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PRECOPULATORY BEHAVIOR AND MATING SUCCESS OF THE EUROPEAN CORN BORER¹ UNDER CONTROLLED CONDITIONS²

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ABSTRACT. Biotic factors exhibited little influence on mating success when adult European corn borers, Ostrinia nubilalis (Hübner), were exposed to a simultaneous 16:8 hr photo-thermoperiod. Adult age or crowding did not affect mating frequency. Female multiple matings were few but males will mate with a virgin female every day. Neither sex, however, will mate more than once in 24 hr.

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The results reported here represent part of a continuing program designed to determine the nature of European corn borer, Ostrinia nubilalis (Hübner), population fluctuations. As part of this program, a study was conducted to determine the influence of biotic factors on mating success of the Iowa strain of the corn borer.

GENERAL METHODS AND MATERIALS

Adults used in this study were reared in the laboratory from larvae maintained on an artificial diet (Guthrie et al. 1965). After 20 days, pupae were removed from the diet dishes and placed in individual 0.5-oz plastic jelly cups, which were then capped with paper lids. These pupae were exposed to simultaneous cycles of light and temperature. A daily photophase of 16 hr was used. Light intensity and temperature during the photophase were 175 ft-c and 85 °C. During the 8-hr scotophase, the temperature was 65 °F. Relative humidity was held at approximately 75% during the 24-hr cycle. Pupae were checked each day to determine the number of adults that had emerged. Pint-sized screen (19-mesh) mating cages were used. The adults were transferred from the 0.5-oz jelly cups to these mating cages in a 60 °F walk-in incubator. After the adults were transferred, the cages were watered with a fine spray and then placed in the test incubator. The test incubator could be programmed to automatically control temperature, humidity, and light for 24 hr. Temperature control was accurate to within ± 0.5 °F, and relative humidity to within 2%. Light was provided by fluorescent lamps.

¹ Lepidoptera: Pyraustidae

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The criterion for successful mating was the presence of a spermatophore in the bursa copulatrix of the female (Pesho 1961).

RESULTS AND DISCUSSIONS

Crowding

This experiment was designed to determine if crowding under caged conditions had any effect on mating. Combinations of 1, 2, 3, 4, and 5 pairs of adults were placed in pint-sized screen mating cages. The adults were exposed for 24 hr to cycling conditions of 16 hr light at 85°F and 8 hr darkness at 67°F. Relative humidity ranged from 75 to 85%.

Table 1. Influence of crowding on mating.^{a/}

Adults per cage (pr)	Percent mating	
	Mean	Range
1	75	0-100
2	63	0-100
3	75	66-100
4	56	25- 75
5	75	60- 80

^{a/}Experiment performed twice using 2 cages of adults per treatment.

Table 1 shows the percentage of females that mated in this investigation. For the purpose of this experiment, it was concluded that there was no difference in mating due to crowding under these conditions.

Adult age

An experiment was designed to determine if the age of the imagoes is a factor in mating success. To determine this, adult males and females were paired by age to determine how long after emergence adults would be able to mate. Age was established as the number of hours after eclosion of the imago. Age groups studied were 1-6, 24-30, and 48-54 hr old at the beginning of the experiment. Adults of the same age were exposed to cycling conditions of 16 hr light at 85°F and 8 hr darkness at 67°F. Relative humidity ranged from 85 to 75%. The experiment was performed 5 times with 5 pair of adults per age group.

The relationship between age and percentage mating is shown in Table 2. The results indicate that there is no difference in the ability of adults, in the age groups tested, to mate successfully.

Mating frequency

An experiment was designed to determine if an adult male or female

will mate more than once during a 24-hr period. Adults were caged in different sex ratios and exposed to cycling conditions of 16 hr light at 85°F and 8 hr darkness at 67°F. Relative humidity ranged from 75 to 85%.

Table 2. Effect of age on mating.

	Age (hr)		
	1-6	24-30	48-54
Females mated	17	18	18
Females unmated	8	7	7

Table 3. Mating frequency in a 24-hr period.

