INTERRELATIONS IN STORED-WHEAT ECOSYSTEMS INFESTED WITH MULTIPLE SPECIES OF INSECTS: A DESCRIPTIVE AND MULTIVARIATE ANALYSIS

by

Noel D. G. White

A Thesis

submitted to the Faculty of Graduate Studies

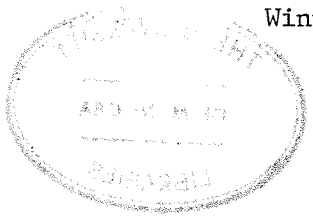
in partial fulfillment of the requirements

for the Degree of Doctor of Philosophy

Department of Entomology

University of Manitoba

Winnipeg, Manitoba



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A dissertation submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

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ABSTRACT

INTERRELATIONS IN STORED-WHEAT ECOSYSTEMS
INFESTED WITH MULTIPLE SPECIES OF INSECTS:
A DESCRIPTIVE AND MULTIVARIATE ANALYSIS

By Noel D. G. White

University of Manitoba, 1979 Supervisor: Dr. R. N. Sinha

The pathways and consequences of multiple insect infestation of bulk-stored wheat, for 60 weeks, under simulated tropical conditions (30±2°C) were determined by classical descriptive, and multivariate statistical methods of ecosystem analysis. Descriptive results relating to interactions among the variables were complimented and quantified by those obtained by multivariate analyses. Eight 204-liter drums containing wheat at 15.5% moisture content were used as three distinct man-made ecosystems: (a) Control system (2 drums), insect-free; (b) RST system (3 drums), infested with naturally-occurring groupings of Rhyzopertha dominica, Sitophilus oryzae, and Tribolium castaneum; and (c) COT system (3 drums), infested with naturally-occurring groupings of Cryptolestes ferrugineus, Oryzaephilus surinamensis, and Tribolium castaneum. The variables measured regularly within each system included carbon dioxide, oxygen, temperature, grain moisture, seed damage, grain weight and volume, dust weight and volume, seed germination, microflora which mainly included Alternaria alternata, Aspergillus glaucus group, Aspergillus candidus, and bacteria, insect numbers, and the numbers of the mite Tarsonemus granarius.

Alternaria infection and seed germination disappeared by week 15 while

Aspergillus and bacterial infection of the seeds increased in all systems. Aspergillus candidus replaced Aspergillus glaucus group as the main fungus for a short time after 30 weeks of storage in the RST system. Fat acidity values of the grain in the same system were highest until week 30 when they began a steady decline although values in the Control system continued to rise followed more slowly by values in the COT system. All insect numbers increased exponentially for nine weeks followed by a sharp decline and oscillations of populations; Sitophilus and Oryzaephilus were unable to survive in the presence of the other insects and Tarsonemus survived only in the Control system in the absence of large insect populations. The quantity of uric acid in the dust of the RST system was seven fold greater than in the COT system.

Highlights of the principal component analyses for each sampling period and for the cumulative data were that Alternaria infection and seed germination declined as seed damage, carbon dioxide levels and bacterial infection increased; and that insect numbers were positively related to Aspergillus but negatively related to bacterial infection of the seeds and, after lengthy storage, with high fat acidity values.

The main relationship found by canonical correlation analysis were that Alternaria and germination were negatively related to high fat acidity values, bacterial infection, and seed damage.

Moderate moisture content of the wheat (16-17%) was positively related to insect numbers and Aspergillus, but when high (>18%) it was negatively related to insect numbers.

Multiple linear regression analysis indicated that the fungus-feeding mite Tarsonemus was positively related to Alternaria, Aspergillus and to carbon dioxide levels.

Generally, Alternaria and germination rapidly declined because of Aspergillus growth; insects and mold produced large amounts of carbon dioxide, damaged the grain, and produced higher moisture levels which stimulated bacterial growth which in turn inhibited insect and fungal growth. Fat acidity values increased with time but the presence of Rhyzopertha sharply counteracted this trend in the RST system in which deterioration of the overall quality of grain was most rapid.

High temperature and high moisture levels accelerated insect and mold growth. The insects played an important role in the loss of grain quality for 40 to 50 weeks after which increasing moisture levels resulting from accelerated metabolic activity of the insects resulted in predominant bacterial infection and further grain spoilage.

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Chapter 1

INTRODUCTION

Wheat grown in western Canada is susceptible to attack by insect pests whether stored at home or shipped abroad. Wheat stored in various climatic regions of the world (Gunn 1968) is subject to invasion by characteristic groups of major pest species of insects. Although individual species overlap among these groups, the impact of collective infestations by a characteristic ecological species group is an important consideration for storage of cereal seeds with minimum quality loss. Canadian wheat is exported to many countries having a low-latitude climate with warm wet and dry seasons where most destructive storage insects remain active in almost all seasons of the year. The common pests in these regions are the lesser grain borer, Rhyzopertha dominica (F.)(Bostrichidae), the rice weevil, Sitophilus oryzae (L.) (Curculionidae), and the red flour beetle, Tribolium castaneum (Herbst) (Tenebrionidae), (Bains et al. 1976). In 1976, wheat exports from Canada to countries in such regions included 22,162 tons to Tanzania, 1,344,121 tons to China, 601,667 tons to India, 1,752,485 tons to Japan, and 178,909 tons to Pakistan (Anonymous 1976). In the Uttar Pradesh State of India, weight losses and a germination decrease of up to 9.7 and 22% respectively, were reported in wheat stored for 6 months and infested with insects, including R. dominica, S. oryzae, and T. castaneum (Girish et al. 1974).

Western Canada has a continental climate which includes a mid-latitude humid zone and a semi-arid zone. Its major insect pests are: the

rusty grain beetle, Cryptolestes ferrugineus (Stephens)(Cucujidae), the saw-toothed grain beetle, Oryzaephilus surinamensis (L.)(Cucujidae), and the red flour beetle, Tribolium castaneum (Sinha 1965a, 1974; Watters 1976).

Most stored-product insects have a world-wide distribution. The reasons for their ubiquitous presence lie both in their evolutionary adaptations (morphological, physiological, and behavioral) and in the actions of modern man who transports insect-infested grain throughout the world (Freeman 1973). Man also provides breeding habitats for these pests by building storage structures within which bulk grain provides both food and protection from atmospheric temperature fluctuations. Sinha (1974) demonstrated close relationships between stored-product insect pests and climate over various regions of the world, and developed a "climatic plasticity index" with which to determine the relative capacity of each major insect species to thrive and cause outbreaks in different countries with distinct climatic conditions. These calculations have shown that the most cosmopolitan pests, C. ferrugineus, O. surinamensis, and T. castaneum have the highest indices, which incorporate laboratory data on a species' intrinsic rate of increase and physical limits.

Most stored product insects reproduce continuously under favorable environmental conditions. The intrinsic rate of increase (maximum), r_m, is a good measure of the reproductive potential of a species (Birch 1948). Howe (1953) expressed it as the number of times a population would increase in four weeks in a non-limiting environment under optimal conditions. The r_m values for R. dominica and S. oryzae are 20X and 25X respectively; both species feed on whole grain (Birch 1953); C. ferrugineus (r_m=60X), feeds

mainly on the damaged germ of seeds (Bishop 1959, Smith 1965); and T. castaneum (r_m=70X) (Leslie and Park 1949; Howe 1956a, 1962) and O. surinamensis (r_m=60X) (Howe 1956b) feeds primarily on broken and ground grain.

The quality of stored grain is important not only to man, but also to insect species. Many stored-grain insects are not whole-grain feeders. Some of them thrive only in the presence of dockage such as broken kernels or weed seeds (Sinha 1975) whereas many others live on fungi associated with decaying stored grain (Sinha 1965, 1966a, 1966b, 1968a, 1971; Sinha and Harasymek 1974).

The rate of grain deterioration is affected by moisture, temperature, oxygen supply, and the condition, or degree of damage, of the seeds. These factors and the physical and biochemical state of the health of the cereal seed (Multon et al. 1973) determine the type of fauna and microflora that can invade the grain (Trisvyatskii 1966, Pixton and Hill 1967). The two key abiotic variables, temperature and moisture (Muir 1973) also affect the rate of biochemical reactions within the seeds. During storage, carbohydrates (starch and sugar) decrease, protein, vitamin, and mineral levels remain fairly constant, and fats are steadily broken down by either oxidative reactions, which lead to odors in the grain, or hydrolytic reactions caused by lipases secreted by molds, which break down the fats to free fatty acids and glycerol. Zeleny (1954) and Christensen (1974) have reviewed the processes involved in grain deterioration.

The stored-grain ecosystem is immature, unstable, and ecologically simple, containing species with high reproductive potential and facultative feeding behavior. However, interactions among abiotic and biotic

variables are often complex (Margalef 1963, Sinha 1973a). Bronswijk and Sinha (1971) have shown that some of the interrelationships among many variables operating within stored-grain ecosystems are not always clear from classical, descriptive studies. Use of multivariate data summarization techniques, such as principal component analysis and canonical correlation analysis (Sinha et al. 1969a, 1969b, 1972) give a more realistic picture of these interrelations of stochastic processes. Although many stored product insects are found together in natural infestations of stored grain, few controlled studies have been carried out to determine their collective impact on the loss of quality of food grain.

The purpose of this investigation was to clarify and quantify the interactions among two common combinations of stored-grain insects; and to describe the ramifications of such multiple species infestations in terms of several biotic and abiotic variables.

Therefore, a multidisciplinary study of naturally occurring complexes of stored-product insects at near optimal environmental conditions (Howe 1965), in storage containers similar to those used at the farm level in parts of Asia (Sinha 1968b), was undertaken to determine their effect on grain deterioration and the succession within the faunal and microfloral communities. Both descriptive and multivariate statistical analyses were used to elucidate the various interrelations among variables and to assist in understanding the structure and function of stored-wheat ecosystems under the specified conditions.

Chapter 2

MATERIALS AND METHODS

Stored-wheat Ecosystems

(A) Experimental Design

Eight 204-liter steel drums, placed on end, were each filled with No. 2 Canada western red spring wheat (Triticum aestivum L., cv Neepawa) grown in 1976 at Glenlea, Manitoba (Figs. 1 and 2). A 157-Kg parcel of grain was placed in each drum to about 5 cm from the top. The moisture content of the wheat, on a wet weight basis, was 15.5±0.10%. Dockage, consisting of small and broken wheat kernels and weed seeds, comprised less than 1% of the weight.

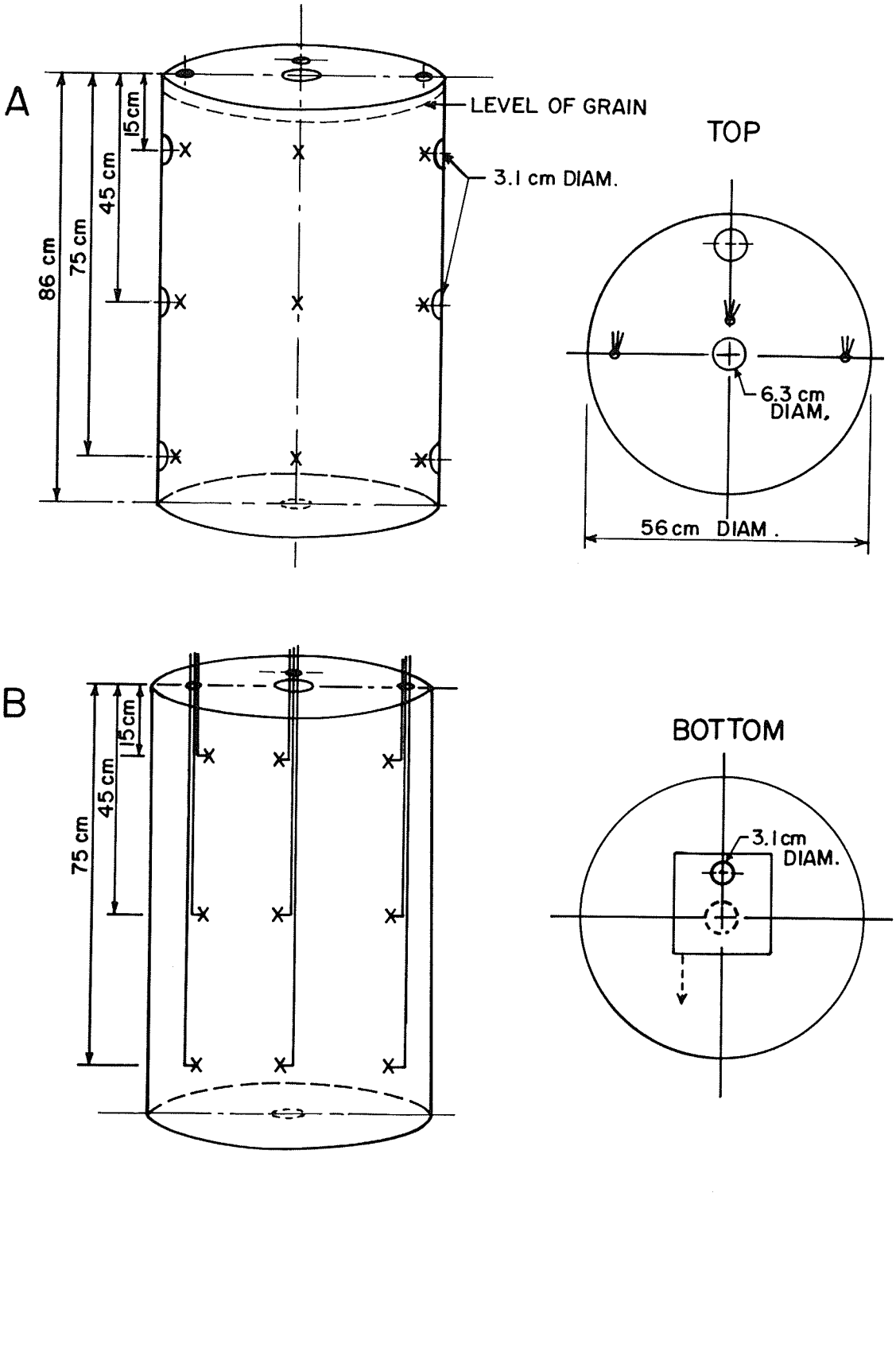
Holes were drilled in each drum at eight points (Fig. 2A). The top outlet (6.3-cm diam) was covered with 0.30-mm diam aperture (50-mesh) stainless steel screen which allowed gas exchange but contained the insects. Another ventilation hole 2 cm in diam. was drilled at the periphery of each top and covered with similar screening. The side outlets (3.1 cm diam) were plugged with rubber stoppers, and a sliding steel plate with a moveable opening covered the bottom outlet (3.1 cm diam). Sampling locations at the sides of the drums were 15, 45, and 75 cm from the top of the drum and central sampling locations were at 15 and 45 cm from the top and at the bottom of the drum.

Copper-constantan thermocouples and plastic Nalgene (Sybron Corp., Rochester, New York) gas tubes (3.5 mm diam), which had the bottom ends covered with 0.42 mm aperture nylon screen (40-mesh) and the upper ends covered with rubber nipples, were placed through the top of the drums in positions corresponding to sampling locations (Fig. 2B). These openings

Figure 1. Experimental wheat-filled drums equipped with thermocouples to record temperature and plastic tubes to extract gas samples for monitoring CO_2-O_2 levels. The first two drums to the left were control and the other 6 contained different combinations of stored-product insects.

Figure 2A. Planviews of experimental drums showing sampling locations and other dimensions.

2B. Planviews of experimental drums showing the location of thermocouples and gas tubes and other dimensions.


A
LEVEL OF GRAIN
15 cm
45 cm
75 cm
86 cm
3.1 cm DIAM.
TOP
6.3 cm DIAM.
56 cm DIAM.
B
15 cm
45 cm
75 cm
BOTTOM
3.1 cm DIAM.

in the drum tops were sealed with Silicone (Canadian General Electric, Toronto, Ontario) caulking compound. The drums were held in a room at 30±2°C and 45±5% relative humidity (RH) for 60 wk.

The drums were numbered from 1 to 8 and the sampling locations from 1 to 72 for nine consecutive locations per drum. The noninfested controls were drums 1 and 2 (Control system). On 25 May, 1977 the other drums were artificially infested with insects (Fig. 3) in the following manner: no. 3, 4, and 5, each with 500 adult lesser grain borers, R. dominica, reared on whole wheat, 500 adult rice weevils, S. oryzae, reared on whole wheat, and 500 adult red flour beetles, T. castaneum, reared on wheat flour and brewer's yeast powder (19:1) (Rhyzopertha-Sitophilus-Tribolium System or RST system); no. 6, 7, and 8 each with 500 adult rusty grain beetles, C. ferrugineus, reared on whole wheat and wheat germ (19:1), 500 adult saw-toothed grain beetles, O. surinamensis, reared on rolled oats, and 500 adult red flour beetles, T. castaneum, reared on wheat flour and brewer's yeast powder (Cryptolestes-Oryzaephilus-Tribolium System or COT system). All insects were taken from laboratory stock cultures maintained at 30±1°C and 70±2% RH. The insects were introduced through the top-central opening in each drum. Two species of common grain mites, Tarsonemus granarius Lindquist, and Lepidoglyphus destructor (Schrank) were initially present in the grain in small numbers. Of these only T. granarius multiplied successfully and only in drums 1 and 2.

To differentiate the various larval instars of each insect species, head capsule widths were measured. To obtain larvae, 2000 adults of four of the five species were permitted to deposit eggs on a flour medium for 24 hr at 30±1°C and 70% RH. After oviposition the eggs were sifted from the flour using a 0.42 mm aperture sieve. Thirty vials, 7 cm high and

Figure 3. Adult insects used to artificially infest the stored-wheat ecosystems:
(A) Rhyzopertha dominica,
(B) Sitophilus oryzae,
(C) Oryzaephilus surinamensis,
(D) Cryptolestes ferrugineus, and
(E) Tribolium castaneum.

A

B

C

D

E

3 cm in diameter and covered with a ventilated (0.30 mm aperture screen) lid, were set up for each species. Ten eggs were placed in each vial containing 20 g of wheat flour for C. ferrugineus, O. surinamensis, and T. castaneum and incubated at 30°C, 70% RH. Every other day two vials per species were removed from the growth cabinet, the flour was sifted, and the larvae mounted on microscope slides in Hoyer's medium (Krantz 1971), and covered with a circular no. 1 cover slip 18 mm in diameter. Ten R. dominica eggs were placed in each of 30 vials containing 10 g of whole wheat and 1 g of wheat germ, held under similar conditions, and the seeds from two vials were dissected every two days and the larvae mounted on microscope slides. An ocular grid in a binocular microscope (Carl Zeiss, Jena, German Democratic Republic) was calibrated with a stage micrometer and the head capsule width of the larvae determined. The head capsule measurements reported by Sharifi and Mills (1971) were used for S. oryzae classification.

In each drum, temperature was recorded to $\pm$ 1°C continuously at the 45 cm depth on the periphery of the bulk grain with copper-constantan thermocouples in conjunction with a multipoint electronic recorder (Model no. 9330FJ, Foxboro Co., Foxboro, Massachusetts) from wk 0-30.

Seventy-two 200-ml grain samples were taken at wk 0, 4, 6 and tri-weekly thereafter for 60 wk; nine locations in each drum. Wheat held in plastic bags at -15°C for the duration of the study served as a base-line control (157 kg). One day prior to each sampling date temperatures at nine locations in each drum were recorded with a Digimite potentiometer (Thermo Electric Co., Saddle Brook, New Jersey), and 30 ml of gas were drawn from each gas tube (Fig. 2B). The percentages of carbon dioxide and oxygen content at each location were determined with a Matheson gas

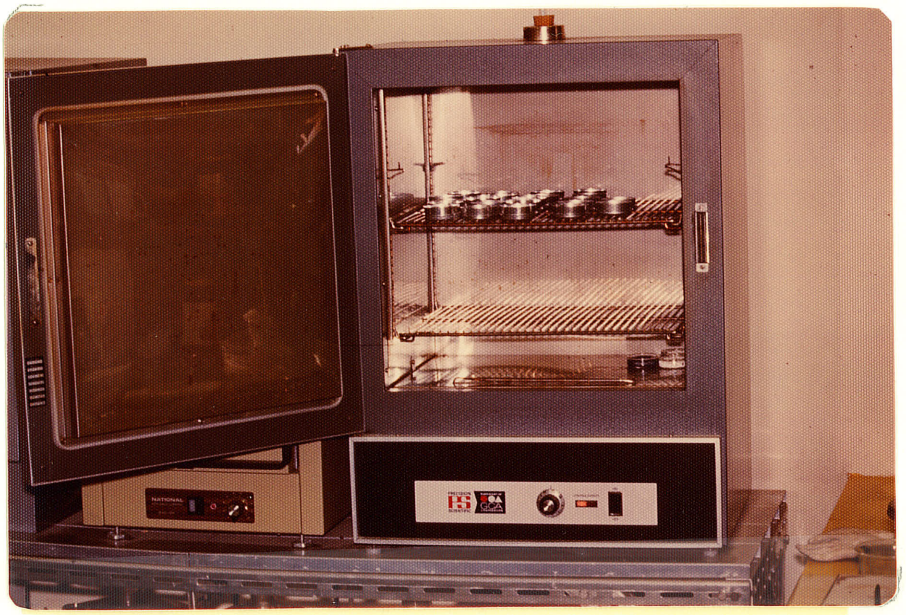
chromatograph (Model 8430, Matheson Gas Products, P. O. Box 85, East Rutherford, New Jersey) (Fig. 4). Grain samples were collected from the side and bottom locations with minimum disturbance of the bulk wheat by removing the stopper or sliding the steel plate respectively, and allowing the grain to flow into the sampling bottle. As it was impossible to remove samples from the two upper central locations in a similar manner, a brass torpedo probe holding 200 ml of wheat was used. Probes were placed in an oven at 100°C for 2 hr several hours before use to prevent accidental insect infestation. At each sampling, approximately 1215 g of grain (9x135g) were removed per drum, or 1.21% of the total mass. Removal of this quantity of wheat at a given time was not considered to influence future sampling appreciably because the systems had three weeks to return to an equilibrium state.

When the wheat had been removed from the drums the grain was sifted through 2 mm aperture (10-mesh) and 0.42 mm aperture (40-mesh) sieves. The various stages of living insects collected on the 0.42 mm aperture sieve were counted, and the dust passing through this sieve was weighed and the volume determined.

Subsamples of 50 ml were taken from every sample, placed in sealed plastic bags and refrigerated at 5°C. Wheat from the subsamples was used in the determination of seed moisture content, by wet weight basis, in duplicate, on 10-g samples by the ASAE oven-dry method, (Fig. 5) no. s352 (Anonymous 1975). Seed germination was determined and the microfloral association noted on 25 randomly chosen kernels by the filter paper method (Wallace and Sinha 1962) after surface sterilization for 1 min in a 1% sodium hypochlorite solution followed by a sterile water rinse (Tuite 1969). The weight and volume of 100 randomly chosen kernels was deter-

Figure 4. Injecting gas samples, taken from various locations in stored-wheat ecosystems, into a gas chromatograph to determine percentages of CO_2 and O_2.

Figure 5. Oven used to dry 10-g lots of wheat for moisture content determinations at 130°C for 19 hours.



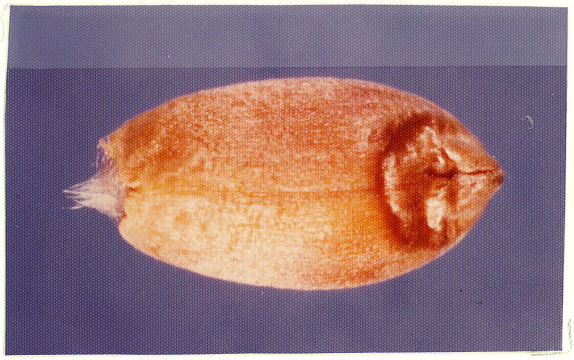
mined and the extent of seed damage classified on a scale of 1 (no damage) to 5 (only a shell remaining) for both germ (Fig. 6) and endosperm (Fig. 7). A further 50 kernels were removed at random from each subsample from drums 3, 4, and 5 and dissected with a scalpel under a binocular microscope to determine the number of R. dominica and S. oryzae larvae that were present.

The remaining 150 ml of wheat, to which the sifted dust was returned, was placed in a Berlese funnel for further extraction of mobile stages of insects and mites (Sinha 1964a). Specimens collected were preserved in 70% alcohol, counted under a microscope, and added to the values obtained previously by sifting. Free fatty acid contents of the samples or fat acidity values (FAV) in the grain were determined (Fig. 8) in duplicate on pooled samples from each drum for wk 0-60, and on pooled top samples only, from each drum for wk 15-60, because grain damage was generally greatest during these periods (AACC method 02-01, Anonymous 1962).

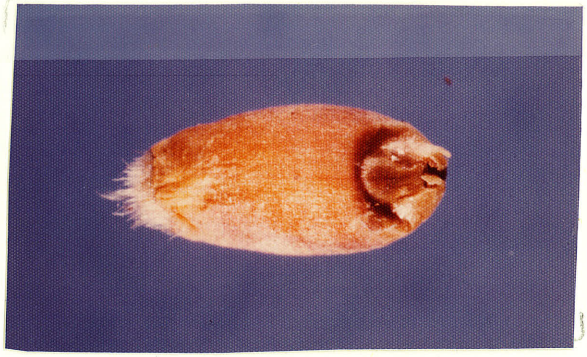
(B) Berlese Extraction of Insects

The efficiency of Berlese extraction for C. ferrugineus, O. surinamensis and T. castaneum was determined by placing separate groups of 25 individuals of each stage (four larval instars and adults), reared at 30°C, 70% RH, in 150 ml of dry (13.5% moisture content), tough (15.5% moisture content), and damp (17.5% moisture content) wheat for 1 hr and then placing the wheat in Berlese funnels for 24 hr. The percentage of each stage recovered was determined. Four replicates were used for each larval and adult group. Incandescent light bulbs (100 W) 6 cm above the grain were used. It was not deemed necessary to determine the efficiency of Berlese extraction for R. dominica or S. oryzae since dissection of kernels gave a good indication of larval numbers.

Figure 6. Wheat kernels showing degrees of germ damage caused by insect feeding:
(A) no damage,
(B) scratches - 1/4 eaten,
(C) 1/2 eaten,
(D) 3/4 eaten, and
(E) practically all eaten (shell left).



A



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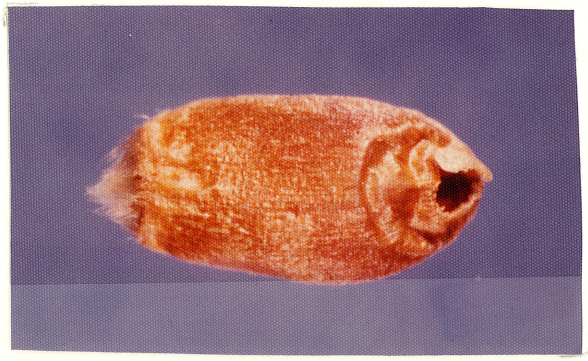


D

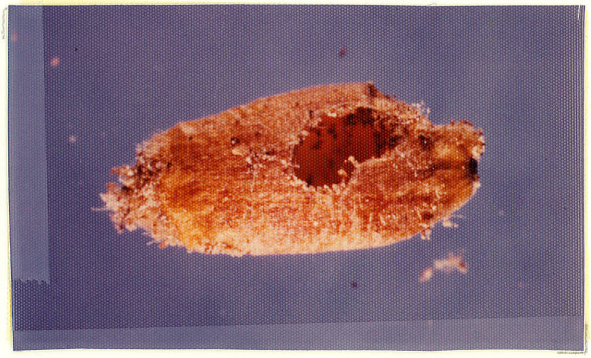
E

Figure 7. Wheat kernels showing degree of endosperm damage caused by insect feeding:
(A) no damage,
(B) scratches - 1/4 eaten,
(C) 1/2 eaten,
(D) 3/4 eaten, and
(E) practically all eaten (shell left).

A

B

C



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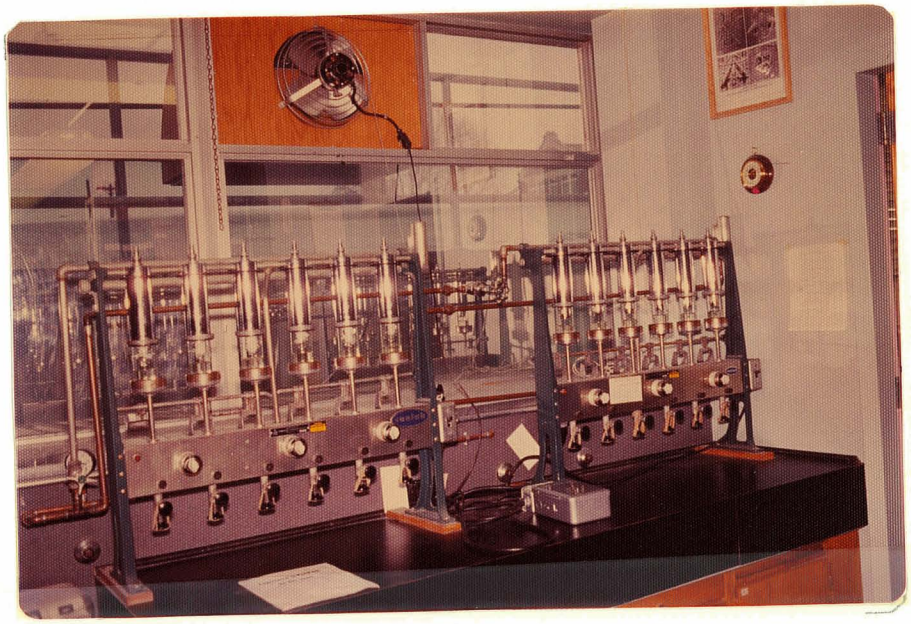
E

Figure 8. Goldfisch fat extractor apparatus used for removal of free fatty acids from samples of ground grain.



(C) Uric Acid Analysis

Uric acid concentration was determined on dust collected from the cold-room controls, and pooled samples from the Control system, the RST system, and the COT system drums on wk 9, 18, 27, 36, 45, 54, and 60 by the enzymatic spectrophotometric method described by Farn and Smith (1963) as modified by Bronswijk and Sinha (1971). The quantity of uric acid per mg dust and the total mean quantity for all samples from each drum, i.e. 9 samples or 1800 ml wheat, from a system on a given date were determined.

(D) Determination of the Distribution of Insects (COT system)

On wk 39 of the study a change in the distribution of T. castaneum and C. ferrugineus adults was noted in the COT system. To determine if the changing distribution patterns were because of the age of the beetles, 100 C. ferrugineus adults and 100 T. castaneum adults were removed from the tops of drums 6, 7, and 8 (total of 300 C. ferrugineus, 300 T. castaneum) and 100 adults of each species were removed from the bottom of drums 6, 7, and 8. Three hundred adults of each species were also taken from stock cultures maintained at 30°C and 70% RH.

Groups of 100 adults were placed in bottles (200 ml) containing a mixture of wheat (15.5% moisture content) and 5% wheat germ for 1 wk at 30°C and 70% RH. Then the adults were removed by sifting and the grain was placed in a Berlese funnel for 24 hr and the number of mobile offspring counted. The old adults were placed on fresh rearing media at that time and successively at tri-weekly intervals and the number living were counted by sifting at 4 and 7 wk after removal from the drums.

(E) Seed Electrolyte Leakage Test

The conductivity (electrolyte leakage in damaged seeds) of a deionized water solution containing 1 g of seeds was determined for the cold-room

controls and pooled samples from each drum at wk 18, 21, 24, 27, 30, and 60 (Mills and Kim 1977).

(F) Milling and Baking Tests

Standard milling and baking analyses (Pomeranz and Shellenberger 1971) were done on 1 kg of wheat from the cold-room controls and pooled wheat for each system at wk 15, 30, and 60. The physical and chemical analyses included: whole wheat (kernel weight, protein content, flour yield); flour (protein content, ash content, amylograph viscosity, baking absorption, sedimentation value); baking (loaf volume); and farinogram tests.

Insect Population Action and Interaction

Although most organisms were investigated at the species level, the arthropods and microflora are generally referred to by their generic names throughout the remaining text for convenience.

To determine the effects of individual and multiple groupings of the five species of insects used in the experimental ecosystem study, the following experiment was designed. One hundred and twenty-six bottles, 5 cm diam and 10 cm high, were filled with 102 g (150 ml) of whole wheat (cv Neepawa) at 15.5% moisture content. Nine bottles were used as replicates for each treatment, one set of nine serving as controls. Each of the remaining bottles received 25 adult insects of each species. All insects were obtained from laboratory stock cultures maintained at 30°C and 70% RH. The insect combinations used were as follows: (1) Tribolium; (2) Rhyzopertha; (3) Sitophilus; (4) Oryzaephilus; (5) Cryptolestes; (6) Tribolium and Rhyzopertha; (7) Tribolium and Sitophilus; (8) Tribolium and Oryzaephilus; (9) Tribolium and Cryptolestes; (10) Rhyzopertha and Sitophilus; (11) Oryzaephilus and Cryptolestes; (12) Rhyzopertha, Sitophilus, and Tribolium; and (13) Cryptolestes, Oryzaephilus, and Tribolium. All bottles were sealed

with ventilated (0.30 mm aperture screen) screw-on lids and placed in growth cabinets at 30°C and 70% RH. Samples of three bottles per treatment were analyzed at wk 5, 10, 15 for insect numbers, dust weight and volume, kernel weight and volume, degree and type of seed damage, seed moisture content, seed germination and microfloral infection, and pooled FAV levels for each treatment. The methodology of sample analysis was similar to that previously outlined.

Measurement of Carbon Dioxide Production and Oxygen Consumption

To measure the quantity of CO_2 produced and O_2 consumed by individuals of each of the five insect species used in the foregoing experiments, under near optimal conditions, a further experiment was designed. Twenty-four bottles (5 cm diam, 10 cm high) were filled with 150 ml of wheat (cv Neepawa) at 15.5% moisture content. Four bottles served as non-infested controls, the remaining bottles held 100 adult insects (Rhyzopertha, Sitophilus, Cryptolestes, Oryzaephilus, or Tribolium), four bottles of each species serving as replicates. The insects had been reared under standard conditions on standard media as previously described. Each bottle was sealed with Parafilm (American Can Co., Neenah, Wisconsin) and a screw-on lid with a 5-mm diam hole in the centre and placed in a tightly sealed plastic bag. The non-infested controls and the bottles with insects were placed in a growth cabinet at 30°C and 70% RH. After 24 hr the amount of CO_2 and O_2 present were determined by extracting 1 ml of gas near the grain surface and doing an analysis with a gas chromatograph.

Multivariate Statistical Analyses

To obtain multivariate summarization of the data, principal component analyses (PCA) were done on samples from each of the three storage systems at each sampling date (Control system, 18 samples; RST system, 27 samples;

COT system, 27 samples) and at the end of 60 wk (Control system, 378 samples; RST system, 567 samples; COT system, 567 samples). An IBM 370-168 digital computer was used with the University of California BMD01M program using Fortran language (Dixon and Brown 1977). The following variables were used in the PCA : FAV (mg KOH/100 g dry wheat), CO_2 (%), O_2 (%), temperature (°C), grain weight (100 kernels in grams), dust weight (g), grain moisture (%), Alternaria alternata (Fr.) Keissler, Aspergillus glaucus group, bacteria, and seed germination. The last four variables were measured in number of seeds infected or sprouted out of 25 seeds. In addition Tarsonemus granarius numbers were used in the Control system; germ damage (%), endosperm damage (%), adult Rhyzopertha, Sitophilus, and Tribolium in the RST system; and germ damage, endosperm damage, adult Cryptolestes, Oryzaephilus, and Tribolium in the COT system. Variables were omitted from the analyses if they were present in fewer than 10% of the samples. An arbitrary principal component (eigenvector) loading cut-off level of 0.30 was used in interpretation of the analyses (Sinha 1977).

