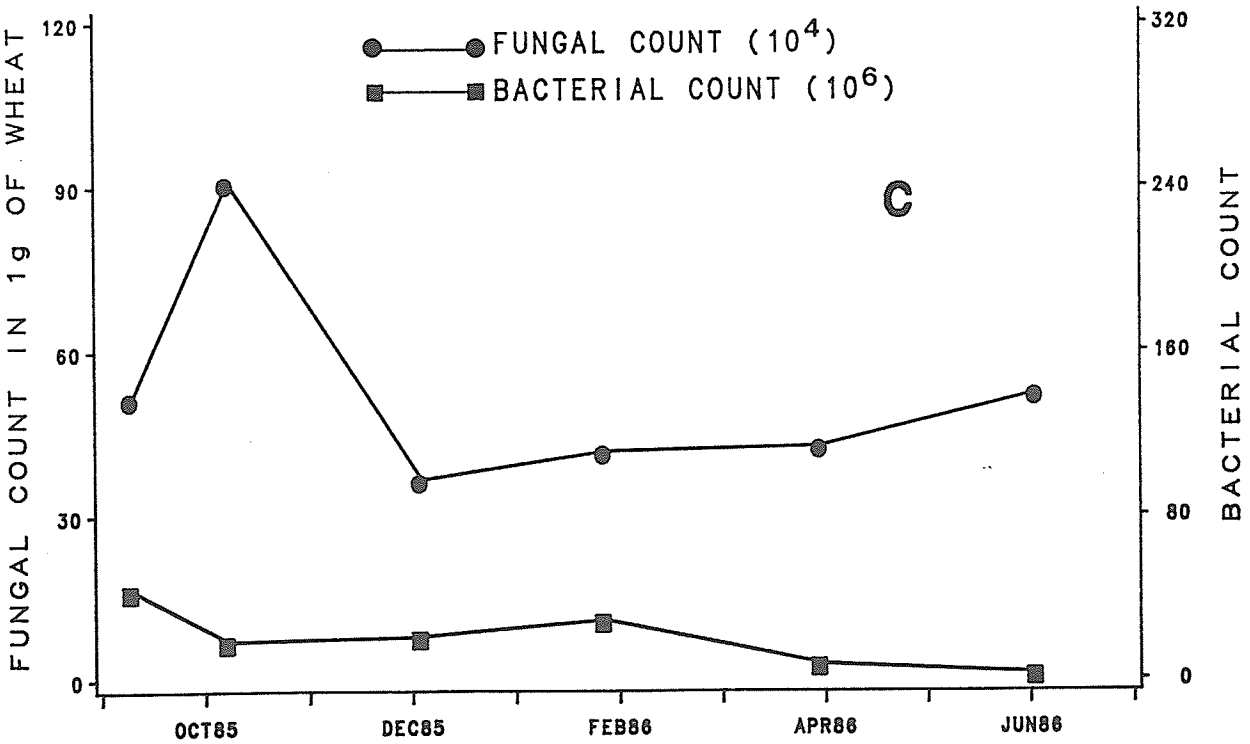

3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
100
90
80
70
60
50
40
30
20
10
0
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 21. Moisture content, temperature and dilution plating of wheat stored at 25% moisture content in low-ventilated bin 6 in 1985-86.


BIN=6
MOISTURE CONTENT, %
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
a
SEP85
DEC85
MAR86
JUN86
WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
b
SEP85
DEC85
MAR86
JUN86
BIN=6

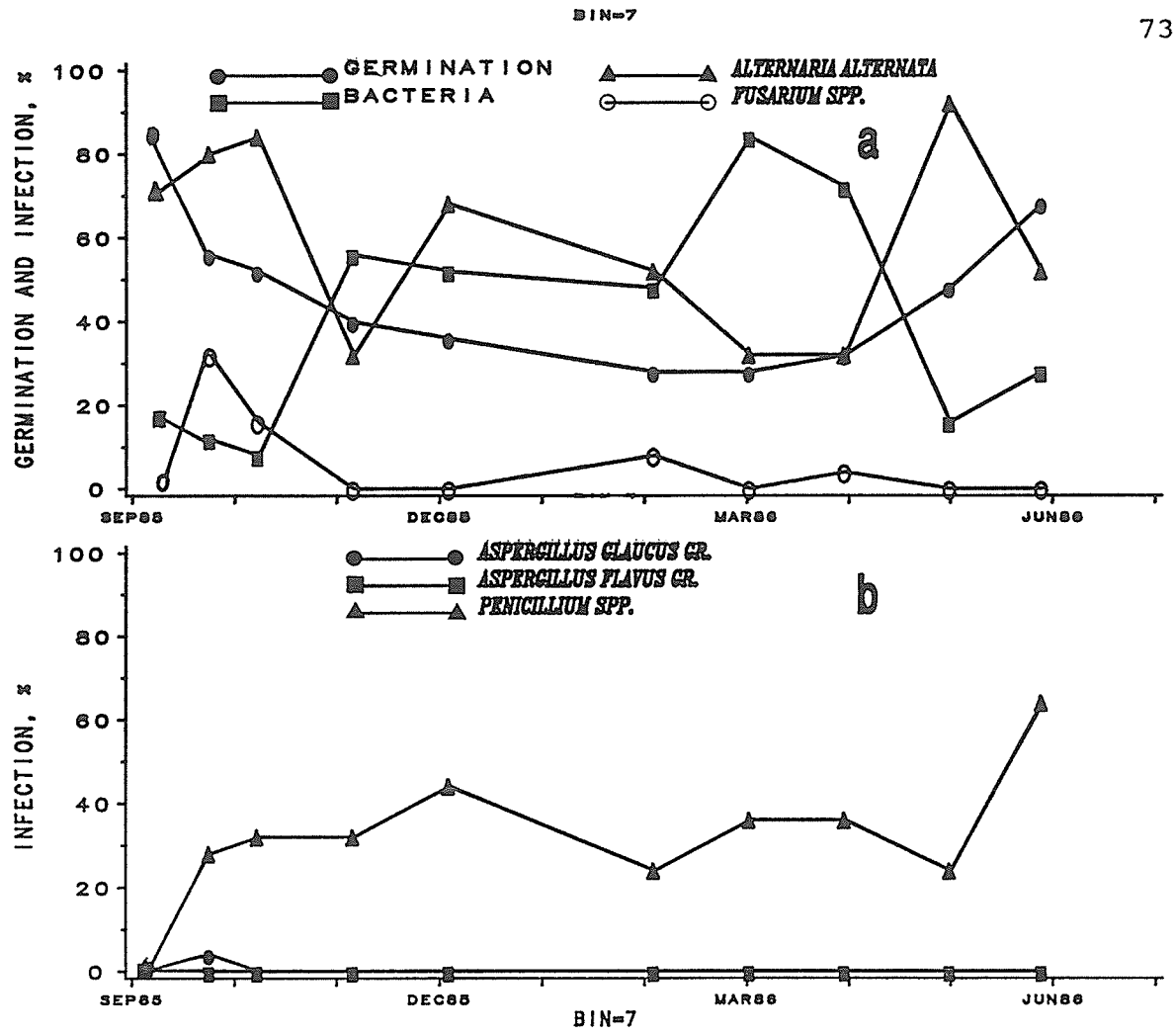

FUNGAL COUNT (10^4)
BACTERIAL COUNT (10^6)
FUNGAL COUNT IN 1g OF WHEAT
120
90
60
30
0
BACTERIAL COUNT
320
240
160
80
0
c
OCT85
DEC85
FEB86
APR86
JUN86

In high-ventilated bins only 3-methyl-1-butanol was detected (Figs. 22c and 24c); its level increased from 58 to 98% in October 1985 in bin 7. This increase coincided with a high infection level of *Alternaria alternata* at 80%, and with a sharp increase in *Penicillium* spp. infection level to 25% (Fig. 22a,b). A gradual increase of *Penicillium* spp. infection seems to have kept 3-methyl-1-butanol at 50% level (av.) despite fall and spring ventilation and low winter temperatures of -10^0C. Resumed ventilation from 26 March 1986 increased the grain temperature and resulted in a sharp decrease in moisture content, from 22% to 16% (Figs. 23a and 25a), and coinciding with the same sharp loss of odor volatiles (Figs. 22c and 24c). Seed germination decreased to a 10% level(av.) during the winter and increased again in the spring to a 50% (av.). This fluctuation could result from shrinkage of grain and the subsequent change in grain levels at sampling ports from which seeds were taken for germination analysis. Fungal dilution counts were ca. 10^4 and bacterial dilution counts were ca. 10^6 per 1 g of grain (Figs. 23c and 25c).

(1) Abiotic variables

Grain moisture content in control bins stayed at the initial 25% level during the first month of storage, then dropped to 22-23% in November 1985. Moisture content leveled off during winter and declined to 14% in May 1986 in control bin 9 (Fig. 15a). In control bin 10, moisture content further declined to 20% in December and to 13% in May (Fig. 17a). In low-ventilated bins the moisture content dropped from 25% to 22-23% during the first month of storage and stayed at the same level until May 1986, when it further declined to 17% (Figs. 19a and 21a). In high-ventilated bins, moisture content decreased slightly to 23-24% in the first month of storage and stayed at the same level until April 1986. At this time it dropped sharply to 16% m.c. remaining at this level until June 1986 (Figs. 23a and 25a).

Figure 22. Microfloral infection, germination and odor volatiles of wheat stored at 25% moisture content in high-ventilated bin 7 in 1985-86; (ventilation periods are designated with broken line).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GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
GERMINATION AND INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
INFECTION, %
BIN=7

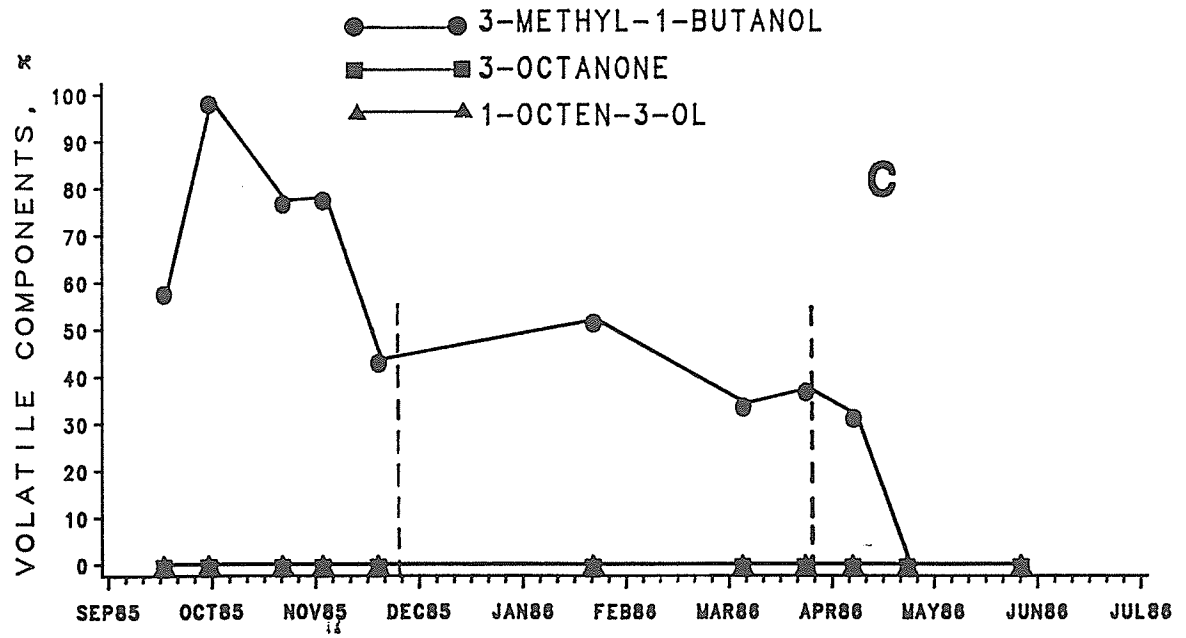
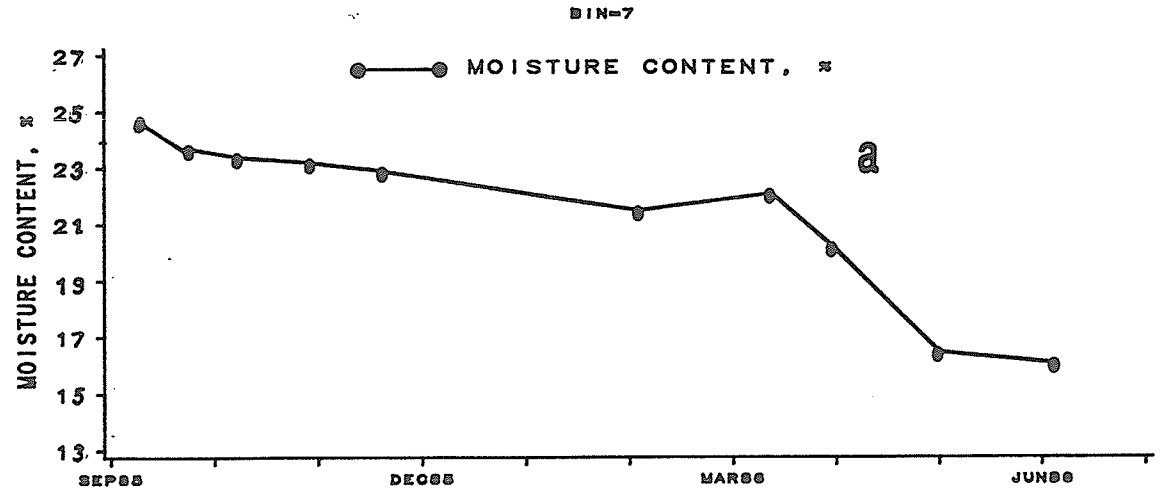
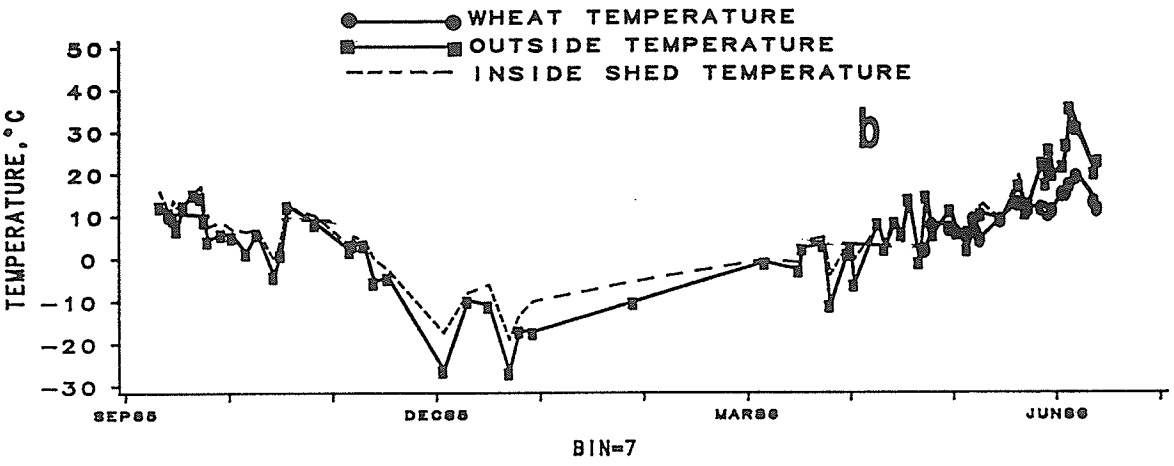

3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
100
90
80
70
60
50
40
30
20
10
0
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 23. Moisture content, temperature and dilution plating of wheat stored at 25% moisture content in high-ventilated bin 7 in 1985-86.