Sex ratio	Spermatophores found
1 male to 5 females ^{a/}	1
5 males to 1 female ^{b/}	1

^{a/}Experiment performed 3 times.

^{b/}Experiment performed 4 times.

Data presented in Table 3 indicate that an individual of either sex mates only once during a 24-hr period.

Multiple mating

In the previous experiment, the paired adults were left together for 24 hr. During this time, neither the male nor the female will mate more than once. This experiment was designed to determine if the female will mate more than once in 48 hr.

Adults of the same age were exposed to a 24-hr cycle of 16 hr light at 85°F and 8 hr darkness at 67°F. Adults were provided water once a day.

Of 41 females examined, none had mated twice. The experiment was repeated, except that the females were examined for spermatophores only after all the females had died. At the end of 11 days, all 17 females had mated, and of these, 2 (11.7%) had mated twice.

To determine how many times a male will mate in a lifetime, a newly emerged male imago was provided with 2 virgin females every 24 hr until the male died. The adults were exposed to a 24-hr cycle of 16 hr light at 85°F and 8 hr darkness at 67°F. Water was provided every day. The male lived 10 days and transferred 9 spermatophores; 1 spermatophore was transferred every 24 hr. On the morning of the 10th day, the

male was dead. Although no measurements were taken, spermatophore size decreased with each successive day past day 3. On days 8 and 9, the spermatophore was observed to be malformed in that it was not of the normal egg shape but, rather, of a dumbbell configuration. The experiment was not repeated because the results have no significance in this study. The observation was included only to serve as a reference to female multiple matings.

Age combination

This experiment was designed to determine if there would be a difference in mating success when adults of different ages were placed together. Adults were mixed in various age combinations ranging from 1-5 days of age. Adult age was the number of days after eclosion of the imago. Adults were exposed to cycling conditions of 16 hr light at 85°F and 8 hr darkness at 67°F. The experiment was performed twice with 5 pr of adults per cage.

Results indicate that there is no difference in mating success when adults of different ages are placed together (Table 4).

Female precopulatory behavior

The female, for the most part, plays a passive role in mating. She probably releases a copulatory stimulant or attractant at falling temperatures and decreasing light intensity. This stimulant probably is distributed throughout the environment by the air. In only one observation was a female seen actively moving her wings in what might be considered a dispersal action. The female is sometimes seen to move her abdomen in a pumping action. Usually the female will rest passively with the tip of her abdomen exposed (Fig. 1) as the male tries to copulate with her.

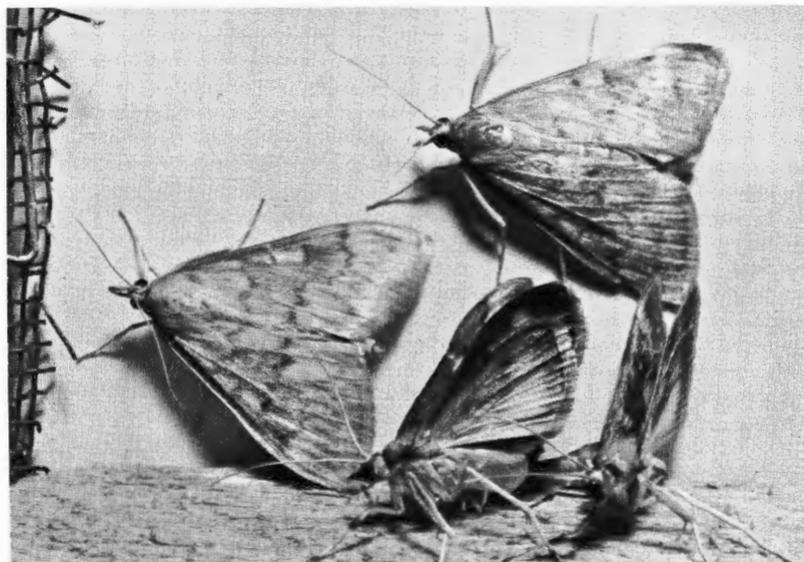


Figure 1. Female with tip of abdomen exposed.