Canonical correlations were calculated for each system at the end of 60 wk using the computer and the University of California BMDP6M program. In the Control system the criterion (dependent) variables were: FAV, CO_2, O_2, dust weight, moisture, and germination; and the predictor (independent) variables were: Alternaria, Aspergillus, bacteria, and Tarsonemus. In the RST system the criterion variables were: FAV, CO_2, moisture, germ damage, endosperm damage, and germination; and the predictor variables were: Alternaria, Aspergillus, bacteria, Tribolium, Rhyzopertha, and Sitophilus. In the COT system the criterion variables were: FAV, CO_2, moisture, dust weight, germ damage, and germination; and the predictor variables were: Alternaria, Aspergillus, bacteria, Tribolium, and Cryptolestes.

Stepwise multiple linear regression was done on variables in the Control system only, with the Statistical Package for the Social Sciences (SPSS) program (Nie et al. 1975) on the computer. Eight independent variables (FAV, CO_2, O_2, temperature, moisture, Alternaria, Aspergillus, and bacteria) were related to the dependent variable Tarsonemus to explore the factors that might act as natural regulating agents on the mite population. The analysis was done on all data from wk 0 - 33 with a total of 198 samples.

All variables were analyzed after $\sqrt{x}$ or $\sqrt{x+1}$ transformations with the exception of mite and insect numbers which received a $\log_{10}(x+1)$ transformation (Goulden 1945; Sinha et al. 1969a).

At the end of 15 wk both drums used in the Control system accidentally became lightly infested with Oryzaephilus. To determine if this infestation was affecting the variables monitored, 1 kg of wheat was taken from both drum 1 and 2 and placed at -15°C for seven days. The grain was then sifted through a 10-mesh screen and placed in plastic vials holding 10 g of wheat and ventilated at both ends with 0.30 mm aperture screening. The vials were then returned to the drums at various depths and retrieved at regular intervals by means of string attached to each vial. The insects did not reproduce extensively in the control drums and were not present after wk 40. No differences, with the exception of 1% grain damage, were observed between the bulk grain in the control drums and that in the vials. The infestation was, therefore, considered to have negligible impact on the functioning of the ecosystems.

Chapter 3

RESULTS AND DISCUSSION

Description of Abiotic and Biotic Changes

The presence, abundance and distribution of insects in the drums representing three systems (Control, RST and COT) affected many of the variables monitored throughout this study, both directly and indirectly.

Physical Changes

Temperature differences among the systems were noticeable only at the upper levels of the bulk grain (Fig. 9). The mean temperatures at the top of the grain masses in the RST system were consistently higher than those in the other systems. The RST system temperatures were approximately 1 - 2°C warmer than those of the Control system and often 1°C warmer than the COT system. The higher temperatures could be the result of heat generated by the metabolic activity of Rhyzopertha and Sitophilus (Howe 1962, Sinha 1973a). A drop in temperatures at all levels at weeks 42 and 54 was the result of environmental temperature changes.

Carbon dioxide levels increased steadily in all three systems until wk 12 when CO_2 concentrations declined to relatively stable levels for the duration of the experiment (Fig. 10). The difference between the CO_2 levels in the Control system and the insect-infested systems indicated that the insects produced a large amount of CO_2 in addition to that produced by microflora alone. This difference was most pronounced in the RST system (Fig. 10B). Carbon dioxide concentration was greatest at the

Figure 9. Mean grain temperature at the top of the bulk wheat in the Control, Rhyzopertha-Sitophilus-Tribolium, and Cryptolestes-Oryzaephilus-Tribolium systems.

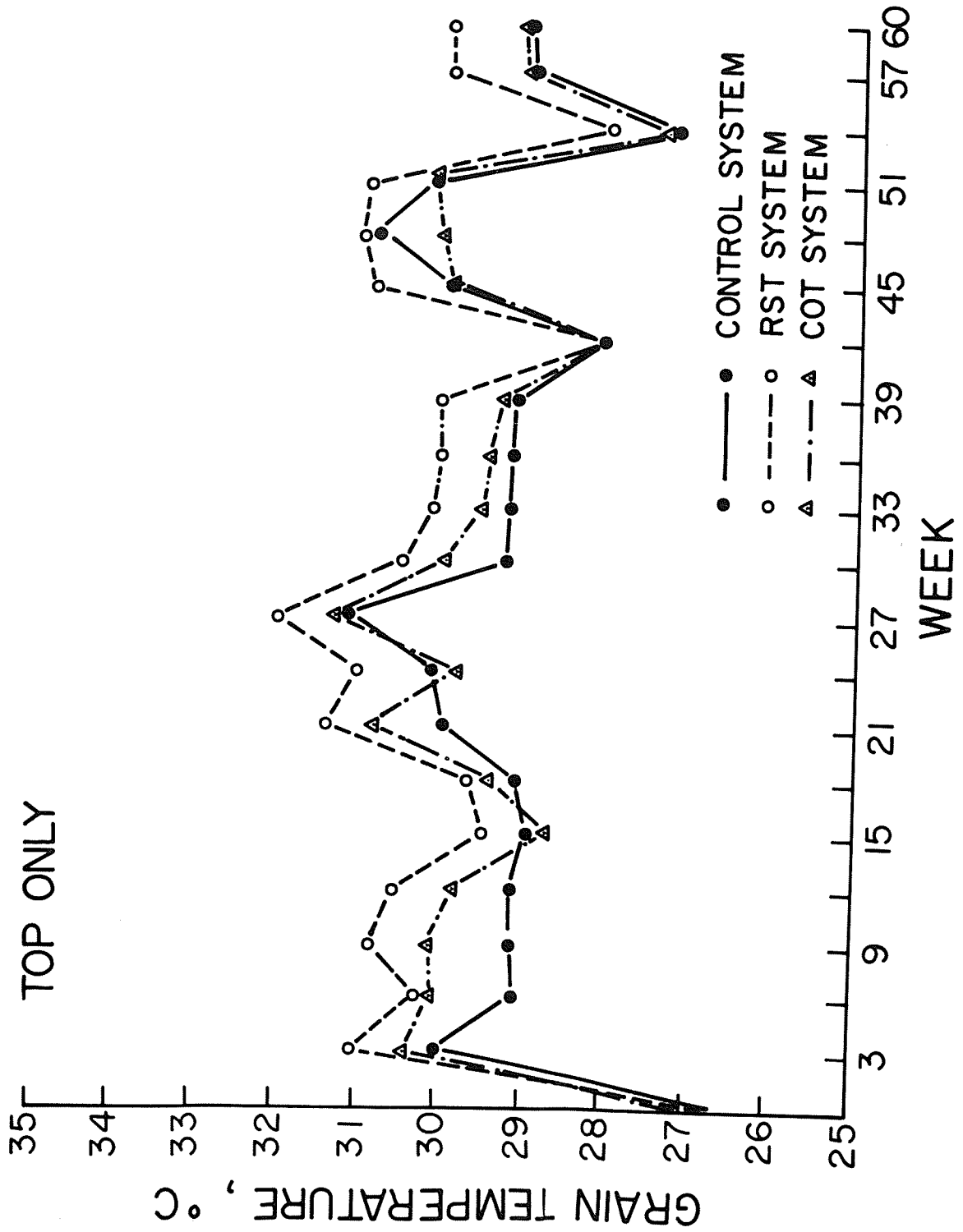
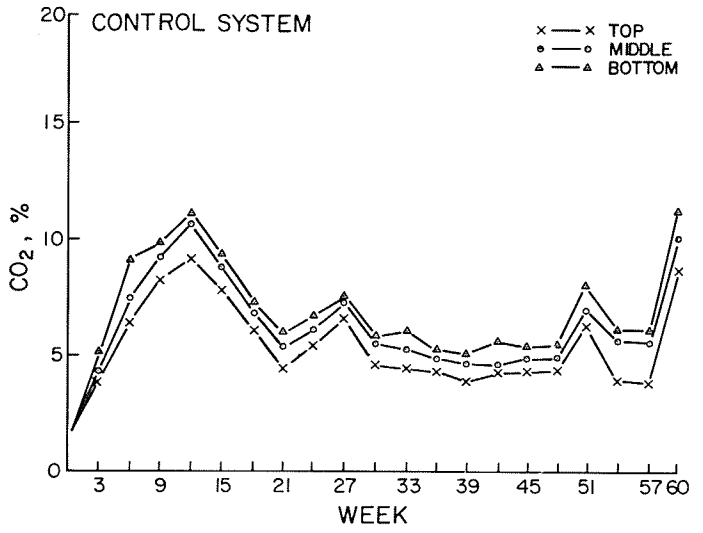
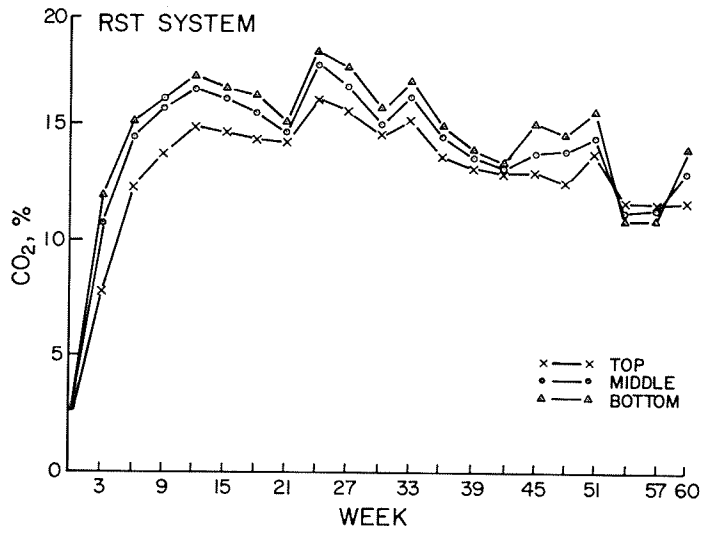

TOP ONLY
GRAIN TEMPERATURE , °C
35
34
33
32
31
30
29
28
27
26
25
3
9
15
21
27
33
39
45
51
57 60
WEEK
CONTROL SYSTEM
RST SYSTEM
COT SYSTEM

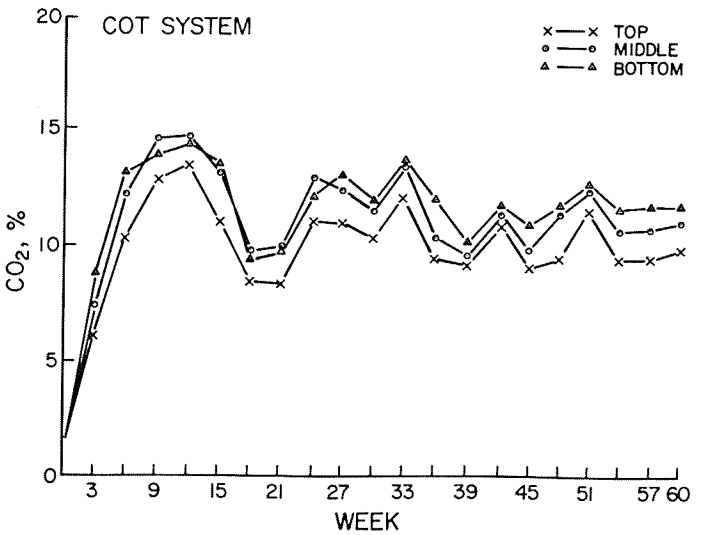
Figure 10. Mean percentage of carbon dioxide present at the top, middle, and bottom of the drums in the
(A) Control,
(B) Rhyzopertha-Sitophilus-Tribolium, and
(C) Cryptolestes-Oryzaephilus-Tribolium systems.


CONTROL SYSTEM
TOP
MIDDLE
BOTTOM
CO_2, %
WEEK
0
5
10
15
20
3
9
15
21
27
33
39
45
51
57 60

A


RST SYSTEM
TOP
MIDDLE
BOTTOM
CO_2, %
WEEK
0
5
10
15
20
3
9
15
21
27
33
39
45
51
57 60

B


COT SYSTEM
TOP
MIDDLE
BOTTOM
CO_2, %
WEEK
0
5
10
15
20
3
9
15
21
27
33
39
45
51
57 60

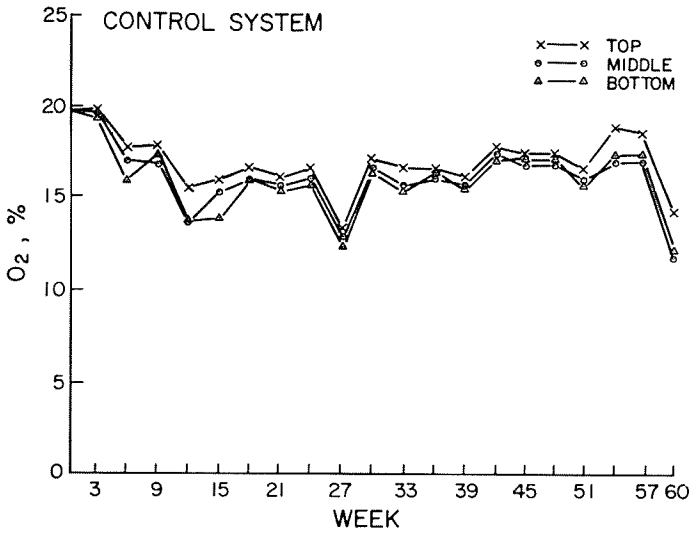
C

bottom of all of the drums, because CO_2 is heavier than air (Spratt 1975) and diffusion of gases into the external air was greatest at the tops of the drums where a provision for limited ventilation was made. During wk 60 the highest mean CO_2 level in the Control system was about 11% (Fig. 10A), in the RST system it was 18% (Fig. 10B), and in the COT system it was 14.5% (Fig. 10C). The initial rapid increase in CO_2 concentrations was closely related to the initial increase in the fungal growth in the Control system and additional rise in insect numbers in the RST and COT systems during the first few weeks of the experiment. Their presence undoubtedly affected the quantity of CO_2 present. The exponential rise in CO_2 levels in the RST and COT systems was followed by a stationary phase with oscillations. Carbon dioxide is produced by respiration of the dormant grain, microflora and insects. Oxley (1948) has determined that weevils produce about 130,000 times more CO_2 than equivalent weights of grain, and Trisvyatskii (1966) has reported that cultures of fungi produce about 1800 mg CO_2/g dry matter in 24 hours as opposed to 0.1 mg CO_2/g dry matter by dry wheat.

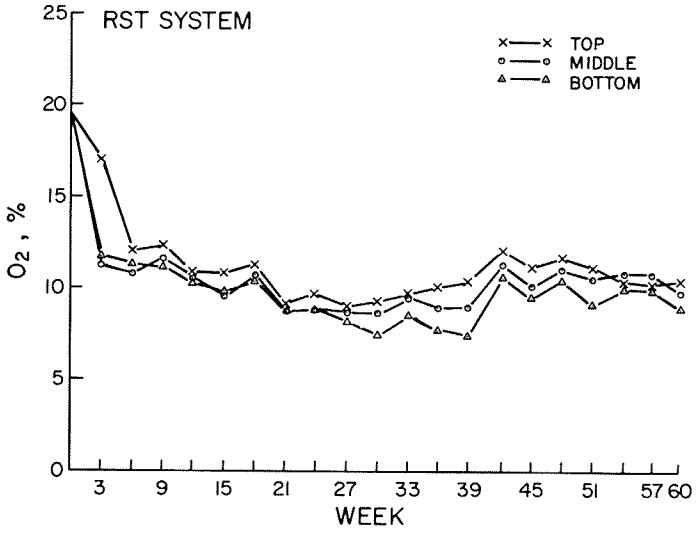
Oxygen concentrations declined sharply during the first few weeks of the study (Fig. 11), although the levels were relatively stable after wk 9. Oxygen was most concentrated at the surface of all drums because of the ventilation holes located there. The lowest mean value for O_2 concentration in the Control system was about 13% (Fig. 11A) at wk 27, 7% in the RST system at wk 39 (Fig. 11B), and 11% in the COT system at wk 12 (Fig. 11C). Insects, particularly those in the RST system, consumed more oxygen than those of the aerobic fungi in the Control system.

Atmospheric concentrations of CO_2 and O_2 are 0.033% and 20.95% respectively. The changes in gaseous ratios in the intergranular spaces were conspicuous and undoubtedly affected the growth and multiplication of

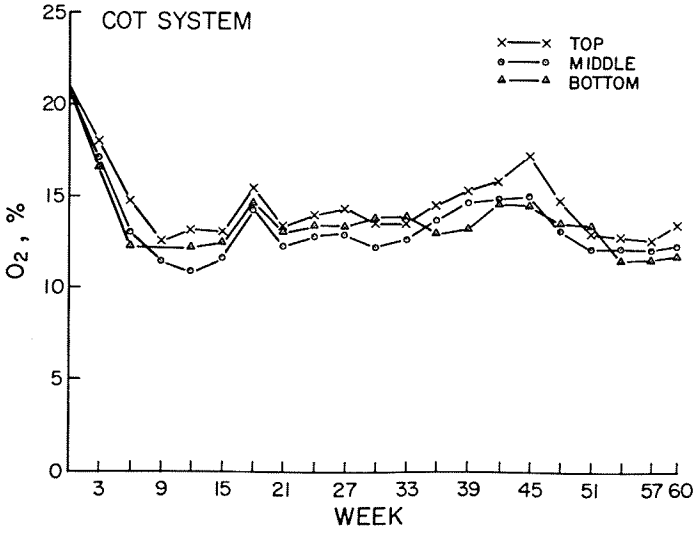
Figure 11. Mean percentage of oxygen present at the top, middle, and bottom of the drums in the
(A) Control,
(B) Rhyzopertha-Sitophilus-Tribolium, and
(C) Cryptolestes-Oryzaephilus-Tribolium systems.


CONTROL SYSTEM
TOP
MIDDLE
BOTTOM
O2, %
25
20
15
10
5
0
3
9
15
21
27
33
39
45
51
57 60
WEEK

A


RST SYSTEM
TOP
MIDDLE
BOTTOM
O2, %
25
20
15
10
5
0
3
9
15
21
27
33
39
45
51
57 60
WEEK

B

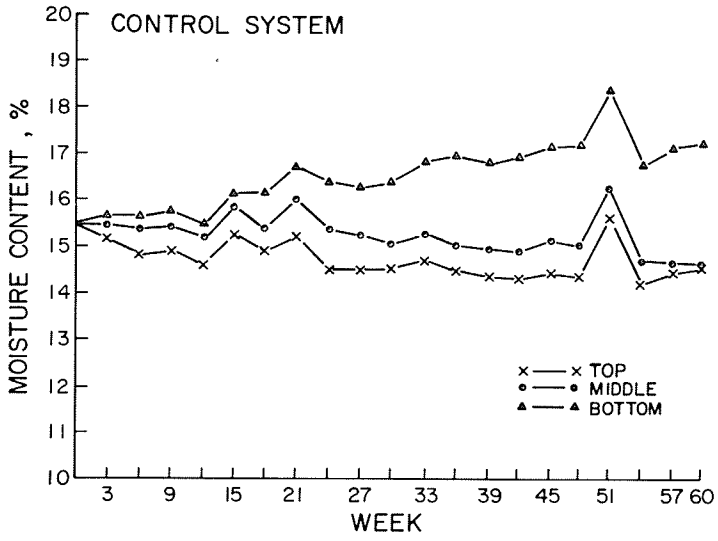

COT SYSTEM
TOP
MIDDLE
BOTTOM
O2, %
25
20
15
10
5
0
3
9
15
21
27
33
39
45
51
57 60
WEEK

C

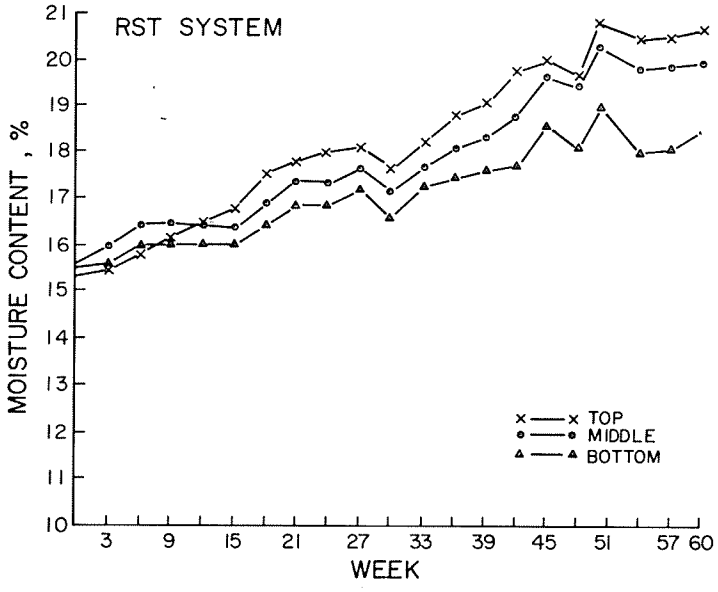
microflora and fauna in all of the drums (Singh et al. 1977). Spratt (1975) has reported that increasing levels of CO_2 cause stored-product insects to consume more O_2 than what they would when exposed to atmospheric air. Harein and Press (1968) have demonstrated that atmospheres containing about 36% CO_2 are lethal to Tribolium castaneum even when 15% O_2 is present. Storey (1977) has reported that O_2 concentrations below 1% and exposure for several days were necessary to kill the larvae of Tribolium spp. at both 18 and 27°C. Exley and Wickendon (1963) have shown that O_2 levels below 2% will kill Sitophilus oryzae, Rhyzopertha dominica, Oryzaephilus surinamensis, and Cryptolestes spp. Navarro (1975) demonstrated that O_2 levels of 0.1% led to mortality in adult T. castaneum after 30 - 48 hr and Peterson et al. (1956) have shown that CO_2 levels of 13.8 to 18.6% inhibit mold growth and respiration.

The moisture content of the wheat changed throughout the 60 wk of observation in all systems (Fig. 12). In the Control system, grain at the surface of the drums became drier, and that at the middle of the drums changed moisture content only slightly throughout 60 wk. However, the grain at the bottom of the drums gradually increased in moisture content (Fig. 12A). In the RST system, grain at all levels increased in moisture content during 60 wk. Grain at the surface of the drums was most moist, reaching levels around 20.5% at wk 51, while grain at the bottom of the drums was drier, reaching 18.5% at wk 51 (Fig. 12B). The moisture levels of grain in the COT system remained fairly stable at the tops of the drums, near 15.5%, and 16% at the middle levels, during 60 wk. However, moisture increased steadily at the bottoms of the drums to more than 18% after wk 45 (Fig. 12C). The pattern of moisture increase in the Control and COT systems was similar but the latter was about 1% more moist than

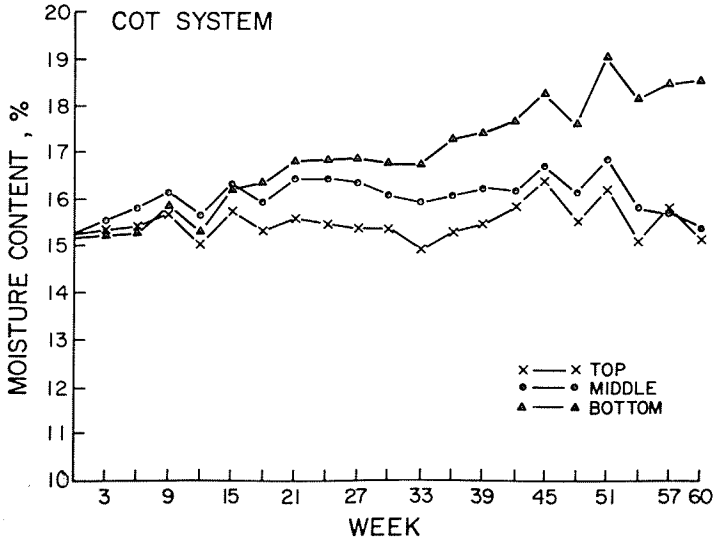
Figure 12. Mean moisture content (percent) of wheat at the top, middle, and bottom of the drums in the
(A) Control,
(B) Rhyzopertha-Sitophilus-Tribolium, and
(C) Cryptolestes-Oryzaephilus-Tribolium systems.


CONTROL SYSTEM
MOISTURE CONTENT , %
WEEK
TOP
MIDDLE
BOTTOM

A


RST SYSTEM
MOISTURE CONTENT , %
WEEK
TOP
MIDDLE
BOTTOM

B


COT SYSTEM
MOISTURE CONTENT , %
WEEK
TOP
MIDDLE
BOTTOM

C

the Control system.

Microbial Changes

The field fungus, Alternaria alternata, initially infected nearly 40% of all seeds used in the study. The level of infection declined rapidly and disappeared by wk 18 (Fig. 13). Alternaria spp. do not increase in storage and are inhibited by the growth of storage fungi such as Penicillium spp. and Aspergillus spp. (Wallace and Sinha 1962). The rate of decline of Alternaria seems to be much faster when stored at constant high temperatures than when stored in unheated granaries (Sinha et al. 1969).

Aspergillus glaucus group was the predominant storage fungus in all systems throughout the study with the exception of Aspergillus candidus Link, which was more prevalent than A. glaucus gr. in the RST system between wk 30 - 39. A. glaucus gr. infected about 50% of the seeds in the Control system by wk 12, gradually declining to insignificant levels by wk 45 (Fig. 14A). In the RST system approximately 35% of the seeds were infected by wk 9, followed by a period of decline until wk 45 (Fig. 14B). The COT system followed a similar pattern to the Control system, although with a more rapid decline in A. glaucus gr. infection (Fig. 14C). Little heating occurred as a result of metabolic activity of these fungi in all three systems. Carter (1950) has reported that A. glaucus gr. produces little heat during reproduction. Armolik and Dickson (1956) have shown that A. glaucus gr. can germinate on wheat at 13 - 14% moisture content. Bacterial infection levels increased steadily and followed similar patterns of growth in all systems during 60 wk (Fig. 15) reaching infection levels of about 80%. Bacteria require a moister environment for multiplication than fungi (Wallace 1973); and

Figure 13. Mean percentage of kernels infected with Alternaria in the Control, Rhyzopertha-Sitophilus-Tribolium, and Cryptolestes-Oryzaephilus-Tribolium systems.

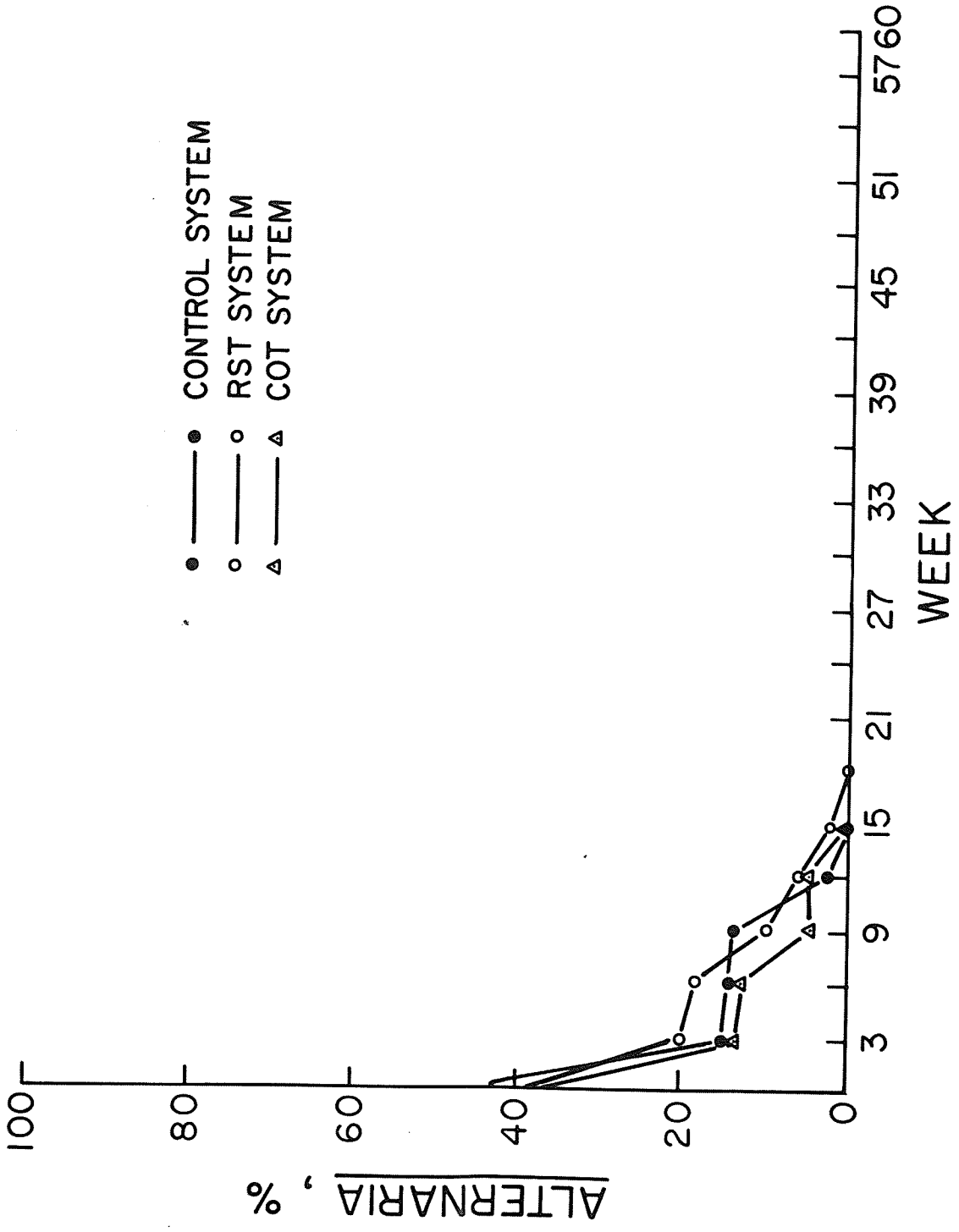
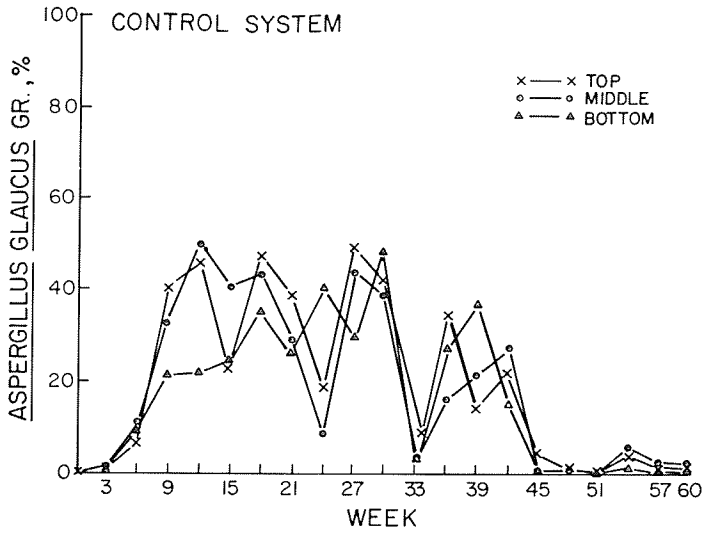
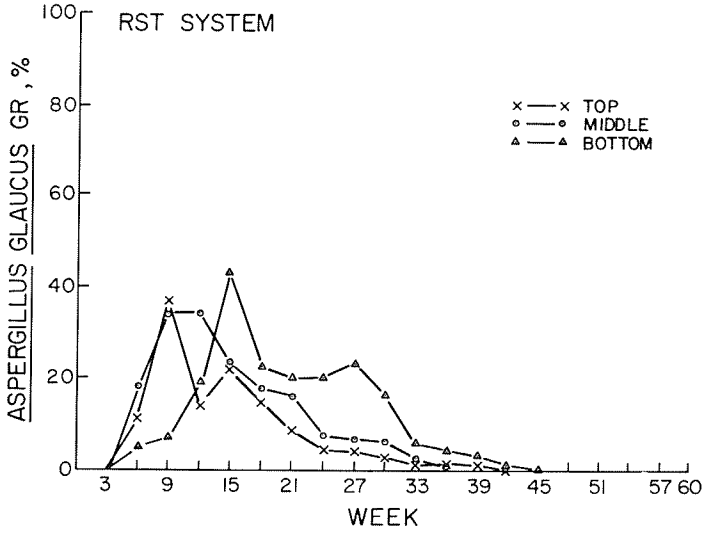

CONTROL SYSTEM
RST SYSTEM
COT SYSTEM
ALTERNARIA , %
100
80
60
40
20
0
3
9
15
21
27
33
39
45
51
57
60
WEEK

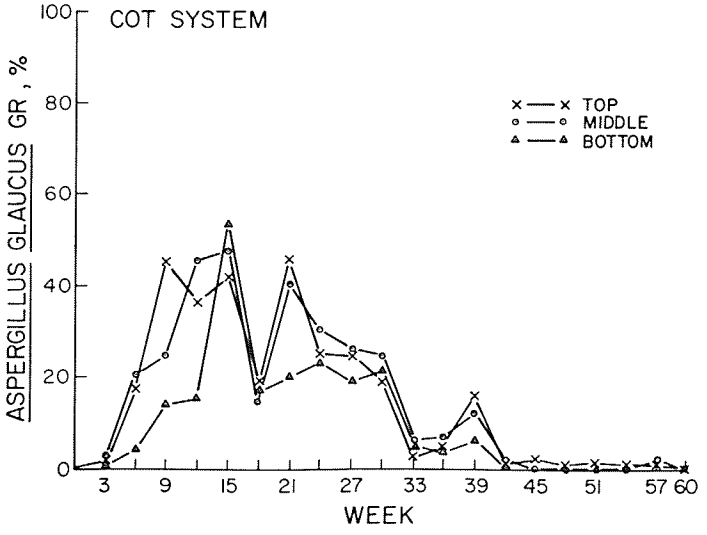
Figure 14. Mean percentage of kernels infected with Aspergillus glaucus group at the top, middle, and bottom of the drums in the (A) Control, (B) Rhyzopertha-Sitophilus-Tribolium, and (C) Cryptolestes-Oryzaephilus-Tribolium systems.