BIN=7
MOISTURE CONTENT, %
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
a
SEP85
DEC85
MAR86
JUN86


WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
b
SEP85
DEC85
MAR86
JUN86

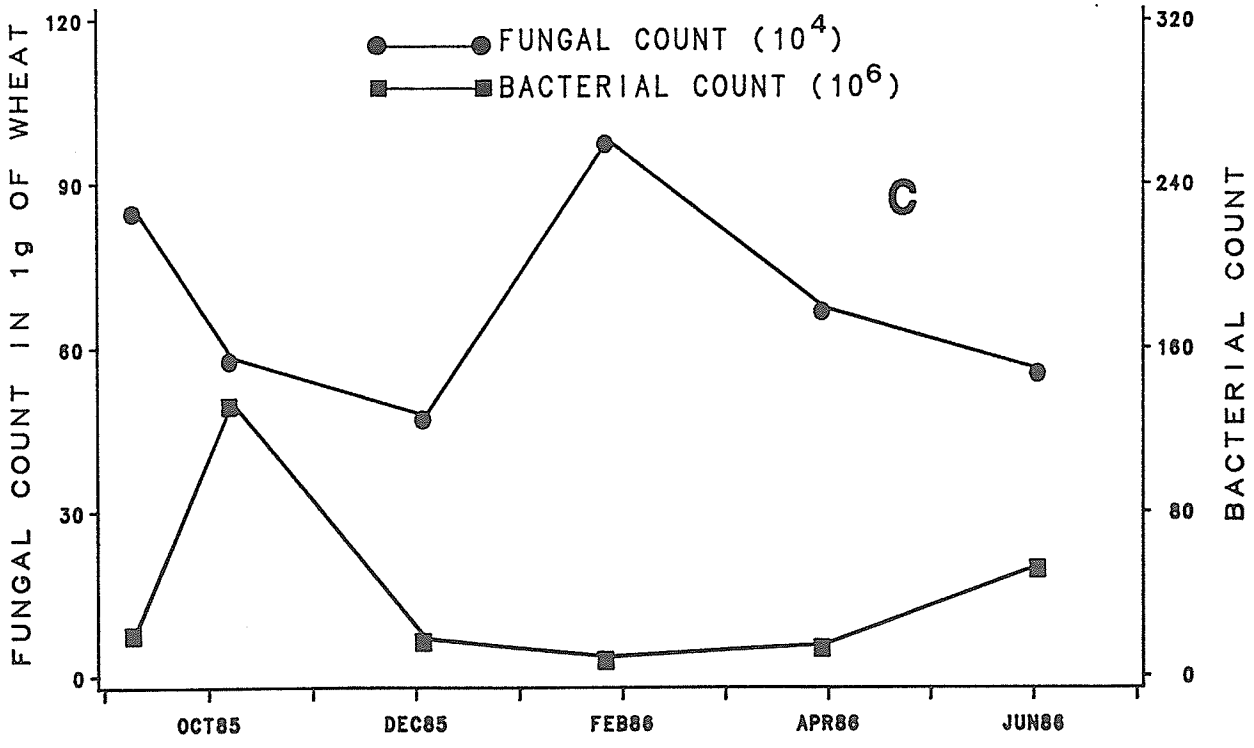

BIN=7
FUNGAL COUNT (10^4)
BACTERIAL COUNT (10^6)
FUNGAL COUNT IN 1g OF WHEAT
BACTERIAL COUNT
120
90
60
30
0
320
240
160
80
0
c
OCT85
DEC85
FEB86
APR86
JUN86

Figure 24. Microfloral infection, germination and odor volatiles of wheat stored at 25% moisture content in high-ventilated bin 8 in 1985-86; (ventilation periods are designated with broken line).

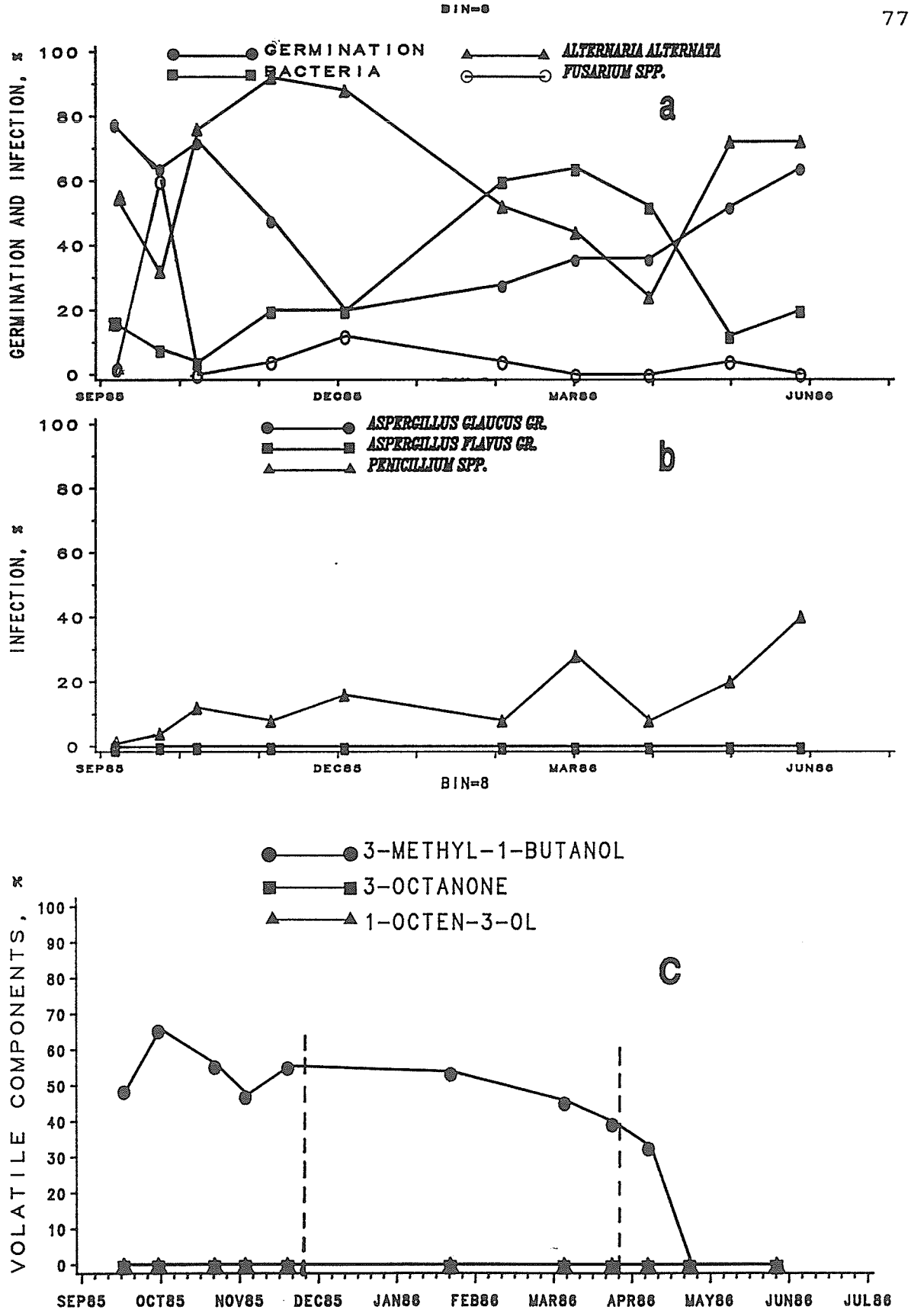
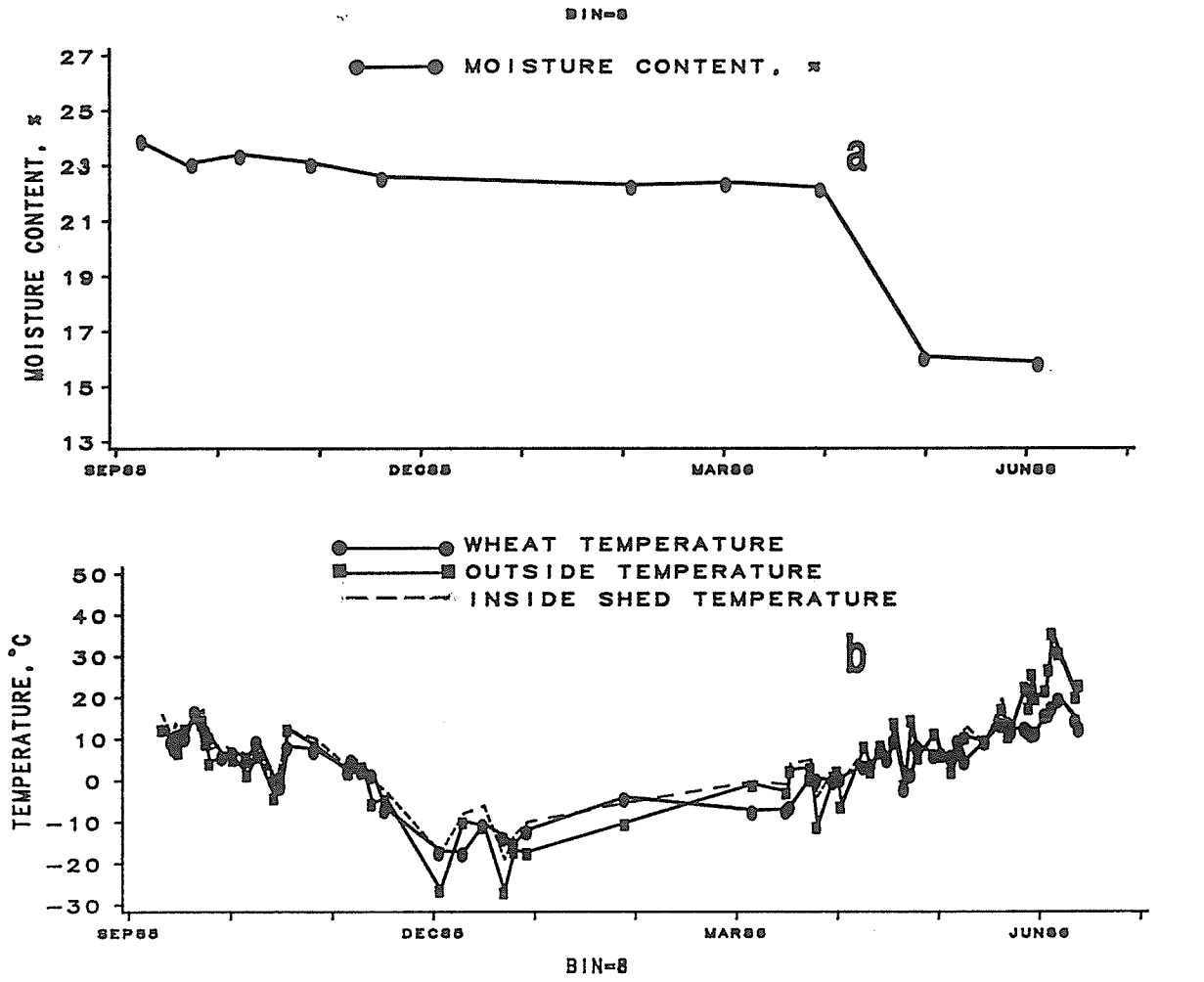
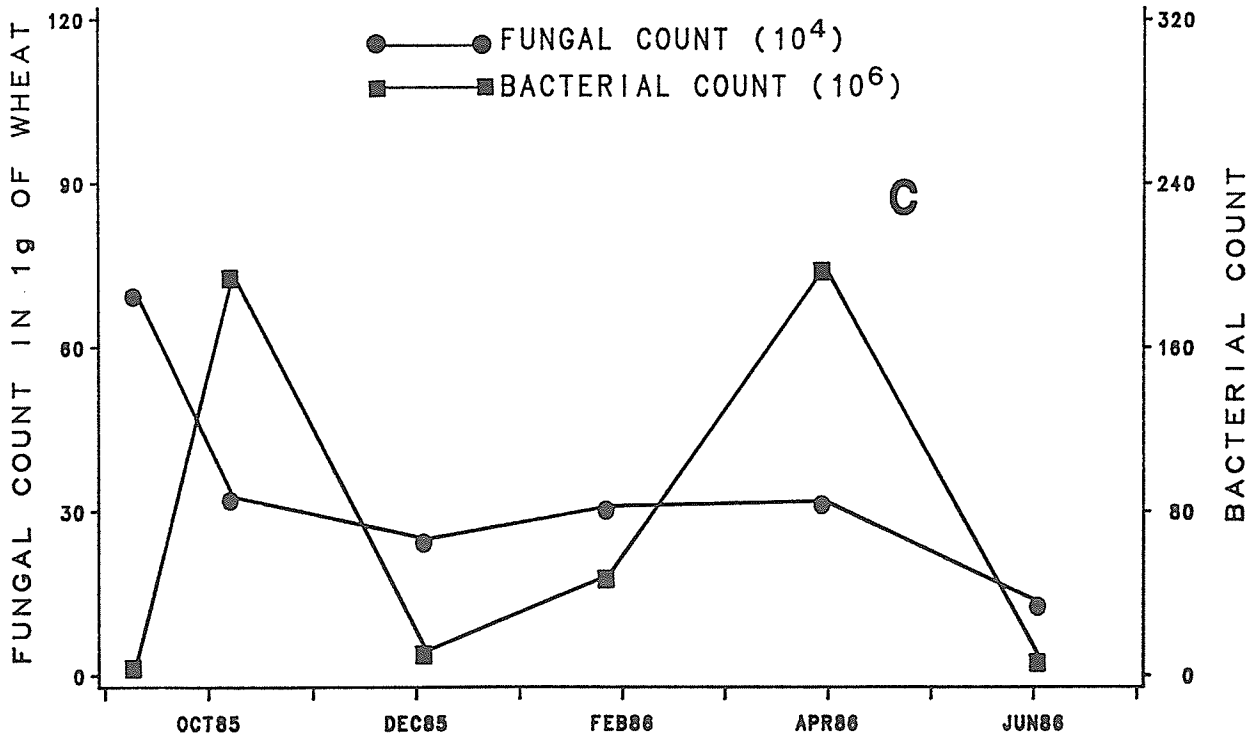

BIN=8
GERMINATION
BACTERIA
ALTERNARIA ALTERNATA
FUSARIUM SPP.
a
GERMINATION AND INFECTION, %
100
80
60
40
20
0
SEP85
DEC85
MAR86
JUN86
ASPERGILLUS GLAUCUS GR.
ASPERGILLUS FLAVUS GR.
PENICILLIUM SPP.
b
INFECTION, %
BIN=8
3-METHYL-1-BUTANOL
3-OCTANONE
1-OCTEN-3-OL
c
VOLATILE COMPONENTS, %
90
70
50
30
10
SEP85
OCT85
NOV85
DEC85
JAN86
FEB86
MAR86
APR86
MAY86
JUN86
JUL86

Figure 25. Moisture content, temperature and dilution plating of wheat stored at 25% moisture content in high-ventilated bin 8 in 1985-86.


BIN=8
MOISTURE CONTENT, %
27
25
23
21
19
17
15
13
a
SEP85
DEC85
MAR86
JUN86
WHEAT TEMPERATURE
OUTSIDE TEMPERATURE
INSIDE SHED TEMPERATURE
TEMPERATURE, °C
50
40
30
20
10
0
-10
-20
-30
b
SEP85
DEC85
MAR86
JUN86
BIN=8


FUNGAL COUNT (10^4)
BACTERIAL COUNT (10^6)
FUNGAL COUNT IN 1g OF WHEAT
120
90
60
30
0
BACTERIAL COUNT
320
240
160
80
0
c
OCT85
DEC85
FEB86
APR86
JUN86

Grain temperature in control bins reached a maximum of 45^{0}C in the fall, this level being 30^{0}C above the inside shed temperature (Figs. 15b and 17b). In November and December 1985 grain temperature slowly decreased to slightly above the inside shed temperature of -10^{0}C. In May-June 1986 grain temperature increased again to 30-40^{0}C while the inside shed temperature fluctuated between 10 and 30^{0}C.

Grain temperature in low- and high-ventilated bins followed closely the outside temperature during the first 3 mo. (Figs. 19b, 21b, 23b, 25b). In December 1985 grain temperature further decreased equal to the lowest inside shed temperature of -15^{0}C. During April-June 1986 grain temperature remained lower (5 to 15^{0}C) and fluctuated less than the outside temperature (15 to 35^{0}C).

(2) Biotic variables

Seed germination in control bins decreased sharply during the first month of storage and remained within 0-35% for the rest of experiment (Figs. 14a and 16a). Microfloral infection in the fall consisted of the field fungi, *Alternaria alternata* and *Fusarium* spp. An initial *A. alternata* infection of 80-90% decreased sharply during the first month of storage to 0-5% for the rest of experiment. Pronounced *Fusarium* spp. infection occurred only once in September 1985 when it reached an infection level of 52% in bin 9 and 68% in bin 10. This field fungus was replaced by bacteria, but it reoccurred in June when m.c. further decreased, thus making conditions unfavorable for bacteria. Bacterial infection increased sharply from an initial <10% during September 1985 to 70%, and remaining between 55-85% during October-April 1986 (Figs. 14a and 16a). In June bacterial infection decreased to 25% and 16% in bins 9 and 10, respectively.

Storage fungi of *Aspergillus* and *Penicillium* genera occurred in both bins (Figs. 14b and 16b). Infection by *Penicillium* spp. increased during the first month to infection levels of 75% and 100% in bins 9 and 10, respectively, a result of high m.c.

and temperature. Bacterial succession kept *Penicillium* spp. infection to 0-20% until the completion of the experiment. Infection by *Aspergillus glaucus* gr. was between 15-20% during November-June 1986 in bin 9 only (Fig. 14b). In November 1985 infection by *Aspergillus flavus* was associated with high temperature and moisture and increased to 95% in bin 9. This fungi remained at levels <10% for the remainder of the experiment in both control bins.