Table 4. Effect of age combinations on mating.

Age combination			
Age (days)	Sex	Rep.	Spermatophores present
1	male	1	3
	X		
1	female	2	4
2	male	1	4
	X		
1	female	2	4
1	male	1	5
	X		
2	female	2	3
2	male	1	4
	X		
2	female	2	3
1	male	1	4
	X		
3	female	2	4
3	male	1	5
	X		
1	female	2	4
1	male	1	4
	X		
5	female	2	4
5	male	1	4
	X		
1	female	2	4

The results of these experiments indicate that biotic factors such as mating age, age preference for a mate, and crowding under the described conditions have no effect on mating success. In this regard, Caffrey and Worthley (1927) reported that the corn borer will mate as soon as 12 hr after eclosion, but no environmental conditions were stated.

The frequency of multiple mating for females was low (11.7%) compared with the 46% (27 of 58) reported by Drecktrah and Brindley (1967). Among feral corn borer adults, Pesho (1961) reported a range of 8 to 43% multiple matings for females. It appears that the current results are close to describing multiple matings under field conditions.

ACKNOWLEDGMENTS

I wish to thank Dr. Tom A. Brindley for his support and assistance during the conduct of this research.

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STERILIZATION TECHNIQUES FOR SEEDS AND
EXCISED EMBRYOS OF CORN (ZEA MAYS L.)¹

W. J. Dahmen and J. J. Mock²

ABSTRACT. Effects of surface-sterilizing treatments on germination, growth, and microbial contamination of seeds and excised embryos of corn (Zea mays L.) were investigated. Treatment sequence, 70% ethanol dip plus a 5-minute exposure to 2.6% sodium hypochlorite, was most effective for both whole seeds and embryos. The procedure should be useful for other monocot species.

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Sterile culture of seeds and excised embryos is becoming a common technique for germination and growth of intergeneric and interspecific hybrid seed. Also, it is used for basic physiological, biochemical, and genetic studies. Use of the technique requires plant material free from microbial contamination; thus, before culture, surface sterilization of potentially infected material is necessary.

Most published procedures give a specific treatment for sterilizing specific material, but there is little data on relative effectiveness of a number of treatments. Smith *et al.* (4), used a 10-minute exposure to merthiolate (1:2000) for surface-sterilizing peach seeds and a 5-minute exposure to 2.6% sodium hypochlorite for excised peach embryos. Various surface sterilizing procedures for corn seeds have been reported; e. g., 70% ethanol dip (6), washings with "Tide" (1), and dip in 1:1 diluted S. T. 37 (4-hexylresorcinol) (5). Miflin (2) surface-sterilized barley seeds with a 1-hour treatment of 3-5% sodium hypochlorite. He used 0.1% HgCl₂ (3 minutes) plus 0.01% H₂O₂ (3 minutes) for barley embryos. Morrison *et al.* (3), used 1:10 diluted "Javex" for 1 minute to surface-sterilize barley seeds.

This paper compares effectiveness of several surface-sterilizing treatments for corn seeds and excised embryos.

MATERIAL AND METHODS

Agar-sucrose basal medium (A-S basal medium) used in these experiments was originally developed by Dure (1) for seeds and excised embryos of corn and is presented in Table 1.

Surface-sterilization treatments (used individually and in combination) were 70% ethanol, 1:2000 merthiolate (obtained from Eli Lilly and Co.),

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Table 1. Synthetic nutrient medium used in culturing seeds and embryos.

Component	Concentration (mg/l)
Sucrose	20,000.0
Agar	7,500.0
$\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$	237.0
KNO_3	85.0
KCL	65.0
$\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$	16.5
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	36.0
FeCitrate (Ferric)	30.0
$\text{MnSO}_4 \cdot 4\text{H}_2\text{O}$	0.4

2.6% sodium hypochlorite (diluted from commercial bleach), and 2.5% Ferbam in water (carbamate powder obtained from Niagara Chemical Division of FMC Corporation).