CONTROL SYSTEM
ASPERGILLUS GLAUCUS GR., %
TOP
MIDDLE
BOTTOM
WEEK
0
20
40
60
80
100
3
9
15
21
27
33
39
45
51
57 60

A

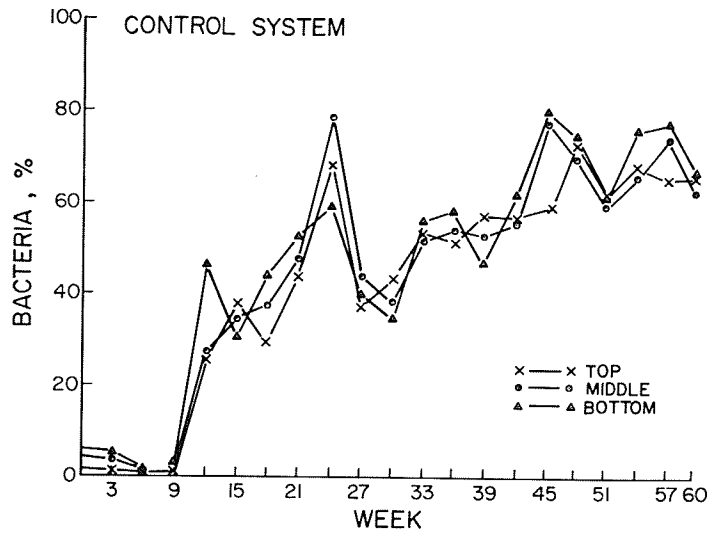

RST SYSTEM
ASPERGILLUS GLAUCUS GR , %
TOP
MIDDLE
BOTTOM
WEEK
0
20
40
60
80
100
3
9
15
21
27
33
39
45
51
57 60

B

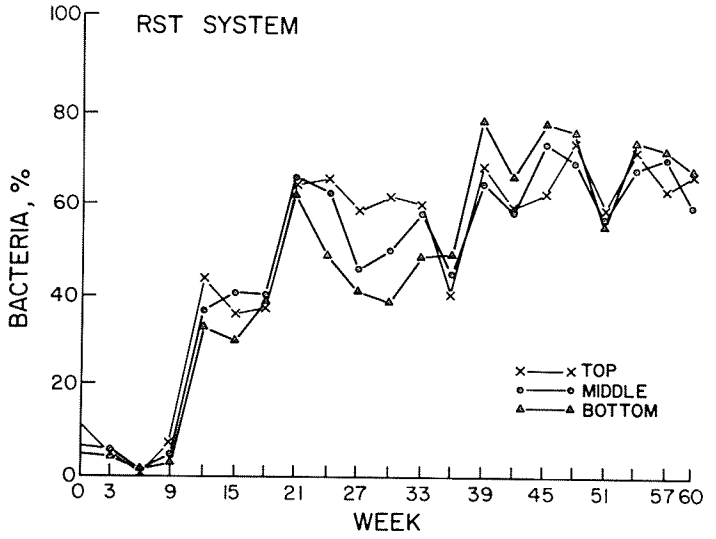

COT SYSTEM
ASPERGILLUS GLAUCUS GR , %
TOP
MIDDLE
BOTTOM
WEEK
0
20
40
60
80
100
3
9
15
21
27
33
39
45
51
57 60

C

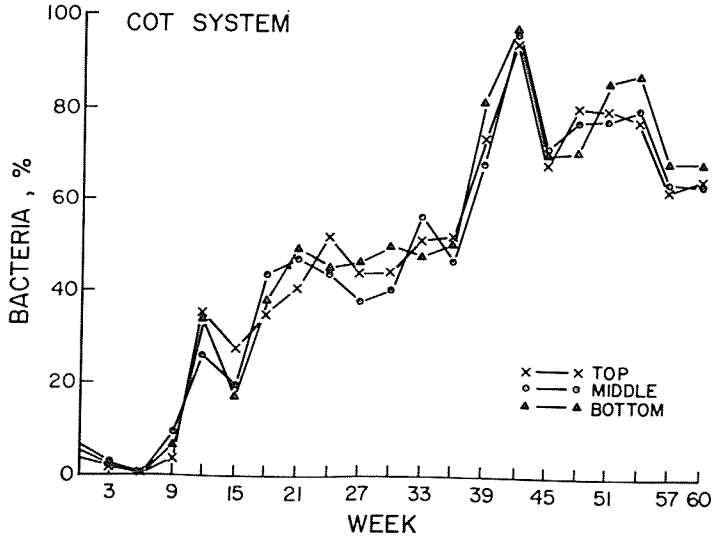
Figure 15. Mean percentage of kernels infected with bacteria at the top, middle, and bottom of the drums in the
(A) Control,
(B) Rhyzopertha-Sitophilus-Tribolium, and
(C) Cryptolestes-Oryzaephilus-Tribolium systems.


CONTROL SYSTEM
BACTERIA , %
WEEK
TOP
MIDDLE
BOTTOM

A


RST SYSTEM
BACTERIA, %
WEEK
TOP
MIDDLE
BOTTOM

B


COT SYSTEM
BACTERIA , %
WEEK
TOP
MIDDLE
BOTTOM

C

bacteria inhibit A. glaucus gr. growth (Lustig et al. 1977). Harein and Casos (1968) have shown that the granary weevil, Sitophilus granarius, is capable of transmitting and infesting grain with several kinds of bacteria.

The patterns of microfloral succession with the systems confirm those outlined in reviews of microflora interacting with stored grains (Wallace 1973, Christensen 1974). The role of the most common seed-borne fungal species as favorable or unfavorable diets of major stored-grain insects are now known from the laboratory studies of Sikorowski (1964), Lenz (1968) and Sinha (1964, 1965, 1966a, 1966b). The effect of mycotoxins produced by storage fungi on some stored-product insects are now known from the work of Harein and his group (Rao et al. 1971).

Acarological and Entomological Changes

The mites Lepidoglyphus destructor and Tarsonemus granarius were both scarce in the wheat initially placed in all drums. Several weeks after insects had been added to the RST and COT systems, mites were common only in the Control system. L. destructor did not multiply extensively, reaching population levels of 3 - 4 individuals per sample. T. granarius, however, multiplied successfully, reaching levels of up to 1500 per sample (Fig. 16). There was a rapid increase in T. granarius numbers at wk 9 followed by a rapid decline and a stabilization period at a low population level after wk 27.

To classify various larval instars of the insects studied, the larval head capsule measurements were taken (Table 1). The values obtained for C. ferrugineus and R. dominica were similar to those found by Campbell and Sinha (1978).

The efficiency of Berlese funnel extraction of Tribolium, Oryzaephilus, and Cryptolestes is presented in Table 2. The moisture content of the grain

Figure 16. Mean number of Tarsonemus granarius per 150 ml sample of wheat in the Control system.

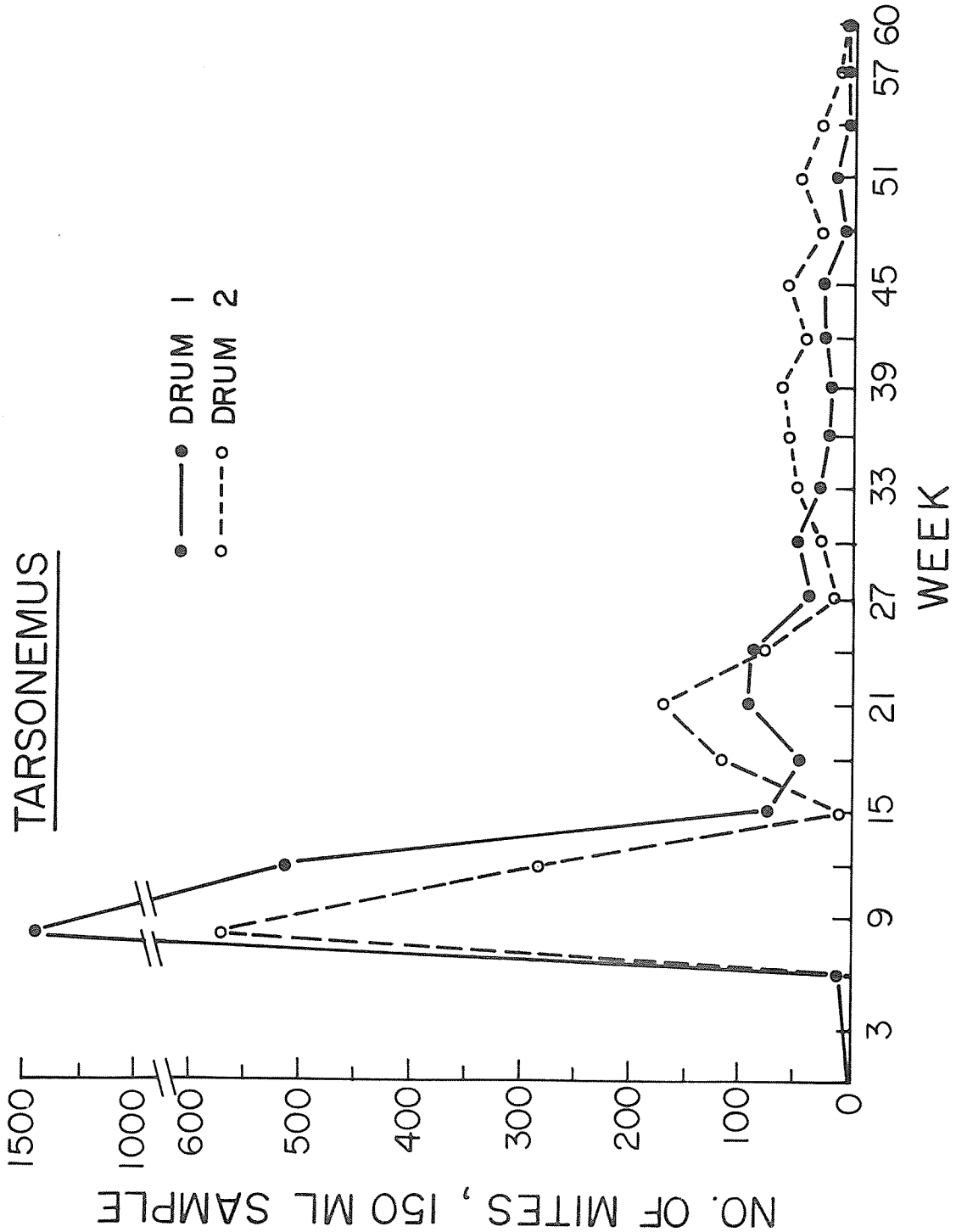

TARSONEMUS
DRUM 1
DRUM 2
NO. OF MITES, 150 ML SAMPLE
1500
1000
600
500
400
300
200
100
0
3
9
15
21
27
33
39
45
51
57 60
WEEK

Table 1. Head capsule widths (millimetres x 10^{-3}) of larvae of *Tribolium castaneum*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*, *Rhyzopertha dominica*, and *Sitophilus oryzae*.

Insect Species	Larval instar											
	L1			L2			L3			L4		
	N	Mean	SE	N	Mean	SE	N	Mean	SE	N	Mean	SE
T. castaneum	55	199.6	2.6	36	284.8	2.7	34	412.0	4.7	28	649.9	6.1
O. surinamensis	39	261.3	2.4	34	346.5	3.0	31	447.5	2.8	26	519.2	5.2
C. ferrugineus	51	161.8	1.3	23	205.1	2.7	24	252.2	3.7	25	277.6	3.1
R. dominica	22	146.0	2.3	21	210.5	4.0	16	282.9	11.0	17	448.5	6.7
S. oryzae[a]	135	200.0		135	280.0		135	380.0		135	530.0	

[a]Sharifi and Mills (1971); standard errors of the means were not determined.

Table 2. Recovery of various developmental stages of *Tribolium castaneum*, *Oryzaephilus surinamensis*, and *Cryptolestes ferrugineus*, from 150 millilitres of wheat at three moisture contents, by means of Berlese funnel extraction.[a]

Moisture Content (%)	Stage[b]	*T. castaneum*			*O. surinamensis*			*C. ferrugineus*		
		Mean no. recovered[c]	SE	% recovery	Mean no. recovered	SE	% recovery	Mean no. recovered	SE	% recovery
13.5										
	L1	18.0	0.41	72	20.3	0.48	81	9.0	0.41	36
	L2	23.0	0.41	92	18.8	1.10	75	11.8	0.63	47
	L3	23.8	0.48	95	20.3	0.48	81	12.8	1.32	51
	L4	24.8	0.25	94	21.5	1.04	86	17.8	0.48	71
	Adult	24.5	0.29	98	15.0	1.08	60	24.0	0.41	96
15.5										
	L1	20.3	0.85	81	15.3	0.85	61	8.5	0.65	34
	L2	24.0	0.41	96	20.0	0.71	80	11.8	0.41	47
	L3	23.8	0.25	95	19.8	0.63	79	14.3	0.85	57
	L4	24.5	0.29	98	20.8	0.41	83	18.3	0.63	73
	Adult	24.8	0.25	99	16.8	1.93	67	24.3	0.41	97
17.5										
	L1	19.8	1.75	79	17.5	0.96	70	9.5	0.65	38
	L2	19.8	0.25	79	21.8	0.85	87	11.0	0.91	44
	L3	23.5	0.65	94	20.0	1.08	80	16.0	1.08	64
	L4	23.0	0.82	92	24.8	0.25	99	17.3	0.85	69
	Adult	23.8	0.41	95	12.3	1.11	49	24.5	0.29	98

[a] Four replicates per developmental stage per species at each moisture content.

[b] Larval instars and adults.

[c] Total of 25 individuals per replicate.

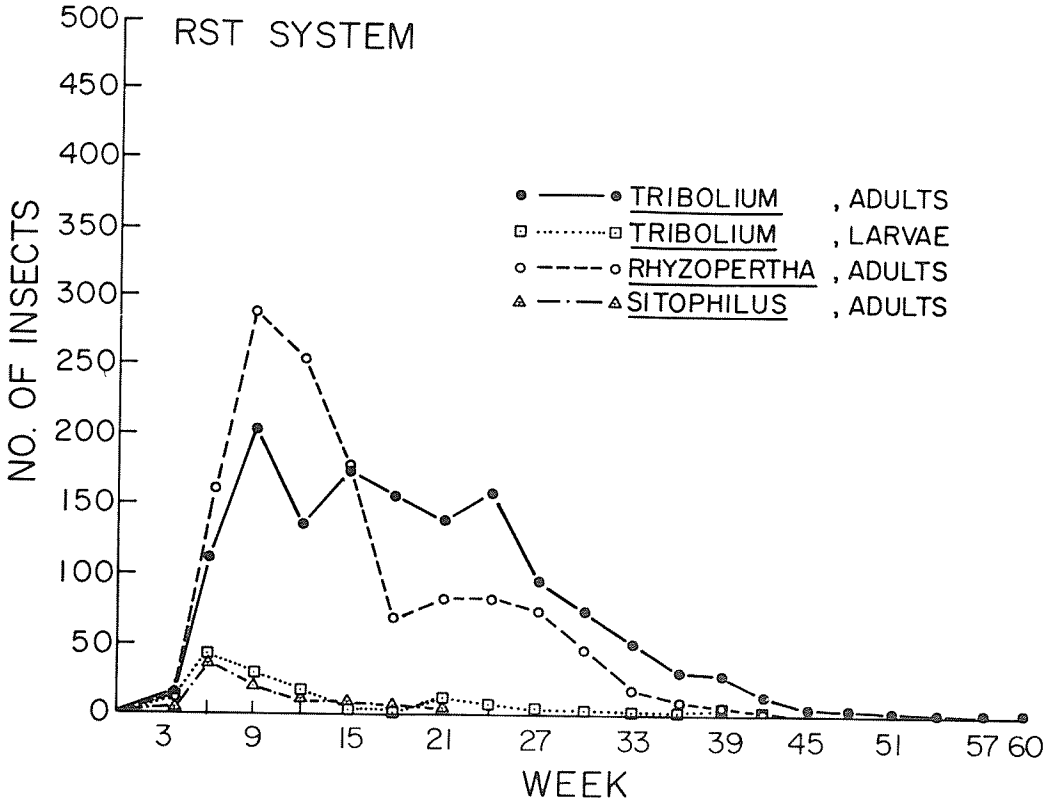
did not have an effect on the extraction of the insects. The recovery of all stages of Tribolium and the larval stages of Oryzaephilus was good although Oryzaephilus adults tended to escape from the top of the funnels, accounting for the low recovery rate of that stage. The recovery of Cryptolestes adults was high with a lower efficiency of larval extraction. Smith (1977) extracted Cryptolestes ferrugineus from 300 g of wheat at 16% moisture content and found that 27.9% of L1 and L2, 53.8% of L4 and 34.2% of adults were recovered. In 150 g of wheat, 88.3% of the adults were recovered.

In the RST system the populations of Rhyzopertha and Tribolium peaked at wk 6-9, began a steady decline, and then virtually disappeared by wk 45. The mean number of Sitophilus per sample rose to 45 adults at wk 6, but rapidly declined to the point where no Sitophilus were found in any samples after wk 21 (Fig. 17A). The mean number of Rhyzopertha and Sitophilus larvae found by kernel dissection is shown in Fig. 17B. Rhyzopertha larvae were most abundant at wk 12 and 27 and Sitophilus larvae were present in small numbers. Both Rhyzopertha and Tribolium congregated primarily at the surface of the drums (Figs. 18A, B).

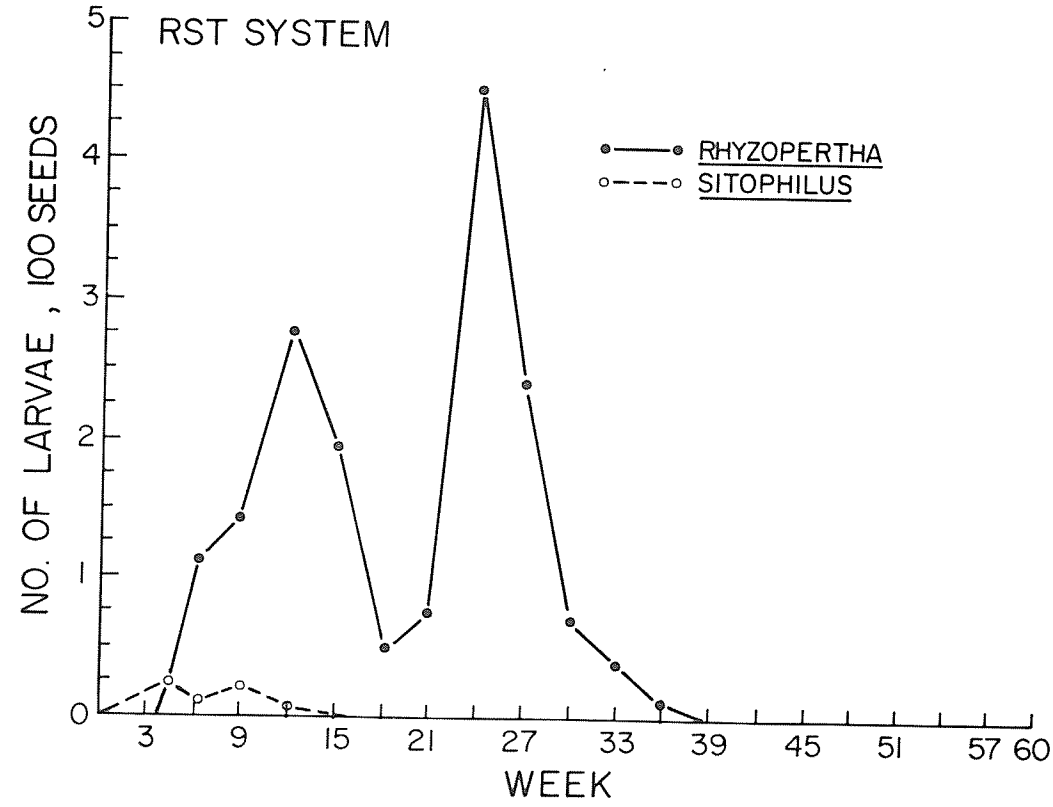
In the COT system, Cryptolestes and Tribolium populations increased in numbers to wk 9 - 15 followed by a decline and a successive increase at wk 27 - 30. Populations of adults remained stable until wk 42 - 45, followed by a rapid decline (Figs. 19A, B). The increase in the number of Cryptolestes and Tribolium adults lagged several weeks behind the increase in the number of larvae. In both the RST and COT systems, the number of Tribolium larvae was considerably smaller than the number of adults possibly indicating adult predation on the immature stages, or that the larvae, which were less mobile than the adults, were developing in localized areas in the drums.

Figure 17A. Mean number of Tribolium adults and larvae, Rhyzopertha adults, and Sitophilus adults per sample in the Rhyzopertha-Sitophilus-Tribolium system.

17B. Mean number of Rhyzopertha and Sitophilus larvae in 100 seeds per sample in the Rhyzopertha-Sitophilus-Tribolium system.


RST SYSTEM
NO. OF INSECTS
500
450
400
350
300
250
200
150
100
50
0
3
9
15
21
27
33
39
45
51
57 60
WEEK
TRIBOLIUM , ADULTS
TRIBOLIUM , LARVAE
RHYZOPERTHA , ADULTS
SITOPHILUS , ADULTS

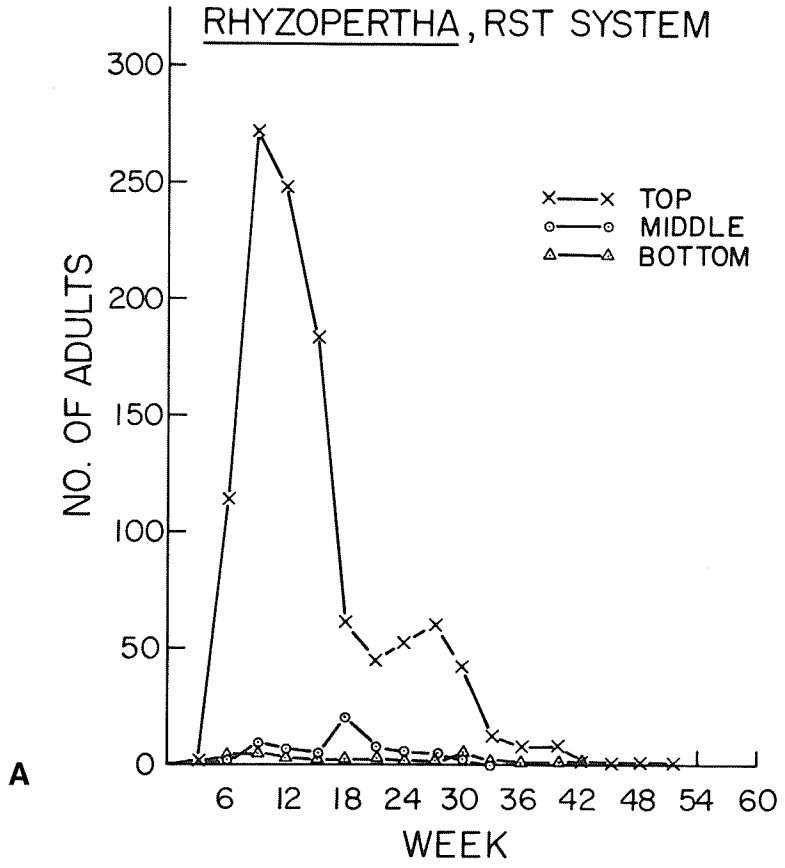
A


RST SYSTEM
NO. OF LARVAE , 100 SEEDS
5
4
3
2
1
0
3
9
15
21
27
33
39
45
51
57 60
WEEK
RHYZOPERTHA
SITOPHILUS

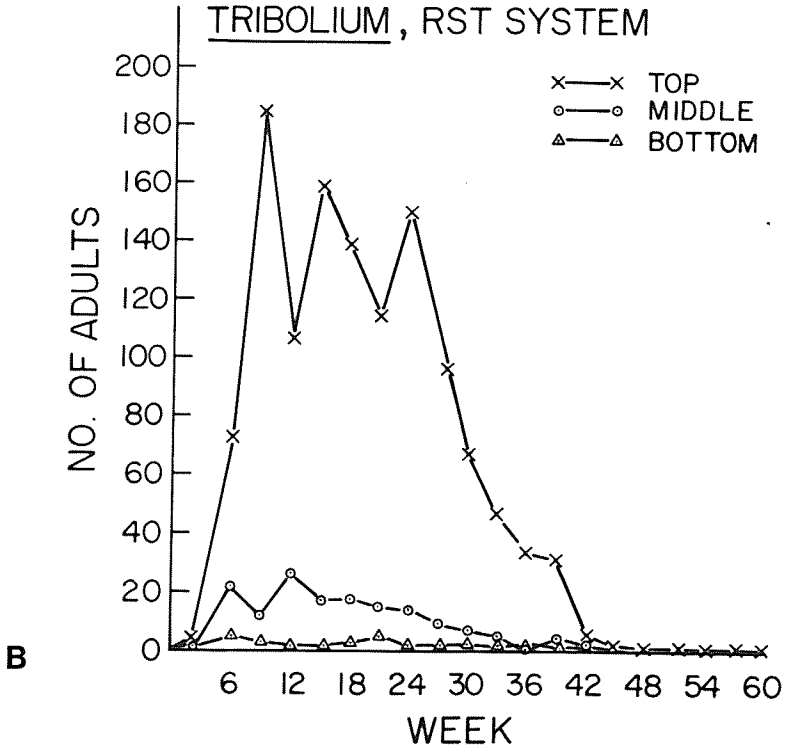
B

Figure 18A. Mean number of Rhyzopertha adults per sample at the top, middle, and bottom of the drums in the Rhyzopertha-Sitophilus-Tribolium system.

18B. Mean number of Tribolium adults per sample at the top, middle, and bottom of the drums in the Rhyzopertha-Sitophilus-Tribolium system.


RHYZOPERTHA, RST SYSTEM
NO. OF ADULTS
300
250
200
150
100
50
0
TOP
MIDDLE
BOTTOM
6 12 18 24 30 36 42 48 54 60
WEEK

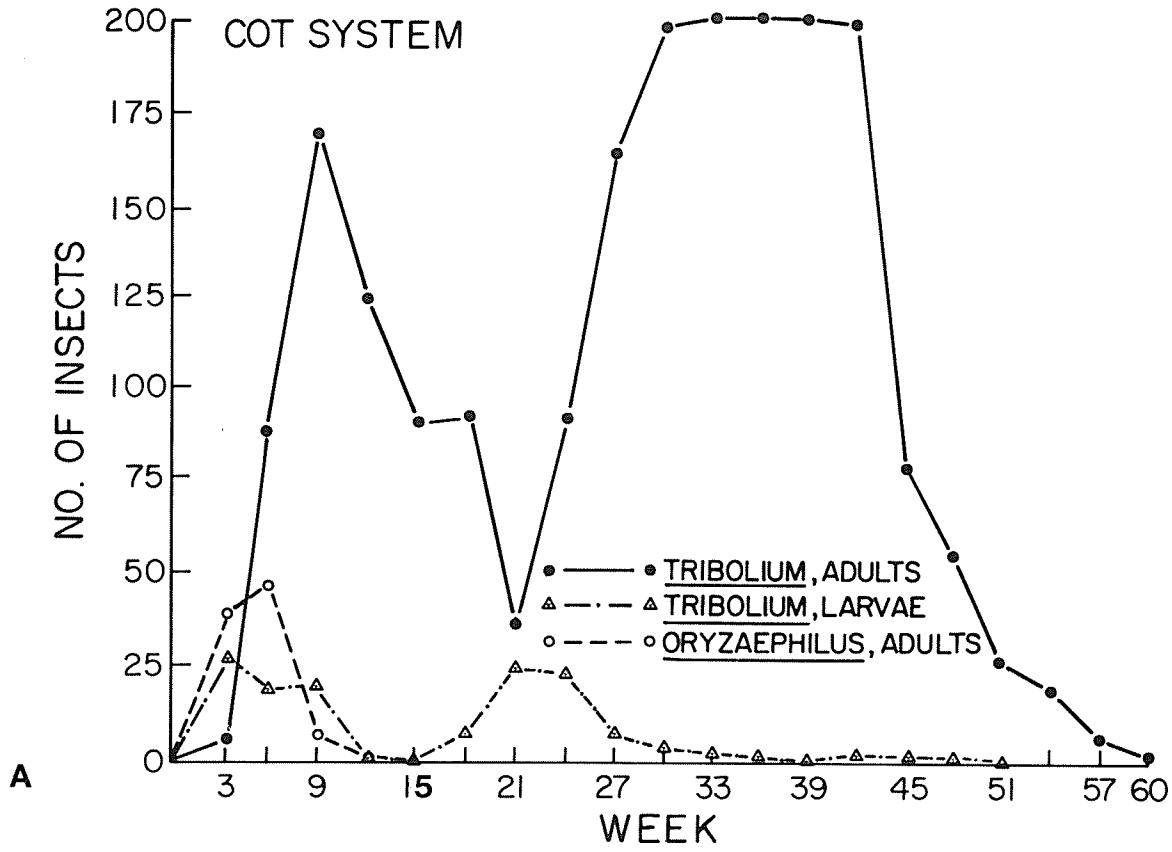
A


TRIBOLIUM, RST SYSTEM
NO. OF ADULTS
200
180
160
140
120
100
80
60
40
20
0
TOP
MIDDLE
BOTTOM
6 12 18 24 30 36 42 48 54 60
WEEK

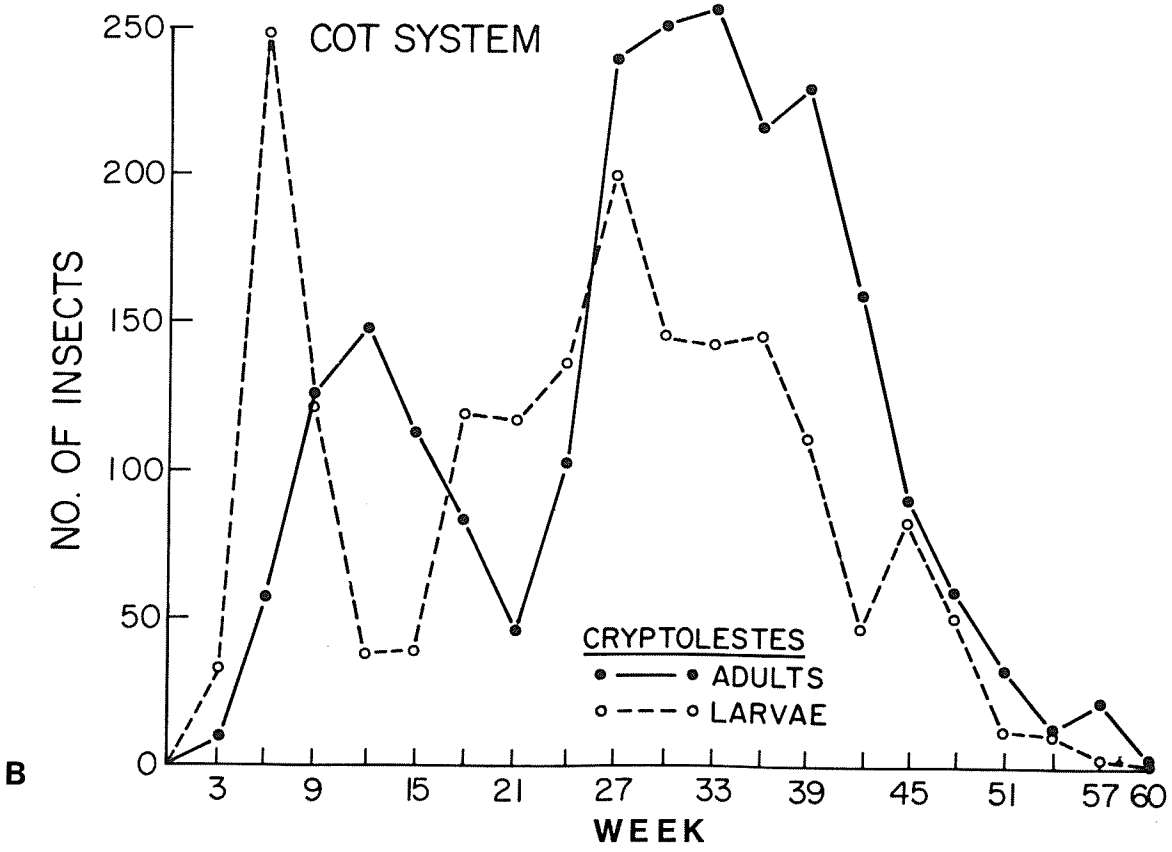
B

Figure 19A. Mean number of Tribolium adults and larvae, and Oryzaephilus adults per sample in the Cryptolestes-Oryzaephilus-Tribolium system.

19B. Mean number of Cryptolestes adults and larvae per sample in the Cryptolestes-Oryzaephilus-Tribolium system.


COT SYSTEM
NO. OF INSECTS
200
175
150
125
100
75
50
25
0
3
9
15
21
27
33
39
45
51
57
60
WEEK
TRIBOLIUM, ADULTS
TRIBOLIUM, LARVAE
ORYZAEPHILUS, ADULTS

A


COT SYSTEM
NO. OF INSECTS
250
200
150
100
50
0
3
9
15
21
27
33
39
45
51
57
60
WEEK
CRYPTOLESTES
ADULTS
LARVAE

B

In the COT system, Oryzaephilus populations did not thrive for very long, reaching their maximum numbers at wk 6 and virtually disappearing by wk 15 (Fig. 19A). In the COT system, adults and larvae of all of the insects were more numerous at the surface of the drums for the first 9-12 wk. However, for the remaining period of the experiment, adults of Cryptolestes and Tribolium were most numerous at the bottoms of the drums (Fig. 20A, B, C). After wk 12, Cryptolestes larvae were evenly distributed throughout the drums (Fig. 20B).

Larvae were not classified according to their stage of development in the summarization of the data in this report since the majority of immature forms counted were first and second instar larvae. The mortality of individuals of these stages in all species was high throughout the study.

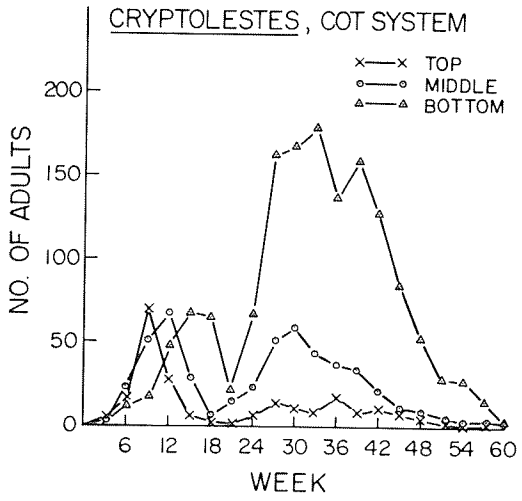
Numerous studies have been carried out on various aspects of the life history of stored-product insects. Tribolium castaneum is one of the most extensively studied species; King and Dawson (1971) and Mertz (1972) have reviewed various aspects of its population dynamics. Fujii (1974) identified by mathematical modelling several factors governing population fluctuations of T. castaneum reared in the laboratory. Estimates of optimal and minimal conditions for population increase and developmental periods of many stored-product insects have been compiled and reviewed by Howe (1965, 1966).

Tribolium castaneum populations typically undergo large cyclic fluctuations because of intense larval and adult cannibalism of eggs (Fujii 1974, Boyer 1976). Fluctuations in adult population size correspond to the average adult longevity of 200 - 300 days at 29°C, 70% RH (Park 1948). When an infestation in a limited environment is started with a small number of adults, new adults of similar age emerge after 3 - 5 wk and exert pressure

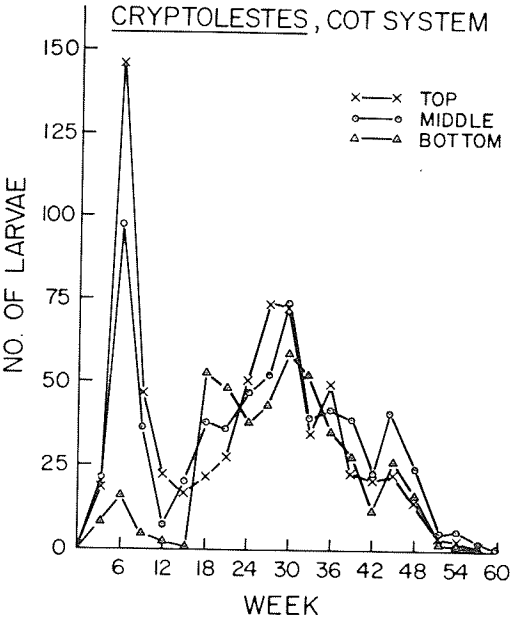
Figure 20A. Mean number of Cryptolestes adults per sample at the top, middle, and bottom of the drums in the Cryptolestes-Oryzaephilus-Tribolium system.