In both control bins fungal dilution counts fluctuated between $20x10^5$-$130x10^5$ per 1 g of grain throughout the experiment; bacterial dilution counts fluctuated between $5x10^6$-$180x10^6$ per 1 g of grain (Figs. 15c and 17c).

In low-ventilated bins, seed germination fluctuated between 25-90% during the first 5 mo. and between 30-60% during the next 6 mo. (Figs. 18a and 20a). Microfloral infection consisted of *Alternaria alternata* spp. ranging from 10-100%, and *Fusarium* spp., which increased to 80% during September 1985 in bin 5. The *Fusarium* spp. infection remained at <30% in both bins for the remainder of the experiment (Figs. 18a and 20a). Bacterial infection ranged from 0 to 80% and fluctuated reciprocally to *Alternaria alternata* infection, probably the result of species competition. Infection by *Penicillium* spp. increased steadily to 40% by the end of the study (Figs. 18b and 20b).

Fungal dilution counts in low-ventilated bins fluctuated between $10x10^4$-$90x10^4$ per 1 g of wheat during the first 2 mo. (Figs. 19c and 21c). During December-June 1986, fungal dilution counts remained between $30x10^4$-$50x10^4$ per 1 g of grain; bacterial dilution counts remained between $4x10^6$-$40x10^6$ per 1 g of grain throughout the experiment.

In high-ventilated bins, seed germination decreased from an initial 78-85% range to 2-10% during the first 4 mo. (Figs. 22a and 24a), then remained between 30-70%. Microfloral infection consisted of *Alternaria alternata* ranging from 30 to 90%, and *Fusarium* spp. which increased during September 1985 to 35 and to 60%

in bins 7 and 8,respectively; it remained <10% in both bins (Figs. 22a and 24a). Bacterial infection was between 0-75%, fluctuated reciprocally to *A. alternata* infection. Infection by *Penicillium* spp. increased gradually to 30-60% during May-June 1986 (Figs. 22b and 24b).

In high-ventilated bins fungal dilution counts fluctuated between $15x10^4$-$100x10^4$, while bacterial dilution counts fluctuated between $2x10^6$-$200x10^6$ per 1 g of grain throughout the experiment (Figs. 23c and 25c).

In control bins three volatiles were detected at different levels (Figs. 14c and 16c). The most common and abundant volatile, 3-methyl-1-butanol, increased in October 1985 from an initial 50% to 930% in bin 9 and from an initial 70% to 550% in bin 10. This volatile remained between 50-250% during November-May 1986. The absence of 3-methyl-1-butanol was noted on the last day of the experiment in June, when m.c. decreased to 13-14%. The second most abundant volatile, 3-octanone, increased in October 1985 to 70% and remained between 20-150% during October-June 1986. A third volatile, 1-octen-3-ol, occurred sporadically at 20-30% levels in October 1985, January and May 1986.

In low-ventilated bins two volatiles, 3-methyl-1-butanol and 1-octen-3-ol, were detected (Figs. 18c and 20c). The most common and abundant volatile, 3-methyl-1-butanol, ranged from 35 to 90% during September-May 1986 and was most abundant in September and March 1986 in bin 5 only (Fig. 18c). The second volatile, 1-octen-3-ol, occurred only once at 15% in September 1985 in bin 6 (Fig. 20c).

In high-ventilated bins the only detected volatile was 3-methyl-1-butanol which occurred abundantly from September 1985 until April 1986 (Figs. 22c and 24c). This volatile increased to 100% in October in bin 7 and remained at 45-55% levels during December-March 1986 in both bins. No odor volatiles were detected

during April-June 1986 presumably because efficient ventilation carried volatiles away.

An unidentified peak occurred unexpectedly in both control bins during the first week and between 5-34 weeks of storage (Table 2); this peak was later identified as tridecane. As stored-product mites are known producers of tridecane, it seemed logical to sample the grain for mites. The tridecane peak was not identified until late February and grain was analyzed for mites in March 1986. At this time the presence of tridecane coincided with the collection of 143 mobile mites. At the end of experiment no tridecane was detected, although grain samples taken a few days before that were infested with *Acarus* spp. mites .

3. Statistical analyses on data from bin experiment 1985-86 (Table 3)

The most common and abundant volatile, 3-methyl-1-butanol, was correlated and associated in control bins with 20% m.c. wheat having *Aspergillus glaucus* gr. and bacterial infections. In control bins with 25% m.c. this volatile was correlated and associated with moisture content and with bacterial infection. The second most abundant volatile, 1-octen-3-ol, was correlated and associated in all control bins with temperature and with *Penicillium* spp. infection.

Ventilation interrupted natural microflora development and subsequent volatile production. Therefore, it was expected that most of the volatiles would be purged from ventilated stored-grain (bins were not airtight), and biotic interrelationships among variables would be less pronounced than those found in non-ventilated control bins. Three statistical methods, sample linear correlation coefficient, principal component, and stepwise multiple regression analyses, were applied on transformed data and presented in Table 3. Correlation matrices and graphical representations of relevant principal components along with associated variables are presented in Appendices 3-7.

Table 2. Acarine volatiles and mite count in 25% initial moisture content wheat stored in two control bins in 1985-86

Storage week-month-year	Odor volatiles tridecane (%)	Mite count (in 500 g) *Acarus* spp.
1-Sept 1985	77.9	n/a
	50.9	n/a
3-Sept 1985	0.0	n/a
	0.0	n/a
5-Oct 1985	0.0	n/a
	34.4	n/a
8-Oct 1985	186.8	n/a
	382.0	n/a
10-Nov 1985	47.2	n/a
	275.2	n/a
12-Nov 1985	42.6	n/a
	274.3	n/a
14-Dec 1985	133.4	n/a
	331.8	n/a
20-Jan 1986	95.7	n/a
	319.0	n/a
26-March 1986	71.0	0
	146.0	143[a]
30-March 1986	29.0	n/a
	94.0	n/a
32-April 1986	21.7	n/a
	65.1	n/a
34-May 1986	0.0	n/a
	44.2	n/a
39-June 1986	0.0	19[b]
	0.0	161[c]

n/a - not available
[a] *Acarus hypopus*, *A. immobilis* (Griffith), *A. farris* (Ond.)
[b] *A. farris*, *A. immobilis*
[c] *A. farris*, *A. siro*, *A. hypopus*

Table 3. Statistical analyses of interrelations of odor volatiles with biotic and abiotic variables according to simple correlation coefficient, principal component analyses and stepwise regression analyses of multivariate data in control and ventilated stored-wheat ecosystems in 1985-86

Odor volatile	Interrelation with, according to: Simple correlation (r)	Principal component analyses[b]	Stepwise regression analyses
Control ecosystem[a] - 20% initial moisture content			
3-Methyl-1-butanol	*Asp. glaucus*(p<0.01) bacteria(p<0.05)	(PC1, var. 32%) 1-octen-3-ol *Penicillium* *-Alternaria* bacteria (PC3, var. 16%) *Asp. glaucus* *Alternaria* -bacteria (PC4, var.12%) germination *-Penicillium*	(R^2=0.57, p<0.01) *Asp. glaucus* bacteria
1-Octen-3-ol	*Penicillium*(p<0.01) -Moist. content(p<0.01) Temperature(p<0.05)	(PC1, var. 32%) *Penicillium* *-Alternaria* bacteria 3-methyl-1-butanol (PC2, var. 24%) Temperature -bacteria -Moist. content	(R^2=0.71, p<0.01) *Penicillium* Temperature
Ventilated ecosystem[a] - 20% initial moisture content			
3-Methyl-1-butanol	-Temperature(p<0.05)	(PC2, var. 24%) *Penicillium* *-Asp. glaucus* -Temperature *-Fusarium* (PC3, var. 18%) Moisture content *Fusarium*	

Table 3. (continued)

Odor volatile	Interrelation with, according to: Simple correlation (r)	Principal component analyses[b]	Stepwise regression analyses
	Control ecosystem[a] - 25% initial moisture content		
3-Methyl-1-butanol	Moist. content($p<0.01$) *Penicillium*($p<0.01$) 1-octen-3-ol($p<0.05$) bacteria($p<0.05$)	(PC2, var. 20%) bacteria *-Fusarium* Moisture content	($R^2=0.71$, $p<0.01$) Moist. content bacteria
3-Octanone	*-Alternaria*($p<0.01$) -germination($p<0.05$) *-Fusarium*($p<0.05$)		($R^2=0.34$, $p<0.01$) *Alternaria*
1-Octen-3-ol	*Penicillium*($p<0.05$) Temperature($p<0.05$) 3-methyl-1-butanol($p<0.05$) *-Asp. glaucus*($p<0.05$)	(PC3, var. 16%) -germination Temperature *-Alternaria* *Fusarium*	($R^2=0.23$, $p<0.05$) *Penicillium*
	Low ventilated ecosystem[a] - 25% initial moisture content		
3-Methyl-1-butanol		(PC3, var. 17%) *Fusarium* *-Alternaria*	
	High ventilated ecosystem[a] - 25% initial moisture content		
3-Methyl-1-butanol	Moist. content($p<0.01$)	(PC1, var.36%) -germination -Temperature Moisture content bacteria (PC2, var. 30%) *Fusarium* -bacteria *-Penicillium* Moisture content	($R^2=0.80$, $p<0.01$) Moist.content($p<0.01$)

[a]Airflow conditions:

ventilated ecosystem - 2 ($L \cdot s^{-1}$) m^{-3}

low ventilated ecosystem - 3.5 ($L \cdot s^{-1}$) m^{-3} (12-h switch off)

high ventilated ecosystem - 7 ($L \cdot s^{-1}$) m^{-3}

control ecosystems - no airflow

[b]PC= principal component, only vector loading higher than 0.30 were considered; var.= variability accounted for eigenvalues.

a) 20% initial moisture content wheat

In the control ecosystem, 3-methyl-1-butanol is associated and correlated with bacteria and *Aspergillus glaucus* gr. in all three statistical analyses. A relatively high R^2 value (R^2=0.57) and high significance level ($p<0.01$) indicate that the presence of bacteria and *A. glaucus* gr. could account for production of 3-methyl-1-butanol. Both species did produce 3-methyl-1-butanol (Table 4), thereby confirming the multivariate interrelationship observed in the bins.

The second detected volatile, 1-octen-3-ol, is correlated significantly and associated with *Penicillium* spp. and temperature in all three statistical analyses. The R^2 value for SMRA is highly significant (R^2=0.71, $p<0.01$) thus indicating a possible temperature effect on *Penicillium* spp. enabling them to produce 1-octen-3-ol; both *P. cyclopium* and *P. chrysogenum* produced 1-octen-3-ol (Table 4). Thus, direct cause-and-effect relationship experiments and objective analyses of field data from undisturbed stored-grain ecosystems have demonstrated that *Penicillium* spp. were the main sources of production of 1-octen-3-ol.

In the ventilated ecosystem, 3-methyl-1-butanol is associated in the second principal component (PC2) with *Penicillium* spp., and inversely associated with *Aspergillus glaucus*, temperature, and *Fusarium* spp. The same volatile is associated in PC3 with moisture content and *Fusarium* spp. Production of 3-methyl-1-butanol appeared to have resulted when grain with high moisture content was infected with *Penicillium* spp. This phenomenon was confirmed by laboratory findings which showed that two *Penicillium* species could produce 3-methyl-1-butanol. Stepwise multiple regression analysis, however, does not reveal any meaningful explanation of the cause-and-effect relationship.

b) 25% initial moisture content wheat

In the control ecosystem 3-methyl-1-butanol is correlated and associated with moisture content and bacteria in all three statistical analyses. The R^2 is highly significant (R^2=0.71, $p<0.01$), indicating that presence of bacteria in a high-moisture ecosystem could account for 3-methyl-1-butanol production. The volatile 3-octanone was significantly correlated with *Alternaria alternata* in SMRA, and with *A. alternata* and *Fusarium* spp. in simple correlation analysis (CA). No association is revealed in any of the first three principal components. The volatile 1-octen-3-ol is positively correlated with *Penicillium* in CA and in SMRA, and with temperature in CA and PCA. Laboratory study also confirmed the production of 1-octen-3-ol by two *Penicillium* species tested (Table 4). The interrelation of 1-octen-3-ol with temperature and *Penicillium* spp. is similar to that in the control ecosystem with 20% initial m.c. wheat.

In the low-ventilated ecosystem, 3-methyl-1-butanol is not significantly correlated with any variables in CA and SMRA, but in PC3 this volatile is directly associated with *Fusarium* spp. and inversely associated with *A. alternata*. Odor volatiles produced by microflora were purged away and natural microfloral associations were disrupted under ventilated conditions.

In the high-ventilated ecosystem, 3-methyl-1-butanol is highly correlated ($p<0.01$) and associated with moisture content in all three statistical analyses. This interrelationship explains the absence of volatiles in spring after ventilation was started and grain moisture content decreased sharply. Again, ventilation seemed to purge volatiles produced by microflora.

4. Study on 15.2% moisture content wheat - 1986-87

The mite-produced volatile, tridecane, was detected in both non-ventilated control and ventilated bins during August 1986-July 1987. Populations of three acarine species *Acarus siro*, *Aeroglyphus robustus* and *Lepidoglyphus destructor* were

present in grain samples taken at this time for counting. Although *Acarus siro* was introduced in the highest number and *Aeroglyphus robustus* in the lowest number (14 times less), the ratio between the two species reversed dramatically as storage proceeded. During winter months when grain temperature reached -10^{0}C, *Aeroglyphus robustus* had the highest survival of the three introduced acarine species. Citral, a mite alarm pheromone, increased in the fall and winter months and disappeared in late spring.

a) Abiotic variables

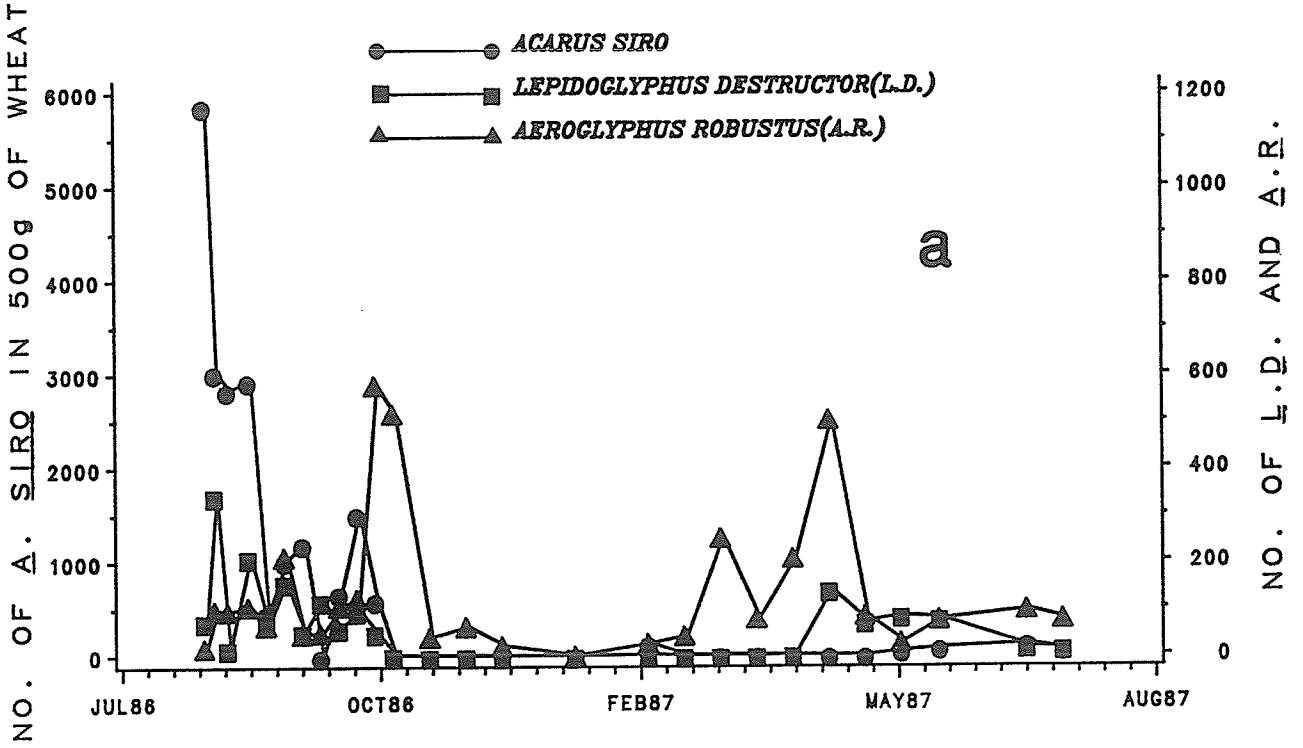
Grain moisture content in control and ventilated bins fluctuated between 14.1 and 15.5% during July 1986-July 1987. The initial grain temperature of 26^{0}C decreased to 18^{0}C by the end of August. Grain temperatures in the ventilated bin were usually a few degrees lower than those in the non-ventilated control bin, because of air movement. Temperatures in both bins generally followed the reduction in ambient temperature to 12-14^{0}C in September, 6-8^{0}C in October, and to -10^{0}C in November, where it remained during the winter months. Grain temperature increased to 3^{0}C by March 1987, and reached 11^{0}C in April. Grain temperatures followed the rise in ambient temperature to 16^{0}C in May, 22-25^{0}C in June, and finally to 26^{0}C at the experiment end on 30 July 1987.

b) Biotic variables

Acarus siro was the most abundant mite species in the control bin during August-October 1986 (Fig. 26a). This mite disappeared during the winter months and stayed relatively low during the rest of the experiment. Two other introduced acarine species, *Lepidoglyphus destructor* and *Aeroglyphus robustus* fluctuated within their original population levels during August-October. In November 1986 the number of *Aeroglyphus robustus* far exceeded the other two mite species with 560 individuals in 500 g of grain; remaining as the only active species during winter. In

Figure 26. Mite volatiles and acarine populations in wheat stored at 15% moisture content in control bin in 1986-87.