Whole corn seeds of the single cross A619 x SD10 were treated (10 seeds per treatment) with the sterilizing solutions, rinsed three times in sterile water, soaked (to soften seeds) for 55 minutes in sterile water, and transferred to 22 x 175 mm culture tubes containing 20 ml A-S basal medium. Tubes were plugged with porous plastic plugs. Excised embryos of A619 x SD10 were allowed to become uniformly contaminated (by handling and air exposure) and then treated similarly, except that they were not soaked. Cultures were incubated in a germination chamber with constant temperature (24°C) and light (700 ft-c). Seedlings were observed after they attained maximum germination and growth (8 and 13 days for whole seeds and excised embryos, respectively) and were evaluated for percentage germination, shoot length, and percentage microbial contamination (subdivided on the basis of colony morphology into bacterial and fungal components). Percentage values were based on number of total cultures per treatment; therefore, only shoot length was replicated and analyzed statistically by student's t test.

RESULTS AND DISCUSSION

In experiment 1, three treatments, 1:2000 merthiolate for 10 minutes, 2.6% sodium hypochlorite for 5 minutes, and 70% ethanol dip plus 2.6% sodium hypochlorite for 5 minutes, resulted in significantly greater shoot growth than did the control (Table 2). Shoot length differences among the three treatments were not statistically different. Combination treatment most effectively removed contamination.

Table 2. Effects of surface-sterilization treatments of whole corn seeds on seed germination, seedling shoot length, and culture contamination.

Treatment	% Germination	Shoot* length(mm)	% Contamination Bact.	Fung.
<u>Experiment 1</u>				
Control (no treatment)	85	26.4 a	30	100
70% ethanol dip	100	58.8 b	50	5
1:2000 merthiolate (10 min.)	100	79.1 c	35	0
2.6% sodium hypochlorite(5 min.)	100	80.9 c	0	10
2.5% Ferbam dip	70	24.6 a	0	100
70% ethanol + 2.6% sodium hypochlorite	100	83.9 c	0	5
<u>Experiment 2</u>				
Control (no treatment)	95	62.2 a	15	50
70% ethanol + 2.6% sodium hypochlorite	100	75.4 b	0	5
2.5% Ferbam + 2.6% sodium hypochlorite	100	76.4 b	0	5
<u>Experiment 3</u>				
Control (no treatment)	90	57.0 b	15	50
70% ethanol + 2.6% sodium hypochlorite	100	75.0 c	0	0
70% ethanol + 1:2000 merthio- late + 2.6% sodium hypochlorite	95	41.7 a	0	0
<u>Experiment 4</u>				
Control (no treatment)	90	48.5 a	20	67
70% ethanol + 2.6% sodium hypochlorite	100	78.1 b	0	3

* Means within an experiment followed by same letter are not-significant at 5% level of probability.

Experiments 2-4 showed 2.5% Ferbam dip plus 2.6% sodium hypochlorite for 5 minutes and 70% ethanol dip plus 2.6% sodium hypochlorite for 5 minutes resulted in equivalent germination, seedling growth, and contamination control (Table 2). A sequence of three treatments, 70% ethanol, 1:2000 merthiolate, and 2.6% sodium hypochlorite, was an effective sterilant, but it substantially retarded growth and reduced germination (Table 2). For all experiments, low germination percentages were associated with high amounts of microbial contamination. Because of consistent results and handling ease, 70% ethanol dip plus 5-minute exposure to 2.6% sodium hypochlorite is suggested as a reliable surface-sterilizing treatment for whole corn seeds.

If excised embryos are internally contaminated or become contaminated during excision, resterilization is necessary. Thus, four re-sterilization treatments were compared (Table 3). Shoot length differences for embryos treated with 70% ethanol plus 2.6% sodium hypochlorite and 2.5% Ferbam plus 2.6% sodium hypochlorite were not statistically significant. Growth of embryos receiving these treatments was greater than for other treatments. Although treatments resulted in decreased seedling growth (compared with control), 70% ethanol plus 2.6% sodium

Table 3. Effects of resterilization treatments of excised corn embryos* on embryo germination, seedling shoot length, and culture contamination.