20B. Mean number of Cryptolestes larvae per sample at the top, middle, and bottom of the drums in the Cryptolestes-Oryzaephilus-Tribolium system.

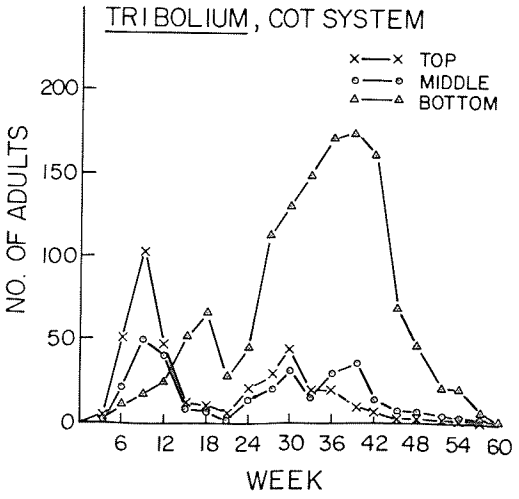
20C. Mean number of Tribolium adults per sample at the top, middle, and bottom of the drums in the Cryptolestes-Oryzaephilus-Tribolium system.


CRYPTOLESTES, COT SYSTEM
TOP
MIDDLE
BOTTOM
NO. OF ADULTS
0
50
100
150
200
6 12 18 24 30 36 42 48 54 60
WEEK

A


CRYPTOLESTES, COT SYSTEM
TOP
MIDDLE
BOTTOM
NO. OF LARVAE
0
25
50
75
100
125
150
6 12 18 24 30 36 42 48 54 60
WEEK

B


TRIBOLIUM, COT SYSTEM
TOP
MIDDLE
BOTTOM
NO. OF ADULTS
0
50
100
150
200
6 12 18 24 30 36 42 48 54 60
WEEK

C

through cannibalism to prevent a new generation from developing. As the second generation adults begin to die, cannibalistic pressure is reduced and a further generation develops, usually to the point of crowding in the limited universe because of the high reproductive potential of this species (Fujii 1978). Crowding affects the adults by reducing fecundity because of the secretion of toxic substances and interference with copulation, feeding, and oviposition (Park 1948). Although the actual rate of population fluctuation depends on specific environmental conditions, the effects of cannibalism are visible. The developmental period of all five insect species used in this study is 4 - 5 wk at the near-optimal temperature of 30°C, yet populations remain constant for long periods.

Interspecific competition further complicates the population dynamics of the insects. LeCato (1975) has shown that Tribolium castaneum populations increase and exhibit more destructive feeding behaviour when in the presence of R. dominica and S. oryzae. The latter two species lay most of their eggs in the first few days after reaching maturity (Golebiowska 1969), whereas Tribolium oviposits consistently throughout the adult life, gradually laying fewer eggs with increasing age (Simwat and Chahal 1975). Ciesielska (1975) has reported that Rhyzopertha has an inhibitory effect on Sitophilus granarius populations at 28°C; a similar phenomenon may partially account for the decline of S. oryzae numbers in the RST system. Interactions of Rhyzopertha dominica, Sitophilus oryzae, and Tribolium castaneum have shown that Tribolium increased in the presence of both of the other insects but Rhyzopertha feeding activity led to extensive larval mortality in the Sitophilus populations (Kabir 1966). Kamal et al. (1976) have reported that Rhyzopertha adults are occasionally cannibalistic on other Rhyzopertha adults and Jacob and Mohan (1973) reported that T. castaneum larvae and

adults are predators of all immature stages of R. dominica.

In bagged wheat in Egypt, Aboul-Nasr et al. (1973) found that R. dominica and T. castaneum were most common at the top of the grain while S. oryzae and O. surinamensis were most common at the bottom. The distribution of Rhyzopertha, Sitophilus, and Tribolium was similar in the RST system of this study.

The depression of the Oryzaephilus populations in the COT system was probably because of predation by Tribolium (Crombie 1946, LeCato 1975b). Cryptolestes is known to inhibit Tribolium populations on wheat at 30°C (Lefkovitch 1968). Such inhibition was not apparent in the COT system. Cryptolestes prefers to aggregate in moist and moldy grain (Loschiavo and Sinha 1966, Dolinski and Loschiavo 1973) which may explain why many adults of this species aggregated at the bottom of the drums in the COT system. Smith (1966) has reported that crowding of Cryptolestes in wheat has deleterious effects on oviposition and development and leads to increased mortality.

Measurement of Carbon Dioxide Production and Oxygen Consumption

To determine the quantity of CO_2 produced and O_2 consumed by individual adults of each of the insect species used in the study, a secondary experiment using bottles filled with wheat was initiated. Trisvyatskii (1966) has stated that a bulk of wheat is composed of 35 - 45% intergranular air. Assuming the intergranular air occupied 40% of the 150 ml of wheat values for CO_2 production and O_2 utilization for one adult beetle were calculated on collective data from 100 insects (Table 3). Tribolium, Rhyzopertha, and Sitophilus individually consumed more oxygen and produced more carbon dioxide than Oryzaephilus or Cryptolestes which may largely be caused by differences in body size of the various insects. The mean

Table 3. Production of carbon dioxide, and oxygen utilization by Tribolium castaneum, Rhyzopertha dominica, Sitophilus oryzae, Oryzaephilus surinamensis, and Cryptolestes ferrugineus in 24 hours[a].

Treatment	CO_2, %		O_2, %		ml/Adult beetle	
	Mean	SE	Mean	SE	CO_2	O_2
Insect-free control	0.11	0.03	19.51	0.12		
T. castaneum	3.18	0.17	15.40	0.35	0.028	0.037
R. dominica	3.27	0.18	15.66	0.36	0.029	0.035
S. oryzae	2.83	0.29	15.54	0.40	0.025	0.036
O. surinamensis	1.41	0.09	17.53	0.11	0.012	0.018
C. ferrugineus	1.15	0.03	17.97	0.18	0.009	0.014

[a]Four replicates per treatment.

Assumption: 40% of wheat mass is intergranular air (Trisvyatskii, 1966); a 200 ml bottle filled with 150 ml of wheat contains 110 ml air and 90 ml wheat.

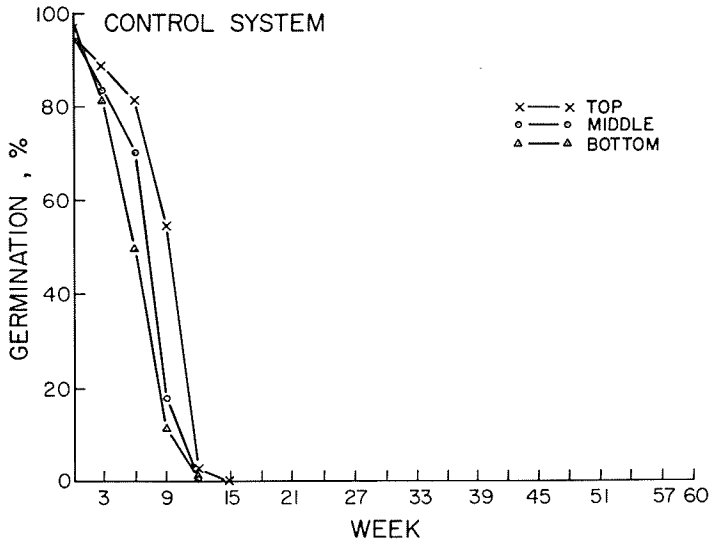
respiratory quotients for Tribolium, Rhyzopertha, Sitophilus, Oryzaephilus, and Cryptolestes were 0.76, 0.83, 0.69, 0.67, and 0.64 respectively. The respiratory quotients for T. castaneum, R. dominica, O. surinamensis, and C. ferrugineus obtained in this study were somewhat lower than those obtained by Bailey (1965). Previously obtained respiratory quotients for adult S. oryzae have been 1.10 (Birch 1947), 0.69 (Oxley and Wickendon 1963) and 0.79 (Singh et al. 1976).

Deteriorative Changes

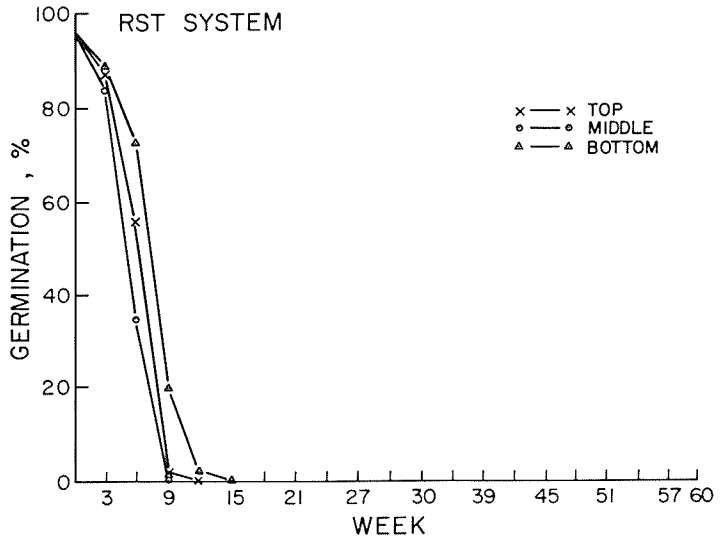
Germination loss in all three systems followed similar trends, with a sharp decrease after the first few weeks. By wk 15, no further seed germination was observed in samples from any system (Figs. 21A, B, C).

The mean weight of 100 kernels of wheat did not change dramatically, at any level, in the Control system during 60 wk (Fig. 22A). Similar results were observed in the COT system, although the mean values for kernel weight were slightly lower (<0.1 g) (Fig. 22C). A sharp decline in grain weight was noted at the tops of the drums in the RST system, with a maximum weight loss of 0.6 g. Grain at the middle of the drums decreased in weight also, although the weight loss was not as severe as at the tops of the drums. Grain at the bottom of the drums decreased in weight gradually and only slightly during 60 wk (Fig. 22B). The increase in mean grain weight at the bottom and middle of the drums from wk 27 - 60 was probably caused by the removal of damaged grain by previous sampling and the rapid decline in Rhyzopertha numbers after wk 27, which would have minimized further grain damage. Campbell and Sinha (1976) have studied the degree of damage done to wheat by Rhyzopertha dominica, Sitophilus granarius, and Cryptolestes ferrugineus. They reported that the weight loss of kernels and the frass production related to the feeding of R. dominica adults was

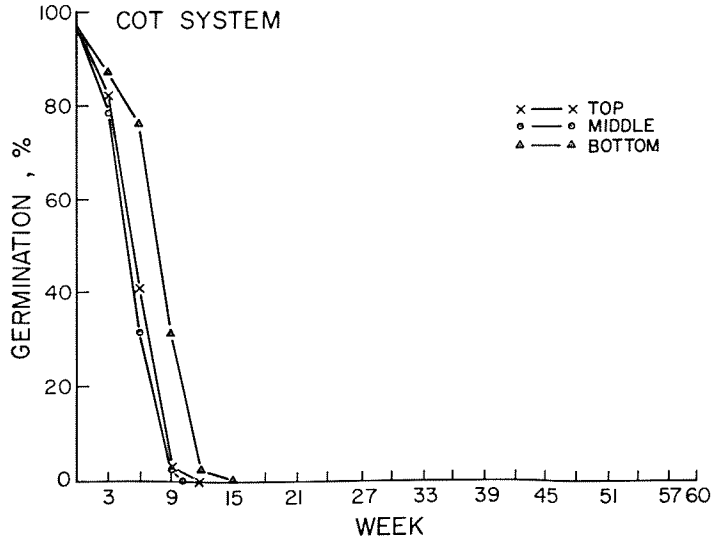
Figure 21. Mean percent germination of seeds at the top, middle, and bottom of the drums in the
(A) Control,
(B) Rhyzopertha-Sitophilus-Tribolium, and
(C) Cryptolestes-Oryzaephilus-Tribolium systems.


CONTROL SYSTEM
GERMINATION , %
WEEK
TOP
MIDDLE
BOTTOM
0
20
40
60
80
100
3
9
15
21
27
33
39
45
51
57 60

A

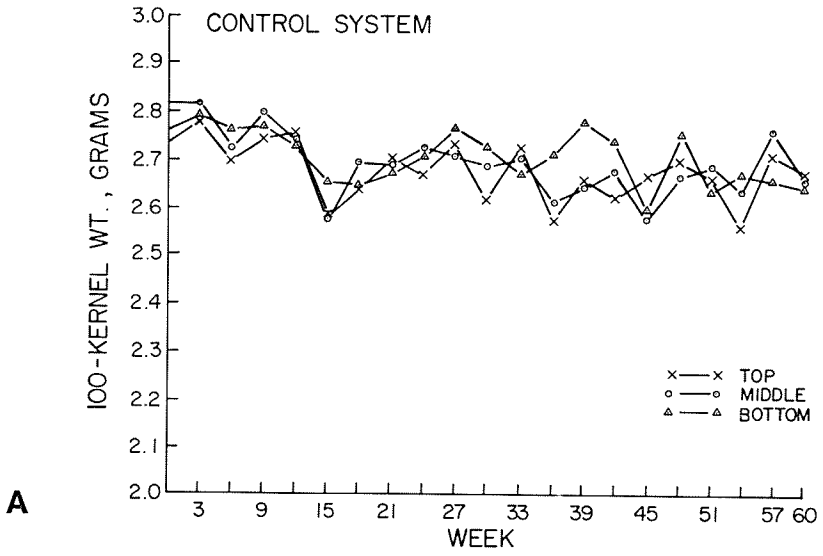

RST SYSTEM
GERMINATION , %
WEEK
TOP
MIDDLE
BOTTOM
0
20
40
60
80
100
3
9
15
21
27
30
39
45
51
57 60

B

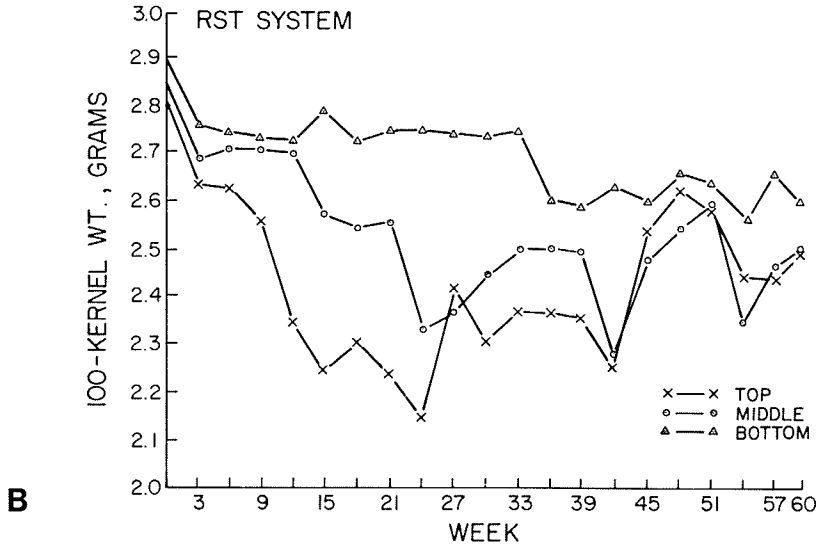

COT SYSTEM
GERMINATION , %
WEEK
TOP
MIDDLE
BOTTOM
0
20
40
60
80
100
3
9
15
21
27
33
39
45
51
57 60

C

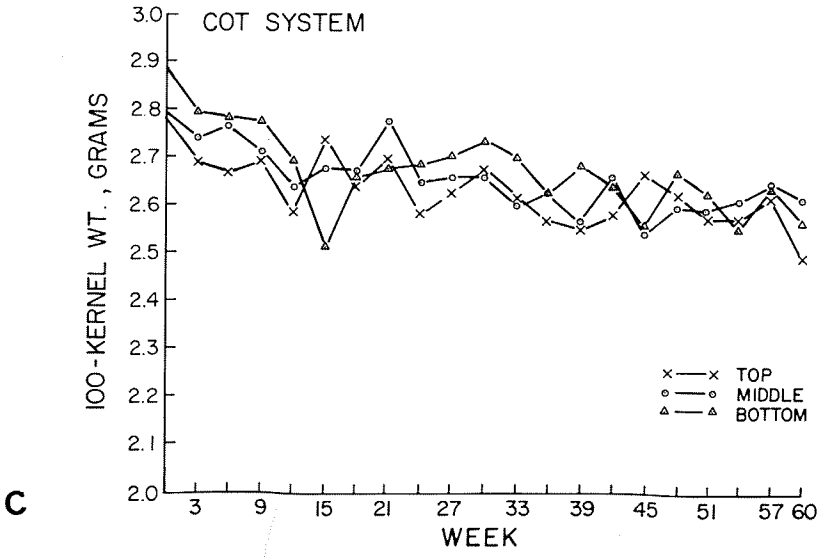
Figure 22. Mean weight of 100 kernels of wheat at the top, middle, and bottom of the drums in the
(A) Control,
(B) Rhyzopertha-Sitophilus-Tribolium, and
(C) Cryptolestes-Oryzaephilus-Tribolium systems.


CONTROL SYSTEM
100-KERNEL WT., GRAMS
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
3
9
15
21
27
33
39
45
51
57 60
WEEK
TOP
MIDDLE
BOTTOM

A


RST SYSTEM
100-KERNEL WT., GRAMS
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
3
9
15
21
27
33
39
45
51
57 60
WEEK
TOP
MIDDLE
BOTTOM

B


COT SYSTEM
100-KERNEL WT., GRAMS
3.0
2.9
2.8
2.7
2.6
2.5
2.4
2.3
2.2
2.1
2.0
3
9
15
21
27
33
39
45
51
57 60
WEEK
TOP
MIDDLE
BOTTOM

C

the greatest of the three species, followed by S. granarius and C. ferrugineus. Howe (1965b) has reviewed the literature on the loss of stored food caused by these insects.

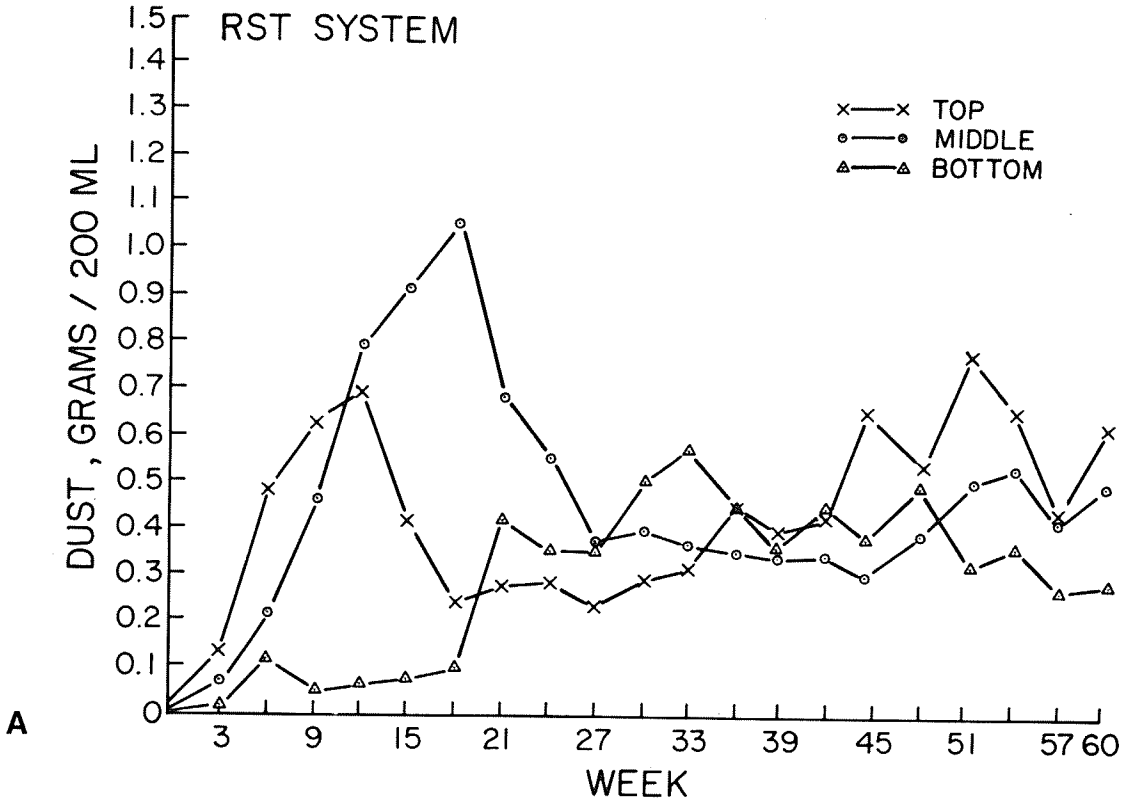
Grain dust in the samples was greatest in the RST system, mainly because of Rhyzopertha feeding. Dust was most concentrated at the tops of the drums during the first 4 wk of the study. From wk 12 - 27, the amount of dust was greatest in the middle samples, and from wk 30 - 36 at the bottom of the drums. After wk 36, dust was again most abundant at the tops of the drums (Fig. 23A). The initial accumulation of dust was probably caused by Rhyzopertha and Tribolium feeding, since the insects were most numerous at the tops of the drums throughout the study. As time progressed, the dust settled toward the bottom of the drums as insect activity mixed the grain. The increase in dust at the tops of the drums late in the experiment may have been caused by the feeding of the small residual populations of Tribolium and microfloral activity.

Dust in the COT system was greatest in the middle of the drums until wk 12 after which the greatest concentrations were at the bottoms of the drums (Fig. 23B). The accumulation of dust at the lower levels of the drums coincided with the occurrence of most adult insects there. A slight decline in the amount of dust present was evident in both the RST and COT systems late in the study and was probably caused by the entrapment of dust in the considerable amounts of fungal mycelia that were present between the wheat seeds.

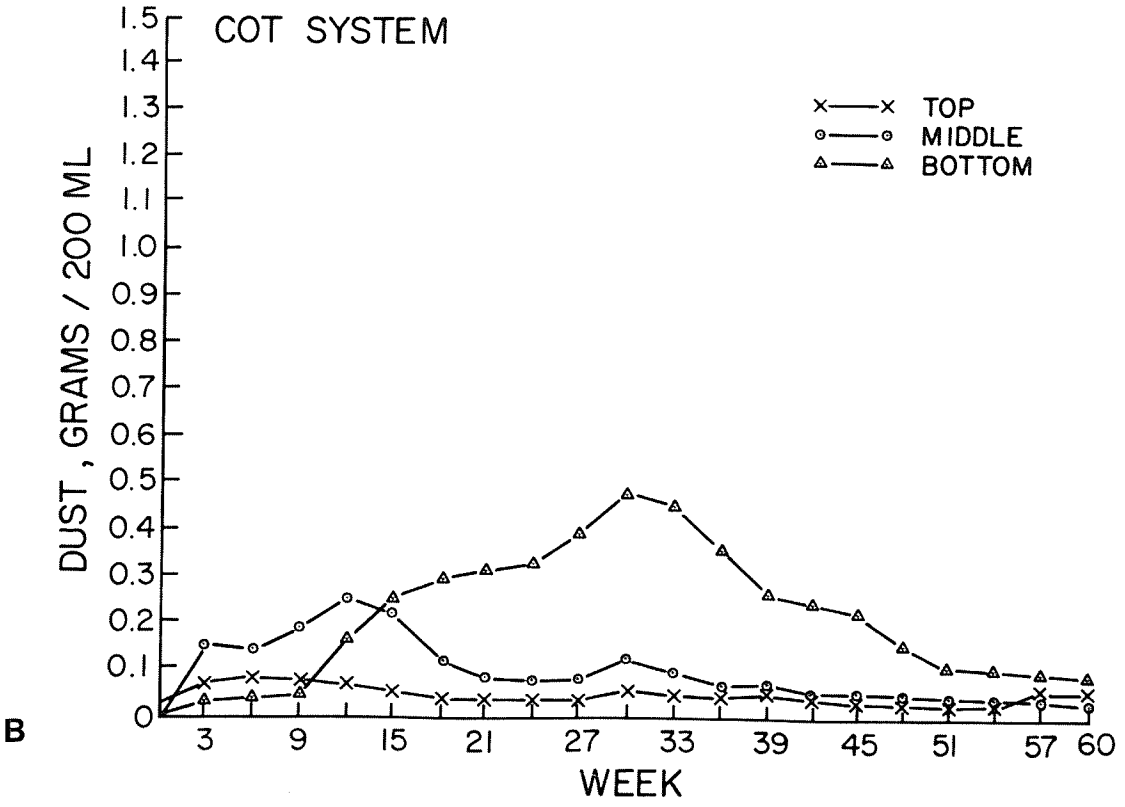
The amount of dust in the Control system was very small even after 60 wk, with a mean weight of < 0.05 g at all levels in the drums.

The volume of 100 kernels and of dust collected from the samples followed patterns similar to those observed in grain and dust weight.

Figure 23. Mean dust weight per 200 ml sample of wheat at the top, middle, and bottom of the drums in the
(A) Rhyzopertha-Sitophilus-Tribolium, and
(B) Cryptolestes-Oryzaephilus-Tribolium systems.


RST SYSTEM
TOP
MIDDLE
BOTTOM
DUST, GRAMS / 200 ML
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3
9
15
21
27
33
39
45
51
57 60
WEEK

A


COT SYSTEM
TOP
MIDDLE
BOTTOM
DUST, GRAMS / 200 ML
1.5
1.4
1.3
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0
3
9
15
21
27
33
39
45
51
57 60
WEEK

B

Grain damage resulting from insect feeding in the RST and COT systems is presented in Fig. 24A, B, C. In the RST system, most germ and endosperm damage reflected the distribution of the insect populations, with maximum damage at the tops of the drums (where over 40% of the germ was damaged) followed by grain at the middle levels. Rhyzopertha fed on whole kernels and Tribolium fed on the germ and broken kernels. Most kernels that had been attacked were severely damaged in both the germ and endosperm regions.

In the COT system only germ damage was evident. The mean level of germ damage was consistent throughout the study at about 20 - 25% and was similar at all levels in the drums (Fig. 24C). The majority of kernels attacked had the entire germ removed.

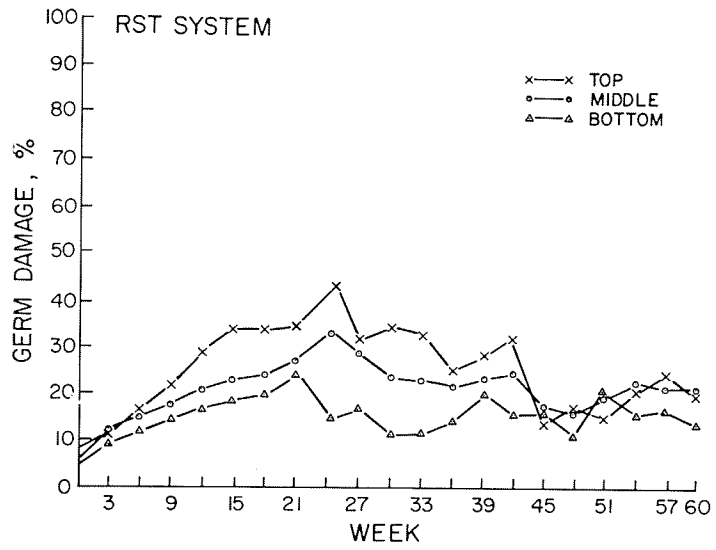
Golebiowska et al. studied the effect of infestation by several species of beetles on grain damage. They found that insects including R. dominica, S. oryzae, and O. surinamensis initially attacked the germ of wheat and thereafter preferred feeding on the dorsal surface of the kernels

The amounts of free fatty acids present in the grain, which result largely from the lypolytic activity of chemicals secreted by fungi, are a recognized measure of deterioration of grain quality (Zeleny 1954). Fat acidity values (FAV), expressed as mg KOH required to neutralize the free fatty acids extracted from 100 g of dry grain, were determined for each drum and the mean values for each system are presented in Fig. 25A. From wk 12-33 the FAV of the RST system was higher than those of the other two systems although it declined sharply after wk 36 in the study. Hayward (1955), Girish et al. (1975) and Lustig et al. (1977) have shown that the presence of stored grain beetles could lead to higher FAV levels than those present in uninfested grain. Pooled samples from each drum did not clearly demonstrate this effect, so grain was taken from the tops, only, of each

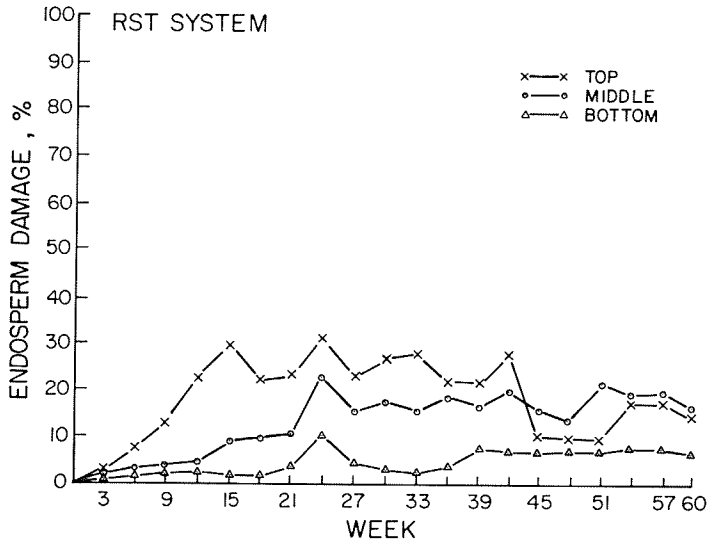
Figure 24A. Mean percent germ damage at the top, middle, and bottom of the drums in the Rhyzopertha-Sitophilus-Tribolium system.

24B. Mean percent endosperm damage at the top, middle, and bottom of the drums in the Rhyzopertha-Sitophilus-Tribolium system.

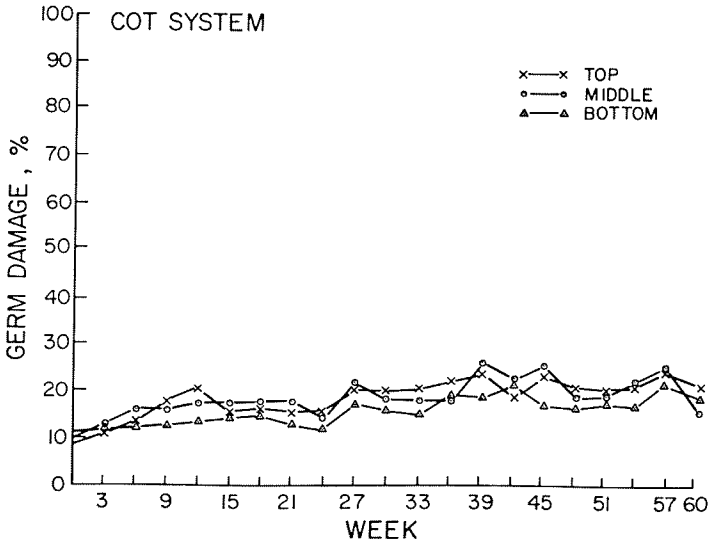
24C. Mean percent germ damage at the top, middle, and bottom of the drums in the Cryptolestes-Oryzaephilus-Tribolium system.


RST SYSTEM
TOP
MIDDLE
BOTTOM
GERM DAMAGE , %
WEEK
0
10
20
30
40
50
60
70
80
90
100
3
9
15
21
27
33
39
45
51
57 60

A


RST SYSTEM
TOP
MIDDLE
BOTTOM
ENDOSPERM DAMAGE , %
WEEK
0
10
20
30
40
50
60
70
80
90
100
3
9
15
21
27
33
39
45
51
57 60

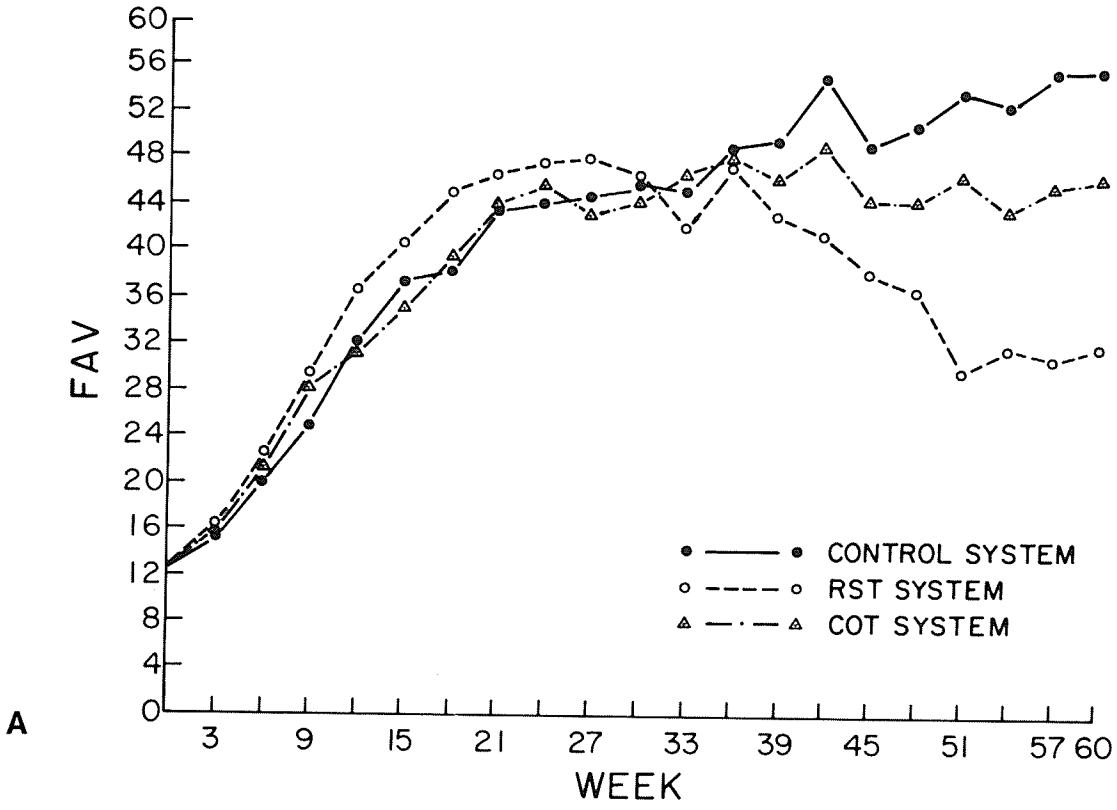
B


COT SYSTEM
TOP
MIDDLE
BOTTOM
GERM DAMAGE , %
WEEK
0
10
20
30
40
50
60
70
80
90
100
3
9
15
21
27
33
39
45
51
57 60

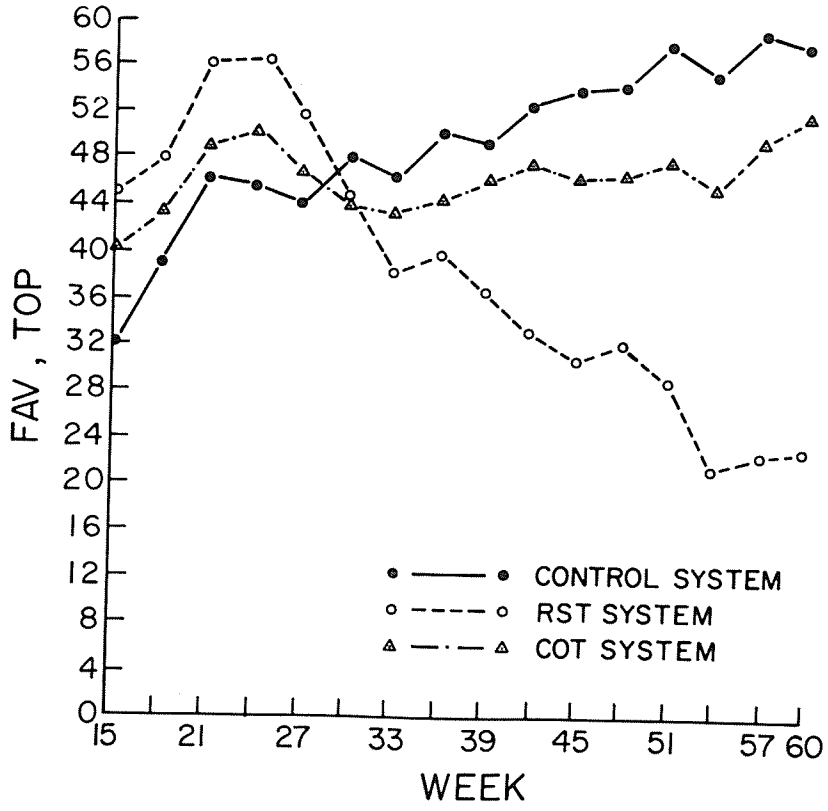
C

Figure 25A. Mean fat acidity values for pooled wheat samples from the Control, Rhyzopertha-Sitophilus-Tribolium, and Cryptolestes-Oryzaephilus-Tribolium systems.

