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PRINCIPAL COMPONENT ANALYSIS OF INTERRELATIONS IN STORED-WHEAT ECOSYSTEMS INFESTED WITH MULTIPLE SPECIES OF INSECTS[1]

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INTRODUCTION

The infestation of stored wheat by insects is a common problem throughout the world (FREEMAN, 1973). Generally, several species of insects invade bulk grain simultaneously or in close succession. Consequently competition for food and space affect insect populations, the quality of the stored grain, and its rate of deterioration (KABIR, 1966; LEFKOVITCH, 1968; CIESIELSKA, 1975; LECATO, 1975). Some of the collective invaders of stored grain in tropical regions of the world 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) (GIRISH *et al.*, 1974). In temperate regions of the world the rusty grain beetle, *Cryptolestes ferrugineus* (STEPH.) (Cucujidae), the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Cucujidae) and the red flour beetle, *Tribolium castaneum* are often associated with each other (SINHA, 1961, 1965, 1974; WATTERS, 1976).

The interaction of these insects with one another and their environment under favourable temperature and grain moisture content usually maximizes biological activity leading to rapid floral and faunal succession and deterioration of wheat (SINHA and WALLACE, 1966). The purpose of this study was to apply a multidisciplinary approach to elucidate the intra- and inter-community relationships among insects and microflora of stored wheat. Using principal component analysis (PCA) the data from insect-free wheat and wheat infested with different insect-species groups were analyzed and summarized to clarify various interrelations among the abiotic and biotic variables that are operative in a stored-wheat ecosystem under simulated tropical conditions.

MATERIALS AND METHODS

Eight 204-liter steel drums were placed on end and each filled with No. 2 Canada Western red spring wheat (*Triticum aestivum* L., cv. Neepawa) grown in 1976 at Glenlea, Manitoba. A 157-kg parcel of grain was placed in each drum filling it to

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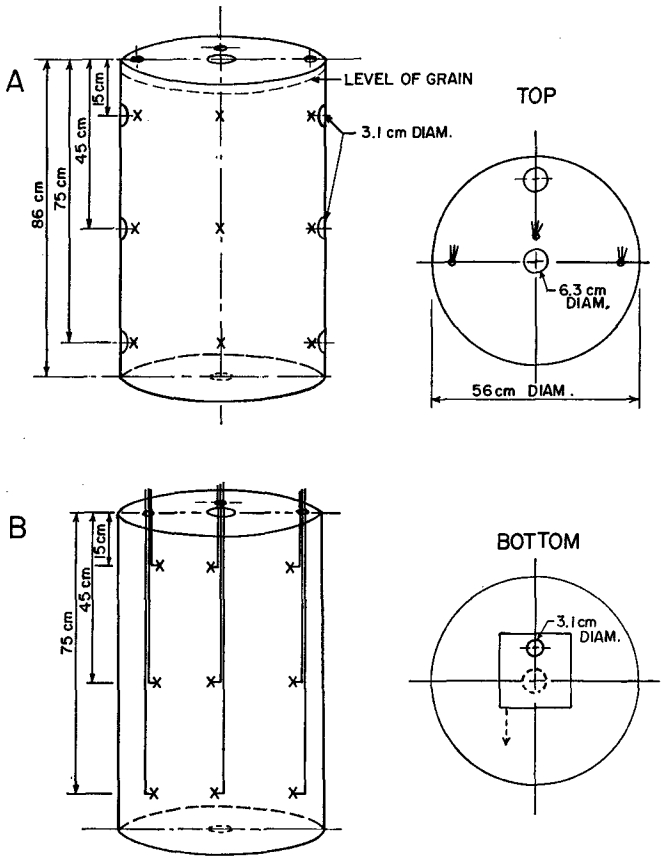

Fig. 1. Planviews of experimental drums showing sampling locations and other dimensions (A), and planviews of experimental drums showing the location of thermocouples and gas tubes and other dimensions (B).

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.

Each drum was sampled through holes drilled at eight points (Fig. 1A). 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 made a 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; 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. 1B). These openings 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 noninfested controls were drums 1 and 2 (Control system). The other drums were artificially infested with adult insects as follows: no. 3, 4, and 5, each with 500 *R. dominica* reared on whole wheat, 500 *S. oryzae* reared on whole wheat, and 500 *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 *C. ferrugineus* reared on whole wheat and wheat germ (19:1), 500 *O. surinamensis* reared on rolled oats, and 500 *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.