Treatment	% Germination	Shoot† length(mm)	% Contamination	
			Bact.	Fung.
Control (no resterilization)	95	59.1 c	25	5
70% ethanol + 1:2000 merthio- late	100	17.6 a	5	0
1:2000 merthiolate + 2.6% sodium hypochlorite	100	14.6 a	10	0
2.5% Ferbam + 2.6% sodium hypochlorite	100	28.9 b	0	10
70% ethanol + 2.6% sodium hypochlorite	100	35.2 b	0	5

* All embryos excised from seeds surface sterilized with 70% ethanol + 2.6% sodium hypochlorite.

† Means followed by same letter are not significant at 5% level of probability.

hypochlorite gave the least inhibition, and these seedlings developed normally after transfer to soil. Also, 70% ethanol plus 2.6% sodium hypochlorite gave the most effective contamination control; therefore, this treatment is recommended for reesterilization of excised embryos.

The sterilization procedure recommended in this paper emphasizes flexibility and handling ease. Both whole seeds and excised embryos can be sterilized with identical materials. Constituents used produce no residual effects on subsequent plant growth, yet are of sufficient potency to control microbial contamination. Data presented are for corn seeds and embryos, but preliminary experiments with sorghum show similar results, suggesting the procedure may be applicable to other monocot species.

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VARIATION AND INTERRELATIONSHIPS OF PROTEIN AND OIL CONTENT, AND SEED WEIGHT, IN GRAIN SORGHUM¹

V. H. Reich and R. E. Atkins

ABSTRACT. Protein and oil percentages and 100-seed weights were determined on parental, F_1 , and F_2 hybrid seed of grain sorghum. Variation in oil content within each population was less than 1%. Protein percentage among the F_1 and F_2 hybrids ranged from 8 to 18 and from 10 to 16, respectively. Correlations among the three characters for F_2 seed were positive, indicating no marked barriers to the simultaneous improvement of protein and oil percentage and seed weight in commercial hybrid sorghums. The heritability of each character seemed sufficient for reasonably effective selection within segregating populations.

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Sorghum grain is used in the United States principally as feed for animals. Composition of the grain is similar to that of corn, except for a higher protein and lower oil content. The content and quality of protein in sorghum grain have been studied considerably, but variability for oil content has received little attention. Sorghum grain also is used by the wet-milling industry for the production of starch and its derivatives, with an edible oil as a principal by-product. Lines with strikingly high percentages of grain protein have been identified recently within the world sorghum collection, and efforts to transfer high protein content into desirable agronomic types are underway. Marked differences in seed size also occur among lines in the collection. Little information has been published on the interrelationships among these attributes in grain sorghums. This paper presents the results of determinations for grain protein and oil percentages and 100-seed weight made on 48 sorghum hybrids and their parental varieties.

REVIEW OF LITERATURE

Several investigations have shown that the protein content of sorghum grain may differ appreciably among varieties or hybrids. Marked effects of cultural practices and environmental conditions on protein percentage have also been reported. Protein percentage among 28 varieties ranged from 7.3 to 10.5 in a 2-year study by Heller and Sieglinger (1944). A range of 11.6 to 15.0% protein was obtained at the same location in a different year, but, at another Oklahoma location, the range among the same varieties was from 8.9 to 11.8%. Drouth stress was observed to decrease yields, but increase protein percentages.

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Hubbard et al. (1950) found a range of only 11.5 to 13.2% protein in the whole grain of five varieties of sorghum. A range in protein content of 8.7 to 16.8% was reported by Miller (1958), but he did not indicate whether varieties or hybrids were evaluated. Grain protein determinations were made on six varieties and 35 sorghum hybrids by Worker and Ruckman (1968). The varieties and hybrids responded alike to different environmental conditions. Mean grain protein content over a 5-year period was 10.1 and 14.8% from plantings in April and July, respectively. The complete range was 8.5 to 21.5% during this period, depending on variety, year, and planting date. Seed size and protein percentage were correlated significantly ($r = 0.55$) in their experiments.