25B. Mean fat acidity values for wheat samples from the tops of the drums in the Control, Rhyzopertha-Sitophilus-Tribolium, and Cryptolestes-Oryzaephilus-Tribolium systems.


FAV
WEEK
CONTROL SYSTEM
RST SYSTEM
COT SYSTEM

A


FAV , TOP
WEEK
CONTROL SYSTEM
RST SYSTEM
COT SYSTEM

B

drum and analyzed. The results (Fig. 25B) revealed a clear differentiation among all three systems, RST having the highest, COT the intermediate, and the Control the lowest FAV levels until wk 27. At that point the Control system values continued to rise steadily, the COT values more or less levelled off with a slight upward trend. The RST values dropped steadily for the remainder of the study (Fig. 25B). Most of the fat in a kernel of wheat is contained in the germ (Pomeranz and Bechtel 1978) and possibly a combination of extensive insect feeding and the resulting intense microbial decomposition of the kernel could account for the decline in FAV levels.

Generally, the type of deteriorative changes were similar to those observed in stored wheat infested with insects under field conditions (Howe 1943, Sinha and Wallace 1966, Coombs and Woodroffe 1973).

Uric Acid Production by Insects

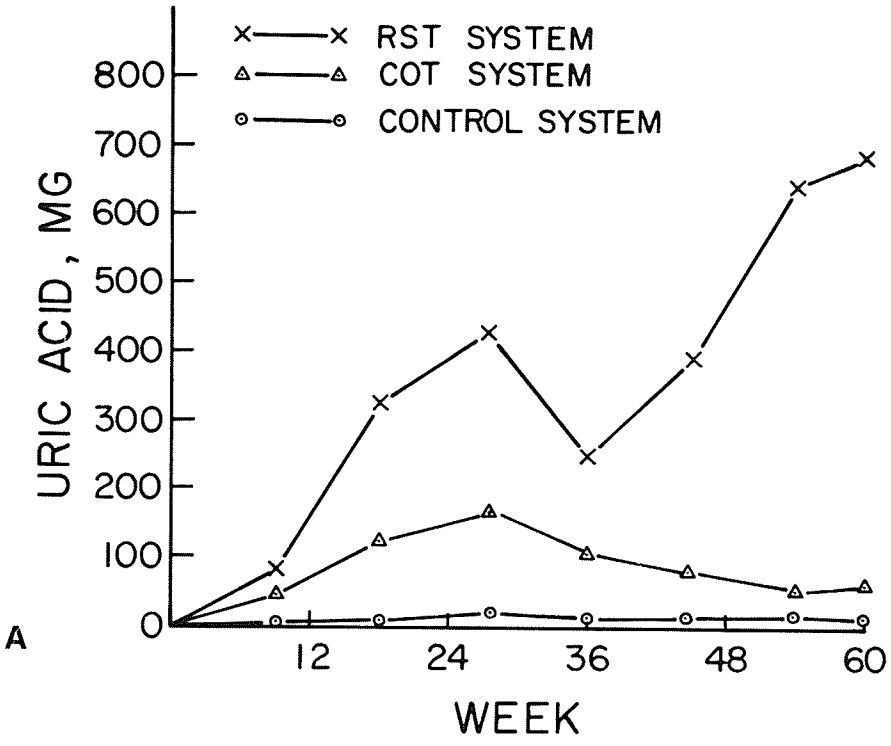
The uric acid in the dust of the RST and COT systems was produced as an excretory product by the insects. Cumulative values, expressed as mg uric acid/mg dust, for the RST and COT systems, were similar after 60 wk (Fig. 26A). However, the total amount of uric acid in nine samples was more than 7-fold greater in the RST system than in the COT system (Fig. 26B). This agrees with the findings of Bronswijk and Sinha (1971).

Determination of the Distribution of Insects (COT system)

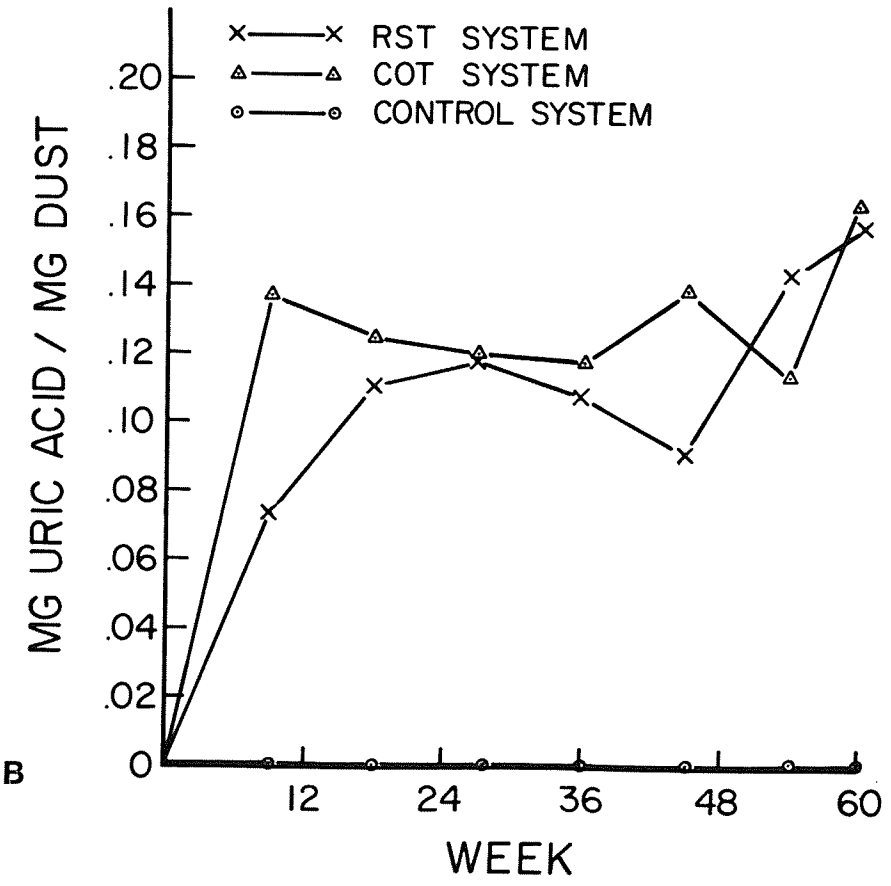
At wk 39 of the drum study most of the adult Cryptolestes and Tribolium were found at the bottoms of the drums in the COT system. To determine if the distribution of these beetles was in part because of an age partition, individuals from tops and bottoms of drums were compared to those taken from cultures. The number of larvae produced in 1 wk and the mortality of the adults during 7 wk were noted (Table 4).

Figure 26A. Mean weight of uric acid in samples of 1800 ml of wheat in the Control, Rhyzopertha-Sitophilus-Tribolium, and Cryptolestes-Oryzaephilus-Tribolium systems.

26B. Mean concentration of uric acid in grain dust in the Control, Rhyzopertha-Sitophilus-Tribolium, and Cryptolestes-Oryzaephilus-Tribolium systems.


RST SYSTEM
COT SYSTEM
CONTROL SYSTEM
URIC ACID, MG
800
700
600
500
400
300
200
100
0
12
24
36
48
60
WEEK

A


RST SYSTEM
COT SYSTEM
CONTROL SYSTEM
MG URIC ACID / MG DUST
.20
.18
.16
.14
.12
.10
.08
.06
.04
.02
0
12
24
36
48
60
WEEK

B

Table 4. Number of living adults after 1, 4, and 7 weeks and the number of larvae produced in 1 week by Tribolium castaneum and Cryptolestes ferrugineus adults taken from the tops and bottoms of drums in the Cryptolestes-Oryzaephilus-Tribolium system at week 39, and from cultures maintained at 30 ± 1°C, 70% RH.

		Tribolium castaneum				Cryptolestes ferrugineus			
		Adults		Larvae		Adults		Larvae	
Week	Location	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1									
	Top	90.7	6.3	1301.7	20.5	52.3	4.1	348.3	51.2
	Bottom	96.7	1.5	1460.0	103.7	45.7	7.0	296.3	25.4
	Culture	97.3	1.5	1438.0	46.2	75.3	3.4	374.7	17.7
4									
	Top	87.7	5.5			30.0	3.9		
	Bottom	94.3	0.9			33.0	3.1		
	Culture	90.3	2.0			62.0	3.9		
7									
	Top	64.3	7.0			18.3	1.5		
	Bottom	55.7	9.8			12.0	2.6		
	Culture	81.7	3.5			21.0	5.2		

Note: Three replicates per location for each species; initially 100 adult insects per replicate.

Two-way analysis of variance of the data collected for adult mortality during seven weeks indicated that there was not a significant difference ($P < 0.05$) between the numbers of adult Tribolium at the tops and bottoms of the drums. However, the number of Tribolium surviving from the bottoms of the drums was significantly different ($P < 0.05$) than those taken from the culture. Students' t tests on the numbers of larvae produced after one week indicated that a significant difference ($P < 0.05$) existed between the bottles containing Tribolium from the tops of the drums and those containing insects from the culture. The lowered number of offspring at the tops of the drums and the higher rate of mortality at the bottoms of the drums may be because of more males near the tops and older females near the bottoms. Cryptolestes adults taken from the tops and bottoms of the drums did not have significantly different ($P < 0.05$) mortality but adults taken from both locations did have a significantly higher mortality ($P < 0.05$) than insects taken from the cultures. No significant differences ($P < 0.05$) were evident in the number of larvae produced by the Cryptolestes from the drums or the culture. Although insects from the drums appeared to be older than those from the culture, the reproductive rate was not different. The use of the number of offspring produced by stored-grain beetles as an indicator of the age of the adult population may be somewhat ambiguous because it is possible that reduced density of the adults resulted in an increased rate of oviposition (Crombie 1943).

Electrolyte Leakage of Seeds

Analysis of wheat from each drum (Table 5) has shown that conductivity decreased with time in both the Control and COT systems. A rise in conductivity was observed at wk 60 in two of the three drums in the RST system. Seed conductivity has been used by Mills and Kim (1977) to measure the

Table 5. Electrical conductivity, in μmho,[a] of wheat-steeped water after 60 minutes and fat acidity values, in mg of KOH required to neutralize the free fatty acids in 100 g of dry wheat, for each experimental drum at weeks 18, 21, 24, 27, 30, and 60.

Origin of Sample	Week											
	18		21		24		27		30		60	
	Cond.[b]	FAV[c]	Cond.	FAV	Cond.	FAV	Cond.	FAV	Cond.	FAV	Cond.	FAV
Cold-room control									93.8	10.3	87.4	10.7
Control system												
Drum 1	65.5	38.5	71.0	43.5	76.4	43.0	65.5	43.1	47.3	46.2	49.1	56.5
Drum 2	71.0	36.9	60.1	42.3	60.1	42.5	58.2	43.8	65.5	42.7	54.6	55.5
Rhyzopertha-Sitophilus-Tribolium system												
Drum 3	76.4	42.8	60.1	46.2	71.1	47.7	71.0	46.9	69.2	45.1	130.1	34.5
Drum 4	65.5	43.9	65.5	44.9	67.3	45.4	61.9	46.9	69.2	45.4	54.6	36.9
Drum 5	103.7	43.8	114.7	45.3	87.4	46.0	51.0	45.6	58.2	42.9	109.2	27.5
Cryptolestes-Oryzaephilus-Tribolium system												
Drum 6	65.5	38.0	54.6	43.1	56.4	43.4	43.7	42.8	45.5	43.5	54.6	47.6
Drum 7	76.4	38.8	60.1	42.5	60.1	42.6	49.1	40.7	52.8	42.4	54.6	47.1
Drum 8	54.6	38.6	38.2	43.4	47.3	44.0	54.6	42.3	54.6	42.9	54.6	47.0

[a] μmho = reciprocal micro ohms per meter [b] Cond. = conductivity of pooled wheat sample from each drum

[c] FAV = fat acidity value of pooled wheat sample from each drum

leakage of electrolytes from damaged rapeseed into aqueous solution. They found that high fat acidity values and physical damage to the seed were related to large conductivity values. The electrolyte leakage in wheat was a poor measure of the difference in seed damage between the Control and COT system.

Milling and Baking Tests

Standard milling and baking analyses were done on wheat from the Control, RST, and COT systems after various periods of storage (Table 6). The flour characteristics were similar in all three systems with the exception of amylograph viscosity and sedimentation value. Amylograph viscosity is determined by placing flour in a buffer and stirring while heat is steadily applied. The viscosity is measured in arbitrary Brabender units (B.U.) (Pomeranz and Shellenberger 1971). The COT system had the highest viscosity of all samples, followed by the RST system and the Control system. The sedimentation value reflects the quality of wheat, and when divided by the percentage of protein in the wheat, gives a specific sedimentation value that can be used as an index of gluten quality. Gluten is the flour protein that makes the framework for bread dough. The gluten quality index in all three systems was low, the lowest being in the COT system. The loaf volume of leavened bread is adversely affected by even incipient insect infestations, and loaf volume reductions greater than 7% are because of unacceptable wheat (Majumder 1975). Insects damage grain by partially eating kernels, leaving excreta and exuviae, and intensifying microfloral growth and succession. Protein is denatured, vitamins are lost, and fats are broken down by these combined effects. In this study, the COT system had very low loaf volume from wk 15 - 60. The RST system had a less dramatic decline in loaf volume during the same period (Fig. 27). The samples

Table 6. Milling and baking quality data for wheat taken from the Control, Rhyzopertha-Sitophilus-Tribolium, and Cryptolestes-Oryzaephilus-Tribolium systems at weeks 15, 30, and 60 of the study.

Property	Sample[a]										
	S	CC	1A	1B	1C	2A	2B	2C	3A	3B	3C
Wheat											
Bushel weight, Kg/hl	80.0	73.0	73.3	73.4	75.6	75.6	70.0	75.0	75.2	70.4	74.6
1000 kernel weight, g	32.0	26.9	26.0	27.0	27.6	26.5	26.5	27.1	26.2	27.0	27.0
Protein, %	14.9	13.5	13.8	13.8	13.7	13.7	14.0	13.7	13.8	14.3	13.9
Flour yield, %	75.6	75.6	76.1	76.3	75.8	76.3	76.6	77.2	76.6	73.8	76.9
Flour											
Protein, %	14.5	13.0	13.1	13.2	13.1	13.0	13.4	13.2	13.0	13.3	13.2
Ash, %	0.44	0.43	0.51	0.53	0.51	0.53	0.60	0.54	0.57	0.69	0.59
Amylograph viscosity	765	700	1000	1000	1000	1080	900	1300	1400	1400	1400
Baking absorption, %	59.2	55.9	54.7	52.5	56.2	54.5	50.1	53.5	54.0	54.4	54.2
Sedimentation value	61	62	40	38	39	36	28	31	28	20	25
Bread											
Loaf volume, cc, remix	920	905	835	750	830	780	360	490	280	240	240
Farinogram											
Absorption, %	63.2	59.9	58.7	56.5	60.2	58.5	58.1	57.5	58.0	58.4	58.2
Development time, min	5.0	5.5	9.5	14.5	14.5	13.0	1.5	1.5	2.0	2.0	2.0
M.T.I., B.U.[b]	30	25	5	5	0	5	75	25	30	95	60

[a] S = standard for analysis, no. 2 grade wheat, cv. Neepawa

CC = cold-room control, stored at -15°C
1 = Control system
2 = RST system
3 = COT system
A = week 15
B = week 30
C = week 60

[b] M.T.I., B.U. = Mixing tolerance index, Brabender units.

Figure 27. Bread baked from insect-infested and insect-free wheat stored for various lengths of time; infestation type (or absence of it) and the length of storage is given with the following numberical codes:

(1) Cold-room control;
(2) Control system - week 15;
(3) Rhyzopertha-Sitophilus-Tribolium system - week 15;
(4) Cryptolestes-Oryzaephilus-Tribolium system - week 15;
(5) Control system - week 30;
(6) Rhyzopertha-Sitophilus-Tribolium system - week 30;
(7) Cryptolestes-Oryzaephilus-Tribolium system - week 30;
(8) Control system - week 60;
(9) Rhyzopertha-Sitophilus-Tribolium system - week 60; and
(10) Cryptolestes-Oryzaephilus-Tribolium system - week 60.


NW, OCT. '78
1
2
3
4
5
6
7
8
9
10

of wheat from each system were pooled from all levels in the drums.

A possible explanation for the more gradual decrease in loaf volume for the more severely damaged wheat from RST system than for that from the COT system is the presence of extensive 'caking' by inter-granular fungal mycelia in the former system. This observation was not evident from surface sterilized seeds. Fungi or bacteria secrete the starch-hydrolyzing enzyme, alpha-amylase, which increases gas production in the dough during baking fermentation leading to increased loaf volume (Pomeranz and Shellenberger 1971). The presence of alpha-amylase results in decreased viscosity of a gelatinized starch by liquefaction which can be measured by the amylograph. The amylograph viscosity (Table 6) indicated that enzymatic activity, probably because of alpha-amylase, was higher in the RST samples than in the COT samples, resulting in lower viscosity levels. The Control system bread had little decrease in loaf volume after 60 wk.

Insect Population Action and Interaction

Populations of all five species of insects increased until weeks 5-10 of this study, and then declined until week 15 (Table 7). Feeding on whole wheat, single populations of Tribolium and Oryzaephilus did not multiply extensively while those of Cryptolestes multiplied rapidly about eight-fold and then remained constant for 10 weeks. Sitophilus, and more noteably Rhyzopertha, became numerous at week 10. Larval numbers were low for all species possibly indicating cannibalism by the adults (Fujii 1974, Boyer 1976). Berlese funnel extraction did not remove any Sitophilus larvae from the wheat; these grubs have no legs and are incapable of moving during the extraction process. Dual combinations of the insects studied showed that Tribolium could not compete well with Rhyzopertha,

Table 7. Mean number of larvae and adults of five species of stored-product insects per bottle when one or more of them infested wheat either singly or collectively after incubation for 5, 10, and 15 weeks at 30°C and 70% RH.

Insect	Week 5				Week 10				Week 15			
	Larvae		Adults		Larvae		Adults		Larvae		Adults	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1. *Tribolium*	3.3	0.9	18.3	3.8	20.7	2.2	20.0	3.6	11.3	6.9	13.3	2.3
2. *Rhyzopertha*	27.3	5.6	394.0	4.6	185.3	13.1	1795.0	128.5	0		108.3	40.2
3. *Sitophilus*	0		37.7	16.8	0		446.3	107.2	0		204.0	132.4
4. *Oryzaephilus*	8.3	3.8	79.7	1.8	23.0	8.1	15.7	7.1	1.3	0.7	6.3	0.9
5. *Cryptolestes*	102.7	18.5	214.0	6.7	34.7	5.8	179.7	45.1	1.7	0.3	19.7	6.7
6. *Tribolium* +	5.3	0.9	46.0	11.9	0		8.7	3.2	0		5.3	0.7
Rhyzopertha	2.0	2.0	157.3	79.0	222.3	27.2	1291.3	263.8	0		29.0	9.7
7. *Tribolium* +	15.3	1.8	54.3	1.2	11.3	8.5	11.0	1.5	8.0	8.0	10.0	5.3
Sitophilus	0		93.7	27.5	0		305.7	67.3	0		483.7	243.4
8. *Tribolium* +	0		37.7	7.5	0.7	0.7	23.0	4.7	1.0	1.0	19.0	2.5
Oryzaephilus	5.3	2.4	46.7	7.5	11.7	3.2	39.3	7.8	1.3	0.8	19.0	6.7
9. *Tribolium* +	3.3	1.5	21.7	4.3	0.3	0.3	17.7	2.4	0		4.0	1.5
Cryptolestes	58.0	2.3	200.3	5.0	67.0	11.1	212.3	28.9	5.7	0.9	21.0	3.0
10. *Rhyzopertha* +	14.7	2.9	272.7	14.2	108.0	49.4	1516.3	42.3	0		12.7	12.2
Sitophilus	0		36.3	11.6	0		59.3	11.9	0		0	
11. *Oryzaephilus* +	0		8.3	0.9	0		0		0		0	
Cryptolestes	103.3	14.6	224.0	35.4	79.3	4.1	294.7	19.7	5.0	1.7	22.3	3.4
12. *Rhyzopertha* +	8.3	2.0	287.0	30.5	253.3	72.3	1030.7	128.7	0		10.7	5.9
Sitophilus +	0		85.3	10.7	0		60.3	22.5	0		0	
Tribolium	1.7	1.2	33.3	4.3	0		9.0	2.1	0		4.7	1.2
13. *Cryptolestes* +	159.3	9.3	203.7	7.7	64.3	13.3	128.7	30.4	3.3	1.2	23.7	4.7
Oryzaephilus +	0		6.7	2.0	0		1.7	0.7	0		0	
Tribolium	6.0	3.2	18.0	0.6	1.0	1.0	41.7	22.2	0		12.0	5.5

[a] *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*.

[b] Mean and standard error, three replicates per treatment.

Sitophilus or Cryptolestes. Tribolium and Oryzaephilus appeared to have a mutually beneficial relationship although the populations of both species remained at low levels. Rhyzopertha inhibited multiplication of Sitophilus, and Cryptolestes inhibited that of Oryzaephilus. In combinations of three species, none of the insects multiplied as extensively as when they were alone in the wheat. In the Rhyzopertha-Sitophilus-Tribolium combination, Rhyzopertha was the dominant species with Sitophilus reaching moderate population levels by week 10 followed by extinction. Tribolium remained at low numbers throughout 15 weeks. In the Cryptolestes-Oryzaephilus-Tribolium combination, Cryptolestes was the dominant species, although Tribolium reached population levels higher than those when it was reared alone. Oryzaephilus declined in numbers rapidly becoming extinct after 10 weeks.

Fat acidity values increased with time and were generally higher in the wheat infested with insects than in the insect-free controls (Table 8). The most dramatic increases in FAV levels typically occurred in wheat which was infested with some combination of Rhyzopertha or Sitophilus. In these treatments, the FAV levels rose until week 10 and then began to decline by week 15. The moisture content of the wheat sharply and steadily increased in the bottles containing Rhyzopertha or Sitophilus, reaching levels of 37.3% in wheat containing Rhyzopertha alone and 36.3% in wheat containing the species combination of Rhyzopertha-Sitophilus-Tribolium (Table 8).

A sharp decrease in grain weight occurred in bottles containing the whole grain feeders, Rhyzopertha and Sitophilus, with corresponding increases in the weight of grain dust (Table 9). Grain weight in the insect-free controls remained constant during 15 weeks and the amount of dust was negligible. The weight of grain dust in the wheat containing Rhyzopertha or

Table 8. Mean fat acidity values (mg KOH/100 g dry seed) and moisture content (%) of wheat in bottles infested singly or collectively by five species of stored-grain insects incubated for 5, 10, and 15 weeks at 30°C and 70% RH.

Insect[a]	Week 5				Week 10				Week 15			
	FAV[b]		Moisture[c]		FAV		Moisture		FAV		Moisture	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1. Tribolium	14.7	0.3	13.4	0.4	14.9	0.3	14.4	0.1	21.3	0.6	12.8	0.1
2. Rhyzopertha	19.8	0.4	14.5	0.2	34.1	0.7	20.3	1.1	24.6	0.2	37.3	1.0
3. Sitophilus	16.9	0.7	14.7	0.1	31.5	2.0	20.3	1.6	22.9	1.9	27.6	0.6
4. Oryzaephilus	14.3	0.2	12.9	0.1	15.6	0.3	14.6	0.1	19.6	0.3	12.8	0.1
5. Cryptolestes	14.5	0.2	12.7	0.1	14.1	1.0	14.2	0.1	21.0	0.3	13.0	0.1
6. Tribolium + Rhyzopertha	18.4	0.2	14.8	0.2	23.2	2.3	20.6	1.0	20.5	0.4	33.3	2.2
7. Tribolium + Sitophilus	16.4	0.2	15.7	0.2	30.3	0.8	19.7	1.3	19.7	0.7	24.7	1.1
8. Tribolium + Oryzaephilus	15.3	0.2	12.9	0.1	16.8	0.2	16.2	1.3	18.7	1.8	13.8	0.1
9. Tribolium + Cryptolestes	15.1	0.2	13.3	0.1	15.1	2.3	14.5	0.2	18.2	0.2	13.3	0.1
10. Rhyzopertha + Sitophilus	18.2	1.3	15.1	0.1	28.3	0.2	27.7	1.2	18.8	0.3	33.3	2.6
11. Oryzaephilus + Cryptolestes	14.1	0.6	13.1	0.1	17.0	0.4	14.8	0.1	20.1	0.9	13.6	0.1
12. Rhyzopertha + Sitophilus + Tribolium	22.4	0.6	16.4	0.1	30.0	0.3	22.1	0.8	25.8	0.5	36.3	1.2
13. Cryptolestes + Oryzaephilus + Tribolium	15.5	0.7	13.6	0.1	17.3	1.3	14.9	0.1	19.7	0.1	13.8	0.1
14. Control	13.0	0.4	13.0	0.1	16.2	0.6	14.1	0.1	19.0	0.2	14.2	0.1

[a] Tribolium castaneum, Rhyzopertha dominica, Sitophilus oryzae, Oryzaephilus surinamensis, Cryptolestes ferrugineus.

[b] Mg KOH/100 g dry wheat; two replicates per treatment [c] %; three replicates per treatment

Table 9. Mean grain weight and dust weight in bottles of wheat infested singly or collectively by five species of stored grain insects incubated for 5, 10, and 15 weeks at 30°C and 70% RH.

Insect [a]	Week 5				Week 10				Week 15			
	Grain wt.[b]		Dust wt.[c]		Grain wt.		Dust wt.		Grain wt.		Dust wt.	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1. *Tribolium*	2.62	0.05	127	16	2.69	0.02	98	10	2.64	0.08	299	60
2. *Rhyzopertha*	2.62	0.01	5850	287	1.67	0.08	20120	4800	1.53	0.11	3475	419
3. *Sitophilus*	2.76	0.03	71	2	2.47	0.10	1053	100	2.01	0.17	641	147
4. *Oryzaephilus*	2.71	0.04	72	9	2.70	0.02	66	11	2.67	0.03	75	1
5. *Cryptolestes*	2.78	0.01	237	2	2.64	0.06	440	15	2.69	0.05	483	39
6. *Tribolium* + *Rhyzopertha*	2.69	0.02	3423	60	1.92	0.07	14530	687	1.73	0.04	3705	482
7. *Tribolium* + *Sitophilus*	2.71	0.02	481	20	2.39	0.21	1094	125	1.89	0.12	1117	167
8. *Tribolium* + *Oryzaephilus*	2.69	0.01	268	32	2.65	0.03	356	65	2.64	0.01	364	64
9. *Tribolium* + *Cryptolestes*	2.68	0.01	356	11	2.65	0.03	498	30	2.65	0.04	545	4
10. *Rhyzopertha* + *Sitophilus*	2.61	0.03	4879	229	1.87	0.08	5677	1414	1.89	0.05	1677	393
11. *Oryzaephilus* + *Cryptolestes*	2.68	0.01	323	28	2.64	0.03	566	71	2.62	0.06	596	119
12. *Rhyzopertha* + *Sitophilus* + *Tribolium*	2.46	0.06	4457	368	1.92	0.06	11099	431	1.77	0.05	2821	227
13. *Cryptolestes* + *Oryzaephilus* + *Tribolium*	2.71	0.04	417	33	2.76	0.04	626	29	2.62	0.05	522	55
14. Control	2.80	0.04	8	1	2.80	0.01	9	1	2.78	0.08	9	1

[a] *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*.

[b] Weight of 100 kernels, in grams.

[c] Dust from 200 ml bottles, in milligrams; three replicates per treatment for each sampling date.

Sitophilus declined between weeks 10 and 15 because of extensive 'caking' of the grain where dust and wheat kernels were bound togehter with an extensive network of fungal mycelia. Considerably more dust was produced by the combination of Cryptolestes-Oryzaephilus-Tribolium than by any of those species individually.

Germ and endosperm damage in the wheat was most extensive when Rhyzopertha occurred alone, with slightly less damage when it was in the presence of other species (Table 10). Sitophilus caused only slightly less damage to both the germ and endosperm. Cryptolestes, whether alone or with other germ-feeding species, consumed the germ in fewer than 24% of the kernels. This level of damage is consistent with that obtained for the COT system in the main experiment using larger containers.

Germination decreased gradually during 15 weeks in wheat infested with the germ-feeders Tribolium, Oryzaephilus, or Cryptolestes (Table 11). Generally, the loss of germination was related to the degree of germ damage caused by the feeding of each combination of insects. By week 10, germination had declined to 0 - 1.2% in wheat which contained either Rhyzopertha or Sitophilus or any combination of Rhyzopertha or Sitophilus.

The fungus Alternaria alternata was present in 52% of the seeds initially used for all insect species combinations. Alternaria declined steadily regardless of insect combinations, virtually disappearing by week 10.

Aspergillus glaucus group did not appear until week 10 and was primarily present on seeds from the insect-free controls, infecting 25% of the seeds. Fewer than 5% of the seeds were infected in the presence of Tribolium and Sitophilus, Tribolium and Oryzaephilus, Tribolium and Cryptolestes, Oryzaephilus and Cryptolestes, and Cryptolestes-Oryzaephilus-

Table 10. Mean numbers per bottle of wheat kernels with germ and endosperm damage caused by infestation of single or multiple species of five stored-grain insects after 5, 10, and 15 weeks of incubation at 30°C and 70% RH.

Insect[a]	Week 5				Week 10				Week 15			
	Germ		Endo.		Germ		Endo.		Germ		Endo.	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1. *Tribolium*	4.3	0.3	0		8.7	1.8	0.7	0.3	12.3	0.3	0	
2. *Rhyzopertha*	9.7	1.8	3.7	0.3	68.3	5.2	57.3	12.2	88.3	3.3	89.0	3.1
3. *Sitophilus*	5.7	2.6	3.0	1.5	18.7	5.2	13.7	8.1	43.0	11.0	47.3	10.4
4. *Oryzaephilus*	5.3	1.2	0		7.7	1.9	0		6.7	0.3	0	
5. *Cryptolestes*	6.3	2.9	0		15.7	0.3	0		23.7	2.0	0	
6. *Tribolium* + *Rhyzopertha*	18.0	2.1	4.3	1.9	66.3	16.4	51.0	5.9	75.7	4.7	75.6	4.7
7. *Tribolium* + *Sitophilus*	17.7	3.4	5.0	1.7	21.0	7.4	13.7	7.4	40.3	4.9	48.0	2.5
8. *Tribolium* + *Oryzaephilus*	10.3	2.3	1.7	0.9	12.7	2.9	0.3	0.3	13.0	3.2	1.3	0.3
9. *Tribolium* + *Cryptolestes*	9.0	1.5	1.0	0.6	19.3	4.5	0		20.0	2.1	0.7	0.7
10. *Rhyzopertha* + *Sitophilus*	11.7	1.5	2.3	1.9	73.7	3.2	71.7	1.2	80.0	8.5	79.0	7.7
11. *Oryzaephilus* + *Cryptolestes*	7.3	0.3	0		19.0	0.6	0		21.0	1.0	0	
12. *Rhyzopertha* + *Sitophilus* + *Tribolium*	20.7	2.3	10.7	2.2	47.3	7.5	47.3	7.5	74.7	4.7	74.6	4.6
13. *Cryptolestes* + *Oryzaephilus* + *Tribolium*	13.7	1.5	0		22.0	1.5	0.3	0.3	21.3	2.4	1.3	1.3

[a] *Tribolium castaneum*, *Rhyzopertha dominica*, *Sitophilus oryzae*, *Oryzaephilus surinamensis*, *Cryptolestes ferrugineus*.

[b] Percentage of kernels with more than 50% damage; three replicates per treatment for each sampling date.

Table 11. The mean percentages of viable seeds and those containing bacteria in bottle samples of wheat infested singly or collectively by five species of stored-grain insects after incubation for 5, 10, and 15 weeks at 30°C and 70% RH.

Insect[a]	Week 5				Week 10				Week 15			
	Germination		Bacteria		Germination		Bacteria		Germination		Bacteria	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1. Tribolium	97.2	1.2	0		72.0	6.8	1.2	1.2	76.0	2.4	2.8	1.2
2. Rhyzopertha	60.0	6.8	14.8	1.2	1.2	1.2	98.8	1.2	0		100.0	0
3. Sitophilus	88.0	4.0	2.8	1.2	1.2	1.2	49.2	15.6	0		100.0	0
4. Oryzaephilus	96.0	2.4	5.2	1.2	86.8	2.8	5.2	1.2	68.0	10.0	4.0	0
5. Cryptolestes	86.8	2.8	4.0	0	81.2	3.6	5.2	1.2	66.8	4.8	5.2	1.2
6. Tribolium + Rhyzopertha	46.8	2.5	10.8	1.2	16.0	12.0	50.8	19.6	0		100.0	0
7. Tribolium + Sitophilus	81.2	1.2	8.0	2.4	0		53.2	7.6	0		100.0	0
8. Tribolium + Oryzaephilus	82.8	1.2	6.8	1.7	65.2	4.8	9.2	1.2	25.2	3.6	9.2	1.2
9. Tribolium + Cryptolestes	68.0	2.4	2.8	1.2	56.0	6.0	5.2	3.6	56.0	8.0	4.0	0
10. Rhyzopertha + Sitophilus	65.2	11.6	13.2	1.2	0		93.2	2.8	0		100.0	0
11. Oryzaephilus + Cryptolestes	81.2	1.2	0		65.2	6.0	2.8	1.2	48.0	2.4	4.0	1.2
12. Rhyzopertha + Sitophilus + Tribolium	65.2	1.2	17.2	1.2	0		98.8	1.2	0		100.0	0
13. Cryptolestes + Oryzaephilus + Tribolium	80.0	2.4	5.2	1.2	74.8	7.2	5.2	2.8	46.8	2.8	8.0	2.4
14. Control	85.2	0.4	0		84.0	1.2	10.8	1.7	72.0	2.0	12.0	1.2

[a] Tribolium castaneum, Rhyzopertha dominica, Sitophilus oryzae, Oryzaephilus surinamensis, Cryptolestes ferrugineus.