Seventy-two 200-ml grain samples were taken at week 0, 4, 6 and tri-weekly thereafter for 60 wk from nine locations in each drum. Wheat held in plastic bags at -15°C for the duration of the study served as a baseline 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. 1B). The carbon dioxide and oxygen contents (%) at each location were determined with a Matheson gas chromatograph (Model 8430, Matheson Gas Products, P. O. Box 85, East Rutherford, New Jersey). 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 for sampling. Probes were placed in an oven at 100°C for 2 hr several hours before use to prevent accidental insect or mite infestation. At each sampling, approximately 1215 g of grain (9×135 g) were removed per drum, or 1.21% of the initial total mass. Removal of this quantity of wheat at a given time was not considered to influence future sampling appreciably.

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 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 with a 1% sodium hypochlorite solution; this step was followed by a sterile water rinse (TUITE, 1969). The weight and volume of 100 randomly chosen kernels were determined; the extent of seed damage was determined for both germ and endosperm. A further 50 kernels were removed at random from each subsample from drums 3, 4, and 5 and dissected with a scalpel under a stereo microscope to determine the number of *R. dominica* and *S. oryzae* larvae 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, 1964). Specimens collected were preserved in 70% ethyl alcohol, identified and counted under a stereo microscope, and added to the values obtained previously by sifting. Free fatty acid content of the samples or fat acidity values (FAV) in the grain were determined in duplicate on pooled samples at tri-weekly intervals from each drum for wk 0-60. Further determinations from each drum for wk 15-60 were also made, because the grain damage was generally greatest during this period (AACC method 02-01, ANONYMOUS, 1962).

To obtain multivariate summarization of the data, PCA was done on samples from each of the three storage systems at each sampling data (Control system, 18 samples; RST system, 27 samples; COT system, 27 samples) and on all of the pooled data 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 BMDO1M program (DIXON and BROWN, 1977). The variables used were: FAV (mg KOH/100 g dry wheat), CO_2 (%), O_2 (%), temperature (°C), grain weight (100 kernels in g), 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 (frequency of occurrence). In addition, *T. granarius* numbers were used in the Control system; germ damage (%), endosperm damage (%), adult *R. dominica*, *S. granarius*, and *T. castaneum* in the RST system; and germ damage, endosperm damage, adult *C. ferrugineus*, *O. surinamensis*, and *T. castaneum* 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).

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.*, 1969).

After 15 wk both drums used in the Control system accidentally became lightly infested with *O. surinamensis*. To determine if this infestation was affecting the variables monitored, 1 kg of wheat was taken from both drums 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 a 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 a negligible effect on the functioning of the ecosystems. Only the generic names of the species of insects, mites and microflora have been used in the Results and Discussion.

Statistical methods

PCA is a statistical method which transforms a given set of variables into a new set of variables (principal components) that are orthogonal (uncorrelated) to one another. No particular assumptions about the underlying structure of the variables is necessary. This analysis determines the best linear 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 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).

PCA performed on the data at each sampling date dealt with smaller numbers of samples and smaller amounts of variation than the overall PCA performed on cumulative data from wk 0-60. The regular tri-weekly analyses were, however, probably more informative for these revealed changing relationships with time.

Component reliability in data from wk 0-60 was determined on all samples from the pooled set after they were randomly assigned to two subsets which were analyzed separately in the Control, RST, and COT systems (LAWLEY and MAXWELL, 1971; SINHA, 1977; MILLS *et al.*, 1978). The loadings of most of the principal components were similar in the split samples and were therefore considered reliable.

RESULTS AND DISCUSSION

Correlation coefficient matrices and principal component matrices of loadings with the proportion of variance accounted for by the loadings are presented for all three

systems for pooled data for wk 0-60 (Tables 1-6). The changing patterns of relationships among variables from one sampling date to another are illustrated for principal components 1 and 2 in all three systems (Figs. 2-7). Correlation coefficient matrices were used during analysis rather than variance-covariance matrices because measurements of variables were taken on different scales (SEAL, 1964; SINHA, 1977; BRONSWIJK and SINHA, 1971).