CONTROL BIN – 15% INITIAL MOISTURE CONTENT WHEAT



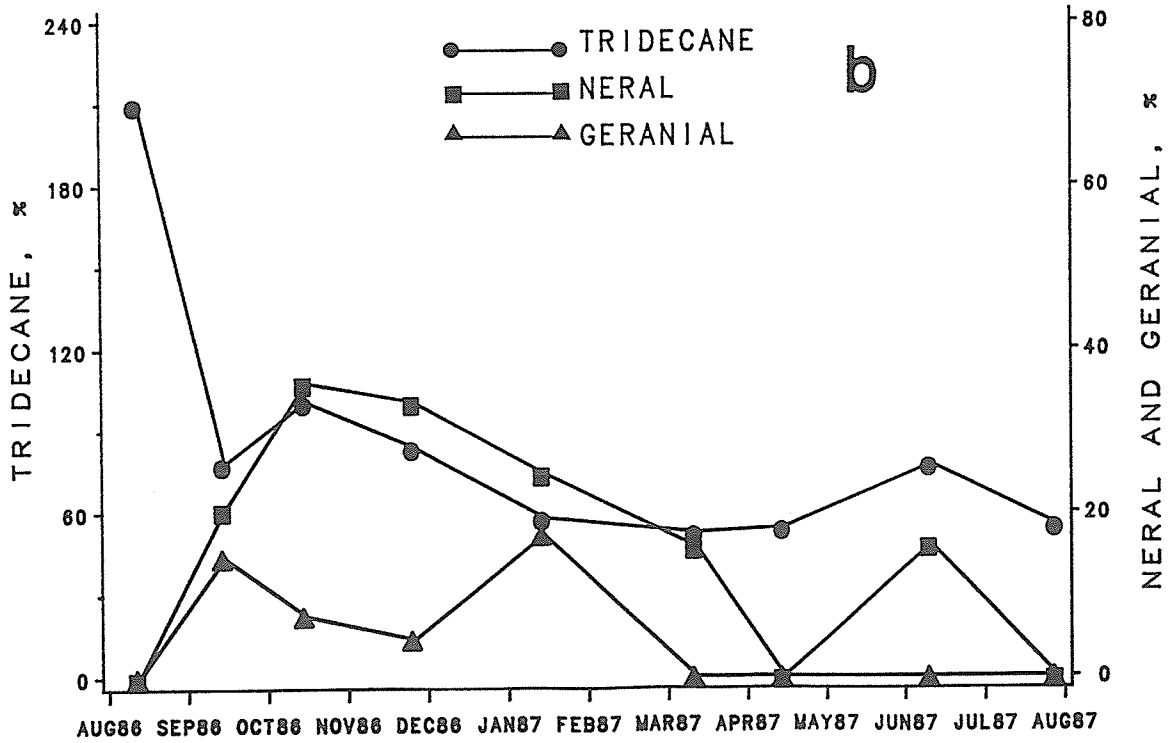
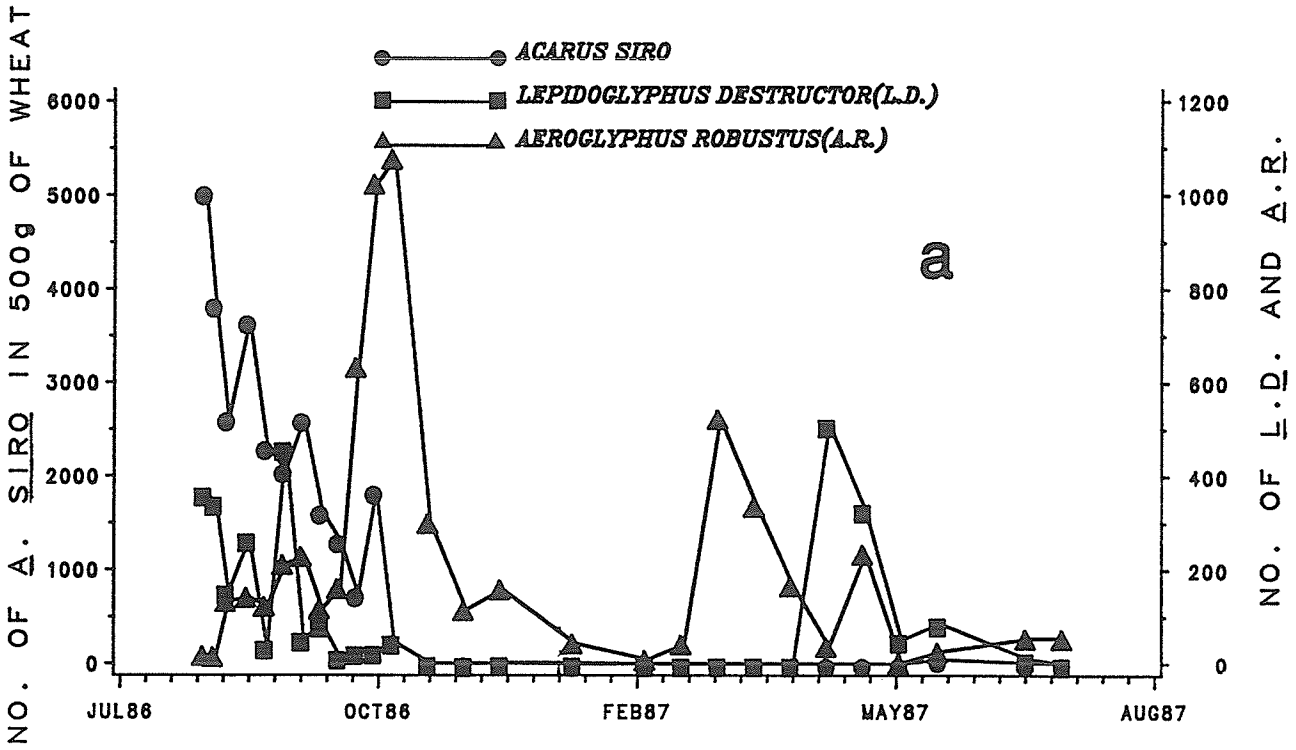
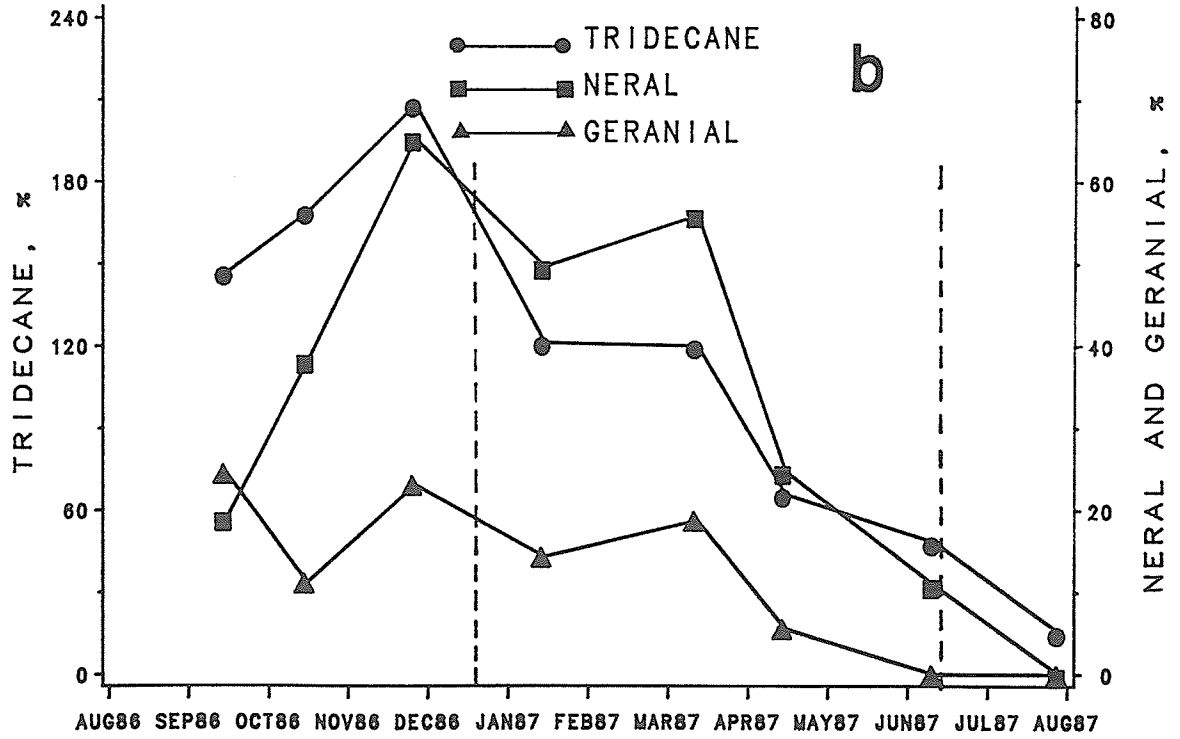


Figure 27. Mite volatiles and acarine populations in wheat stored at 15% moisture content in ventilated bin in 1986-87; (ventilation periods are designated with broken line).

VENTILATED BIN – 15% INITIAL MOISTURE CONTENT WHEAT





the spring and summer, *A. robustus* was the most common and abundant acarine species, increasing once in March and then again in April 1987; it had the highest survival rate.

Tridecane, initially recorded at 210% in August 1986, occurred within a 50-110% range during September 1986-July 1987 in the control bin (Fig.26b). The neral and geranial components of the citral alarm pheromone were detected during September 1986-January 1987. The levels of neral ranged from 17 to 35%, and geranial from 4 to 17%. Only neral was detected twice at the 15% during March-July 1987.

In the ventilated bin, *Acarus siro* was the most abundant acarine species during August-October 1986 (Fig. 27b). This acarine species almost disappeared during winter and spring, but maintained low levels in the summer. *Aeroglyphus robustus* and *L. destructor*, fluctuated within the original population levels during August-September. In November 1986 the number of *A. robustus* far exceeded those of the other two species with 1100 individuals in 500 g of grain; it remained the only active species during winter. In spring this species increased in March and again in May 1987 while *L. destructor* was most abundant during April-May 1987. Of the three introduced acarine species, *A. robustus* had again the highest survival rate.

In the ventilated bin, tridecane occurred at 20-200% levels during September 1986-July 1987 with its highest level recorded in November 1986; after this the level declined steadily to 20% during winter, spring, and summer (Fig. 27b). Neral and geranial were detected during September 1986-April 1987, but both components disappeared in July. Neral increased from 17% in September to 65% in November 1986 and remained high until March 1987.

B. Laboratory experiments on microfloral and mite odor volatiles

1. Volatiles from non-inoculated and from fungus-inoculated wheat

Non-inoculated wheat that was moisturized to 20% m.c. and sterilized for initial assessment, 3-methyl-1-butanol was detected at a 121% av. level (Table 4). Incidence of wheat-borne microfloral infection (plated seeds were not surface-sterilized) consisted of bacteria at 60% and *Penicillium* spp. at 8%. Non-inoculated wheat had 3-methyl-1-butanol detected at a 89% av. after seven days incubation; plated seeds revealed an increased *Penicillium* spp. infection of 88% and bacterial infection at 44% incidence.

After 1 wk the most common and abundant volatile, 3-methyl-1-butanol, was produced at 39-191% levels in wheat inoculated with every fungal species tested (Table 4). The highest level of this volatile was at a 185% av., and occurred in wheat inoculated with *Fusarium semitectum* (ANOVA, $p<0.05$). The lowest level was at a 31% av. occurring in wheat inoculated by *Aspergillus flavus*. The second volatile detected was 1-octen-3-ol ranging from 12 to 31% (in all but the *Fusarium* spp.-inoculated wheat) after 1 wk. All of the fungal species grown on wheat showed total coverage of grain by mycelia, except *Alternaria alternata* and both *Fusarium* spp. Such low mycelial production on wheat inoculated with *A. alternata* and both *Fusarium* spp. probably affected the levels of volatiles produced by these species.

Non-inoculated wheat at the end of the wheat assessment had *Aspergillus repens* infection growing through the cotton plug after 5-wk incubation. In such contaminated wheat, 3-methyl-1-butanol occurred at a 24% av. level, the same level found in wheat inoculated by *A. repens* (Table 4). There was no significant difference in the 3-methyl-1-butanol levels in these two treatments (ANOVA, $p<0.05$). The levels of 3-methyl-1-butanol in wheat inoculated with *A. repens* and with *Penicillium cyclopium* after 5 wk, were not significantly different (ANOVA, $p<0.05$) from those after 1-wk incubation (Table 4).

Table 4. Odor volatiles from non-inoculated wheat and wheat infected with fungal species after 1 and 5 weeks

Wheat inoculated with	Incubation period (weeks)	Odor volatiles[a] (%) 3-methyl-1-butanol	3-octanone	1-octen-3-ol	Tridecane
non-inoculated (initial wheat assessment)	0	138.8 102.3			
non-inoculated (7-day wheat assessment)	1	79.9 97.1			
Aspergillus repens	1	41.3 39.0		13.0 24.0	
Penicillium cyclopium	1	118.1 126.5		30.5 20.8	
Penicillium chrysogenum	1	120.9 98.8		19.4 17.6	
Aspergillus versicolor	1	61.4 59.8		24.2 20.6	
Aspergillus flavus	1	36.3 26.3		13.0	
Fusarium monoliforme	1	93.8 97.7			
Fusarium semitectum	1	190.6 179.6			
Arthrobotrys	1	82.0 91.6		12.2 13.4	
Alternaria alternata	1	59.4 67.7		18.1	

[a]blanks are zero values

Table 4. (continued)

Wheat inoculated with	Incubation period (weeks)	Odor volatiles[a] (%) 3-methyl-1-butanol	3-octanone	1-octen-3-ol	Tridecane
non-inoculated (end-wheat assessment)	5	27.0 21.4		27.4 22.7	
Aspergillus repens	5	28.5 24.2	146.2	17.5 134.8	78.4
Penicillium cyclopium	5	132.5 133.8	10.7 12.7		
P.cyclopium (OA)[b] (disturbed)	5	688.0 662.1	133.8 130.3		

[a]blanks are zero values

[b]OA: previously analyzed for odor volatiles

One of the two flasks containing *A. repens*-inoculated wheat was turned upside down to ease the introduction of teflon tubing for the collection gas. The content of this flask showed previously undetected 3-octanone and 1-octen-3-ol at 146 and at 135% levels, respectively. This simple flask manipulation resulted in an unexpected 10-fold increase of 1-octen-3-ol and detection of 3-octanone, compared to that produced by the undisturbed replicate. Handling possibly facilitated the release of trapped volatiles in intergranular spaces of the caked grain allowing purging of these volatiles with the collection gas. Handling could also increase the surface area for active transfer of volatiles, resulting in the higher levels of volatiles detected. The occurrence of tridecane in one flask with *A. repens*-inoculated wheat is also mentioned in part IV.B.2.

In *Penicillium cyclopium*-inoculated wheat 3-methyl-1-butanol occurred at a 134% av. level and 3-octanone at a 12% av. level after 5 wk (Table 4). These previously analyzed flasks (after 2 days storage at 2.5^{0}C) were shaken to ease volatile transfer and analyzed again, yielding a 675% av. level of 3-methyl-1-butanol and a 132% av. level of 3-octanone; this was a 5- and 10-fold increase in volatiles, respectively. In this treatment 3-methyl-1-butanol level was significantly different (ANOVA, $p<0.05$) from those from all other treatments .

2. Volatiles from acarine species reared on non-inoculated and on fungus-inoculated wheat

Tridecane was detected at a 78.4% level in one flask containing *A. repens*-inoculated wheat (Table 4); this flask was found to be contaminated with a stored-product mite, *Tyrophagus putrescentiae* (Schrank). When *Acarus siro* was introduced into flasks with non-inoculated wheat and with previously-analyzed non-inoculated 7-day wheat (AO), the mites contaminated the wheat with *Aspergillus repens* and sporadically with *Penicillium* spp. Tridecane was detected on "wheat" at 388% av. and on "7-day wheat" at 457% av. levels after 4-wk incubation (Table 5).