[b] Percent; surface sterilized seeds; three replicates per treatment for each sampling date.

Tribolium. Rhyzopus spp. also infected a few seeds in each treatment.

Bacterial infection of the seeds was related to both seed damage and the moisture content of the wheat; such infection was conspicuous only when Rhyzopertha, Sitophilus, or both were present. Nearly 100% seed infection by bacteria was noted by week 10 when Rhyzopertha or Sitophilus were present while less than 10% of the seeds were infected by week 15 with combinations of the germ-feeding insects (Table 11).

Lefkovitch (1968) reported laboratory interactions among S. oryzae, Lasioderma serricorne (F.), T. castaneum, and C. ferrugineus. He found that C. ferrugineus had a harmful effect on T. castaneum populations and C. ferrugineus was the species most likely to persist in a system. Ciesielska (1975) studied effects of interspecific competition on oviposition and population size when combinations of S. granarius, R. dominica, and O. surinamensis were placed together on wheat. It was concluded that R. dominica had a strong competitive effect on the other two species. Kabir (1966) observed interactions among S. oryzae, R. dominica, and T. castaneum on sorghum in the laboratory. He found that R. dominica and S. oryzae mutually inhibited development whereas T. castaneum benefitted by the presence of either or both species. LeCato (1975) found that populations of C. pusillus, O. surinamensis, and T. castaneum reacted favorably in the presence of either R. dominica or S. oryzae.

Extrapolation of data obtained in small-scale laboratory studies to studies involving larger storage containers must be interpreted with caution. The understanding of insect interactions under laboratory conditions, however, in conjunction with experiments using larger containers is likely to shed light on the effects of one species on another and on their environment.

Multivariate Statistical Analyses

(A) Principal Component Analysis

A mathematical description of a real world system is often referred to as a mathematical model, of which there are two basic types. Correlative models reflect observed relations among several variables, and describe and summarize those relationships so that relations can be verified and used for prediction. Explanatory models reflect observed relationships among variables and also reflect some concept of the causal mechanism that underlies the relations (Gold 1977).

In an applied context, multivariate analysis is concerned with discrete samples which possess values for various numbers of variables. This group of statistical methods studies the interrelationships among those variables, looks for sample differences in terms of those variables, and draws inferences relevant to those variables concerning the populations from which the samples were chosen (Tatsuoka 1971).

Principal component analysis is a statistical method which transforms a given set of variables into a new set of composite variables (principal components) that are orthogonal (uncorrelated) to one another. No particular assumption about the underlying structure of the variables is necessary. This analysis determines the best linear combination of variables - the combination of variables that would account for more of the variance in the data as a whole than any other linear combination of variables. The first principal component is usually the single best summary of linear relationships evident in the data. The second component is the second best linear combination of variables with the second component being orthogonal to the first. The second component accounts for the most residual variance after the effects of the first component are removed from the

data (Kim 1975). It is important to note that it is, of course, possible that some principal components will have no physical meaning (Kendall 1965). The use of PCA in interpretation of ecological problems has been discussed by Seal (1964), Pearse (1965), Orloci (1975), and Sinha (1977).

Correlation coefficient matrices and principal component matrices with the proportion of variance accounted for and loadings for all principal components are presented for all systems, for wk 0, 9, 21, 60, and for cumulative data for wk 0 - 60 (Tables 12 - 37). The changing patterns of relationships among variables from one sampling date to another are illustrated diagrammatically for principal components 1 and 2 in all three systems (Figs. 28 - 33). Correlation coefficient matrices were used during analysis rather than variance-covariance matrices because measurements of variables were taken on different scales (Bronswijk and Sinha 1971).

Principal component analysis done on data at each sampling date dealt with smaller numbers of samples and small amounts of variation as compared to the overall PCA on cumulative data from wk 0 - 60. However, the regular analyses were probably more meaningful in that these revealed changing relationships with time.

To determine component reliability in data from wk 0 - 60, PCA was separately undertaken on randomly split samples in the Control, RST, and COT systems, and the results compared (Lawley and Maxwell 1971, Sinha 1977, Mills et al. 1978). Since all of the principal components remained basically the same in the split samples, they were considered highly reliable.

PCA of Data From Each Sampling Date

Correlation coefficient matrices for variables in the Control system for wk 0, 9, 21, and 60 (Tables 12, 14, 20, 26) and principal component matrices showing principal component loadings and the percent variability

Table 12. Correlation coefficient matrix for all systems at week 0, prior to insect introduction.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11
1. FAV											
2. CO_2	-46										
3. O_2	36	-66									
4. Temperature	01	-08	06								
5. Grain weight	-05	05	-12	-09							
6. Dust weight	01	-04	02	30	-23						
7. Moisture	-13	36	-31	-21	11	-15					
8. Alternaria	11	-03	-09	-12	14	-07	21				
9. Aspergillus	-03	-05	-05	-07	-01	26	11	13			
10. Bacteria	-03	32	-17	04	08	-01	-07	01	05		
11. Germination	24	-14	23	-10	14	-06	-05	-11	-08	11	

[a]Decimal points are omitted.

Table 13. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for all systems at week 0, prior to the introduction of insects into the wheat.

Variable	Principal component										
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}
FAV	39	-23	-12	-22	-06	-42	-26	-31	-58	-06	-21
CO_2	-55	12	20	-07	10	-14	-13	07	-04	-01	-76
O_2	53	-10	-01	04	07	06	11	-10	46	50	-46
Temperature	13	47	13	-09	-52	11	-44	-33	29	-26	-04
Grain weight	-14	-39	10	-24	-42	66	-02	-02	-27	25	-08
Dust weight	11	54	-25	-28	13	10	-29	45	-25	43	06
Moisture	-36	-25	-29	10	21	-05	-55	-38	22	35	23
Alternaria	-10	-28	-51	-16	-48	-31	-02	45	29	-10	-07
Aspergillus	-05	13	-57	-40	28	31	27	-33	08	-32	-15
Bacteria	-17	06	31	-67	-08	-35	29	-19	16	29	25
Germination	19	-32	31	-40	38	14	-40	31	24	-35	03
Variability accounted for (%)	22	14	12	11	9	8	7	6	5	4	2

[a]Decimal points are omitted.

Table 14. Correlation coefficient matrix for the Control system at week 9.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12
1. FAV												
2. CO_2	-46											
3. O_2	69	-60										
4. Temperature	0	-28	-02									
5. Grain weight	28	-11	32	01								
6. Dust weight	-18	18	-25	31	-52							
7. Moisture	18	66	03	-57	17	-08						
8. Alternaria	-12	57	-03	-53	-12	-12	69					
9. Aspergillus	-03	-03	-14	13	-03	10	-12	-18				
10. Bacteria	0	-09	-01	13	10	19	-19	-55	-20			
11. Germination	07	-76	29	24	-12	08	-66	-44	-06	10		
12. Tarsonemus	39	-35	42	11	02	01	-04	-07	-36	20	18	

[a]Decimal points are omitted.

Table 15. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Control system at week 9.

Variable	Principal component											
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}
FAV	20	-44	-02	-04	-46	-12	-04	-51	-31	-17	-32	-22
CO_2	-48	13	-12	21	-09	11	-04	07	-01	-22	15	-77
O_2	26	-46	-02	-15	-16	-08	-28	19	63	13	29	-33
Temperature	29	27	04	10	-32	70	-15	-07	18	-41	06	15
Grain weight	05	-33	37	51	04	22	-27	44	-30	20	-21	-02
Dust weight	01	37	-41	-13	-46	-12	-44	25	-17	38	-15	04
Moisture	-41	-31	-14	08	-25	-12	-14	10	-21	-32	50	46
Alternaria	-41	-21	-11	-35	09	15	-10	24	24	-29	-63	14
Aspergillus	-01	19	54	-13	-51	-31	38	36	08	-16	-05	-02
Bacteria	19	11	-33	63	02	-42	04	07	30	-32	-24	10
Germination	40	07	-01	-31	32	-20	-28	30	-36	-50	10	-19
Tarsonemus	21	-25	-50	-05	-08	26	61	38	-18	06	04	-07
Variability accounted for (%)	29	22	12	10	9	6	4	3	3	1	<1	<1

[a]Decimal points are omitted.

Table 16. Correlation coefficient matrix for the Rhyzopertha-Sitophilus-Tribolium system at week 9.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. FAV																
2. CO_2	-25															
3. O_2	-03	-18														
4. Temperature	03	-41	19													
5. Grain weight	-13	18	-04	-54												
6. Dust weight	-07	-42	28	88	-51											
7. Moisture	21	14	03	-11	21	-31										
8. Germ damage	-15	-07	-28	53	-36	36	07									
9. Endosperm damage	20	-27	22	72	-67	75	-10	52								
10. Alternaria	-14	33	03	-65	19	-64	05	-46	-47							
11. Aspergillus	-29	-38	07	58	04	52	-26	26	15	-33						
12. Bacteria	27	-01	27	-16	20	-16	46	-22	-04	04	-29					
13. Germination	01	41	-15	-68	25	-58	-27	-52	-42	58	-37	-10				
14. Tribolium	-04	-26	40	81	-59	75	10	50	78	-44	27	07	-53			
15. Rhyzopertha	-14	-23	26	75	-62	69	-03	51	75	-34	28	-04	-41	91		
16. Sitophilus	-02	-30	16	70	-39	57	28	61	72	-43	28	08	-51	81	82	

[a]Decimal points are omitted.

Table 17. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Rhyzopertha-Sitophilus-Tribolium system at week 9.

Variable	Principal Component															
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}
FAV	01	35	-11	-11	-67	-07	-19	-40	-13	-25	-12	-24	-02	-06	-24	-05
CO_2	17	01	49	07	27	59	-08	-35	-31	-16	03	-06	-17	-03	-12	-11
O_2	-10	22	-30	-48	40	12	21	-27	37	-31	-22	10	-15	04	-07	13
Temperature	-37	-07	-10	02	-04	07	-01	-19	-28	03	-07	-25	-16	46	57	31
Grain weight	23	02	-36	31	27	10	-43	-12	31	-09	27	-45	20	-06	13	01
Dust weight	-34	-14	-15	-14	-02	26	01	01	-12	16	41	-02	31	19	-55	33
Moisture	03	54	05	38	15	-20	15	-38	-06	27	05	32	23	-06	04	29
Germ damage	-24	-09	32	43	-02	-06	03	15	27	-58	-25	-02	19	14	-15	26
Endosperm damage	-33	09	19	-19	-15	09	-16	-03	15	-28	54	32	09	-30	41	-06
Alternaria	25	04	20	-27	26	-57	13	-01	-31	-33	34	-24	12	16	-04	05
Aspergillus	-18	-35	-39	17	20	-20	-27	-20	-43	-26	-14	36	-01	-21	-09	-11
Bacteria	03	55	-19	-01	09	21	-23	61	-35	-18	-07	09	-03	06	02	07
Germination	27	-12	23	-33	-03	-07	-62	-06	11	14	-24	28	21	23	05	29
Tribolium	-35	15	11	-13	18	-01	-04	-05	-04	11	-25	-15	58	14	06	-58
Rhyzopertha	-33	05	21	-14	19	-15	-18	08	-06	20	-20	-39	-10	-61	-05	31
Sitophilus	-31	18	14	12	15	-27	-35	-02	20	13	19	05	-53	33	-26	-26
Variability accounted for (%)	42	13	10	9	8	4	3	3	2	2	1	1	<1	<1	<1	<1

[a]Decimal points are omitted.

Table 18. Correlation coefficient matrix for the Cryptolestes-Oryzaephilus Tribolium system at week 9.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. FAV																
2. CO_2	-41															
3. O_2	13	-80														
4. Temperature	09	04	-20													
5. Grain weight	-11	02	20	-55												
6. Dust weight	17	16	-17	54	-37											
7. Moisture	50	-25	16	03	-03	29										
8. Germ damage	41	-22	08	36	-11	24	24									
9. Endosperm damage	05	-12	01	20	-29	16	08	26								
10. Alternaria	-50	23	01	-38	17	-33	-36	-35	-22							
11. Aspergillus	-13	04	-12	29	-20	-03	17	01	23	-18						
12. Bacteria	-18	34	-16	04	-11	12	-03	-10	34	07	04					
13. Germination	-10	04	20	-73	56	-42	-24	-30	-24	28	-31	04				
14. Tribolium	29	-38	21	45	-30	06	49	53	22	-33	52	-26	-61			
15. Oryzaephilus	10	-35	24	33	-43	-01	27	32	42	-10	23	-19	-48	66		
16. Cryptolestes	04	-17	11	41	-23	12	46	46	28	-19	46	-14	-44	83	69	

[a]Decimal points are omitted.

Table 19. Principal component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Cryptolestes-Oryzaephilus-Tribolium system at week 9.

Variable	Principal Component															
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}
FAV	-18	24	49	01	08	03	11	43	26	11	20	49	05	26	09	20
CO_2	16	-49	-02	-18	27	-26	-08	10	-07	01	-16	10	26	34	-50	28
O_2	-05	48	-08	27	-20	18	-17	-44	07	08	-06	01	18	25	-38	37
Temperature	-30	-32	08	-11	-27	04	11	-33	16	-11	-09	54	-18	-38	-30	-06
Grain weight	24	27	01	-12	39	-26	07	-40	-17	-47	21	34	15	01	16	-10
Dust weight	-18	-25	40	02	-10	04	-39	-35	-38	32	17	03	17	21	25	-19
Moisture	-24	18	21	-04	47	15	-53	15	-05	-10	02	-15	-05	-44	-30	01
Germ damage	-27	10	18	02	01	-69	21	-17	24	24	10	-32	21	-24	-02	09
Endosperm damage	-19	-08	-09	65	09	-12	23	13	-44	-07	34	06	-24	03	-22	02
Alternaria	24	-01	-40	-01	-17	-23	-47	11	24	23	54	23	-08	-09	-03	-03
Aspergillus	-20	-14	-34	-11	41	42	31	-14	06	36	28	05	31	-13	10	13
Bacteria	06	-30	03	60	27	04	-17	-14	55	-14	-20	03	08	06	22	-11
Germination	33	23	03	14	20	-10	09	-01	-13	59	-38	28	-08	-14	-14	-35
Tribolium	-40	13	-19	-17	12	-03	02	-02	20	-04	06	-08	-10	47	-25	-63
Oryzaephilus	-32	10	-32	12	-19	-13	-14	30	-20	-13	-28	24	60	-12	14	-13
Cryptolestes	-35	05	-29	-10	22	-23	-18	-10	-09	12	-31	15	-47	16	35	37
Variability accounted for (%)	31	16	11	8	7	6	5	4	4	2	2	2	1	1	1	1

[a]Decimal points are omitted.

Table 20. Correlation coefficient matrix for the Control system at week 21.[a]

Variable	1	2	3	4	5	6	7	8	9	10
1. FAV										
2. CO_2	-09									
3. O_2	-02	-82								
4. Temperature	06	-64	48							
5. Grain weight	01	11	01	08						
6. Dust weight	22	10	08	14	-10					
7. Moisture	07	78	-52	-83	06	26				
8. Aspergillus	03	-18	13	-01	-26	05	05			
9. Bacteria	-20	06	03	03	07	-29	-22	-85		
10. Tarsonemus	-01	-15	08	30	31	-01	-21	60	-57	

[a]Decimal points are omitted.

Table 21. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Control system at week 21.

Variable	Principal Component									
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
FAV	02	14	40	48	75	03	10	09	06	10
CO_2	-52	06	-13	10	-11	-26	-09	65	14	41
O_2	44	-05	17	-02	-20	64	07	55	08	09
Temperature	48	-05	02	24	-12	-47	-33	13	54	-24
Grain weight	-01	-05	-55	63	-07	35	-28	-23	-01	20
Dust weight	-03	20	53	42	-59	-12	19	-22	-14	19
Moisture	-50	19	10	08	-11	35	07	-05	52	-55
Aspergillus	12	58	-02	-33	05	10	-09	-28	44	51
Bacteria	-03	-61	-03	01	01	-02	53	-19	45	31
Tarsonemus	21	43	-45	15	-02	-17	68	15	-05	-18
Variability accounted for (%)	31	24	14	12	8	7	2	1	<1	<1

[a]Decimal points are omitted.

Table 22. Correlation coefficient matrix for the Rhyzopertha-Sitophilus-Tribolium system at week 21.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1. FAV													
2. CO_2	-55												
3. O_2	-18	07											
4. Temperature	23	-29	-96										
5. Grain weight	-19	41	79	-88									
6. Dust weight	-01	-07	18	-12	12								
7. Moisture	-22	20	97	-97	76	16							
8. Germ damage	-23	-03	80	-75	46	07	83						
9. Endosperm damage	-21	-17	67	-55	18	17	71	81					
10. Aspergillus	-29	34	68	-71	63	03	69	43	28				
11. Bacteria	13	-06	37	-36	28	06	40	40	32	-28			
12. Tribolium	15	-40	-08	21	-48	17	-01	16	53	-30	11		
13. Rhyzopertha	-03	-40	-08	18	-38	-20	-11	09	32	-23	-16	57	

[a]Decimal points are omitted.

Table 23. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Rhyzopertha-Sitophilus-Tribolium system at week 21.

Variable	Principal Component												
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}
FAV	-13	15	56	33	-40	07	-45	38	13	09	07	01	05
CO_2	12	-41	-22	-36	30	06	-59	37	15	05	02	03	20
O_2	41	08	08	10	-11	-15	08	-18	16	05	-27	14	79
Temperature	-42	03	-05	-09	01	14	12	-03	-05	02	48	-54	51
Grain weight	35	-24	14	09	-02	-39	-13	-13	-58	33	39	-06	-03
Dust weight	07	03	15	-74	-53	-28	16	12	08	-12	07	-01	-06
Moisture	42	08	03	03	-02	04	-14	-08	13	-03	-28	-81	-21
Germ damage	35	24	-04	04	12	22	29	65	-38	-30	06	01	09
Endosperm damage	27	42	-16	-11	03	27	07	03	31	64	33	11	-11
Aspergillus	31	-19	-29	21	-38	25	-13	-22	19	-46	46	11	-06
Bacteria	15	18	55	-16	54	-06	-05	-22	23	-34	30	07	-03
Tribolium	-06	52	-13	-25	-07	22	-49	-32	-45	-13	-13	09	07
Rhyzopertha	-08	41	-38	19	07	-70	-17	18	18	-16	15	-03	-02
Variability accounted for (%)	43	20	12	8	8	3	3	1	1	< 1	< 1	< 1	< 1

[a]Decimal points are omitted.

Table 24. Correlation coefficient matrix for the Cryptolestes-Oryzaephilus-Tribolium system at week 21.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1. FAV													
2. CO_2	-54												
3. O_2	26	-83											
4. Temperature	10	-17	03										
5. Grain weight	-25	06	-20	11									
6. Dust weight	-08	17	11	-66	-24								
7. Moisture	-02	17	04	-71	-17	64							
8. Germ damage	-30	24	-19	26	07	-20	12						
9. Endosperm damage	01	-17	07	-08	-28	-29	15	04					
10. Aspergillus	-06	09	-26	51	21	-31	-46	02	-03				
11. Bacteria	05	-22	20	-44	19	12	37	-16	12	-68			
12. Tribolium	-15	16	02	-51	-22	77	35	-07	-27	-26	-03		
13. Cryptolestes	-09	05	01	-03	30	51	04	-01	-52	09	-08	49	

[a]Decimal points are omitted.

Table 25. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Cryptolestes-Oryzaephilus-Tribolium system at week 21.

Variable	Principal Component												
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}
FAV	04	37	-25	-16	-23	-59	-47	-18	-15	-05	-06	-21	-24
CO_2	-06	-50	33	-08	-19	-17	12	08	24	-10	-16	-22	-63
O_2	-07	44	-31	01	39	30	14	36	04	-11	-07	-15	-53
Temperature	46	-02	-19	-01	25	-17	11	-10	40	19	-65	01	15
Grain weight	11	-20	-09	69	-10	20	-30	05	-33	-22	-32	-25	02
Dust weight	-47	-12	-19	-17	01	07	-09	16	23	19	-06	-66	36
Moisture	-42	03	24	-03	14	-04	-52	35	14	-03	-34	47	04
Germ damage	10	-20	26	04	78	-26	-20	-07	-21	12	25	-20	-03
Endosperm damage	07	28	44	-21	03	49	-26	-51	14	-16	-13	-21	-04
Aspergillus	34	-24	-18	-21	-15	36	-42	16	-06	57	15	06	-20
Bacteria	-24	28	18	55	-08	-08	09	-22	21	63	06	01	-17
Tribolium	-39	-19	-21	-22	11	10	19	-40	-51	21	-38	15	-15
Cryptolestes	-17	-28	-48	16	15	07	-22	-41	45	-24	27	21	-12
Variability accounted for (%)	27	20	15	11	8	6	5	3	2	1	1	< 1	< 1

[a]Decimal points are omitted.

Table 26. Correlation coefficient matrix for the Control system at week 60.[a]

Variable	1	2	3	4	5	6	7	8	9	10
1. FAV										
2. CO_2	-19									
3. O_2	29	-65								
4. Temperature	15	-44	34							
5. Grain weight	44	-46	31	10						
6. Dust weight	-23	05	12	07	-34					
7. Moisture	17	70	-13	-49	-34	02				
8. Aspergillus	03	-03	-10	18	32	-47	-11			
9. Bacteria	30	-10	24	18	-08	42	-04	-74		
10. Tarsonemus	25	-08	34	26	-16	19	29	-41	40	

[a]Decimal points are omitted.

Table 27. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Control system at week 60.

Variable	Principal Component									
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}
FAV	-26	-04	-62	-11	-32	-27	-25	-49	-14	-21
CO_2	53	-09	-17	09	-25	-24	11	44	-12	-58
O_2	-44	-12	-11	12	62	-11	-36	36	-03	-34
Temperature	-39	-01	20	56	-46	-17	-05	26	-37	21
Grain weight	-34	29	-26	-39	04	-18	64	32	-14	11
Dust weight	02	-41	36	-02	20	-72	26	-29	-01	01
Moisture	36	-19	-51	19	25	-18	-09	16	-10	63
Aspergillus	01	53	-08	37	-01	-36	-01	-02	66	-01
Bacteria	-19	-49	-02	-32	-37	-04	-15	35	56	16
Tarsonemus	-14	-41	-26	47	06	35	54	-17	22	-17
Variability accounted for (%)	28	26	15	10	7	6	4	2	<2	<1

[a]Decimal points are omitted.

Table 28. Correlation coefficient matrix for the Rhyzopertha-Sitophilus-Tribolium system at week 60.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11
1. FAV											
2. CO_2	36										
3. O_2	04	-70									
4. Temperature	01	-49	43								
5. Grain weight	-02	36	-19	-48							
6. Dust weight	02	-61	43	49	-16						
7. Moisture	-58	-69	33	70	-47	38					
8. Germ damage	-02	-39	36	50	-85	23	48				
9. Endosperm damage	-17	-54	45	59	-85	24	63	82			
10. Bacteria	-55	-53	22	30	-25	28	56	21	35		
11. Tribolium	10	03	-20	-13	-13	09	-12	15	-04	08	

[a]Decimal points are omitted.

Table 29. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Rhyzopertha-Sitophilus-Tribolium system at week 60.

Variable	Principal Component										
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}
FAV	-14	-55	43	-09	-11	-39	-01	-25	-20	-43	-17
CO_2	-37	-23	-21	15	-28	-20	-01	54	35	-20	42
O_2	27	03	50	06	56	-10	-34	22	12	-03	41
Temperature	35	-09	20	11	-60	-17	-46	07	06	46	-09
Grain weight	-33	41	27	-06	-02	08	-28	38	12	-25	-58
Dust weight	25	10	44	-43	-31	19	58	20	18	-04	12
Moisture	39	23	-15	11	-30	24	-25	-10	-18	-68	22
Germ damage	34	-39	-18	03	14	15	09	61	-44	-01	-28
Endosperm damage	39	-24	-16	18	15	01	08	-12	72	-19	-37
Bacteria	26	38	-25	-22	05	-79	14	11	-08	-08	-06
Tribolium	-02	-21	-27	-82	06	12	-41	-03	13	-01	04
Variability accounted for (%)	44	16	13	10	6	4	3	2	1	<1	<1

[a]Decimal points are omitted.

Table 30. Correlation coefficient matrix for the Cryptolestes-Oryzaephilus-Tribolium system at week 60.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13
1. FAV													
2. CO_2	28												
3. O_2	18	-79											
4. Temperature	-31	-48	34										
5. Grain weight	-01	12	-01	15									
6. Dust weight	-27	19	-31	-41	-16								
7. Moisture	01	35	-33	-52	13	71							
8. Germ damage	-03	-13	08	-04	-33	09	07						
9. Endosperm damage	-18	-33	08	-03	-52	21	02	37					
10. Aspergillus	-15	08	04	07	-07	26	16	-14	-12				
11. Bacteria	13	24	-13	-31	-10	24	18	14	07	28			
12. Tribolium	-01	29	-35	-37	15	37	27	-12	32	10	16		
13. Cryptolestes	03	28	-34	-48	-07	73	80	12	20	20	33	49	

[a]Decimal points are omitted.

Table 31. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Cryptolestes-Oryzaephilus-Tribolium system at week 60.

Variable	Principal Component												
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}
FAV	-02	-21	-60	-33	-29	-15	-17	-32	-28	-13	-18	-01	-36
CO_2	-31	-40	-21	11	35	05	01	-24	-05	03	-32	-16	61
O_2	31	22	01	-44	-46	-07	-05	03	17	-08	-15	-01	61
Temperature	36	05	36	03	13	01	22	-35	-66	-19	-14	22	07
Grain weight	03	-44	29	04	-42	-01	55	-03	10	16	-19	-37	-16
Dust weight	-39	21	27	-02	-07	18	-15	16	01	-58	-52	-15	-15
Moisture	-41	02	12	-07	-32	36	-06	-04	-12	47	-15	56	02
Germ damage	-03	40	-29	01	07	42	50	-45	32	-12	04	02	-05
Endosperm damage	-07	57	-10	25	-03	-35	01	-04	-19	47	-35	-32	-02
Aspergillus	-11	01	41	-56	33	-16	-17	-43	25	22	-01	-12	-17
Bacteria	-22	05	-16	-48	28	-20	54	48	-19	01	-03	13	01
Tribolium	-30	-02	08	24	-17	-67	16	-24	22	-26	09	39	07
Cryptolestes	-44	13	09	-08	-24	06	01	-12	-38	-09	61	-39	18
Variability accounted for (%)	30	16	12	9	8	8	6	4	3	2	1	< 1	< 1

[a]Decimal points are omitted.

Table 32. Correlation coefficient matrix for the Control system, weeks 0-60.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12
1. FAV												
2. CO_2	01											
3. O_2	-27	-73										
4. Temperature	26	27	-26									
5. Grain weight	-39	-08	15	-08								
6. Dust weight	60	-15	-08	07	-25							
7. Moisture	08	13	-12	-28	02	17						
8. Alternaria	-80	-14	31	-44	36	-47	-02					
9. Aspergillus	-04	29	19	13	-10	-08	-10	-09				
10. Bacteria	82	-10	-17	18	-29	51	08	-69	-32			
11. Germination	-88	-21	40	-31	39	-49	-08	78	-22	-73		
12. Tarsonemus	-21	40	-13	22	03	-12	05	05	42	-25	-03	

[a]Decimal points are omitted.

Table 33. Correlation coefficient matrix for the Rhyzopertha-Sitophilus-Tribolium system, weeks 0 - 60.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. FAV																
2. CO_2	69															
3. O_2	-40	-53														
4. Temperature	24	40	-13													
5. Grain weight	-34	-18	14	-37												
6. Dust weight	51	40	-29	31	-27											
7. Moisture	13	04	-20	23	-35	28										
8. Germ damage	53	60	-32	54	-70	36	32									
9. Endosperm damage	43	31	-20	51	-84	41	46	80								
10. Alternaria	-72	-55	40	-31	40	-65	-43	-57	-53							
11. Aspergillus	15	25	-05	02	12	05	-42	10	-14	02						
12. Bacteria	58	30	-31	17	-33	45	64	36	46	-67	-41					
13. Germination	-83	-63	45	-25	36	-63	-45	-58	-51	82	-12	-72				
14. Tribolium	25	24	02	52	-35	17	-15	46	43	-13	26	-15	-12			
15. Rhyzopertha	22	22	05	41	-26	17	-29	34	30	-09	34	-16	-10	76		
16. Sitophilus	-34	01	11	14	12	-15	-28	02	-11	34	25	-50	35	35	38	

[a]Decimal points are omitted.

Table 34. Correlation coefficient matrix for the Cryptolestes-Oryzaephilus-Tribolium system, weeks 0 - 60.[a]

Variable	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1. FAV																
2. CO_2	41															
3. O_2	-33	-78														
4. Temperature	26	43	-27													
5. Grain weight	-43	-23	27	-17												
6. Dust weight	15	36	-33	-01	-01											
7. Moisture	34	19	-11	-10	-05	39										
8. Germ damage	65	60	-42	52	-39	11	11									
9. Endosperm damage	54	32	-24	17	-43	10	23	58								
10. Alternaria	-65	-54	40	-41	41	-22	-27	-69	-55							
11. Aspergillus	-10	27	-18	22	09	23	-08	02	-14	-08						
12. Bacteria	61	17	-15	04	-37	04	28	46	53	-63	-44					
13. Germination	-90	-51	39	-34	43	-23	-27	-64	-54	86	-13	-70				
14. Tribolium	14	47	-18	16	-04	65	39	26	15	-27	33	-03	-28			
15. Oryzaephilus	-58	02	04	17	21	-06	-14	-06	-19	42	09	-57	53	07		
16. Cryptolestes	15	51	-26	16	-03	65	35	26	11	-25	29	-01	-26	84	03	

[a]Decimal points are omitted.

Table 35. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Control system, weeks 0 - 60.

Variable	Principal Component											
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}
FAV	-46	12	01	03	02	13	11	05	14	21	52	64
CO_2	-09	-54	-26	-28	-13	-05	-07	11	-34	61	-17	09
O_2	21	40	28	37	23	-21	27	-08	-26	57	-11	01
Temperature	-19	-26	47	-31	29	-33	-08	-53	29	07	-07	01
Grain weight	24	01	-05	-34	73	53	01	10	-06	03	03	01
Dust weight	-31	20	-11	27	24	-02	-83	02	-07	10	-10	-06
Moisture	-04	02	-76	16	24	-18	22	-49	11	-03	-04	01
Alternaria	43	02	-12	01	-10	-03	-19	18	76	36	-05	09
Aspergillus	-02	-42	17	56	-04	52	05	-24	09	-03	-32	20
Bacteria	-40	24	-03	-16	09	-06	24	33	18	-08	-71	18
Germination	45	07	-01	-12	-05	-19	-23	-14	-26	-26	-20	70
Tarsonemus	04	-45	02	34	41	-45	08	48	03	-20	14	08
Variability accounted for (%)	35	20	11	8	8	5	4	4	2	2	< 1	< 1

[a]Decimal points are omitted.

Table 36. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Rhyzopertha-Sitophilus-Tribolium system, weeks 0 - 60.

Variable	Principal Component															
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}
FAV	32	-01	-29	24	-11	-26	-01	-17	-05	-04	-24	-37	-20	-30	-38	-42
CO_2	27	11	-35	-30	-05	-23	22	-17	-34	-04	-12	38	-32	08	43	-01
O_2	-19	08	28	61	21	10	45	-37	-32	-03	-04	07	-09	02	06	01
Temperature	21	24	21	-24	37	-25	54	38	01	24	04	-29	02	-04	-10	08
Grain weight	-26	-06	-39	-08	45	-16	15	-15	16	-22	03	23	48	-37	-03	-09
Dust weight	27	-03	-13	15	50	39	-29	41	-36	-06	-24	15	02	09	-07	-09
Moisture	19	-29	32	-23	21	30	16	-18	47	-13	-10	24	-38	-09	-09	-25
Germ damage	33	17	13	-14	-29	09	18	-15	-17	-12	08	34	44	22	-52	-07
Endosperm damage	31	06	35	03	-21	12	-07	05	-09	-01	-10	01	31	-63	43	-05
Alternaria	-34	11	12	-11	-13	-07	03	07	06	16	-86	14	06	01	-11	07
Aspergillus	-01	32	-42	13	-20	55	34	10	32	15	-04	-10	10	10	19	-21
Bacteria	28	-33	06	08	16	-18	-07	-27	13	34	-16	-15	40	45	30	-20
Germination	-35	11	21	-12	-04	-13	-05	23	-16	-03	15	08	-01	10	11	-80
Tribolium	14	45	13	14	11	-21	-13	01	30	-67	-13	-11	03	26	18	03
Rhyzopertha	11	46	03	24	15	-20	-30	-08	28	49	15	43	-12	-10	-09	-03
Sitophilus	-10	38	04	-44	25	29	-24	-52	-20	10	-01	-35	-01	-03	-03	-02
Variability accounted for (%)	39	19	11	6	5	4	4	3	2	2	1	1	1	1	<1	<1

[a]Decimal points are omitted.

Table 37. Principal-component matrix with loadings[a] showing the effects of the principal components on the variables and the percentage of variability accounted for by each principal component for the Cryptolestes-Oryzaephilus-Tribolium system, weeks 0 - 60.

Variable	Principal Component															
	C_1	C_2	C_3	C_4	C_5	C_6	C_7	C_8	C_9	C_{10}	C_{11}	C_{12}	C_{13}	C_{14}	C_{15}	C_{16}
FAV	-36	-21	08	13	-06	-13	-01	-07	-01	-18	-08	-08	-08	-09	-39	-76
CO_2	-29	25	-23	-06	34	-17	-06	06	-15	01	-03	13	26	72	02	-11
O_2	23	-14	26	01	-71	14	01	01	-04	-15	04	07	08	54	-02	-05
Temperature	-17	10	-47	-01	-43	-21	-10	-43	30	47	-01	-10	08	-03	04	-02
Grain weight	20	16	12	16	-15	-80	01	46	02	08	-02	01	-01	-07	-03	02
Dust weight	-15	35	34	-01	06	10	-21	08	80	-17	10	-03	01	06	-01	-01
Moisture	-14	09	45	-25	06	-27	63	-44	-04	05	16	-06	01	04	04	08
Germ damage	-32	-02	-26	-20	-19	-08	-04	17	-11	-41	68	-18	01	-11	07	13
Endosperm damage	-26	-14	-02	-35	-11	25	36	59	13	46	-06	-01	-03	02	-04	-06
Alternaria	37	08	04	-14	13	08	-07	01	-05	15	13	-71	38	05	-32	-10
Aspergillus	-03	34	-16	59	-09	21	49	12	-01	-12	-06	-29	-02	01	28	-11
Bacteria	-28	-34	19	-08	-02	-14	-21	02	-01	-10	-37	-44	13	01	59	05
Germination	37	10	-02	-25	09	01	-05	-02	02	12	29	11	-13	-02	55	-59
Tribolium	-18	43	22	-07	-22	14	-13	03	-31	04	-10	24	57	-37	06	-10
Oryzaephilus	17	27	-31	-52	-13	-06	17	-01	07	-46	-49	-06	-12	-06	-08	-03
Cryptolestes	-16	42	21	-07	-11	08	-30	-01	-35	20	-03	-25	-63	09	-05	05
Variation accounted for (%)	37	18	11	6	6	5	4	4	2	2	1	1	1	1	<1	<1

[a]Decimal points are omitted.

Figure 28. Diagram illustrating the changing relationships among variables for principal component 1 in the Control system as revealed by tri-weekly principal component analyses of multivariate data.