PCA of data from each sampling date

Figures 2 and 3 show that principal components 1 and 2 accounted for approxi-

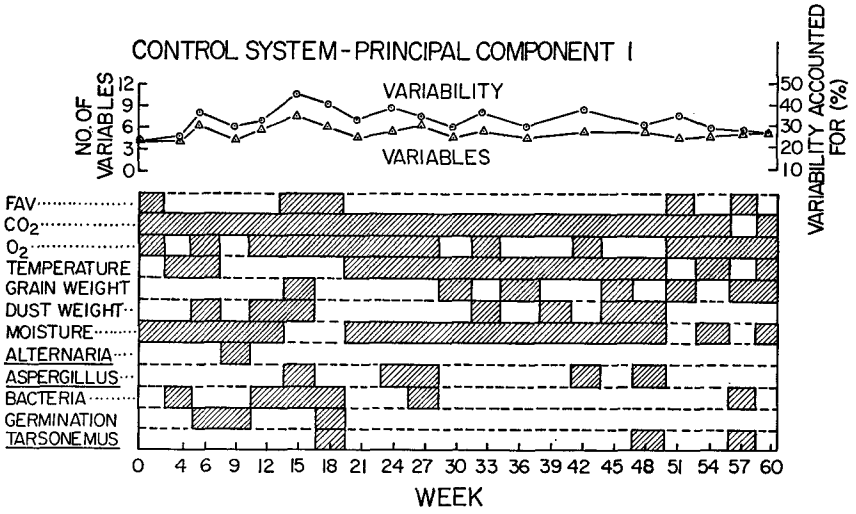

Fig. 2. Diagram illustrating the changing relationships among variables for principal component 1 in the Control system as revealed by tri-weekly principal component analyses of multivariate data.

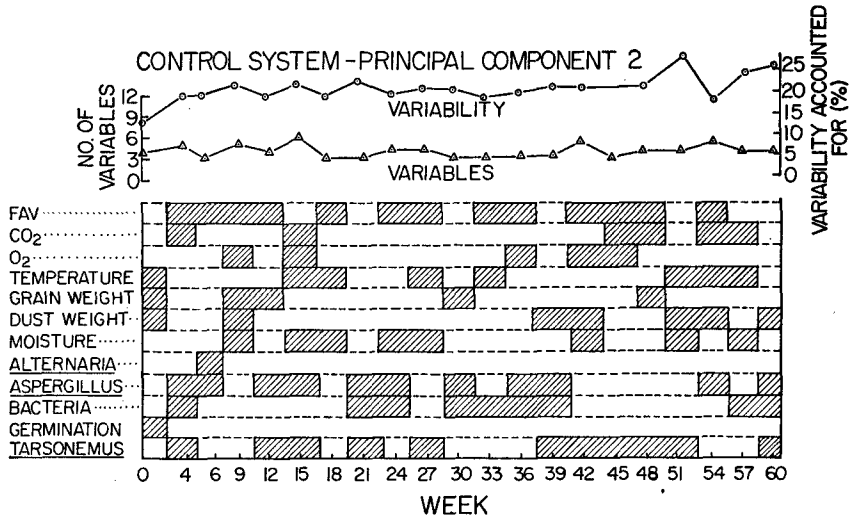

Fig. 3. Diagram illustrating the changing relationships among variables for principal component 2 in the Control system as revaled by tri-weekly principal component analyses of multivariate data.

mately 50% of the variability in the system. The number of variables describing the variation remained fairly constant throughout the study. Principal component 1 (C1) was essentially an abiotic component involving long-term relationships among CO_2, O_2, temperature, and moisture (Fig. 2). High temperature and moisture levels were related to high CO_2 and low O_2 concentrations. Principal component 2 mainly demonstrated somewhat fleeting relationships among FAV, moisture, *Aspergillus*, bacteria, and *Tarsonemus* (Fig. 3). High moisture content of the wheat was associated with large populations of *Aspergillus*, bacteria, and *Tarsonemus* at different points of time. 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 with loadings greater than 0.30 for each PCA ranged from four to six.