Similar ranges in grain protein (6.6 to 12.8% and 5.9 to 12.1%) among the entries in Kansas hybrid sorghum trials in two seasons were reported by Miller et al. (1964). Significant differences among locations and hybrids were found, and fertilizer applications resulted in both increased yields and protein percentages. In tests where check varieties were included, the standard varieties were higher in protein percentage than the hybrids. In other tests in Kansas, Deyoe and Shellenberger (1965) found a range of 8.6 to 12.5% in protein content among 15 sorghum hybrids grown at three locations. Significant differences were noted for the hybrids, locations, and hybrids x location sources of variation.

Analyses of the grain of entries in the world sorghum collection by Pickett (1969a) showed a range of 7 to 26% in crude protein. Values above 20% were obtained mostly with grassy-types, but a range of 8 to 20% protein was obtained among grain types. In a subsequent study by Pickett (1969c) with hybrids among some of the entries, positive correlations of seed size with grain yield ($r = 0.42$) and seed size with protein yield ($r = 0.51$) were obtained. With hybrids among a different group of lines, Pickett (1969b) found a negative association ($r = -0.57$) between yield and protein percentage.

Oil content of the whole grain ranged only from 3.2 to 3.9% among five sorghum varieties analyzed by Hubbard et al. (1950). The range in oil percentage among the 28 varieties evaluated by Heller and Sieglinger (1944) at two locations in two years was 1.4 to 3.9. A somewhat greater range (1.2 to 5.7%) was reported by Pickett (1969a) among diverse entries of the world sorghum collection.

Seed protein and oil content are correlated negatively in some crop species. Simultaneous increases of both constituents by plant breeders, therefore, have been difficult with these crops. According to Wilcox (1969), soybeans consistently have shown negative correlations between oil and protein content, ranging from -0.48 to -0.77. Associations of both oil and protein content with seed size in soybeans have been variable. Some studies associate high-oil and high-protein percentage with large seed, but others indicate that high values for both constituents are correlated with small seed. Brown et al. (1966) found significant negative correlations of -0.31 and -0.48 between oil and protein content in spring and winter oats, respectively. Correlations of 0.72 and 0.79 were obtained between oil percentage and 100-seed weight in two experiments with flax by Johnson (1932).

Oil and protein content of the corn kernel were not significantly correlated ($r = 0.23$) in the experiments of Brunson et al. (1948). They

also observed that kernel size had little influence on composition of the kernel. Hopkins *et al.* (1903) reported that the correlation between protein and oil content of corn was very low. In two synthetic varieties of corn, previously selected for oil content in the grain, Alexander and Seif (1963) found oil content independent of kernel weight. Miller and Brimhall (1951) found that variation in total-oil percentage was not closely associated with the variation in total-protein percentage of corn kernels. Correlations between these attributes were not significant, ranging from -0.04 to 0.33 in F_2 and backcross populations.

In sorghum, oil and protein content of the grain were significantly correlated ($r = 0.44$) in an experiment reported by Pickett (1969c). The experiment involved six genotypes planted at three population densities. Additional evaluations of associations between these attributes in sorghum were not found in the literature.

EXPERIMENTAL PROCEDURE

Sorghum grain samples of 14 parental varieties, plus the F_1 and F_2 seed of 48 hybrids, were obtained from plantings at Ames, Iowa. The F_1 hybrid seed was produced by pollinating each of six male-steriles (A-lines) with eight different pollen fertility restorers (R-lines). F_2 seed was obtained the same year from an adjacent experiment by bagging heads of F_1 plants of these hybrids. Seed of the parents was obtained from bagged heads of the R-lines and B-lines (fertile, nonrestorer counterparts of each male-sterile).