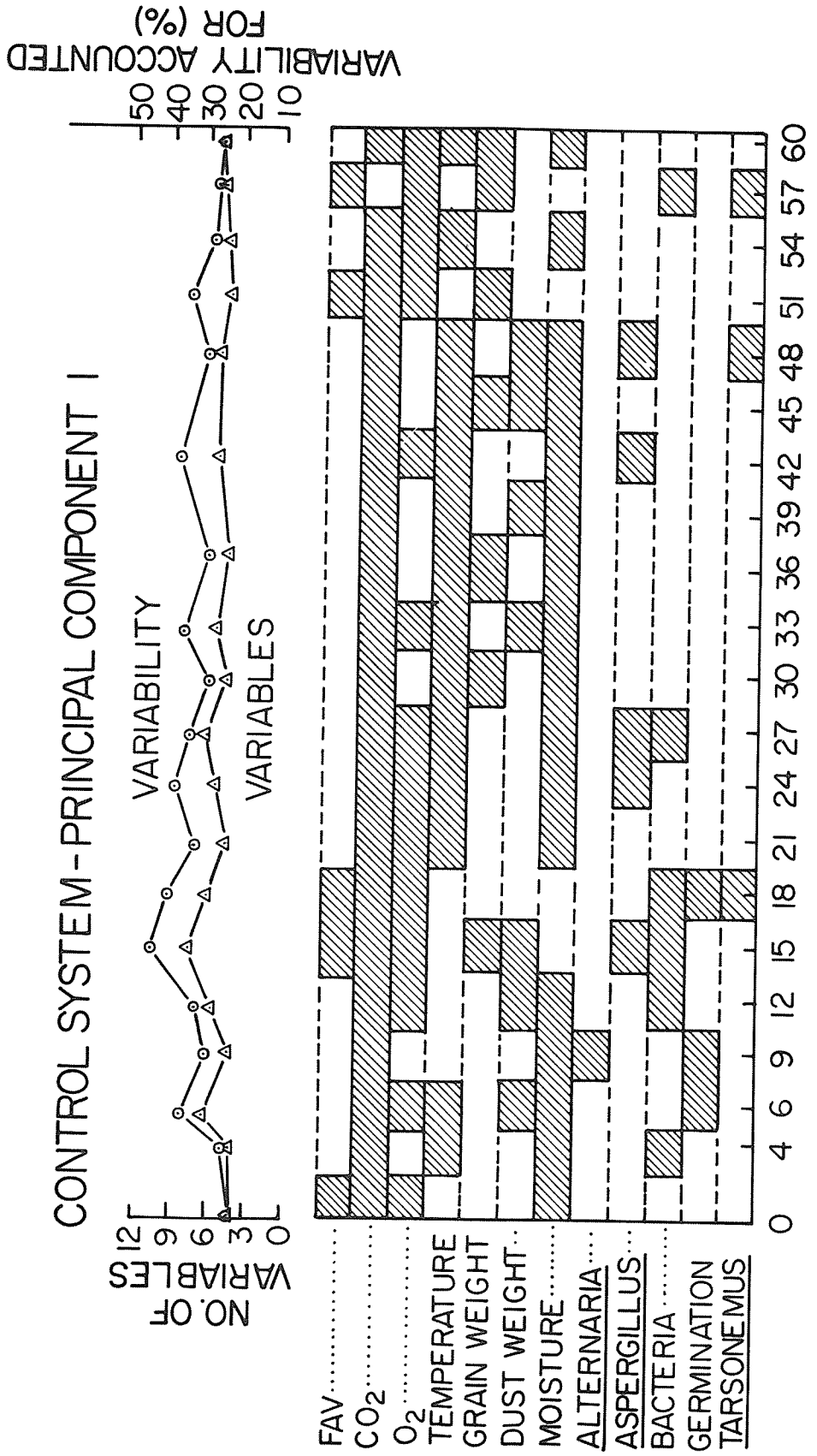

CONTROL SYSTEM-PRINCIPAL COMPONENT I
VARIABILITY ACCOUNTED FOR (%)
50 40 30 20 10
NO. OF VARIABLES
12 9 6 3 0
VARIABILITY
VARIABLES
FAV
CO2
O2
TEMPERATURE
GRAIN WEIGHT
DUST WEIGHT
MOISTURE
ALTERNARIA
ASPERGILLUS
BACTERIA
GERMINATION
TARSONEMUS
0 4 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60
WEEK

Figure 29. Diagram illustrating the changing relationships among variables for principal component 2 in the Control system as revealed by tri-weekly principal component analyses of multivariate data.

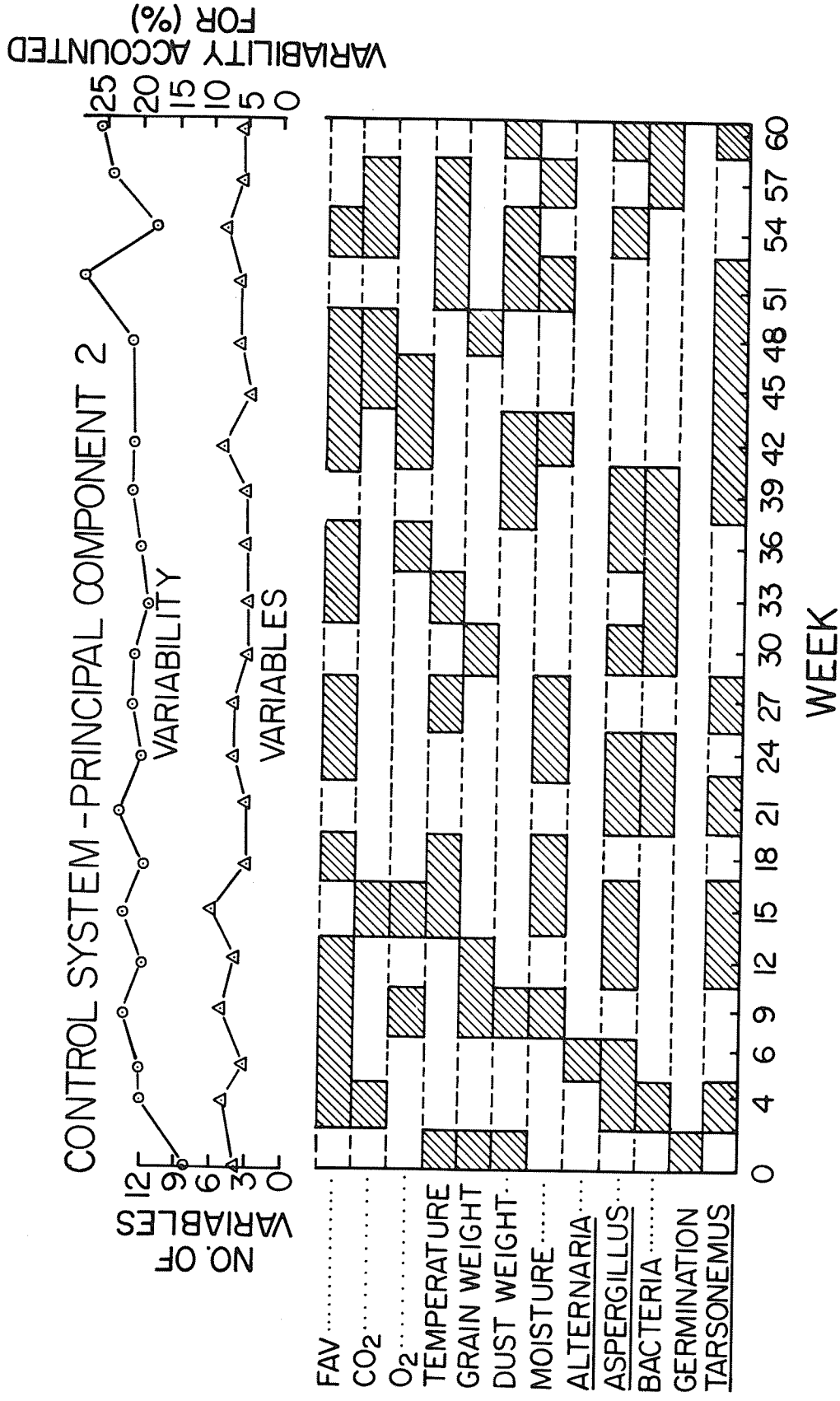

CONTROL SYSTEM - PRINCIPAL COMPONENT 2
VARIABILITY ACCOUNTED FOR (%)
25
20
15
10
5
0
VARIABILITY
VARIABLES
NO. OF VARIABLES
12
9
6
3
0
FAV
CO2
O2
TEMPERATURE
GRAIN WEIGHT
DUST WEIGHT
MOISTURE
ALTERNARIA
ASPERGILLUS
BACTERIA
GERMINATION
TARSONEMUS
0 4 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60
WEEK

Figure 30. Diagram illustrating the changing relationships among variables for principal component 1 in the Rhyzopertha-Sitophilus-Tribolium system as revealed by tri-weekly principal component analyses of multivariate data.

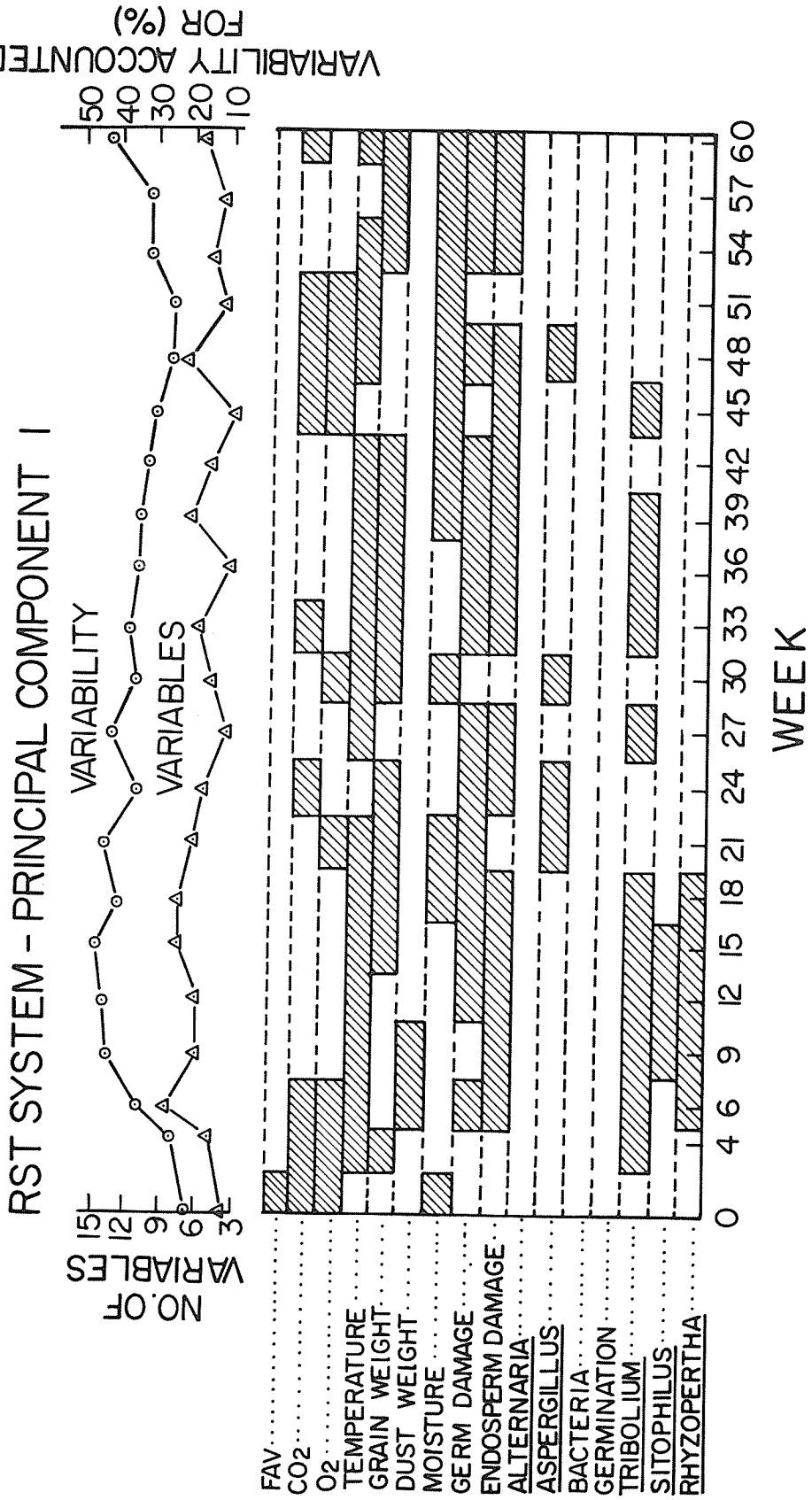

RST SYSTEM - PRINCIPAL COMPONENT I
VARIABILITY ACCOUNTED FOR (%)
50 40 30 20 10
NO. OF VARIABLES
15 12 9 6 3
VARIABILITY
VARIABLES
FAV
CO2
O2
TEMPERATURE
GRAIN WEIGHT
DUST WEIGHT
MOISTURE
GERM DAMAGE
ENDOSPERM DAMAGE
ALTERNARIA
ASPERGILLUS
BACTERIA
GERMINATION
TRIBOLIUM
SITOPHILUS
RHYZOPERTHA
0 4 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60
WEEK

Figure 31. Diagram illustrating the changing relationships among variables for principal component 2 in the Rhyzopertha-Sitophilus-Tribolium system as revealed by tri-weekly principal component analyses of multivariate data.

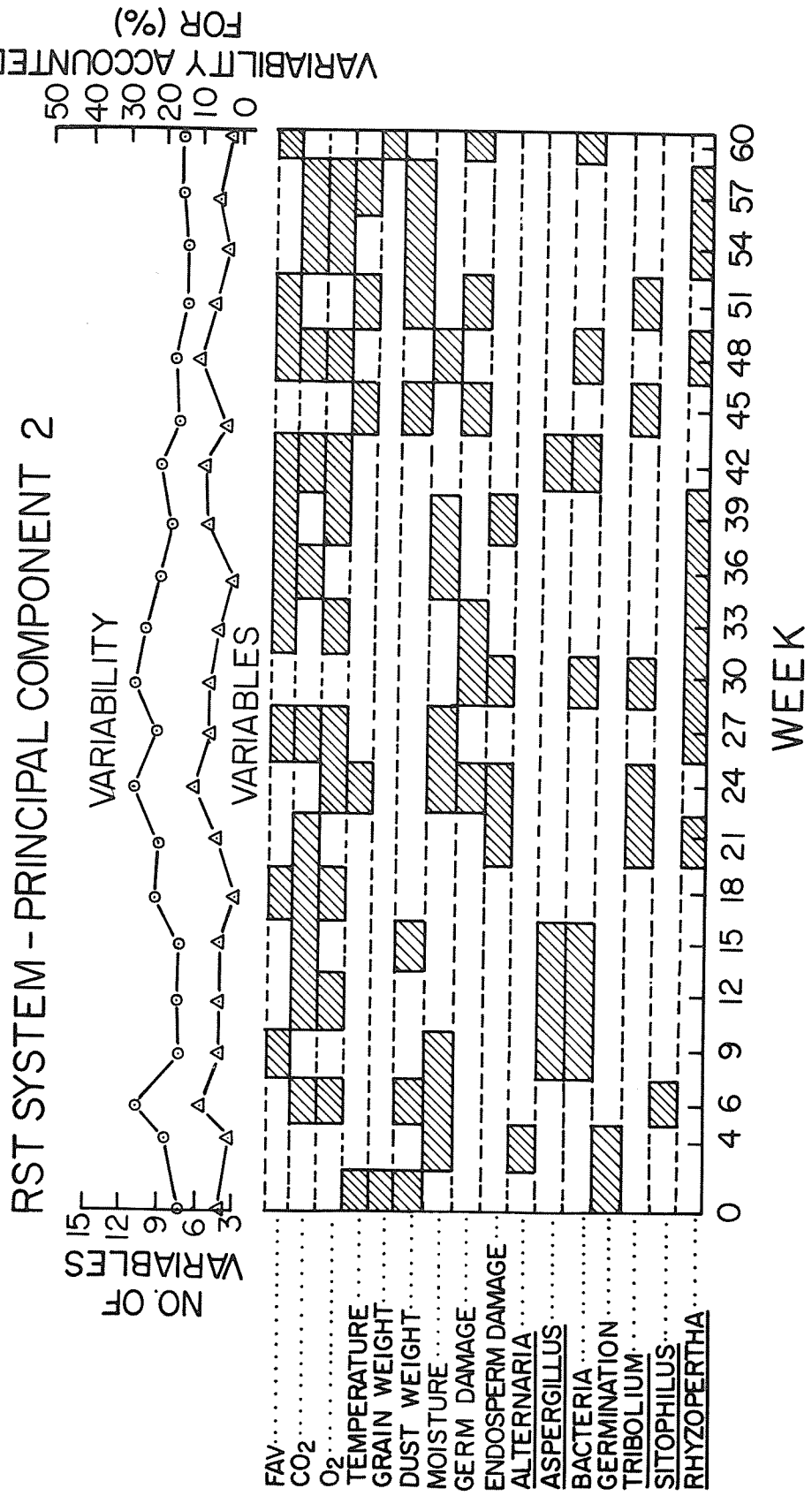

RST SYSTEM - PRINCIPAL COMPONENT 2
VARIABILITY
VARIABLES
NO. OF VARIABLES
15 12 9 6 3
VARIABILITY ACCOUNTED FOR (%)
50 40 30 20 10 0
FAV
CO2
O2
TEMPERATURE
GRAIN WEIGHT
DUST WEIGHT
MOISTURE
GERM DAMAGE
ENDOSPERM DAMAGE
ALTERNARIA
ASPERGILLUS
BACTERIA
GERMINATION
TRIBOLIUM
SITOPHILUS
RHYZOPERTHA
0 4 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60
WEEK

Figure 32. Diagram illustrating the changing relationships among variables for principal component 1 in the Cryptolestes-Oryzaephilus-Tribolium system as revealed by tri-weekly principal component analyses of multivariate data.

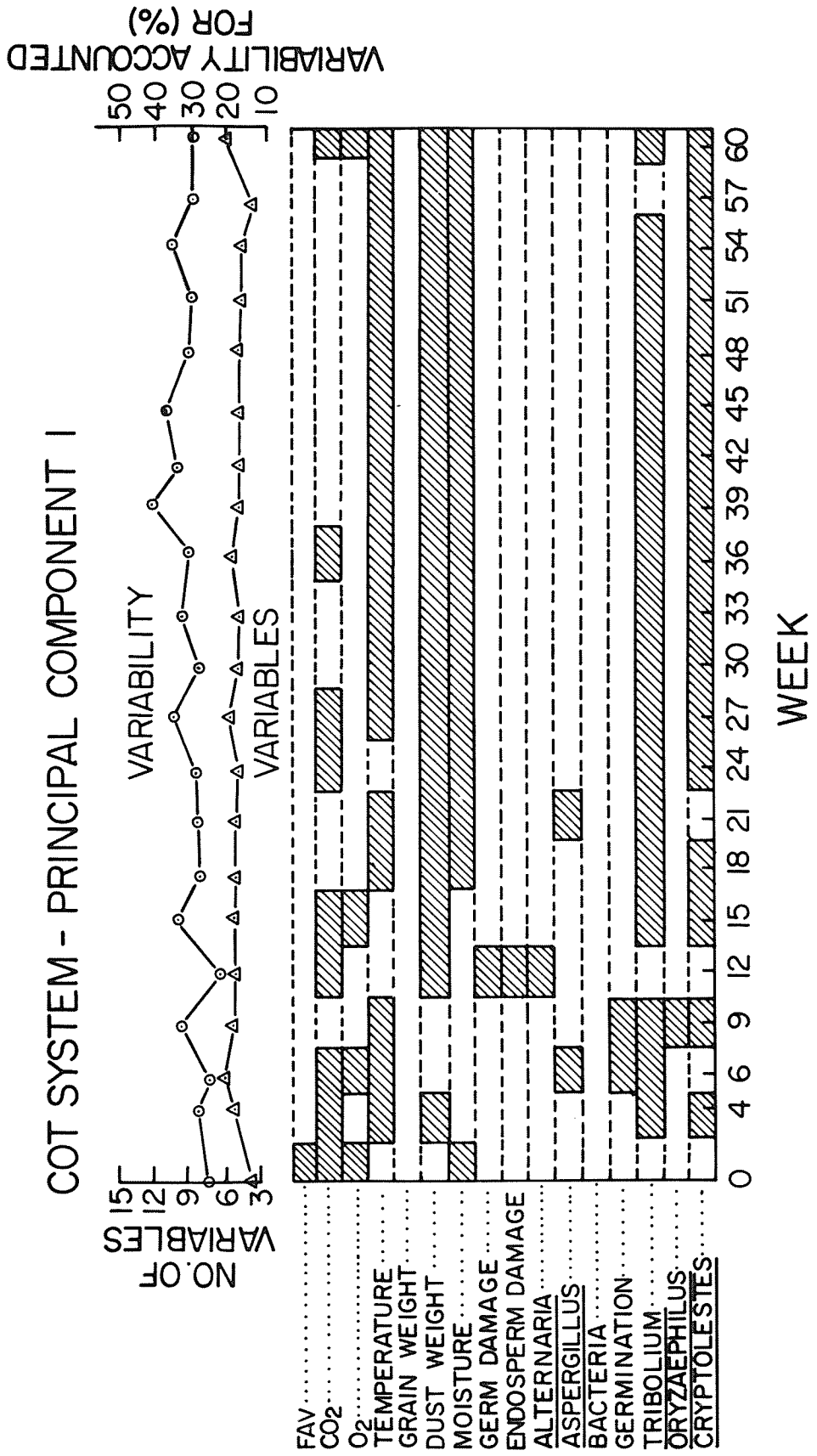
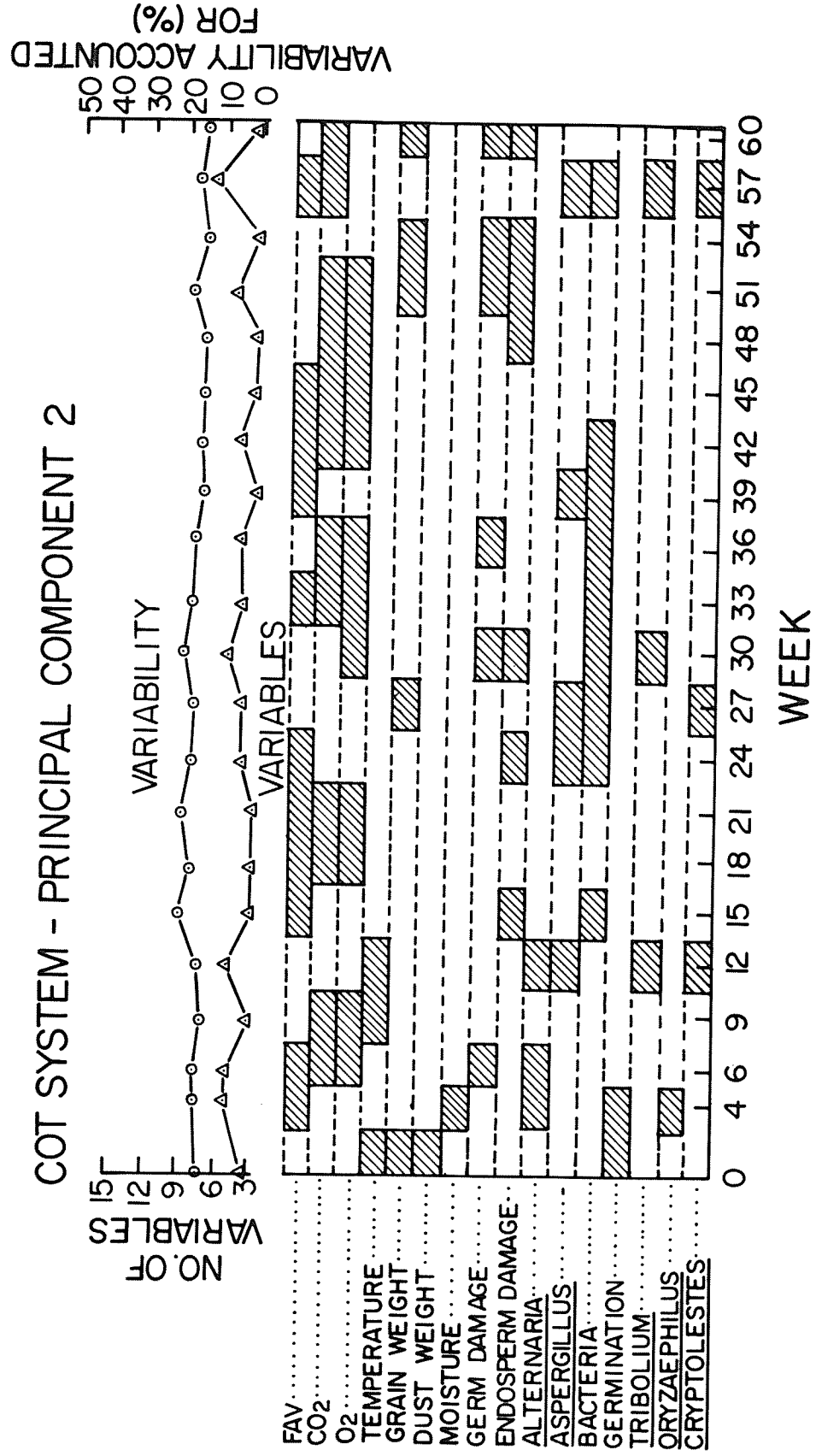

COT SYSTEM - PRINCIPAL COMPONENT I
VARIABILITY ACCOUNTED FOR (%)
50 40 30 20 10
NO. OF VARIABLES
15 12 9 6 3
VARIABILITY
VARIABLES
FAV
CO2
O2
TEMPERATURE
GRAIN WEIGHT
DUST WEIGHT
MOISTURE
GERM DAMAGE
ENDOSPERM DAMAGE
ALTERNARIA
ASPERGILLUS
BACTERIA
GERMINATION
TRIBOLIUM
ORYZAEPHILUS
CRYPTOLESTES
0 4 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60
WEEK

Figure 33. Diagram illustrating the changing relationships among variables for principal component 2 in the Cryptolestes-Oryzaephilus-Tribolium system as revealed by tri-weekly principal component analyses of multivariate data.


COT SYSTEM - PRINCIPAL COMPONENT 2
VARIABILITY ACCOUNTED FOR (%)
0 10 20 30 40 50
NO. OF VARIABLES
3 6 9 12 15
VARIABILITY
VARIABLES
FAV
CO2
O2
TEMPERATURE
GRAIN WEIGHT
DUST WEIGHT
MOISTURE
GERM DAMAGE
ENDOSPERM DAMAGE
ALTERNARIA
ASPERGILLUS
BACTERIA
GERMINATION
TRIBOLIUM
ORYZAEPHILUS
CRYPTOLESTES
0 4 6 9 12 15 18 21 24 27 30 33 36 39 42 45 48 51 54 57 60
WEEK

accounted for by each component for wk 0, 9, 21, and 60 (Tables 13, 15, 21, 27) give a complete summary of analysis at the beginning of the study, at a point during the rapid increase in insect numbers, at a point during the initial decline in insect numbers, and at the end of the study. Figures 28 and 29 show that principal components 1 and 2 accounted for approximately 50% of the variation in the system. The number of variables describing the variation remained fairly consistent throughout the study. Principal component 1 was essentially an abiotic component involving long-term relationships among CO_2, O_2, temperature, and moisture (Fig. 28). High temperature and moisture levels were related to high CO_2 and low O_2 concentrations. Principal component 2 mainly demonstrated relationships among FAV, moisture, Aspergillus, bacteria, and Tarsonemus. High moisture content of the wheat was associated with large populations of Aspergillus, bacteria, and Tarsonemus. It appeared that such collective invasion of organisms resulted in increasing FAV levels. Aspergillus and Tarsonemus were usually positively correlated to one another and negatively correlated to bacterial infection. The number of variables involved at the time of each PCA ranged from four to six.

RST system - Principal components 1 and 2 together accounted for about 55 - 60% of the variation in this system with fewer than half the variables monitored playing a meaningful role in each component (Fig. 30 and 31). The main variables interacting in principal component 1 were temperature, grain weight, moisture, germ damage, endosperm damage, Tribolium; and Sitophilus and Rhyzopertha only between wk 4 and wk 18. Populations of the three insect species were positively correlated to germ damage, endosperm damage, and grain weight loss obviously because of their feeding activity. Insect numbers were positively related to

higher temperatures and increased moisture levels. This is explainable because all three insect species thrive at high temperature and relatively high relative humidity (Howe 1965b).

Principal component 2 showed intermittent but steady interactions among FAV, CO_2, O_2, moisture, occasionally Aspergillus, and bacteria. After wk 18, Tribolium, Rhyzopertha, germ and endosperm damage were often also involved. Aspergillus and bacteria both increased early in the study and microfloral metabolism seemed to have led to increased CO_2 and moisture levels, lowered O_2 levels and were correlated with an FAV increase. As the variables, Tribolium and Rhyzopertha, increased in importance germ and endosperm damage in the grain, which are directly related to insect feeding, became more important. Correlation coefficient matrices for variables in the RST system for wk 0, 9, 21, and 60 (Tables 12, 16, 22, 28) and principal component matrices with principal component loadings and the percent variability accounted for by each component for wk 0, 9, 21, and 60 (Tables 13, 17, 23, 29) are of assistance in visualizing the interaction of variables represented in Figures 30 and 31.

COT system - The first two principal components accounted for about 50% of the variation in the system and involved only 3 - 6 variables at any one time (Figs. 32 and 33). Principal component 1 was usually explained for long segments of the time span by the relation among temperature, dust weight, moisture, Tribolium, and Cryptolestes (Fig. 32). Insect numbers were positively correlated to dust weight and moisture obviously because of feeding activity and increased metabolic activity. The total number of variables involved at each time of analysis remained remarkably uniform throughout the study period, although alignment of individual variables varied.

Principal component 2 is less consistent over time but its main variables were FAV, CO_2, O_2, germ damage, endosperm damage, Aspergillus, and bacteria (Fig. 33). Generally, Aspergillus and bacteria were negatively correlated to each other and both were negatively correlated to O_2, germ damage, and endosperm damage but positively correlated to CO_2. Aspergillus declined late in the study and bacteria increased as the FAV increased. Correlation coefficient matrices for the COT system at wk 0, 9, 21, and 60 (Tables 12, 18, 24, 30) and principal component matrices with principal component loadings and the variation account for by each component at wk 0, 9, 21, and 60 (Tables 13, 19, 25, 31) give more detailed results of the interactions among the variables at critical times.

(C) PCA of Cumulative Data

Principal component analysis of the cumulative data from wk 0 -60 for all three systems gave a somewhat different view of the changing patterns of relationships during the study, and should be considered along with the regular analyses to understand the systems. Correlation coefficient matrices (Tables 32, 33, 34), and principal component matrices with the proportion of total variance accounted for by each component, and the loadings for each principal components (Tables 35, 36, 37) are presented.

Control System - Principal component 1 involved FAV, dust weight, Alternaria, bacteria, and germination. An interpretation of the results (Table 35) indicates that a rise in FAV is related to a rise in dust weight and bacteria, and a decline in Alternaria infection and seed germination. Principal component 2 was explained by CO_2, O_2, Aspergillus, Tarsonemus, and possibly temperature; even though the last variable has a relatively low loading (-26). A positive correlation was found between Aspergillus, Tarsonemus, and CO_2 levels, all of which were negatively correlated to O_2 levels.

RST system - Principal component 1 involved FAV, germ damage, endosperm damage, Alternaria, and germination (Table 36). As FAV levels, germ and endosperm damage increased with time, Alternaria infection and seed germination decreased. Principal component 2 is predominantly an insect component and is described by the variables Tribolium, Rhyzopertha, Sitophilus, moisture, Aspergillus, and bacteria. The insect variables were positively related to Aspergillus but negatively related to bacterial infection. It appears that prolonged activity and interacting of the three species of insects generate additional moisture in the environment, which in turn allows greater microbial activity.

COT System - Principal component 1 was described by the variables FAV, germ damage, Alternaria, and germination; the last one is probably the most important variable in this group. Loss of seed germination and a decline in Alternaria infection appear to be related to increased FAV levels and germ damage. Principal component 2 is an insect component and is explained by the variables Aspergillus, bacteria, Tribolium, and Cryptolestes. When Tribolium, Cryptolestes, Aspergillus, and dust weight decline presumably because of overcrowding and pollution of the environment, bacterial infection increases.

Ecological Interpretation - The Control system underwent abiotic and biotic changes somewhat similar to those in the RST and COT systems, although the magnitude of those changes was smaller in the former system. As time progressed, seed germination and Alternaria infection declined. This relationship was previously reported by Wallace and Sinha (1962). Aspergillus glaucus and bacterial infection increased as did the CO_2 and FAV levels, while O_2 concentrations declined. FAV has previously been shown to increase steadily with time as the fungus-infected grain deteriorates

(Bronswijk and Sinha 1971, Lustig et al. 1977). Dust weight increased slightly probably as a result of microbial action; the composition of dust included fungal spores. The mite Tarsonemus granarius was abundant in the Control system which was free from competing insect pests, but reached large populations only when associated with the fungus Alternaria. This mite did not reproduce in the RST or COT systems possibly because of direct competition with the insects or indirect competition such as the presence of toxic secretions from the insects. Tribolium secretes quinones which partially inhibit fungal growth when insect populations are dense (Wyk et al. 1959).

The RST and COT systems followed similar patterns of changing relationships. The rapid population growth of Rhyzopertha and Tribolium, and the extensive germ and endosperm damage that resulted, allowed the microflora to proliferate and the moisture levels to rise. The rapid grain deterioration and high moisture levels allowed bacteria to replace fungi as the predominant form of microflora. The fat acidity levels increased until deterioration became advanced, when the FAV levels began a steady decline possibly because of advanced decomposition of the fats by bacteria and complete consumption by insects of much of the germ. The COT system maintained large populations of Cryptolestes and Tribolium until late in the study, but moisture levels in the grain did not rise sharply. Less extensive grain damage did not allow bacteria to invade the seed extensively and to decompose the wheat; and the FAV, however, increased steadily. The effect of the location of grain in the bulk wheat was often large and of great importance in the deterioration of the grain.

(D) Canonical Correlation Analysis - Canonical correlation analysis uses two sets of variables as basic input, each of which can be given theoretical

meaning as a set. Sinha et al. (1969b) designated these sets as predictor (independent variable) and criterion (dependent variable) groupings. The basic purpose of canonical correlation analysis is to form a linear combination from each of the sets of variables so that the correlation between the two linear combinations is maximized. Several pairs of linear groupings can be derived and are called canonical variates. In some ways, they are basically equivalent to principal components formed in PCA except that the criterion for their selection is different. Both techniques form linear combinations of the original variables but canonical correlation analysis accounts for a maximum amount of the relationship between two sets of variables rather than accounting for as much variance as possible within one set of variables (Warwick 1975; Sinha 1977).

Correlation coefficient matrices for this analysis are identical to those for PCA, wk 0 - 60 (Tables 32, 33, 34). The squared multiple correlations of each criterion variable with all of the other criterion variables, the squared multiple correlations of each predictor variable with all of the other predictor variables, the canonical correlation analysis, and the correlation of the canonical variables with the original variables (canonical variable loadings) are presented for the Control (Tables 38 - 40), RST (Tables 41 - 43), and COT systems (Tables 44 -46). The squared multiple correlations of variables determine the effective rank of a variable within its set. In the canonical correlation analysis, the eigenvalues are calculated, the canonical correlations which are the square roots of the eigenvalues are determined, and the maximum number of eigenvalues needed to explain the variation are determined by Bartlett's test. The canonical variable loadings are the correlations of the original variables with the canonical variables and are of value in interpreting the interrelations

Table 38. Squared multiple correlations of each criterion variable with all other criterion variables and of each predictor variable with all other predictor variables in the Control system, weeks 0 - 60.