RST system. Principal components 1 and 2 together accounted for about 55-60% of the variability in this system with 4-8 of the 16 variables monitored playing a meaningful role in each component (Figs. 4 and 5). The main variables interacting in principal component 1 between wk 4 and wk 18 were temperature, grain weight, moisture, germ damage, endosperm damage, *Tribolium*, *Sitophilus* and *Rhyzopertha*. Populations of the three insect species were positively correlated to germ damage, endosperm damage, and grain weight loss because of their feeding activity. Insect numbers were positively related with higher temperatures and increased moisture levels. This is explainable because all three insect species thrive at high temperature and relatively high relative humidity (Howe, 1965).

Principal component 2 showed intermittent but steady interactions among FAV, CO_2, O_2, moisture, occasionally *Aspergillus*, and bacteria. After wk 18, *Tribolium*,

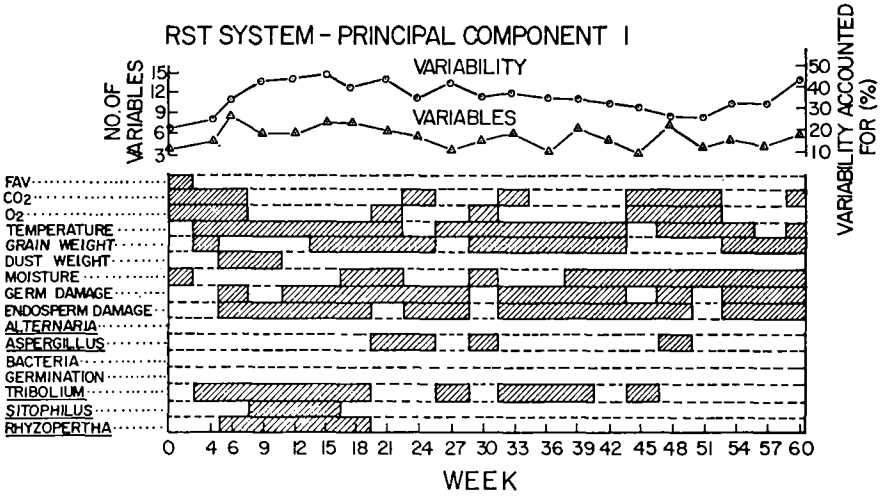

Fig. 4. Diagram illustrating the changing relationships among variables for principal component 1 in the *Rhyzopertha-Sitophilus-Tribolium* system as revealed by tri-weekly principal component analyses of multivariate data.

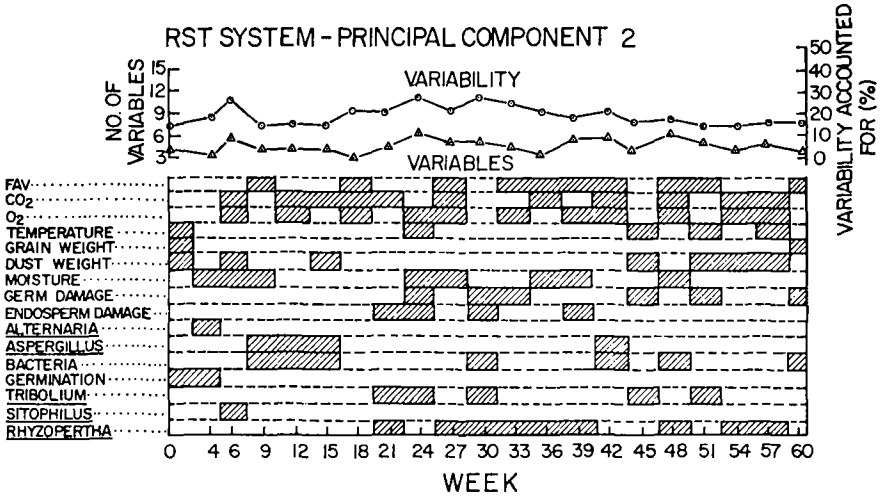

Fig. 5. Diagram illustrating the changing relationships among variables for principal component 2 in the *Rhyzopertha-Sitophilus-Tribolium* system as revealed by tri-weekly principal component analyses of multivariate data.