Table 5. Odor volatiles from acarine species reared on non-inoculated wheat and wheat inoculated with fungal species after 4 weeks

Wheat with		Odor volatiles[c] (%)			
mite[a] species	fungal[b] species	3-methyl-1-butanol	3-octanone	1-octen-3-ol	Tridecane
A.siro (non-inoculated)		44.2 54.5		30.4 29.6	448.1 327.0
A.siro 7-day wheat(OA)[d]		67.1 51.9		25.3 28.6	323.7 590.1
A.siro	*A.repens*	47.9 40.0		26.1 25.9	416.1 380.5
A.siro	*A.repens* (OA)[d]	41.9 38.6	20.7 18.3	25.6 23.7	464.4 368.8
Aer.rob.	*A.repens*	29.8 21.9	15.4	12.0 12.9	66.5 47.3
A.siro	*P.cycl.*	80.8 61.8	11.7 11.5		
A.siro	*P.cycl.* (OA)[d]	136.0 132.6	2.3 10.8		
Aer.rob.	*P.cycl.*	94.1 84.4	14.8 11.6		8.4
A.siro	*P.chrys.* (OA)[d]	203.9 188.1	26.4 24.1		
A.siro	*A.versi.* (OA)[d]	24.7 23.3		18.3 18.0	
A.siro	*A.flavus* (OA)[d]	159.4 152.7	28.7 30.2		

Table 5. (continued)

Wheat with		Odor volatiles[c] (%)			
mite[a] species	fungal[b] species	3-methyl-1-butanol	3-octa none	1-octen-3-ol	Tridecane
A.siro	*Fus.monil.*	252.5			326.8
	(OA)[d]	272.7			336.7
A.siro	*Fus.semi.*	181.9			1031.4
	(OA)[d]	227.0			1029.6
A.siro	*Arthrob.*	81.6		13.7	210.9
	(OA)[d]	71.9		15.6	240.4
A.siro	*Alter.alt.*	42.0			642.3
	(OA)[d]	44.0			337.4

[a] *A.siro-Acarus siro*; *Aer.robustus-Aeroglyphus robustus*

[b] *A.repens-Aspergillus repens*; *A.versi.-Aspergillus versicolor*; *A.flavus-Aspergillus flavus*; *P.cycl.-Penicillium cyclopium*; *P.chrys.-Penicillium chrysogenum*; *Alter.alt.-Alternaria alternata*; *Fus.monil.-Fusarium moniliforme*; *Fus.semi.-Fusarium semitectum*; *Arthrob.-Arthrobotrys*

[c] blanks are zero values

[d] OA: previously analyzed for odor volatiles

In wheat with *A. repens* and *Acarus siro*, tridecane occurred at a 417% av. level. In wheat with *A. repens* and *Aeroglyphus robustus*, tridecane was detected at much lower levels of 57% av. These flasks had lower *A. robustus* survival as compared to *Acarus siro*. In wheat with *Penicillium* spp. and *Acarus siro*, and those with *Aeroglyphus robustus*, no tridecane occurred except in one flask at a 8.4% level; the acarine populations in all these flasks were declining and in a poor condition . Excessive condensation in flasks with both *Penicillium* spp. could negatively affect the survival of both acarine species.

In wheat with *Fusarium semitectum* and *Acarus siro*, tridecane occurred at a 1031% av.; this level was significantly different (ANOVA, $p<0.05$) from those in all other treatments . In all wheat flasks inoculated with fungal species and infested with *Acarus siro*, mite survival and tridecane production was higher than in those infested with *Aeroglyphus robustus*. Fungal spores were eaten by mites in all flasks in which high levels of tridecane were recorded. Based on tridecane levels, fungal media which provide best for *Acarus siro* include: *Fusarium semitectum*, *Alternaria alternata*, *Aspergillus repens*, *Fusarium moniliforme* and *Arthrobotrys* spp.

3. Volatiles from microfloral species grown on synthetic media

Only one volatile, 1-octen-3-ol, was detected from three inoculation media (potato dextrose agar, yeast malt agar, plate count agar) having 12-25% levels (Table 6). *Fusarium semitectum* grown on the PDA produced 3-methyl-1-butanol at a 397% av. level. This culture contained heavy mycelial growth and good sporulation on the PDA substrate, possibly producing the higher levels of volatiles detected as compared to the same species grown on wheat (Table 4). *Penicillium cyclopium* grown on YMA produced 3-methyl-1-butanol at a 37% av. level (Table 6). The formation of a compact mycelium mat characteristic for *Penicillium* growth as compared to "airy" *Fusarium* growth, could result in a smaller surface area thus limiting transfer of volatiles during collection. This phenomenon was further

Table 6. Odor volatiles from fungal and bacterial species grown on synthetic media after 1 week

Agar media	Microbial species	Odor volatiles[a] (%) 3-methyl-1-butanol	1-octen-3-ol
Potato dextrose agar(PDA)			24.9
PDA	*Fusarium semitectum*	387.9 405.3	
Yeast malt agar(YMA)			22.8
YMA	*Penicillium cyclopium*	33.1 40.0	
Plate count agar(PCA)			12.2 14.9
PCA	Bacterium 1	50.0 35.5	
PCA	Bacterium 2	25.1 16.0	

[a]blanks are zero values

complicated because collection gas was purged over the *Penicillium* mat as compared to purging gas through the *Penicillium*-infected wheat mass (Table 4). Two bacterial isolates grown on PCA produced 3-methyl-1-butanol within a 16-50% level (Table 6).

V. DISCUSSION

This study has demonstrated that two odor volatiles, 3-methyl-1-butanol and 1-octen-3-ol, are characteristically produced in non-ventilated bin-stored wheat at 15.6 to 25% initial moisture content. A third volatile, 3-octanone, occurred only in wheat of 18 to 25% initial m.c. All three volatiles were detected year-round i.e. during fall, winter, spring and summer seasons, provided that moisture content and the nature of microfloral infection of wheat were suitable. The highest levels of 3-methyl-1-butanol were recorded in fall and then diminished in late spring, when grain moisture content decreased. In contrast, 1-octen-3-ol, had a tendency to increase over time.

The most relevant microfloral species significantly associated with odor volatiles production were: *Aspergillus glaucus* gr. and bacteria with 3-methyl-1-butanol, *Penicillium cyclopium* and *P. chrysogenum* with 1-octen-3-ol, and *Alternaria alternata* with 3-octanone. A cause-and-effect study based on laboratory experiments confirmed these associations, except for *Alternaria alternata* with 3-octanone.

Ventilation of bin-stored wheat at airflow rates of 3.5 and 7 $(L{\cdot}s^{-1})\ m^{-3}$ purged 3-octanone and 1-octen-3-ol, but not 3-methyl-1-butanol. Ventilation at 2 $(L{\cdot}s^{-1})\ m^{-3}$ did not purge any of aforementioned volatiles or tridecane at 1 $(L{\cdot}s^{-1})\ m^{-3}$. There was no significant association between microfloral species and 3-methyl-1-butanol produced in these ventilated bins. The only meaningful relationship with moisture content (in high-ventilated bins) suggests that the disappearance of volatiles in spring was affected by a decrease in grain moisture content through resumed ventilation.

In winter, when temperature dropped below -5^0C, all volatile levels plateaued. At this time microbial activity was negligible, however accumulated

levels of volatiles produced earlier were still detected in freshly removed samples. The low gas diffusion of the grain prevented existing volatiles from dissipating.

Tridecane was exclusively detected in bin-stored wheat infested with the stored-product mites, *Acarus siro*, *Aeroglyphus robustus*, and *Lepidoglyphus destructor*. The laboratory study demonstrated that tridecane could be produced by *Acarus siro* and *Aeroglyphus robustus*.

A. Bin-stored wheat ecosystem

This 2-year bin study has shown that 3-methyl-1-butanol is the most common and abundant odor volatile in bin-stored hard red spring wheat. Two other volatiles, 3-octanone and 1-octen-3-ol, occurred more sporadically and in less abundance. Fungal and bacterial species isolated from bin-stored wheat produced 3-methyl-1-butanol as a major odor volatile. The microfloral origins of this component, initially detected on non-inoculated wheat, were identified by growing isolated microbial species on synthetic media to eliminate the interference of "natural" multiple infections on grain.

These odor volatiles in stored grain are the same as those recorded by Richard-Molard *et. al.* (1976) and by Abramson *et al.* (1980, 1983). The difference between the latter studies and the results reported here lies in the relative proportions of volatiles, which could be affected by differences in collection methods and in cereal cultivars. Vacuum-steam extraction of homogenized grain used by Richard-Molard *et. al.* (1976) and Kaminski *et al.* (1973) was discussed by the latter authors for the origin of 1-octen-3-ol, which was detected as a major volatile in both studies. Kaminski *et. al.* (1973) suggested possible artifact production of 1-octen-3-ol from enzymatic cleavage of unsaturated fatty acids. Hamilton-Kemp and Andersen (1984, 1986) demonstrated an increase of such cleavage products in disrupted plant tissues.

Seasonal variations in profiles of the odor volatiles closely followed climatic changes, especially in high moisture content non-ventilated bin-stored wheat. This correspondence can be associated with increased microfloral activity during the first stages of storage in fall, and again in spring as temperature increased. The highest levels of 3-methyl-1-butanol in the 1985-86 bin study occurred after 4 and 5 wk of storage. This finding generally agrees with those of Abramson *et al.* (1980, 1983), who observed the highest detected levels of volatiles at 6-7 wk of storage.

Ventilation of bin-stored wheat in the 1985-86 study interrupted the natural progression of microflora and subsequent production of odor volatiles. Only few measured variables were correlated or associated with volatiles, therefore making it difficult to estimate cause-and-effect relationships with the microflora present. The only significant association of 3-methyl-1-butanol with moisture content was found in the high-ventilated bins (airflow rate of 7 $(L{\cdot}s^{-1})$ m^{-3}). Resumed ventilation in the spring decreased grain moisture content from 22 to 16%, thus reducing microfloral activity and possibly purging 3-methyl-1-butanol to undetectable levels.

In the 1985-86 bin study, tridecane was detected and identified for the first time in bin-stored grain. Later, its presence was confirmed by finding a natural mite infestation in control bins with wheat at 25% initial m.c. In the follow-up 1986-87 study, tridecane was monitored bimonthly in acarine-infested bin-stored wheat for one year. The highest levels of tridecane occurred during the first 4 mo. of storage when the mite counts from grain samples were also high. The initially most-numerous *Acarus siro* population was replaced by the initially least-numerous *Aeroglyphus robustus* population at that time. *Aeroglyphus robustus* had the highest survival under these storage conditions and possibly accounted for most of the tridecane detected during winter. Because both *Acarus siro* and *L. destructor* overwinter as immobile, cold-tolerant hypopus stage, the numerical dominance of *Aeroglyphus robustus* could be an erroneous observation if all stages of the mite

populations were considered (Sinha 1965). Citral, detected in acarine-infested grain, probably resulted from disturbing mites during handling in the laboratory (Baker and Krantz 1984, Kuwahara *et al.* 1980).

B. Laboratory experiments

All isolated fungal species grown on wheat and synthetic media in the laboratory produced 3-methyl-1-butanol as the major odor volatile. The two other volatiles, 1-octen-3-ol and 3-octanone, were produced less frequently. These laboratory findings support field results by confirming that the same volatiles were produced by individual fungal species and bacteria; these detected odor volatiles are qualitatively similar to those detected by Kaminski *et al.* (1972, 1973, 1974). The quantitative difference in the most common and abundant volatile could be caused by isolation and concentration techniques.

Two unidentified bacterial species, isolated on agar from control bins with 25% initial m.c. wheat, produced 3-methyl-1-butanol; however, they did not produce 1-octen-3-ol as found by Kaminski *et al.* (1974, 1979). The production of 3-methyl-1-butanol by bacterial species is reported for the first time in this study. This finding complicates the interpretation of 3-methyl-1-butanol quantities detected on fungus-inoculated wheat. In the laboratory experiment fungal inocula were found to be contaminated with bacteria using dilution plating. This contamination, however, seemed not to affect the spreading of mycelia and sporulation of most fungal species.

Three field-fungal species, *Alternaria alternata* and two *Fusarium* spp., showed minimal spreading and sporulation on wheat. Heavy mycelial growth and good sporulation by *F. semitectum* occurred on agar, possibly resulting in higher production of 3-methyl-1-butanol on agar than that on wheat.

Laboratory-incubated acarine species, *Acarus siro* and *Aeroglyphus robustus*, reared on wheat and wheat inoculated with fungal species both produced tridecane.

This finding is in agreement with Curtis *et al.* (1981) for *Acarus siro*, but the production of tridecane by *Aeroglyphus robustus* has not been previously reported. *Aeroglyphus robustus* is a species native to Canada; it has not been tested by other researchers. The abundance of tridecane produced by mites differed substantially on wheat infected with fungi. *Acarus siro*, as a fungivorous mite, had better survival on fungus-inoculated wheat than *Aeroglyphus robustus*. The preferred fungal diet according to tridecane level are *Fusarium semitectum*, *F. moniliforme*, *Aspergillus glaucus*, and *Arthrobotrys* spp. No citral component was detected from mites in these laboratory experiments; this could result from not disturbing flask contents and mites prior to analysis (Baker and Krantz 1984, Kuwahara *et al.* 1980).

Stored grain is meant to be preserved for human consumption, and as such it must be monitored to detect spoilage. Microbial decomposers can reduce the available nutrients and energy in grain, thus reducing its value. A stored-grain ecosystem is unstable especially at elevated moisture contents and temperature when relatively harmless field fungi are succeeded by storage fungi. The species of *Aspergillus* and *Penicillium* genera degrade the grain by enzymatic activity and further may produce mycotoxins hazardous to human and animal consumption. At higher grain moisture contents, storage fungi can be replaced by bacteria during ecological succession.

This dynamic ecosystem must be monitored if methods to prevent deterioration are to be applied. Detection of odor volatiles can serve as a new monitoring variable specific to microfloral spoilage and mite infestation. Possibility now exists for detecting the succession of storage fungi, or predicting potential grain spoilage. Further, the use of volatiles as a bioindicator could prove important in prevention of "hot spots" in stored grain. Ventilation is used for cooling and drying grain. Although it does not carry away all volatiles, ventilation does disrupt the development of microflora.

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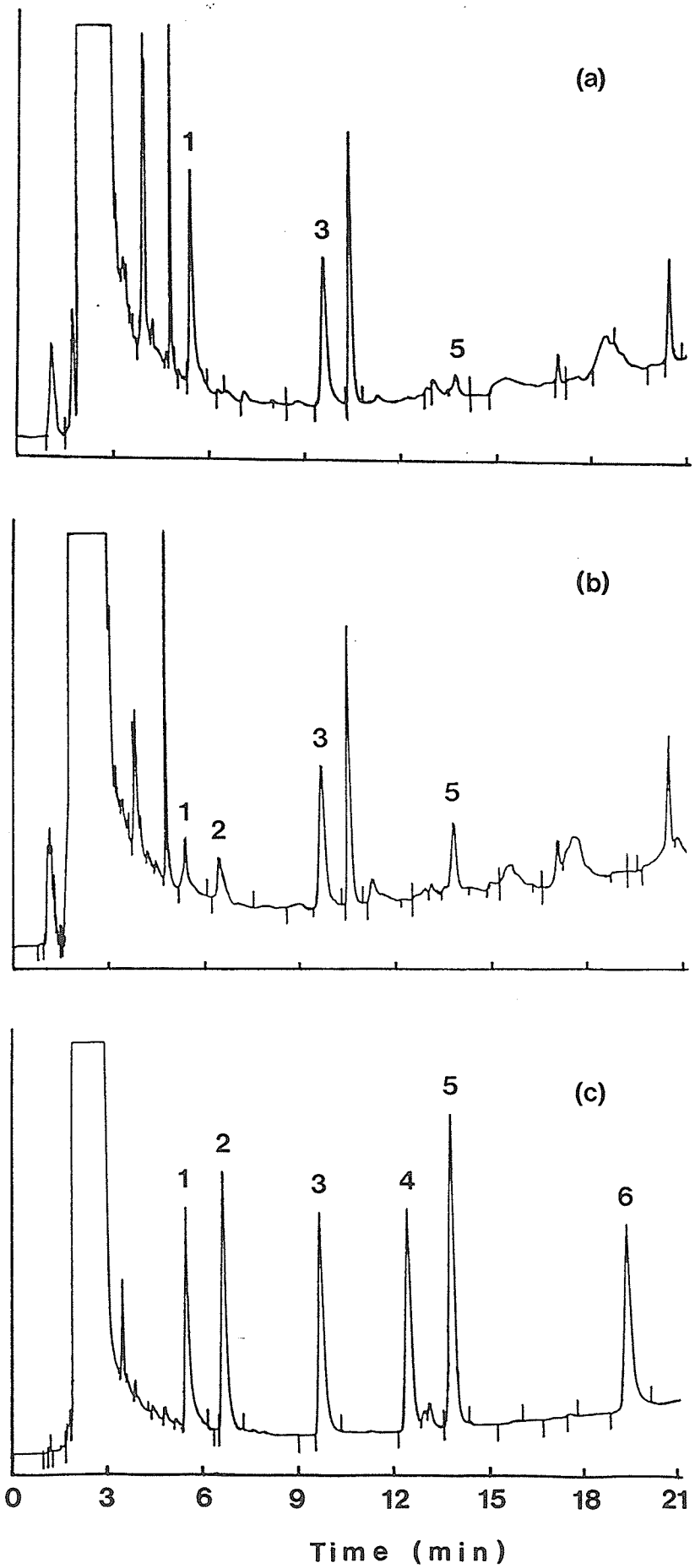
APPENDICES

Appendix 1. Gas chromatographs of volatiles from wheat stored in control bins in 1984-85: (a) bin location 260 cm from 48th week at 15.2% moisture content (Table 1); (b) bin location 230 cm from 48th week at 18.1% moisture content (Table 1); (c) odor volatile standards (1), 3-methyl-1-butanol; (2), 3-octanone; (3), 4-hydroxy-4-methyl-2-pentanone (diacetone alcohol); (4), 2-octanol; (5), 1-octen-3-ol; (6), 1-octanol.