Criterion variable	R^2	Predictor variable	R^2
FAV	0.838	Alternaria	0.587
CO_2	0.623	Aspergillus	0.367
O_2	0.607	Bacteria	0.631
Dust weight	0.407	Tarsonemus	0.194
Germination	0.822		

Table 39. Canonical correlation analysis for Control system variables, weeks 0 - 60.

Eigenvalue	Canonical correlation (R_c)	No. of eigenvalues	Bartlett's test for remaining eigenvalues		
			Chi-square	DF[a]	Significance level
		0	929.68	24	<0.001
0.834	0.913	1	263.18	15	<0.001
0.427	0.653	2	56.61	8	<0.001
0.114	0.338	3	11.64	3	<0.01
0.31	0.176				

[a]DF = degrees of freedom.

Table 40. Correlations of canonical variables with original variables in the Control system, weeks 0 - 60.[a]

Variable	Canonical variable 1	2	3	4
FAV	97	-20	-15	01
CO_2	04	63	-60	-30
O_2	-32	-30	21	46
Dust weight	57	-15	-20	28
Moisture	04	-03	-24	77
Germination	-96	-26	-01	-03
Alternaria	-89	-03	38	26
Aspergillus	09	81	37	-45
Bacteria	89	-29	-02	36
Tarsonemus	-13	87	-35	32

[a]Decimal points are omitted.

Table 41. Squared multiple correlations of each criterion variable with all other criterion variables and of each predictor variable with all other predictor variables in the Rhyzopertha-Sitophilus-Tribolium system, weeks 0 - 60.

Criterion variable	R^2	Predictor variable	R^2
FAV	0.729	Alternaria	0.570
CO_2	0.646	Aspergillus	0.262
Moisture	0.205	Bacteria	0.652
Germ damage	0.789	Tribolium	0.576
Endosperm damage	0.745	Rhyzopertha	0.581
Germination	0.721	Sitophilus	0.369

Table 42. Canonical correlation analysis for Rhyzopertha-Sitophilus-Tribolium system variables, weeks 0 - 60.

Eigenvalue	Canonical correlation (R_c)	No. of eigenvalues	Bartlett's test for remaining eigenvalues		
			Chi-square	DF[a]	Significance level
		0	1456.12	36	<0.001
0.821	0.906	1	491.96	25	<0.001
0.364	0.603	2	238.65	16	<0.001
0.203	0.450	3	111.97	9	<0.001
0.129	0.359	4	34.64	4	<0.001
0.059	0.243	5	0.64	1	0.425
0.001	0.034				

[a]DF = degrees of freedom.

Table 43. Correlations of canonical variables with original variables in the Rhyzopertha-Sitophilus-Tribolium system, weeks 0 - 60.[a]

Variable	Canonical variable					
	1	2	3	4	5	6
FAV	-86	15	06	46	-12	07
CO_2	-58	24	64	13	-25	35
Moisture	-42	-36	03	-23	73	32
Germ damage	-64	59	35	-17	29	-11
Endosperm damage	-64	58	-20	-32	31	14
Germination	97	06	-10	07	20	06
Alternaria	90	-08	-08	09	29	-28
Aspergillus	-12	-05	75	56	24	-21
Bacteria	-81	-28	-42	-29	01	-04
Tribolium	-18	89	17	10	36	-12
Rhyzopertha	-12	73	19	19	-13	-60
Sitophilus	34	39	62	-50	10	-30

[a]Decimal points are omitted.

Table 44. Squared multiple correlations of each criterion variable with all other criterion variables and of each predictor variable with all other predictor variables in the Cryptolestes-Oryzaephilus-Tribolium system, weeks 0 - 60.

Criterion variable	R^2	Predictor variable	R^2
FAV	0.852	Alternaria	0.579
CO_2	0.199	Aspergillus	0.402
Moisture	0.260	Bacteria	0.625
Dust weight	0.262	Tribolium	0.715
Germ damage	0.442	Cryptolestes	0.700
Germination	0.848		

Table 45. Canonical correlation analysis for *Cryptolestes-Oryzaephilus-Tribolium* system variables, weeks 0-60.

Eigenvalue	Canonical correlation (R_c)	No. of eigenvalues	Bartlett's test for remaining eigenvalues		
			Chi-square	DF[a]	Significance level
		0	1778.26	30	<0.001
0.877	0.936	1	606.28	20	<0.001
0.529	0.727	2	185.16	12	<0.001
0.239	0.489	3	32.25	6	<0.001
0.054	0.233	4	0.90	2	0.637
0.002	0.040				

[a]DF = degrees of freedom.

Table 46. Correlations of canonical variables with original variables in the Cryptolestes-Oryzaephilus-Tribolium system, weeks 0 - 60.[a]

Variable	Canonical variable				
	1	2	3	4	5
FAV	98	-16	-07	04	-06
CO_2	-41	14	17	14	53
Moisture	27	87	-28	21	-17
Dust weight	33	27	-59	28	41
Germ damage	70	12	07	-70	05
Germination	-96	09	-24	05	-10
Alternaria	-93	-03	-11	33	-09
Aspergillus	03	51	83	02	-21
Bacteria	83	-36	-30	31	01
Tribolium	30	90	-10	11	29
Cryptolestes	30	88	-27	01	-25

[a]Decimal points are omitted.

within the canonical variables (Frane 1977).

Control System - FAV and germination were the variables most strongly correlated to the entire criterion set (Table 38) while bacteria and Alternaria were the variables most strongly correlated to the entire predictor set (Table 38). Three canonical correlation coefficients are significant at the 1% level (Table 39). The first grouping of canonical variables indicated that Alternaria and bacteria predict 83% variation of FAV, O_2, dust weight, and germination. As the grain ages Alternaria, seed germination, and O_2 levels decline while bacteria increase, as do dust weight and FAV (Fig. 34A). The second grouping of canonical variables indicates that Aspergillus and Tarsonemus predict 43% variation of CO_2 and O_2 levels. When Aspergillus and Tarsonemus increase, CO_2 concentration increases and O_2 concentrations decline (Fig. 35B). The third grouping of variables indicated that Alternaria, Aspergillus, and Tarsonemus predicted 11% variation of CO_2 levels (Table 40).

RST System - The criterion variables FAV, CO_2, germ damage, endosperm damage, and germination were strongly correlated to the total criterion set. The predictor variables Alternaria, bacteria, Rhyzopertha, and Tribolium were strongly correlated with the entire predictor set (Table 41). Four canonical correlation coefficients were significant at the 1% level (Table 42). The first set of canonical variables indicated that Alternaria, bacteria, and Sitophilus predict 82% variation of FAV, CO_2, moisture, germ damage, endosperm damage, and germination (Fig. 35A). The loadings indicate that as Alternaria and Sitophilus decrease and bacteria increases, FAV, CO_2, moisture, germ damage, and endosperm damage increase as germination declines. The second set of canonical variables indicate that Rhyzopertha, Sitophilus, and Tribolium account for 36% variation of moisture,

Figure 34. Diagram representing canonical correlation between two sets of variables in the Control system, with canonical correlation coefficients of different magnitudes.

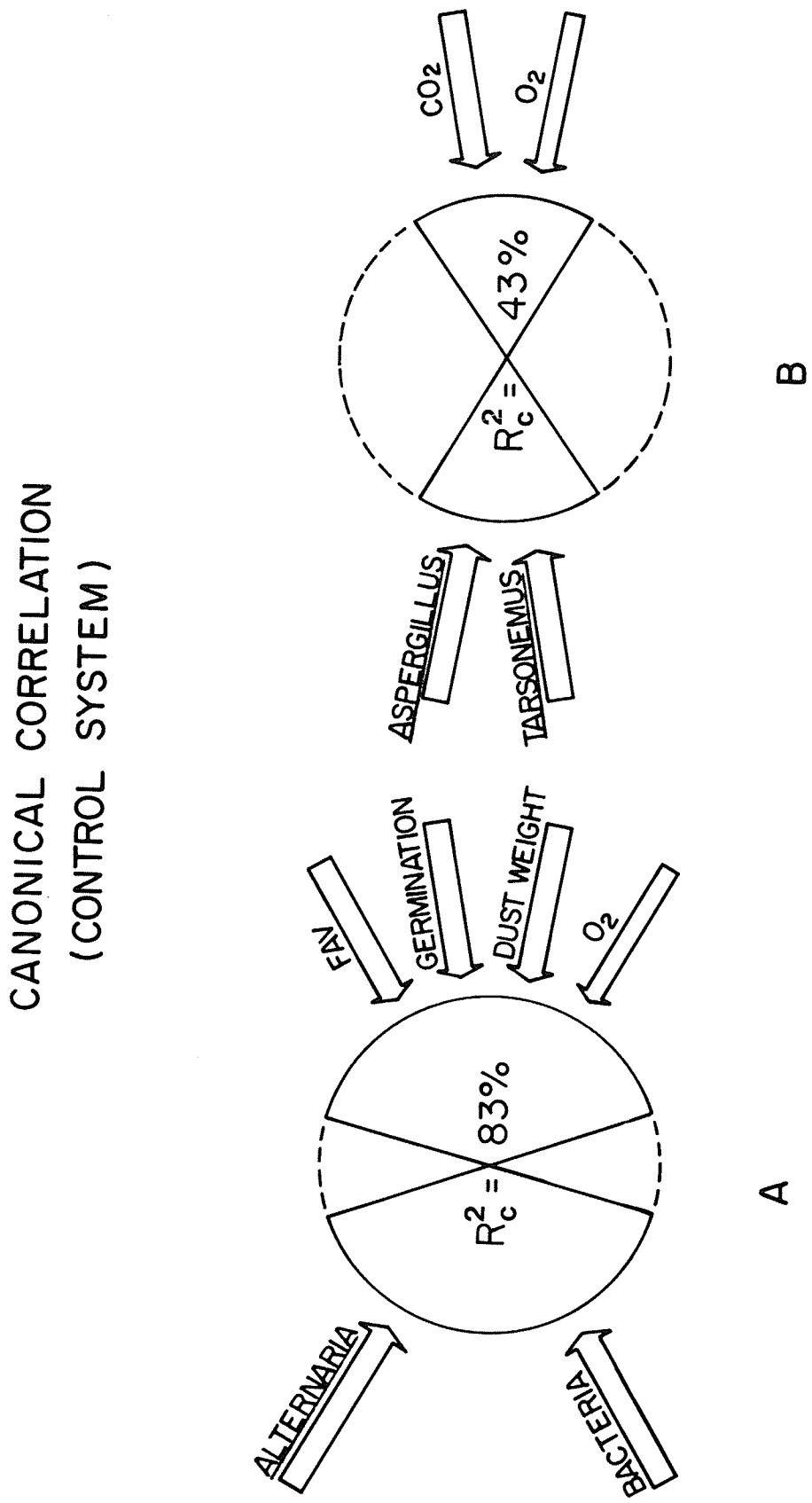
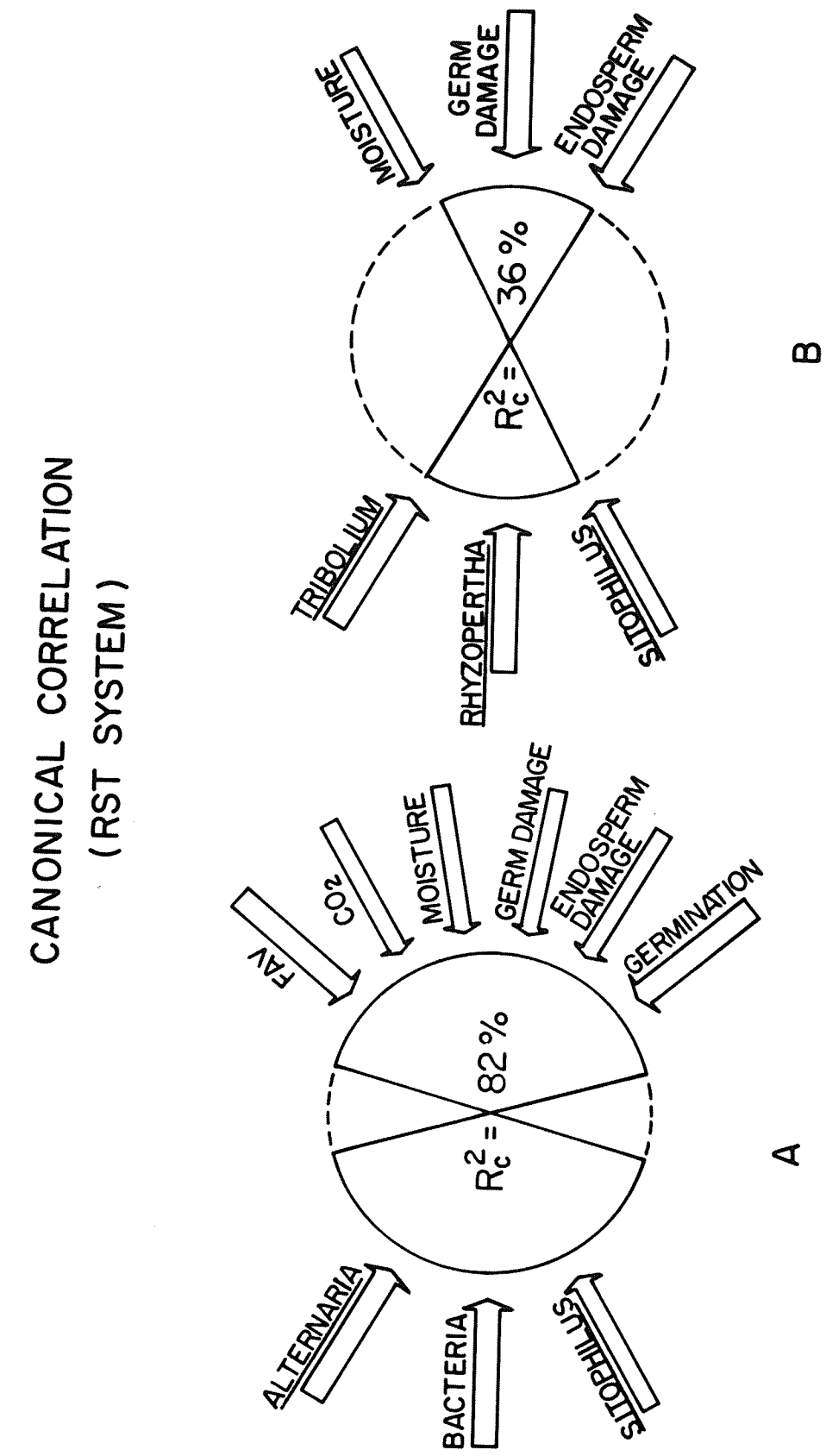

CANONICAL CORRELATION
(CONTROL SYSTEM)
ALTERNARIA
BACTERIA
FAV
GERMINATION
DUST WEIGHT
O2
R_c^2 = 83%
A
ASPERGILLUS
TARSONEMUS
CO2
O2
R_c^2 = 43%
B

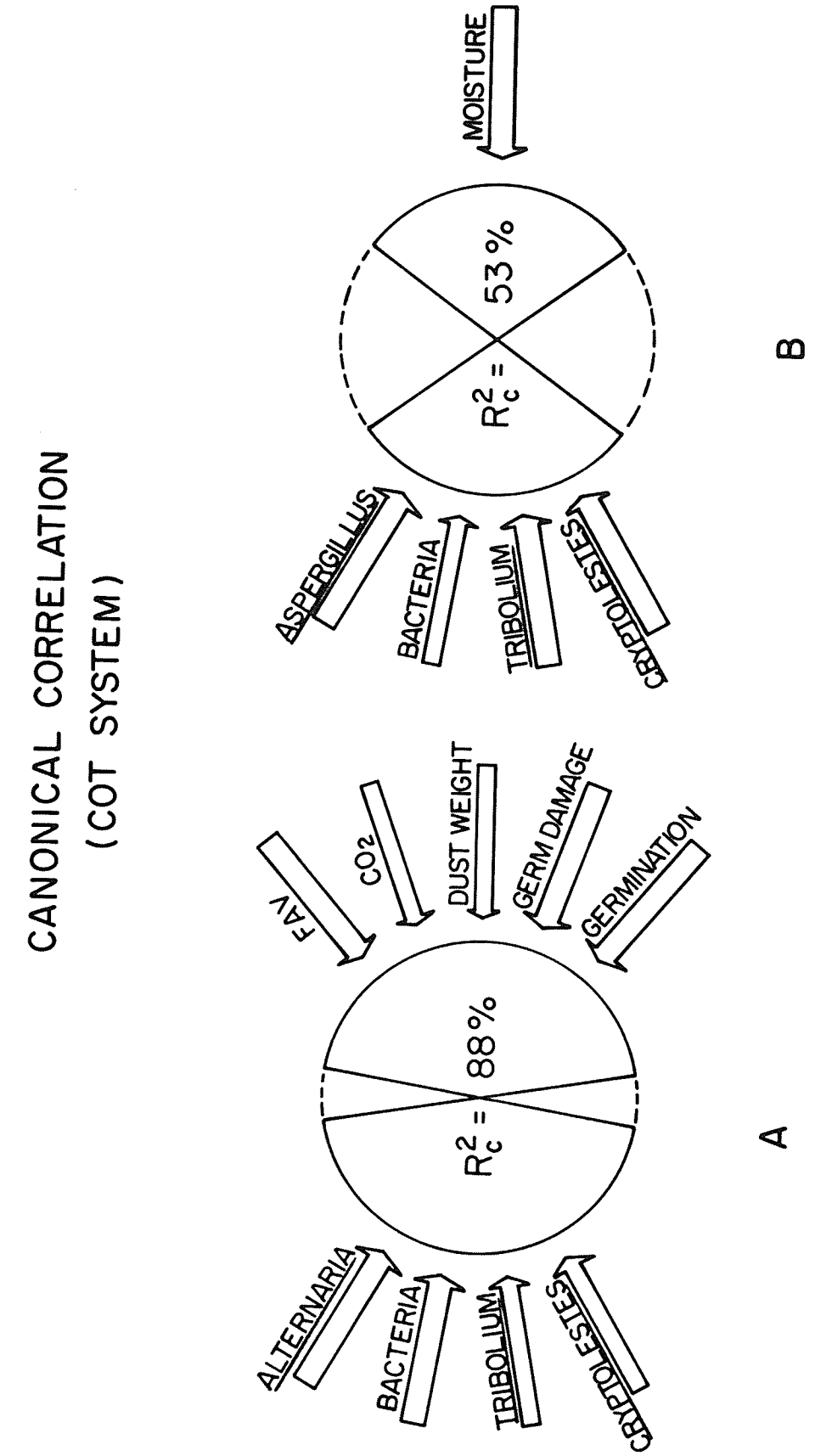
Figure 35. Diagram representing canonical correlation between two sets of variables in the Rhyzopertha-Sitophilus-Tribolium system with canonical co-relation coefficients of different magnitudes.


CANONICAL CORRELATION
(RST SYSTEM)
MOISTURE
GERM DAMAGE
ENDOSPERM DAMAGE
36 %
R_c^2 =
TRIBOLIUM
RHYZOPERTHA
SITOPHILUS
B
FAV
CO2
MOISTURE
GERM DAMAGE
ENDOSPERM DAMAGE
GERMINATION
82 %
R_c^2 =
ALTERNARIA
BACTERIA
SITOPHILUS
A

germ damage, and endosperm damage (Fig. 35B). The loadings indicate that as insect numbers increase, germ and endosperm damage increase and moisture levels decline. The third set of canonical variables indicate that Aspergillus, bacteria, and Sitophilus predict 20% variation of CO_2, and germ damage. The positive and negative signs on the loadings indicate that as Aspergillus and Sitophilus decline and bacteria increases, CO_2 levels and germ damage increase. The fourth set of canonical variables reveal that Aspergillus and Sitophilus predict 13% variation of FAV, and endosperm damage. While Aspergillus increases and Sitophilus decreases, FAV increases and endosperm damage decreases (Table 43).

COT System - The criterion variables FAV and germination are strongly correlated to the entire criterion set (Table 44). The predictor variables Cryptolestes and Tribolium are most strongly correlated to the entire predictor set (Table 44). Three canonical correlation coefficients were significant at the 1% level (Table 45). The first set of canonical variables revealed that Alternaria, bacteria, Cryptolestes, and Tribolium predict 88% variation of FAV, CO_2, dust weight, germ damage, and germination (Fig. 36A). The positive and negative loadings indicate that as Alternaria decreases, bacteria, Cryptolestes and Tribolium increase and FAV, dust weight, and germ damage increase, while CO_2 and germination decline. The second set of canonical variables reveals that Aspergillus, bacteria, Cryptolestes, and Tribolium predicted 53% variation of moisture (Fig. 36B). The loading indicated that a decline in Aspergillus, Cryptolestes, and Tribolium and an increase in bacterial infection was related to higher moistures. The third set of canonical variables revealed that Aspergillus and bacteria predicted 24% variation of dust weight (Table 46). Dust weight increases were related to an increase in bacteria and a decrease in Aspergillus.

Figure 36. Diagram representing canonical correlation between two sets of variables in the Cryptolestes-Oryzaephilus-Tribolium system with canonical correlation coefficients of different magnitudes.


CANONICAL CORRELATION
(COT SYSTEM)
ALTERNARIA
BACTERIA
TRIBOLIUM
CRYPTOLESTES
R_c^2 = 88 %
FAT
CO_2
DUST WEIGHT
GERM DAMAGE
GERMINATION
A
ASPERGILLUS
BACTERIA
TRIBOLIUM
CRYPTOLESTES
R_c^2 = 53 %
MOISTURE
B

A comparison of interrelations among variables in all three systems as revealed by PCA and canonical correlation is summarized in Table 47. As expected, the two techniques revealed similar patterns of interaction and agreed with the descriptive data summary although in a more clearly defined manner.

(E) Multiple Linear Regression Analysis

A problem that had only been partially resolved by descriptive summary and PCA involved the factors regulating populations of Tarsonemus granarius in the Control system. To resolve this question, multiple linear regression was undertaken on Control system data from wk 0 - 33, with Tarsonemus as the dependent variable and CO_2, Alternaria, Aspergillus, moisture, FAV, O_2, bacteria, and temperature as independent variables (White and Sinha 1978). The general form of the unstandardized multiple regression equation is:

$$Y' = A + B_1X_1 + B_2X_2 + ... B_kY_k$$

with Y' representing the estimated value for Y; A is the y-intercept, and B_i are regression coefficients. The multiple regression analysis is summarized in Table 48. The equation resulting from the analysis is:

$$Y'(\text{Tarsonemus}) = 0.539 + 1.044(CO_2) + 0.379(\text{Alternaria}) + 0.082(\text{Aspergillus}) - 1.078(\text{moisture}) + 0.140(\text{FAV}) + 0.682(O_2) + 0.046(\text{bacteria}) - 0.329(\text{temperature}).$$

The values associated with the independent variables are unstandardized partial regression coefficients. The total equation is significant ($P<.01$) and accounts for 25.7% of the variation in Tarsonemus numbers.

The unstandardized regression coefficients (B) for CO_2 and Alternaria were significant ($P<.01$) using a standard regression method while those of CO_2 and Alternaria ($P<.01$), and Aspergillus and O_2 ($P<.05$) were significant

Table 47. Comparison of the interrelations[a] among variables within the Control, Rhyzopertha-Sitophilus-Tribolium, and Cryptolestes-Oryzaephilus-Tribolium systems as revealed by principal components 1 and 2 and canonical variables 1 and 2.

	Principal component				Canonical variable			
	1		2		1		2	
System	Variable	Variability accounted for (%)	Variable	Variability accounted for (%)	Variable	R_c^2	Variable	R_c^2
Control	FAV Dust weight Bacteria Alternaria Germination	35	CO_2 Aspergillus Tarsonemus O_2	20	FAV Dust weight Bacteria O_2 Germination	0.83	CO_2 Aspergillus Tarsonemus O_2	0.43
RST	FAV Germ damage Endosperm damage		Moisture Bacteria		FAV CO_2 Germ damage Endosperm damage Moisture Bacteria		Moisture	
COT	Alternaria Germination	39	Aspergillus Tribolium Rhyzopertha Sitophilus	19	Alternaria Germination Sitophilus	0.82	Germ damage Endosperm damage Tribolium Rhyzopertha	0.36
	FAV Germ damage Alternaria Germination	37	Bacteria Dust weight Aspergillus Tribolium Cryptolestes	18	FAV Germ damage Dust weight Bacteria Tribolium Cryptolestes		Moisture Aspergillus Tribolium Cryptolestes	
					CO_2 Alternaria Germination	0.88	Bacteria	0.53

[a]Positively related variables grouped together, data from weeks 0 - 60 inclusive; loading cutoff level of 0.30.

using a hierarchical method (Kim and Kahout 1975) (Table 48, col. F^f, F^g).

Tarsonemus granarius is a mycophagous mite which feeds and multiplies on many types of fungi (Sinha 1964b). The rapid increase in Tarsonemus numbers between wk 6 - 9 and the sharp decline between wk 9 - 15 was probably related to the presence and decline of Alternaria, which is a favored diet of this mite (Sinha 1964b). Microbial activity results in increased CO_2 levels (Trisvyatskii 1966) which could explain the positive correlation between CO_2 and Tarsonemus. As Alternaria declined, Aspergillus glaucus gr. and bacteria infected more seeds. Aspergillus spp. are an alternate diet for this mite and probably permitted the populations to maintain themselves at lower levels. Further deterioration of the grain and an increase in bacterial infection, with a decrease in Aspergillus infection, led to a decline in mite numbers.

Ecological Processes Involved in stored-grain Ecosystems

In this study, arthropod populations were introduced to discrete ecosystems containing limited and non-renewable resources in the form of dormant wheat seeds. Adult insects were introduced and dispersal out of the systems was prevented. Populations were not started with a stable age distribution and it could be predicted that a very steep growth curve (exponential) would initially be evident, climbing to excessive density near the carrying capacity of the system, followed by a series of fluctuations, in some circumstances approaching an assymptote by a series of damped oscillations. This type of growth curve was, in fact, typical. The classical logistic curve (sigmoid) of population growth is rarely applicable to animals with complex life-histories such as insects (Andrewartha and Birch 1954).

Niche overlap was extensive throughout the various communities and

Table 48. The multiple regression summary table of the prediction equation for Tarsoneumus granarius numbers in a model ecosystem.

Variable	Multiple R[a]	R^2[b]	Simple R[c]	B[d]	Beta[e]	Std. error of B	F[f]	F[g]
CO_2	0.407	0.166	0.407	1.044	0.467	0.249	17.567**	42.150**
Alternaria	0.450	0.203	0.304	0.379	0.301	0.124	9.272**	9.415**
Aspergillus	0.471	0.221	0.177	0.082	0.131	0.061	1.794	4.580*
Moisture	0.481	0.231	-0.081	-1.078	-0.125	0.634	2.886	2.546
FAV	0.489	0.240	-0.164	0.140	0.156	0.141	0.986	2.290
O_2	0.505	0.255	-0.118	.682	0.184	0.380	3.222	3.995*
Bacteria	0.506	0.256	-0.194	0.046	0.071	0.089	0.270	0.254
Temperature	0.507	0.257	-0.098	-0.329	-0.035	0.739	0.198	0.254

*Significant at the .05 level, $F_{.05}$ (1,189) = 3.84; d.f. = N - k - 1 where N = sample size, k = number of independent variables.

**Significant at the .01 level, $F_{.01}$ (1,189) = 6.63.

[a]Multiple correlation coefficient.

[b]Coefficient of determination.

[c]Correlation coefficient.

[d]Unstandardized regression coefficient; constant = 0.539.

[e]Standardized regression coefficient.

[f]F statistic for standard regression, d.f. = 1,189.

[g]F statistic for hierarchial analysis, d.f. = 1,189.

competitive exclusion was likely to be an important factor in the extinction of certain species from the various systems.

Density-dependent population responses were of greatest importance in the systems studied because external environmental factors were maintained at favorable levels (Price 1975). In the insect populations cannibalism, predation, competition, and contamination of the environment acted as the major regulating factors with behavioral factors limiting distribution within a system leading to intensified population interaction. Facultative feeding on fungi by the insects would have slightly decreased competition for food in the form of wheat seeds (Sinha 1966b). However, both larvae and adults of all of the insect species were in direct competition for food, removing the possibility of a partitioning of resources.

The gradual succession of predominant microflora from Alternaria to Aspergillus to bacteria was caused mainly by both competition and the changing conditions within each system; primarily, increasing moisture content of the wheat and a steady deterioration of the substrate.

Energy transfer in the short food-chains found in stored-grain ecosystems is of importance to our understanding of the dynamic interactions involved. The interspecific relationship between daily net production efficiency of the adults of five stored-product beetles has been given by Campbell and Sinha (1978). The comparison of the net production energy, or the production of growth and reproduction/assimilation of energy by feeding, indicated that species with higher rates of population increase had higher production efficiencies. This conclusion implies that the higher the rate of reproduction of a species, the more efficiently it uses food energy. Tribolium castaneum had the highest net production efficiency, followed by Cryptolestes ferrugineus and, at considerably lower levels, Sitophilus

oryzae and Rhyzopertha dominica. This observation is of importance in visualizing the strategies used by each species in the colonization of a habitat. It is probable that the larger the value of r, the more rigorous the environment is for a population (Kormondy 1969) and the greater the need for efficient utilization of energy.

The flora and fauna invading stored-grain ecosystems must utilize the limited resources rapidly and, once large populations have been attained, disperse rapidly in search of new resources. Reproductive strategies to maximize survival under these conditions were shown by all of the species studied.

Chapter 4

SUMMARY AND CONCLUSIONS

The infestation of stored wheat by Rhyzopertha dominica, Sitophilus oryzae, and Tribolium castaneum or Cryptolestes ferrugineus, Oryzaephilus surinamensis, and Tribolium castaneum at 15.5% moisture content and 30°C led to rapid deterioration of the grain. Temperatures in the RST system were slightly higher than those of the COT or insect-free Control systems. Carbon dioxide levels were highest in the RST system and oxygen levels the lowest. The moisture content of the wheat in the RST system was considerably higher than either the COT or Control systems.

Seed germination and infection by the field fungus Alternaria alternata declined rapidly in all systems to 0% by week 15. The storage fungus Aspergillus glaucus group increased as Alternaria decreased and gradually Aspergillus was replaced by bacterial infection which increased steadily in all systems until the end of the study after 60 weeks. The high moisture content of the wheat in the RST system allowed Aspergillus candidus to replace A. glaucus for a short time prior to the decline of the fungi and increase in bacteria.

The mite Tarsonemus granarius reproduced extensively only in the Control system, possibly because of absence of competition from the insects (and also absence of metabolic by-products of insects) as found in the RST and COT systems. In the RST system, Sitophilus failed to multiply extensively, gradually becoming extinct. Rhyzopertha and Tribolium were found mainly at the top of the bulk grain. In the COT system, Oryzaephilus

failed to thrive and became extinct rapdily. After several weeks, Cryptolestes and Tribolium adults were most abundant at the bottom of the bulk grain. Larval mortality was high in both systems, probably because of cannibalism and predation. All arthropod species multiplied at an exponential rate initially followed by sharp oscillations.

Grain dust was produced extensively in the RST system and moderately produced in the COT system. Grain damage was greatest in the RST system, with both germ and endosperm being severely damaged especially near the top of the bulk grain. The germ of the wheat was consumed to a lesser extent in the COT system at all levels of the grain.

Free fatty acid levels rose steadily in all systems until week 30, with the RST system having the highest values. After week 30, wheat in the RST system had steadily declining fat acidity values probably because of complete removal of much of the wheat germ by insect feeding and intense microbial action, while the levels in the Control system steadily increased followed by the levels in the COT system.

Uric acid levels were considerably higher in the RST system than in the COT system.

A study of the distribution of Cryptolestes and Tribolium adults in the COT system indicated that Cryptolestes at the tops and bottoms of the drums were the same age. The number of offspring produced by Tribolium from the tops of the drums was lower than the number produced by adults from the bottoms of the drums. Possibly, a higher ratio of males to females was present at the tops of the drums because the mortality of adults from both the tops and bottoms was similar.

The measurement of electrolyte leakage from wheat seeds as an indication of damage was found to be ineffective.

Milling and baking tests on wheat from all systems at weeks 0, 15, 30, and 60 indicated that the poorest baking tests resulted from flour from the COT system although the RST system wheat was more severely damaged. It is thought these results could be attributed to greater intergranular microbial activity which increased the enzyme alpha amylase levels leading to greater loaf volume in the RST system.

In laboratory competitive studies involving various combinations of storage insects, it was found that the species Rhyzopertha-Sitophilus-Tribolium mutually inhibited reproduction to some extent. Rhyzopertha was the dominant species and was responsible for the greatest and most rapid damage to the wheat. The combination of Cryptolestes-Oryzaephilus-Tribolium was more favorable for Tribolium multiplication than most other combinations. Cryptolestes was the dominant species, while Tribolium was less abundant. In the larger drums, Tribolium and Cryptolestes adults were equally abundant, indicating the danger of extrapolation of data from small containers to far larger structures.

Carbon dioxide production and oxygen consumption were determined for all five species of insects. Carbon dioxide production ranged from .029 ml/adult/day for Rhyzopertha to .009 ml/adult/day for Cryptolestes. Oxygen consumption ranged from 0.037 ml/adult/day for Tribolium to 0.014 ml/adult/day for Cryptolestes.

Multivariate statistical analysis revealed several important relationships among the variables monitored. Principal component analysis was used at regular intervals and compared to cumulative analyses to find the sequence of interactions among variables. It was found that high bacterial counts were associated with high FAV levels; Tarsonemus numbers were related to Aspergillus; Alternaria and seed germination were negatively related

to FAV, bacteria and grain damage; and that the number of insects was related to the presence of Aspergillus and negatively related to the presence of bacteria.

Canonical correlation analyses complemented the results obtained from principal component analyses. High bacterial counts predicted high FAV, CO_2, moisture, grain damage and low seed germination, O_2 levels and Alternaria infection. High insect numbers predicted high moisture levels and extensive grain damage.

Stepwise multiple linear regression analysis indicated that the mite Tarsonemus granarius multiplied extensively on the food source Alternaria and maintained lower population levels on Aspergillus glaucus.

Multivariate analyses indicate that seed germination and Alternaria infection decrease rapidly, under the specified conditions, because of Aspergillus glaucus group infection of the seed. Insects, especially Rhyzopertha, and Aspergillus, increase seed damage and moisture content which favors bacterial growth which in turn inhibits insect and mold growth. Fat acidity values increase with time unless seed damage and bacterial infection are extensive as in the RST system.

Grain stored at 15.5% moisture content under warm conditions is most severely damaged by the insect combination of Rhyzopertha dominica, Sitophilus oryzae and Tribolium castaneum. Less obvious damage is done by the species combination of Cryptolestes ferrugineus, Oryzaephilus surinamensis and Tribolium castaneum. Although grain damage was more localized in the RST system, deterioration of all of the grain was rapid. It is probable that after 15 weeks, even the grain in the Control system was not fit for human consumption. Under warm, moist conditions infestation of wheat by Rhyzopertha dominica and the whole-grain feeders is more hazardous from

the human viewpoint than by germ-feeders such as Cryptolestes ferrugineus and Tribolium castaneum.

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