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 was 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.

COT system. The first two principal components accounted for about 50% of the variability in the system and involved only 3-6 variables at any one time (Figs. 6

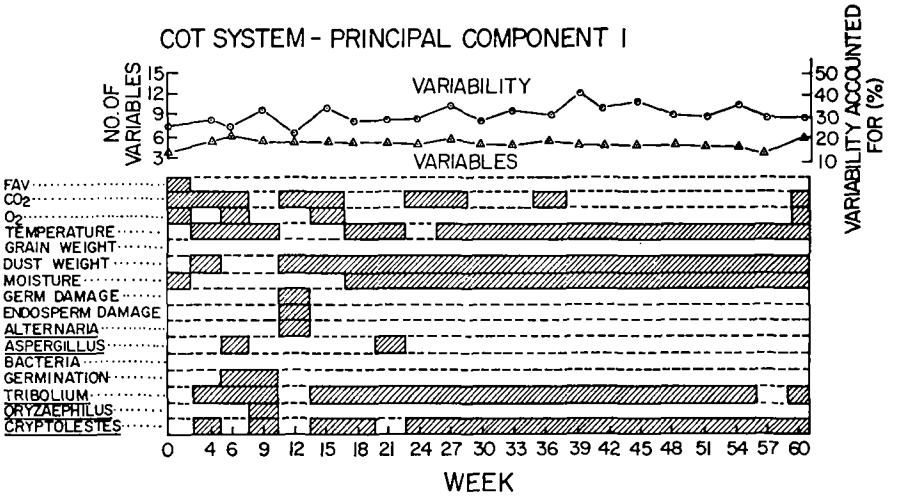

Fig. 6. Diagram illustrating the changing relationships among variables for principal component 1 in the *Cryptolestes-Oryzaephilus-Tribolium* system as revealed by tri-weekly principal component analyses of multivariate data.

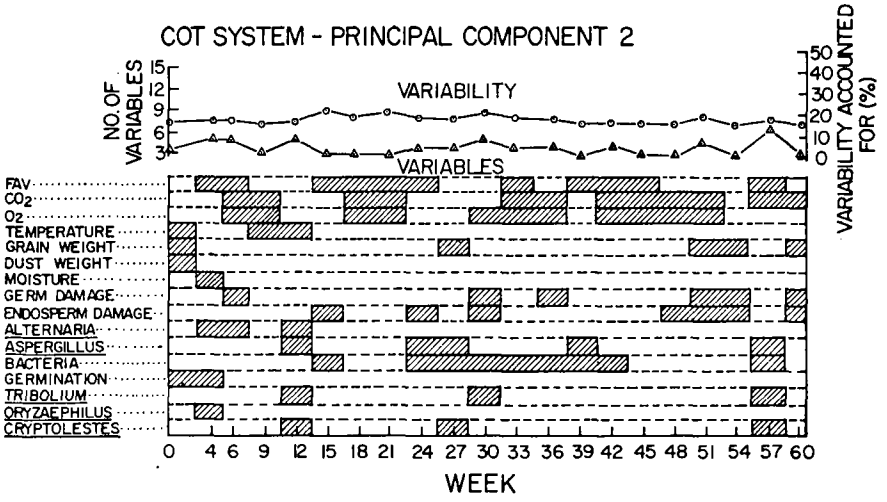

Fig. 7. Diagram illustrating the changing relationships among variables for principal component 2 in the *Cryptolestes-Oryzaephilus-Tribolium* system as revealed by tri-weekly principal component analyses of multivariate data.

and 7). Principal component 1 was usually explained for long segments of the time span by the relation among temperature, dust weight, moisture, *Trbiolium*, and *Cryptolestes* (Fig. 6). Insect numbers were positively correlated with 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; its main variables were FAV, CO_2, O_2, germ damage, endosperm damage, *Aspergillus* and bacteria (Fig. 7). Generally, *Aspergillus* and bacteria were negatively correlated with each other and both were negatively correlated with O_2, germ damage, and endosperm damage but positively correlated with CO_2. *Aspergillus* declined late in the study and bacteria increased as the FAV increased.