(a)
1
3
5
(b)
1
2
3
5
(c)
1
2
3
4
5
6
0
3
6
9
12
15
18
21
Time (min)

Appendix 2. Data distributions and transformations used for biotic and abiotic variables measured in control and ventilated stored wheat ecosystems in 1985-86 experiment.

		20% moisture content		25% moisture content		
Variable	Transf. applied	Control	Ventilated	Control	High	Low
(n - p)[a]		18 - 9	18 - 9	24 - 12	18 - 9	20 - 8
3-Methyl-1-butanol	log(x+1)	normal[b]	normal	transf.[c]	normal	normal
3-Octanone		(n/a)[d]	n/a	normal	n/a	n/a
1-Octen-3-ol		OK[e]	n/a	OK	n/a	n/a
Germination	$\arcsin(x)^{-2}$	normal	normal	transf.	normal	normal
Alternaria	$\arcsin(x)^{-2}$	normal	normal	transf.	normal	normal
Asp. glaucus	$\arcsin(x)^{-2}$	normal	transf.	OK	n/a	n/a
Asp. flavus	$\arcsin(x)^{-2}$	n/a	n/a	transf.	n/a	n/a
Bacteria	$\arcsin(x)^{-2}$	normal	transf.	OK	normal	normal
Fusarium	$\arcsin(x)^{-2}$	n/a	OK	transf.	transf.	transf.
Penicillium	$\arcsin(x)^{-2}$	transf.	OK	transf.	normal	normal
Moisture		OK	OK	normal	OK	normal
Temperature		normal	normal	OK	normal	normal

[a](n-p): number of observations - number of variables

[b]normal: normal distribution

[c]transf.: transformation applied

[d](n/a): variable not available, n<25%

[e]OK: not normal, but no transformation applicable

Appendix 3. Correlation matrix and principle components of data from control bins with 20% moisture content wheat in 1985-86.

CONTROL BINS WITH 20% INITIAL MOISTURE CONTENT

PRINCIPAL COMPONENT ANALYSIS

17 OBSERVATIONS
9 VARIABLES

SIMPLE STATISTICS

	ODOR1	ODOR3	GERM	ALTER	ASPGL	PENIC	BACTER	MOIST	TEMP
MEAN	16.4765	9.3412	18.1765	11.5294	2.76471	0.250282	8.00000	19.0706	8.4118
ST DEV	4.6893	20.0626	3.9723	7.0189	2.13686	0.179907	7.09753	1.1373	10.2256

CORRELATIONS

	ODOR1	ODOR3	GERM	ALTER	ASPGL	PENIC	BACTER	MOIST	TEMP
ODOR1	1.0000	0.2671	-0.0464	-0.2816	0.6200	0.1109	0.4646	0.0466	-0.0586
ODOR3	0.2671	1.0000	-0.1483	-0.2082	0.2767	0.6871	0.0952	-0.6439	0.5342
GERM	-0.0464	-0.1483	1.0000	0.1534	-0.1421	-0.3124	-0.3857	-0.2727	0.1281
ALTER	-0.2816	-0.2082	0.1534	1.0000	0.0380	-0.2198	-0.8456	0.2072	0.0180
ASPGL	0.6200	0.2767	-0.1421	0.0380	1.0000	0.4141	0.0536	-0.0185	-0.1578
PENIC	0.1109	0.6871	-0.3124	-0.2198	0.4141	1.0000	0.1989	-0.4118	0.0754
BACTER	0.4646	0.0952	-0.3857	-0.8456	0.0536	0.1989	1.0000	0.0116	-0.3095
MOIST	0.0466	-0.6439	-0.2727	0.2072	-0.0185	-0.4118	0.0116	1.0000	-0.3785
TEMP	-0.0586	0.5342	0.1281	0.0180	-0.1578	0.0754	-0.3095	-0.3785	1.0000

	EIGENVALUE	DIFFERENCE	PROPORTION	CUMULATIVE
PRIN1	2.83707	0.662419	0.315230	0.31523
PRIN2	2.17465	0.730437	0.241627	0.55686
PRIN3	1.44421	0.324641	0.160468	0.71732
PRIN4	1.11957	0.340426	0.124397	0.84172
PRIN5	0.77914	0.486576	0.086571	0.92829
PRIN6	0.29257	0.059350	0.032507	0.96080
PRIN7	0.23322	0.143993	0.025913	0.98671
PRIN8	0.08922	0.058869	0.009914	0.99663
PRIN9	0.03036	.	0.003373	1.00000

CONTROL BINS WITH 20% INITIAL MOISTURE CONTENT

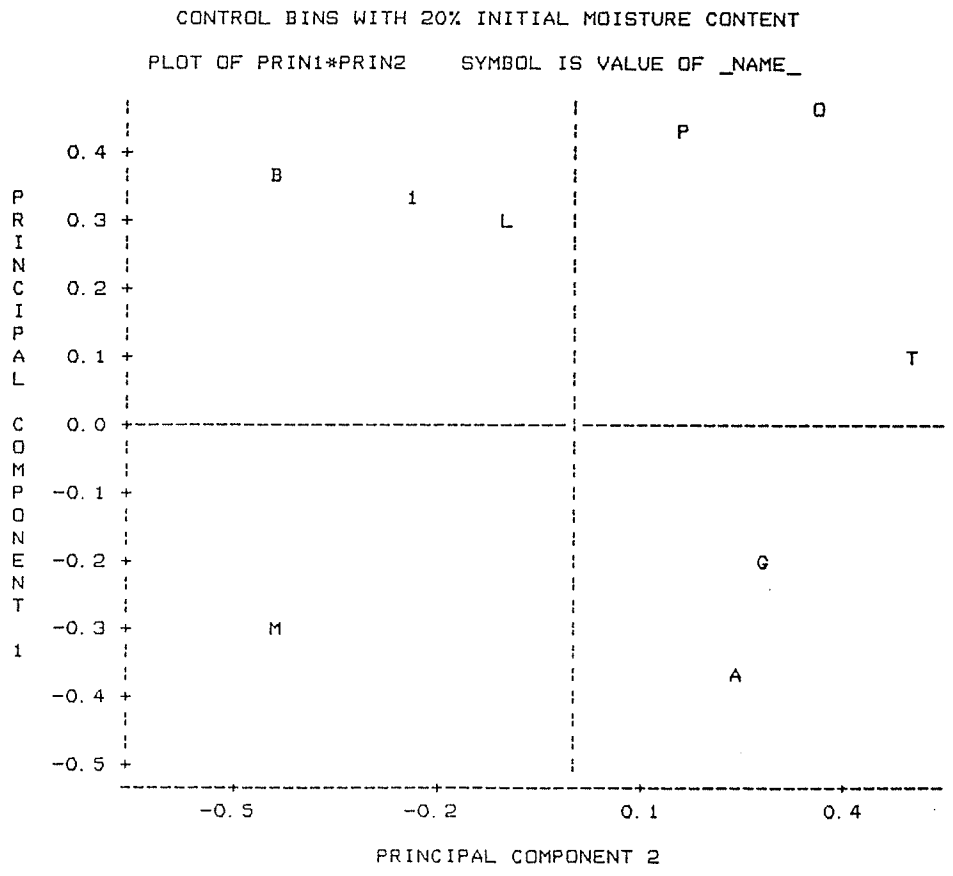
PRINCIPAL COMPONENT ANALYSIS

EIGENVECTORS

	PRIN1	PRIN2	PRIN3	PRIN4	PRIN5	PRIN6	PRIN7	PRIN8	PRIN9
ODOR1	0.334014	-.241559	0.321829	0.493603	0.326272	-.211957	0.344010	0.263067	-.379394
ODOR3	0.463602	0.364891	0.031056	-.088141	0.134853	-.156082	0.339620	-.685663	0.123180
GERM	-.199826	0.271054	-.041839	0.720160	-.331380	0.382989	0.238973	-.080750	0.214702
ALTER	-.362375	0.248878	0.511299	-.179356	0.009187	-.366230	0.345052	0.267672	0.433855
ASPGL	0.295386	-.093018	0.653214	0.134455	-.096101	0.146967	-.585765	-.133259	0.260027
PENIC	0.444087	0.150700	0.136671	-.375242	-.363725	0.478924	0.310356	0.388920	-.106523
BACTER	0.355542	-.447540	-.348210	0.075237	0.001757	-.102996	0.120656	0.185674	0.697670
MOIST	-.285798	-.443780	0.212299	-.174512	0.393816	0.558736	0.302429	-.281167	0.089771
TEMP	0.098513	0.494467	-.143510	0.052812	0.684718	0.275947	-.211386	0.316144	0.181099

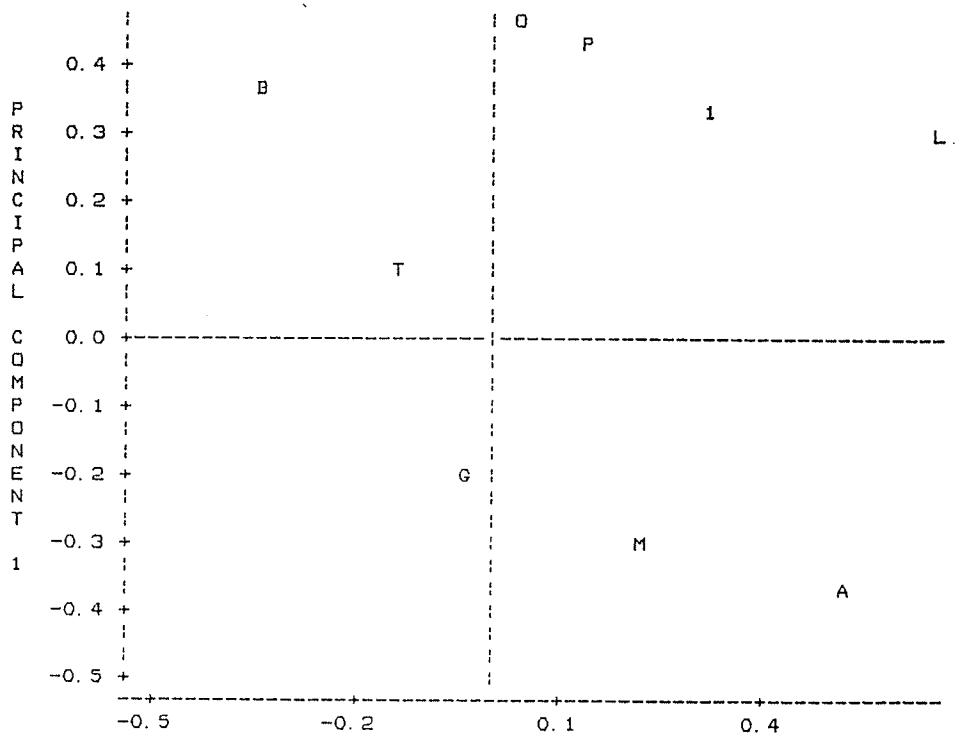
CONTROL BINS WITH 20% INITIAL MOISTURE CONTENT

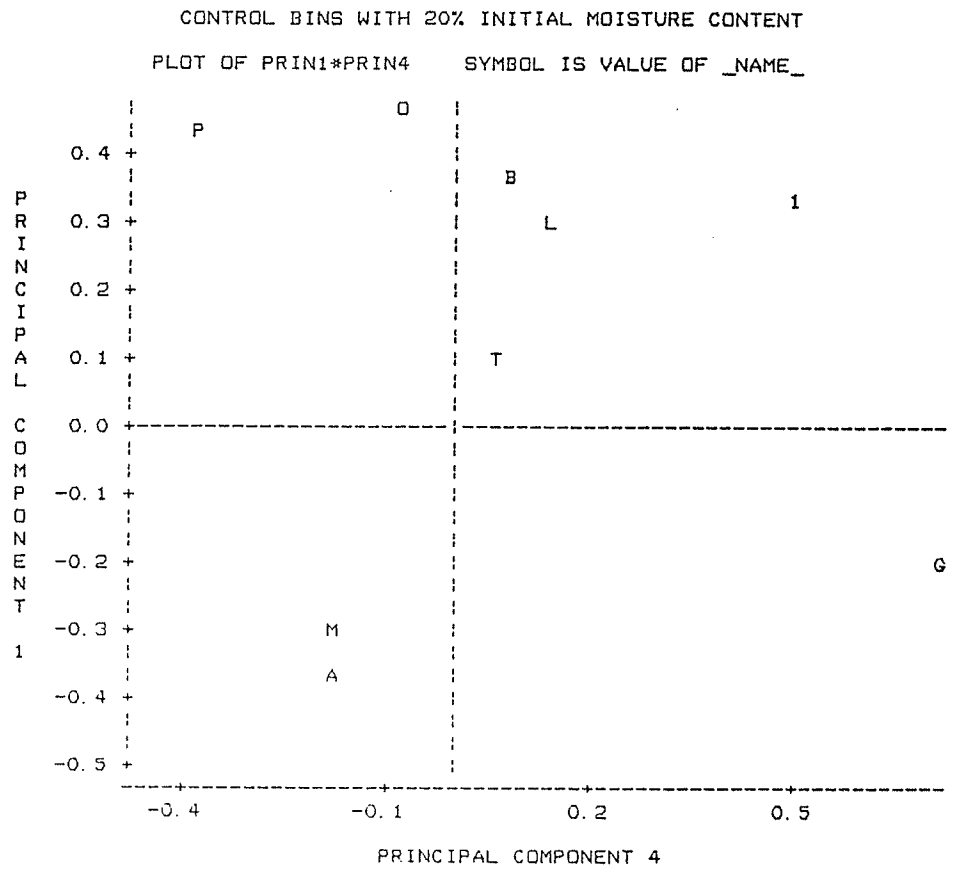
OBS	_TYPE_	_NAME_	ODOR1	ODOR3	GERM	ALTER	ASPGL	PENIC	BACTER	MOIST	TEMP
1	SCORE	PRIN1	0.33401	0.463602	-0.19983	-0.36238	0.295386	0.44409	0.35554	-0.28580	0.09851
2	SCORE	PRIN2	-0.24156	0.364891	0.27105	0.24888	-0.093018	0.15070	-0.44754	-0.44378	0.49447
3	SCORE	PRIN3	0.32183	0.031056	-0.04184	0.51130	0.653214	0.13667	-0.34821	0.21230	-0.14351
4	SCORE	PRIN4	0.49360	-0.088141	0.72016	-0.17936	0.134455	-0.37524	0.07524	-0.17451	0.05281


CONTROL BINS WITH 20% INITIAL MOISTURE CONTENT
PLOT OF PRIN1*PRIN2 SYMBOL IS VALUE OF _NAME_
PRINCIPAL COMPONENT 1
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.5
-0.2
0.1
0.4
PRINCIPAL COMPONENT 2
O
P
B
1
L
T
G
M
A

CONTROL BINS WITH 20% INITIAL MOISTURE CONTENT

PLOT OF PRIN1*PRIN3 SYMBOL IS VALUE OF _NAME_




CONTROL BINS WITH 20% INITIAL MOISTURE CONTENT
PLOT OF PRIN1*PRIN4 SYMBOL IS VALUE OF _NAME_
PRINCIPAL COMPONENT 1
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
-0.5
-0.4
-0.1
0.2
0.5
PRINCIPAL COMPONENT 4
O
P
B
1
L
T
G
M
A

Appendix 4. Correlation matrix and principle components of data from ventilated bins with 20% moisture content wheat in 1985-86.

VENTILATED BINS WITH 20% INITIAL MOISTURE CONTENT

PRINCIPAL COMPONENT ANALYSIS

18 OBSERVATIONS
9 VARIABLES

SIMPLE STATISTICS

	ODOR1	GERM	ALTER	ASPGL	PENIC	BACTER	FUSAR	MOIST	TEMP
MEAN	19.4333	20.2778	11.0000	0.086772	0.444444	0.427465	0.388889	18.5500	3.45000
ST DEV	5.5729	2.7824	5.0293	0.117041	0.511310	0.299524	0.849837	0.5316	8.60180

CORRELATIONS

	ODOR1	GERM	ALTER	ASPGL	PENIC	BACTER	FUSAR	MOIST	TEMP
ODOR1	1.0000	0.0244	0.1373	-0.0904	0.2525	0.1544	0.1325	0.2797	-0.4177
GERM	0.0244	1.0000	0.1093	0.0307	-0.1746	-0.5485	0.0511	-0.0179	-0.0834
ALTER	0.1373	0.1093	1.0000	-0.0348	0.1830	-0.5801	0.1514	-0.2882	-0.3596
ASPGL	-0.0904	0.0307	-0.0348	1.0000	-0.2865	-0.2875	0.6256	0.0363	0.1353
PENIC	0.2525	-0.1746	0.1830	-0.2865	1.0000	-0.0017	-0.2858	0.1731	-0.5029
BACTER	0.1544	-0.5485	-0.5801	-0.2875	-0.0017	1.0000	-0.3179	0.1446	0.3605
FUSAR	0.1325	0.0511	0.1514	0.6256	-0.2858	-0.3179	1.0000	0.3450	0.1461
MOIST	0.2797	-0.0179	-0.2882	0.0363	0.1731	0.1446	0.3450	1.0000	-0.2419
TEMP	-0.4177	-0.0834	-0.3596	0.1353	-0.5029	0.3605	0.1461	-0.2419	1.0000

	EIGENVALUE	DIFFERENCE	PROPORTION	CUMULATIVE
PRIN1	2.22405	0.061208	0.247116	0.24712
PRIN2	2.16284	0.504699	0.240315	0.48743
PRIN3	1.65814	0.648214	0.184238	0.67167
PRIN4	1.00993	0.236483	0.112214	0.78388
PRIN5	0.77344	0.242608	0.085938	0.86982
PRIN6	0.53084	0.117530	0.058982	0.92880
PRIN7	0.41331	0.254193	0.045923	0.97473
PRIN8	0.15911	0.090760	0.017679	0.99241
PRIN9	0.06835		0.007595	1.00000

VENTILATED BINS WITH 20% INITIAL MOISTURE CONTENT

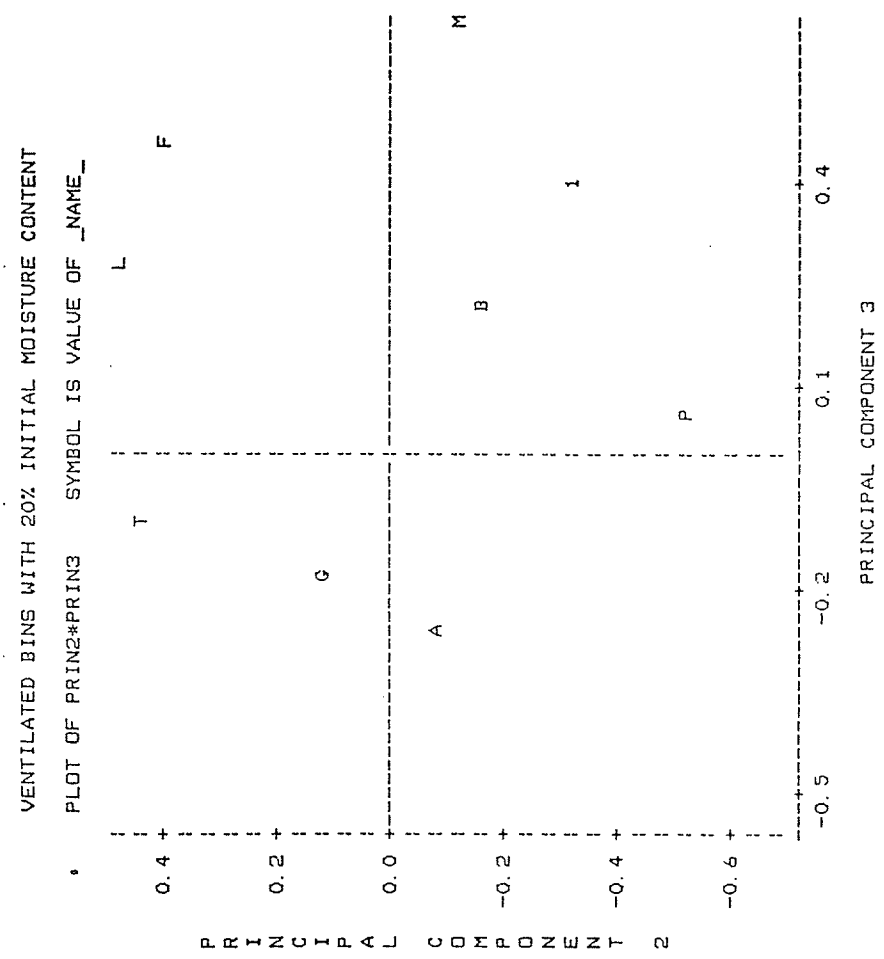
PRINCIPAL COMPONENT ANALYSIS

EIGENVECTORS

	PRIN1	PRIN2	PRIN3	PRIN4	PRIN5	PRIN6	PRIN7	PRIN8	PRIN9
ODOR1	0.166658	-.317202	0.401263	-.034830	0.722107	-.254031	0.125002	-.105968	-.310738
GERM	0.335930	0.136040	-.178521	-.762786	0.118367	-.097347	0.308324	0.211257	0.302906
ALTER	0.481968	-.099080	-.253312	0.413991	0.247672	0.408501	0.012343	0.540011	0.048532
ASPGL	0.192171	0.460184	0.273045	0.262364	-.180731	-.634618	0.047837	0.407517	-.064420
PENIC	0.115244	-.521759	0.059874	0.188535	-.415485	-.115655	0.692519	-.059887	0.078636
BACTER	-.587488	-.156407	0.214275	0.073366	0.244714	-.037451	0.049115	0.401392	0.595217
FUSAR	0.276539	0.398028	0.450505	0.191896	0.081807	0.313874	0.175636	-.429238	0.452790
MOIST	0.027930	-.109694	0.637131	-.313381	-.320113	0.411309	-.119537	0.356278	-.264902
TEMP	-.393035	0.438334	-.100043	0.027587	0.169907	0.271860	0.599782	0.102850	-.411734

VENTILATED BINS WITH 20% INITIAL MOISTURE CONTENT

OBS	_TYPE_	_NAME_	ODOR1	GERM	ALTER	ASPGL	PENIC	BACTER	FUSAR	MOIST	TEMP
1	SCORE	PRIN2	-0.31720	0.13604	-0.09908	0.460184	-0.52176	-0.15641	0.398028	-0.10969	0.43833
2	SCORE	PRIN3	0.40126	-0.17852	-0.25331	0.273045	0.05987	0.21427	0.450505	0.63713	-0.10004


VENTILATED BINS WITH 20% INITIAL MOISTURE CONTENT
PLOT OF PRIN2*PRIN3 SYMBOL IS VALUE OF _NAME_
PRINCIPAL COMPONENT 2
0.4
0.2
0.0
-0.2
-0.4
-0.6
-0.5
-0.2
0.1
0.4
PRINCIPAL COMPONENT 3
L
F
T
G
M
B
A
1
P

Appendix 5. Correlation matrix and principle components of data from control bins with 25% moisture content wheat in 1985-86.

CONTROL BINS WITH 25% INITIAL MOISTURE CONTENT

PRINCIPAL COMPONENT ANALYSIS

22 OBSERVATIONS
12 VARIABLES

SIMPLE STATISTICS

	ODOR1	ODOR2	ODOR3	GERM	ALTER	ASPGL	ASPFL	PENIC	BACTER	FUSAR	MOIST	TEMP
MEAN	1.88144	52.1364	3.30000	0.324578	0.181155	1.81818	0.163193	0.396901	13.5455	0.248641	20.2545	15.5636
ST DEV	0.68482	36.3300	7.60964	0.226643	0.287780	2.23897	0.297930	0.355541	6.7240	0.321984	3.4178	19.2012

CORRELATIONS

	ODOR1	ODOR2	ODOR3	GERM	ALTER	ASPGL	ASPFL	PENIC	BACTER	FUSAR	MOIST	TEMP
ODOR1	1.0000	0.0043	0.3826	0.2979	0.1485	-0.2879	-0.1413	0.5398	0.3659	-0.2195	0.7504	-0.1869
ODOR2	0.0043	1.0000	0.2262	-0.4508	-0.5799	-0.0195	-0.0064	-0.3425	0.3490	-0.3709	-0.2694	-0.3005
ODOR3	0.3826	0.2262	1.0000	-0.0852	0.0967	-0.3689	-0.1338	0.4809	0.0111	0.1544	0.2977	0.4452
GERM	0.2979	-0.4508	-0.0852	1.0000	0.5766	-0.0800	0.0153	0.0270	-0.3574	0.1025	0.5252	0.0425
ALTER	0.1485	-0.5799	0.0967	0.5766	1.0000	-0.0227	-0.1995	0.2740	-0.5576	0.2111	0.4504	0.2173
ASPGL	-0.2879	-0.0195	-0.3689	-0.0800	-0.0227	1.0000	0.1085	-0.3211	0.0638	-0.0806	-0.5487	-0.4199
ASPFL	-0.1413	-0.0064	-0.1338	0.0153	-0.1995	0.1085	1.0000	-0.1749	0.2750	-0.2812	-0.1942	0.1230
PENIC	0.5398	-0.3425	0.4809	0.0270	0.2740	-0.3211	-0.1749	1.0000	-0.0027	0.5087	0.5825	0.4253
BACTER	0.3659	0.3490	0.0111	-0.3574	-0.5576	0.0638	0.2750	-0.0027	1.0000	-0.6057	-0.0190	-0.3951
FUSAR	-0.2195	-0.3709	0.1544	0.1025	0.2111	-0.0806	-0.2812	0.5087	-0.6057	1.0000	0.0841	0.6339
MOIST	0.7504	-0.2694	0.2977	0.5252	0.4504	-0.5487	-0.1942	0.5825	-0.0190	0.0841	1.0000	0.0944
TEMP	-0.1869	-0.3005	0.4452	0.0425	0.2173	-0.4199	0.1230	0.4253	-0.3951	0.6339	0.0944	1.0000

	EIGENVALUE	DIFFERENCE	PROPORTION	CUMULATIVE
PRIN1	3.73112	1.28434	0.310927	0.31093
PRIN2	2.44678	0.54405	0.203899	0.51483
PRIN3	1.90274	0.73121	0.158562	0.67339
PRIN4	1.17153	0.22723	0.097627	0.77101
PRIN5	0.94430	0.23754	0.078691	0.84971
PRIN6	0.70676	0.23750	0.058896	0.90860
PRIN7	0.46926	0.19891	0.039105	0.94771
PRIN8	0.27035	0.11848	0.022529	0.97024
PRIN9	0.15187	0.04683	0.012656	0.98289
PRIN10	0.10504	0.03297	0.008753	0.99165
PRIN11	0.07206	0.04386	0.006005	0.99765
PRIN12	0.02820	.	0.002350	1.00000

CONTROL BINS WITH 25% INITIAL MOISTURE CONTENT

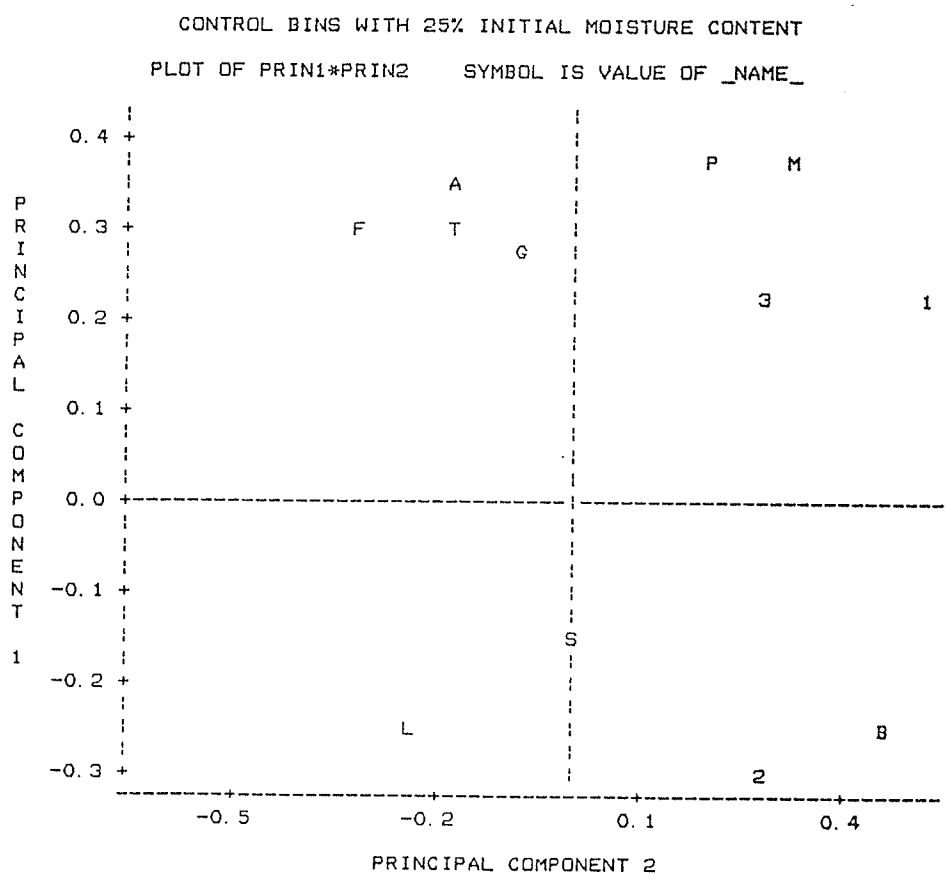
PRINCIPAL COMPONENT ANALYSIS

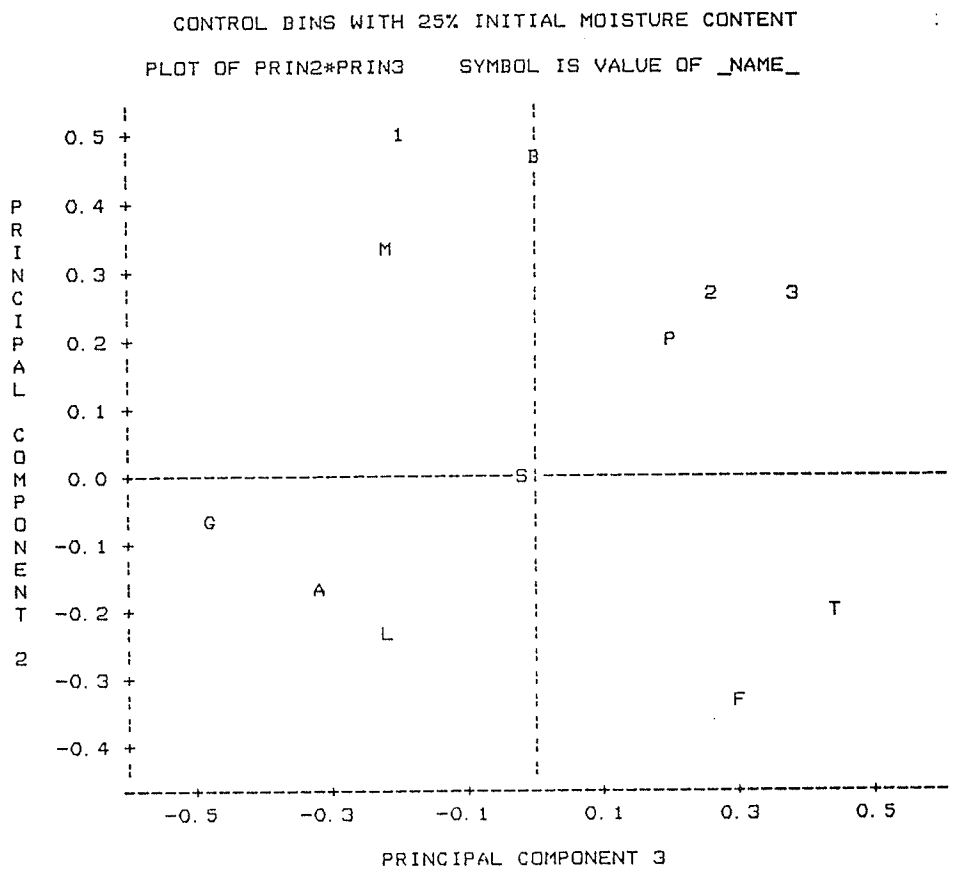
EIGENVECTORS

	PRIN1	PRIN2	PRIN3	PRIN4	PRIN5	PRIN6	PRIN7	PRIN8	PRIN9	PRIN10	PRIN11	PRIN12
ODOR1	0.218045	0.514030	-.202218	-.048547	0.150806	0.089616	0.171910	0.004576	-.272946	0.586578	0.232597	0.334602
ODOR2	-.289449	0.272014	0.257630	-.249207	-.370140	0.232639	0.366085	0.419957	0.405698	0.163903	-.127936	-.066485
ODOR3	0.225427	0.272446	0.383269	-.045561	-.146456	0.623594	-.058707	-.292195	-.275693	-.381838	-.035143	0.061650
GERM	0.271734	-.076362	-.476513	0.141249	-.274473	0.097435	0.519069	-.377549	0.099100	0.016086	-.362985	-.186200
ALTER	0.351250	-.181174	-.318060	-.053361	-.067480	0.381224	-.481382	0.277297	0.371994	0.075744	-.185212	0.320722
ASPGL	-.251002	-.239185	-.219322	-.114905	0.536959	0.544204	0.225205	-.025561	0.130365	-.018647	0.341907	-.219968
ASPFL	-.150328	-.002245	-.021876	0.844743	-.009922	0.175987	0.146035	0.390129	-.160012	-.101170	-.021811	0.149718
PENIC	0.381186	0.193187	0.196422	0.028883	0.511635	-.035112	-.000352	0.286061	-.022388	0.067920	-.461446	-.463766
BACTER	-.247912	0.458853	-.007787	0.213216	0.324649	-.095832	-.094341	-.390964	0.539815	-.153466	-.174306	0.246583
FUSAR	0.301414	-.325376	0.308020	-.125006	0.252606	-.142671	0.490518	0.071330	0.111980	-.177249	-.027610	0.564090
MOIST	0.386886	0.319420	-.215402	0.008649	-.087530	-.184519	0.085689	0.241670	0.231213	-.472454	0.532843	-.183762
TEMP	0.296223	-.189525	0.434874	0.349105	-.108873	0.046959	-.054097	-.260326	0.369058	0.428822	0.341847	-.208896