PCA of cumulative data

PCA of the cumulative data from wk 0-60 for all three systems gave a somewhat different view of the patterns of relationships during the study, and should be considered along with the regular analyses to understand the systems. Tables 1-3 give the correlation coefficient matrices and the principal component matrices of loadings with the proportion of total variance accounted for by each component.

Control system. Principal component 1 involved FAV, dust weight, *Alternaria*, bacteria, and germination. Interpretation of the results listed in Table 4 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

Table 1. 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 2. 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.

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 are negatively correlated with O_2 levels.

RST system. Principal component 1 involved FAV, germ damage, endosperm damage, *Alternaria*, and germination (Table 5). As FAV levels, germ and endosperm

Table 3. 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 4. Principal-component matrix of loadings[a] showing the relative importance of the variables on each principal component and the percentage of variability accounted for by each principal component for the Control system, weeks 0-60.

	Princical Component											
Variable	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 5. Principal-component matrix of loadings[a] showing the relative importance of the variables on each principal component 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.

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 with bacterial infection. It appears that prolonged activity of the three insect species and their interactions generate additional moisture in the environment, which in turn promotes 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 (Table 6). 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.

Changes in the ecosystems

Infestation of stored wheat by *Rhyzopertha dominica*, *Sitophilus oryzae*, and *Tribolium castaneum* or *Cryptolestes ferrugineus*, *Oryzaephilus surinamensis*, and

Table 6. Principal-component matrix of loadings[a] showing the relative importance of the variables on each principal component 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.

Tribolium castaneum at 15.5% moisture content and 30°C led to rapid chemical and physical deterioration of the grain (WHITE and SINHA, unpublished). Differences among variables within each system were minimal and did not approach the differences between systems. Temperatures in the RST system were slightly higher than those of the COT or insect-free Control system. Carbon dioxide levels were highest at the lower levels of the RST system and oxygen levels the lowest. The moisture content of the wheat in the RST system especially at the surface 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 wk 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 at 60 wk. 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 their metabolic by-products, found in the RST and COT systems. In the RST system, *Sitophilus*

failed to multiply extensively and gradually became extinct. *Rhyzopertha* and *Tribolium* were found mainly at the top of the grain. In the COT system, *Oryzaephilus* failed to thrive and rapidly became extinct. After several wk, *Cryptolestes* and *Tribolium* adults were most abundant at the bottom of the 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 oscillation in numbers.

Grain dust was produced extensively in the RST system and moderately in the COT system. Grain damage was greatest in the RST system, with up to 40% of both germ and endosperm being severely damaged especially near the top of the grain. The germ of the wheat was consumed to a lesser extent (about 20%) in the COT system at all levels of the grain.

Fat acidity values rose steadily in all systems until wk 30, with the RST system having the highest values. After wk 30, the FAV in the RST system declined steadily while the FAV levels in the Control and COT systems steadily increased.

Ecological interpretation

The Control system generally underwent abiotic and biotic changes similar to those in the RST and COT systems, although the magnitude of those changes was smaller in the Control system. As time progressed, seed germination and *Alternaria* infection declined. This relationship was previously reported by WALLACE and SINHA (1962) and SINHA and WALLACE (1965). *Aspergillus glaucus* group and bacterial infection increased as did CO_2 and FAV levels, while O_2 concentrations declined. FAV has previously been shown to increase steadily with time as 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. *Tarsonemus* was abundant in the Control system which was free from competing insect pests; but this mite reached large populations only when it was associated with the fungus *Alternaria* (WHITE and SINHA, 1979). *Tarsonemus* 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 metabolic by-products from the insects and microflora. *Tribolium* secretes quinones which partially inhibit fungal growth when insect populations are dense (WYK *et al.*, 1959).

The RST and COT systems each 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 FAV levels increased until deterioration became advanced and then they declined steadily, possibly because of advanced decomposition of the fats by bacteria and complete consumption of much of the germ by insects. 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; the FAV, however, increased steadily. The effect of the depth of the grain from the top surface of the bulk wheat was often large and of importance in the deterioration of the grain and was related to insect distribution (WHITE and SINHA, unpublished).

Wheat stored at 15.5% moisture content at warm temperatures (30 ± 2°C) deteriorates more rapidly and is more severaly damaged by the combination of insects in the RST system than by those in the COT system. The presence of insects which feed on whole grain and produce large amounts of metabolic moisture, notably *R. dominica*, creates a more suitable environment for microfloral growth and succession, leading to grain deterioration.

A stored-grain ecosystem is a closed one with a non-regenerating food supply and no detoxifying processes for accumulated waste; therefore, the initial high productivity of an immature ecosystem declines as the invading species saturate the environment at characteristic densities at the carrying capacity of the system followed by the eventual death or inactivation of most biotic agents.

This study has demonstrated that multivariate analyses (PCA) of both time series and cumulative data collected from a closed man-made ecosystem, such as bulk stored grain can reveal and quantify many of the fleeting relationship patterns among a variety of opportunistic species representing acarine, entomological and microbial communities within the ecosystem.

SUMMARY

Principal component analysis (PCA) was used to determine the effects of infestation of bulk stored wheat by multiple species of insects at 30±2°C for 60 wk. 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), artificially infested with *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.), and *Tribolium castaneum* (HERBST); and (c) COT system (3 drums), infested with *Cryptolestes ferrugineus* (STEPHENS), *Oryzaephilus surinamensis* (L.), and *Tribolium castaneum*. The variables measured tri-weekly within each system included carbon dioxide, oxygen, temperature, grain moisture, seed damage, grain weight and volume, dust weight and volume, fat acidity values (FAV) of the wheat, seed germination, microflora including *Alternaria alternata* (FR.) KEISSLER, *Aspergillus glaucus* group, *Aspergillus candidus* LINK, and bacteria, insects and the mite *Tarsonemus granarius* LINDQUIST. PCA provided multivariate synopsis of the data quantifying several important relationships among the variables monitored. Tri-weekly and cumulative 60-wk analyses of each system showed that high bacterial counts were associated with high FAV levels; *Tarsonemus* numbers were positively 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. Seed germination and *Alternaria* infection often decrease rapidly presumably because of infection by fungi of the *Aspergillus glaucus* group. The combined action of *R. dominica* and *Aspergillus* spp. enhanced seed damage and increased grain moisture content thus promoting bacterial growth which in turn inhibited insect and mold growth. Fat acidity values increased with time unless seed damage and bacterial infection were extensive as in the RST system.

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多種類の昆虫の寄生を受けた貯蔵小麦生態系における相互作用の要因分析

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多種類の昆虫類が30±2°Cの条件で60週間にわたって加害した場合の貯蔵小麦が受ける影響を調べた。15.5%の湿度を含んだ小麦が204 *l* のドラムカン（8個）に貯蔵されている。このうち2個はコントロールで，3個は人工的に *Rhyzopertha dominica*, *Sitophilus oryzae*, *Tribolium castaneum* を寄生させた（RST システム）。のこりの3個は，*Cryptolestes ferrugineus*, *Oryzaephilus surinamensis*, *Tribolium castaneum* を人工的に寄生させたものである（COT システム）。3週間毎に，各システムの CO_2, O_2, 温度，穀物湿度，種子の被害程度，穀物重量と体積，クズの重量と体積，小麦の脂肪酸量（FAV），発芽の程度，微生物・バクテリア・昆虫・ダニ類の発生程度を調べた。因子分析の結果，モニターされた変量の間においていくつかの重要な関係が量的にうらづけられた。

3週間毎，60週にわたる分析の結果，バクテリアのカウント増は高水準の FAV と関係していた。ダニ個体数は *Aspergillus* と関係していた。*Alternaria* と種子発芽は FAV，バクテリアと穀物の損傷と負の相関があった。昆虫の数は *Aspergillus* の存在と関係していたが，バクテリアの存在と負の相関があった。種子発芽と *Alternaria* の発生はしばしば急速に減少した。これは多分 *Aspergillus glaucus* の菌グループが発生したためと思われる。*R. dominica* と *Aspergius* spp. が一緒に作用した結果，種子損傷で助長して，穀物湿度を上昇させ，バクテリアの成長を助けた。さらにバクテリアは昆虫と細菌の成長を抑制した。もし種子の損傷やバクテリアの発生がRSTシステムにおけるように苦しくなければ，脂肪酸は時間とともに増加した。