CONTROL BINS WITH 25% INITIAL MOISTURE CONTENT

OBS	_TYPE_	_NAME_	ODOR1	ODOR2	ODOR3	GERM	ALTER	ASPGL	ASPFL	PENIC	BACTER	FUSAR	MOIST	TEMP
1	SCORE	PRIN1	0.21805	-0.28945	0.225427	0.27173	0.35125	-0.25100	-0.15033	0.381186	-0.24791	0.30141	0.38689	0.29622
2	SCORE	PRIN2	0.51403	0.27201	0.272446	-0.07636	-0.18117	-0.23918	-0.00225	0.193187	0.45885	-0.32538	0.31942	-0.18953
3	SCORE	PRIN3	-0.20222	0.25763	0.383269	-0.47651	-0.31806	-0.21932	-0.02188	0.196422	-0.00779	0.30802	-0.21540	0.43487


CONTROL BINS WITH 25% INITIAL MOISTURE CONTENT
PLOT OF PRIN1*PRIN2 SYMBOL IS VALUE OF _NAME_
PRINCIPAL COMPONENT 1
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.5
-0.2
0.1
0.4
PRINCIPAL COMPONENT 2
P
M
A
F
T
G
3
1
S
L
B
2


CONTROL BINS WITH 25% INITIAL MOISTURE CONTENT
PLOT OF PRIN2*PRIN3 SYMBOL IS VALUE OF _NAME_
PRINCIPAL COMPONENT 2
0.5
0.4
0.3
0.2
0.1
0.0
-0.1
-0.2
-0.3
-0.4
1
B
M
2
3
P
S
G
A
L
T
F
-0.5
-0.3
-0.1
0.1
0.3
0.5
PRINCIPAL COMPONENT 3

Appendix 6. Correlation matrix and principle components of data from low-ventilated bins with 25% moisture content wheat in 1985-86.

LOW-VENTILATED BINS WITH 25% INITIAL MOISTURE CONTENT

PRINCIPAL COMPONENT ANALYSIS

19 OBSERVATIONS
8 VARIABLES

SIMPLE STATISTICS

	ODOR1	GERM	ALTER	PENIC	BACTER	FUSAR	MOIST	TEMP
MEAN	49.8684	11.0526	15.5263	5.42105	8.21053	0.165945	22.5105	3.57895
ST DEV	15.6878	3.8366	5.4095	3.87751	5.58350	0.290703	0.8582	9.17258

CORRELATIONS

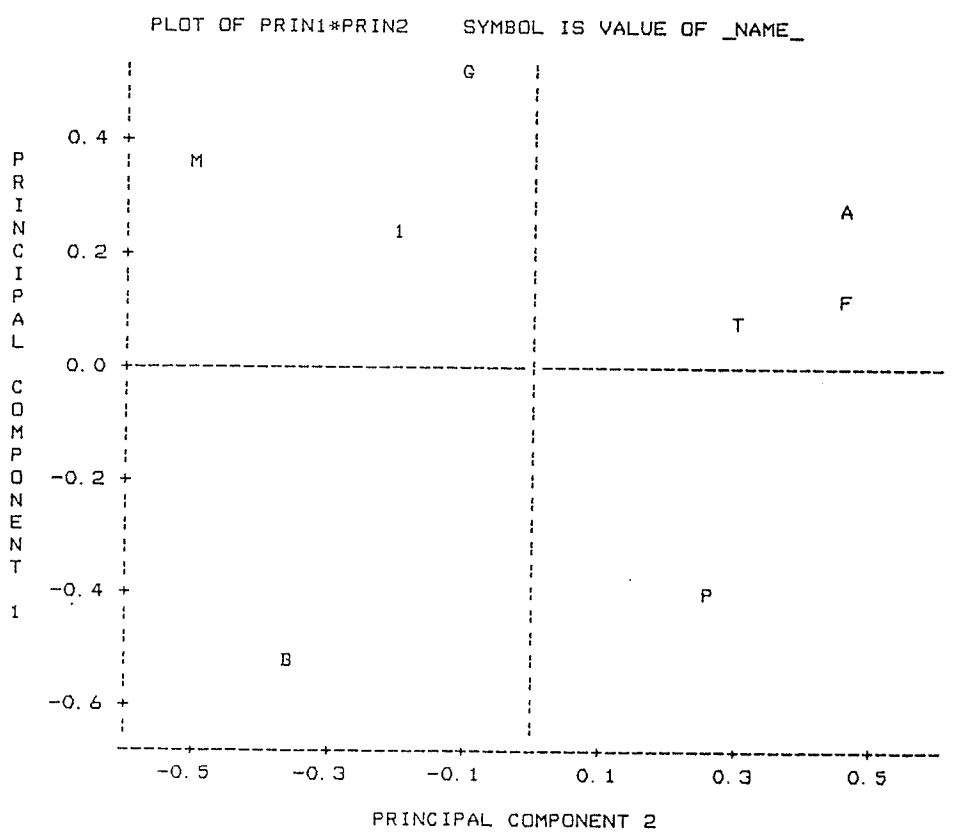
	ODOR1	GERM	ALTER	PENIC	BACTER	FUSAR	MOIST	TEMP
ODOR1	1.0000	0.1809	-0.2037	-0.2263	-0.2856	0.3311	0.3242	-0.1885
GERM	0.1809	1.0000	0.2850	-0.5356	-0.6152	-0.0806	0.4874	0.1323
ALTER	-0.2037	0.2850	1.0000	0.0789	-0.7083	0.0904	0.0526	0.0744
PENIC	-0.2263	-0.5356	0.0789	1.0000	0.2651	-0.0803	-0.4739	-0.1559
BACTER	-0.2856	-0.6152	-0.7083	0.2651	1.0000	-0.3590	-0.1686	-0.0966
FUSAR	0.3311	-0.0806	0.0904	-0.0803	-0.3590	1.0000	-0.3423	0.2132
MOIST	0.3242	0.4874	0.0526	-0.4739	-0.1686	-0.3423	1.0000	-0.1280
TEMP	-0.1885	0.1323	0.0744	-0.1559	-0.0966	0.2132	-0.1280	1.0000

	EIGENVALUE	DIFFERENCE	PROPORTION	CUMULATIVE
PRIN1	2.60016	0.896202	0.325021	0.32502
PRIN2	1.70396	0.325527	0.212995	0.53802
PRIN3	1.37843	0.239809	0.172304	0.71032
PRIN4	1.13863	0.630429	0.142328	0.85265
PRIN5	0.50820	0.111959	0.063525	0.91617
PRIN6	0.39624	0.193853	0.049530	0.96570
PRIN7	0.20238	0.130389	0.025298	0.99100
PRIN8	0.07200	.	0.008999	1.00000

EIGENVECTORS

	PRIN1	PRIN2	PRIN3	PRIN4	PRIN5	PRIN6	PRIN7	PRIN8
ODOR1	0.257821	-.190341	0.623670	-.287365	0.430264	-.123032	-.381005	0.283420
GERM	0.524155	-.097407	-.182085	0.116382	-.078953	-.656166	0.369991	0.308857
ALTER	0.288662	0.456139	-.423982	-.295201	0.059932	0.371433	-.168350	0.521355
PENIC	-.403740	0.267186	-.133611	-.410688	0.584835	-.296373	0.376622	-.085886
BACTER	-.510470	-.354309	0.022897	0.223390	0.010167	0.125531	0.201236	0.712110
FUSAR	0.111040	0.462244	0.600340	0.042663	-.194865	0.236865	0.557900	0.080269
MOIST	0.364412	-.495420	-.136981	-.058974	0.360816	0.505894	0.426601	-.177249
TEMP	0.082179	0.300311	-.023930	0.769978	0.542911	0.026790	-.118404	-.011460

LOW-VENTILATED BINS WITH 25% INITIAL MOISTURE CONTENT



LOW-VENTILATED BINS WITH 25% INITIAL MOISTURE CONTENT

OBS	_TYPE_	_NAME_	ODOR1	GERM	ALTER	PENIC	BACTER	FUSAR	MOIST	TEMP
1	SCORE	PRIN1	0.25782	0.524155	0.288662	-0.40374	-0.51047	0.111040	0.36441	0.082179
2	SCORE	PRIN2	-0.19034	-0.097407	0.456139	0.26719	-0.35431	0.462244	-0.49542	0.300311

Appendix 7. Correlation matrix and principle components of data from high-ventilated bins with 25% moisture content wheat in 1985-86.

HIGH-VENTILATED BINS WITH 25% INITIAL MOISTURE CONTENT

PRINCIPAL COMPONENT ANALYSIS

18 OBSERVATIONS
8 VARIABLES

SIMPLE STATISTICS

	ODOR1	GERM	ALTER	PENIC	BACTER	FUSAR	MOIST	TEMP
MEAN	42.6667	11.2222	14.9444	6.44444	8.83333	0.185822	21.1500	3.3056
ST DEV	27.8112	3.9041	5.8458	3.85353	6.35471	0.250177	2.8667	10.6456

CORRELATIONS

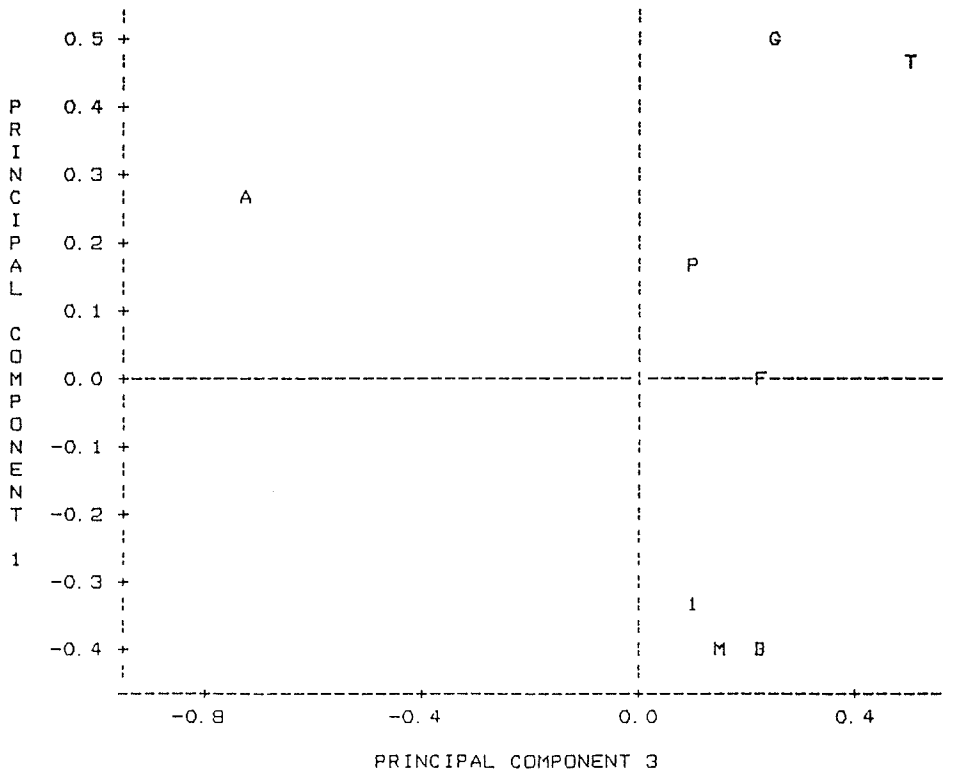
	ODOR1	GERM	ALTER	PENIC	BACTER	FUSAR	MOIST	TEMP
ODOR1	1.0000	-0.2057	-0.0097	-0.3307	0.0139	0.3432	0.8971	-0.2277
GERM	-0.2057	1.0000	0.2506	0.1338	-0.7050	0.1348	-0.3022	0.7963
ALTER	-0.0097	0.2506	1.0000	-0.0589	-0.6922	0.0710	-0.1367	0.0263
PENIC	-0.3307	0.1338	-0.0589	1.0000	0.2266	-0.3910	-0.4329	0.1409
BACTER	0.0139	-0.7050	-0.6922	0.2266	1.0000	-0.4322	0.1594	-0.4703
FUSAR	0.3432	0.1348	0.0710	-0.3910	-0.4322	1.0000	0.3458	0.1973
MOIST	0.8971	-0.3022	-0.1367	-0.4329	0.1594	0.3458	1.0000	-0.3570
TEMP	-0.2277	0.7963	0.0263	0.1409	-0.4703	0.1973	-0.3570	1.0000

	EIGENVALUE	DIFFERENCE	PROPORTION	CUMULATIVE
PRIN1	2.87887	0.49203	0.359859	0.35986
PRIN2	2.38684	1.26027	0.298355	0.65821
PRIN3	1.12657	0.35253	0.140821	0.79903
PRIN4	0.77403	0.25275	0.096754	0.89579
PRIN5	0.52128	0.32798	0.065160	0.96095
PRIN6	0.19330	0.10206	0.024162	0.98511
PRIN7	0.09124	0.06335	0.011404	0.99651
PRIN8	0.02789	.	0.003486	1.00000

EIGENVECTORS

	PRIN1	PRIN2	PRIN3	PRIN4	PRIN5	PRIN6	PRIN7	PRIN8
ODOR1	-.337570	0.421280	0.112469	0.498000	-.089371	0.198799	-.549849	0.313169
GERM	0.497157	0.171606	0.260174	0.219840	-.283144	-.524576	0.197750	0.461422
ALTER	0.252755	0.239492	-.727834	0.240005	0.072945	0.345668	0.339228	0.227003
PENIC	0.163113	-.409575	0.099243	0.669269	0.558616	-.096526	-.005846	-.162694
BACTER	-.414435	-.411410	0.232872	0.002578	-.001296	0.271059	0.424766	0.592326
FUSAR	0.015971	0.487996	0.233755	-.316808	0.744999	-.029497	0.106275	0.198516
MOIST	-.415159	0.387660	0.138649	0.308446	-.133018	-.151847	0.583519	-.426326
TEMP	0.451687	0.101431	0.501970	0.051292	-.147482	0.677566	0.106003	-.197493

HIGH-VENTILATED BINS WITH 25% INITIAL MOISTURE CONTENT



HIGH-VENTILATED BINS WITH 25% INITIAL MOISTURE CONTENT

OBS	_TYPE_	_NAME_	ODOR1	GERM	ALTER	PENIC	BACTER	FUSAR	MOIST	TEMP
1	SCORE	PRIN1	-0.33757	0.497157	0.25276	0.163113	-0.41443	0.015971	-0.41516	0.451687
2	SCORE	PRIN3	0.11247	0.260174	-0.72783	0.099243	0.23287	0.233755	0.13865	0.501970