Samples for determining the oil percentage of each F_2 entry were obtained by bulking seed from three bagged heads from each of two field replicates. For the parental and F_1 seed, three heads of each entry were bulked from controlled self- and cross-pollinations, respectively. Twenty-five grams from each bulked sample were used to determine oil percentage of the whole seed. Oil percentages were determined³ by wide-line nuclear magnetic resonance (NMR) using a Varian PA-7 Process Analyzer equipped with integrator V4221. Conway and Earle (1963) have shown that oil percentages by NMR and by gravimetric analysis are highly correlated ($r = 0.99$).

Duplicate 100-seed lots were taken from each sample after oil percentages were determined and seed weights in centigrams were recorded. These samples were ground in a Wiley mill (40-mesh screen), and 0.1 gram of the pulverized seed from each sample was used for the crude protein analyses. Nitrogen determined by the micro-Kjeldahl procedure was converted to protein percentage by multiplying by a 6.25 factor. Analyses of variance of the data for each attribute, and correlations among attributes, were calculated in accordance with the methods of Snedecor (1956).

³ Thanks are extended to Dr. D. E. Alexander, Agronomy Department, University of Illinois, for the oil percentage determinations.

RESULTS AND DISCUSSION

Oil and protein percentages, plus 100-seed weights, of the 14 parental varieties are listed in Table 1. In the development of these lines, selection has been directed largely toward their value as either seed or pollen parents and their combining ability for grain yield in hybrid combinations. Seed size and desirability for other agronomic and disease characteristics also have been considered, but protein and oil content of the grain have received little attention. Significant differences in oil content, within either the male or female parent groups, were not indicated by the analysis of variance. The range among all parents was less than 1%. Protein percentages among the parents differed more than did their oil content, with a range of 11.1 to 14.4% among all parents. Differences in protein percentages among the female parents exceeded the 5% probability level. Similarly, the female parents differed significantly (1% level) for 100-seed weight, but the male parents did not.

Mean oil percentages for F_1 and F_2 seed of the 48 hybrids are given in Table 2. Comparable data for protein percentage are given in Table 3.

Table 1. Mean oil and protein percentage, and 100-seed weight, of parental varieties of grain sorghum.

	Character ^{1/}		
	Oil (%)	Protein (%)	100-seed wt (g)
<u>Male parents</u>			
Norghum	3.38	12.0	2.46
Texas 7078	3.17	12.6	2.85
Texas 04	2.92	12.7	2.65
Texas 07	3.30	12.1	2.13
Texas 74	3.35	11.5	2.55
Redbine 60	2.93	13.3	2.80
Plainsman	3.01	11.1	2.39
Caprock	3.15	11.4	2.66
Mean of male parents	3.15	12.1	2.56
<u>Female parents</u>			
Reliance	3.31	14.4	2.39
Martin	3.45	13.3	3.01
Combine Kafir 60	3.05	11.8	3.02
Westland	3.10	11.3	2.50
Wheatland	3.05	11.9	3.52
Redlan	2.77	11.3	3.14
Mean of female parents	3.12	12.3	2.93
Mean (all parents)	3.14	12.2	2.72

^{1/} LSD_{.05} (among individual parent means), oil percentage = 0.45, protein percentage = 2.1, 100-seed wt. = 0.58.

Table 2. Mean oil percentage of F_1 and F_2 hybrid seed of grain sorghums.

Male parents	Female parents												Means of hybrids	
	Reliance		Martin		Kafir 60		Westland		Wheatland		Redlan		F_1	F_2
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2		
Norghum	3.53	^{1/} 3.63	3.64	3.50	3.26	3.27	3.36	3.27	3.37	3.14	3.45	3.19	3.43	3.33
Texas 7078	3.54	3.46	3.64	3.59	3.55	3.35	3.26	3.45	3.27	3.38	3.51	3.41	3.46	3.44
Texas 04	3.32	3.38	3.28	3.60	3.38	3.28	3.44	3.43	3.03	3.59	3.16	3.51	3.27	3.46
Texas 07	3.57	3.53	3.63	3.56	3.38	3.43	3.68	3.56	3.03	3.54	3.25	3.66	3.42	3.55
Texas 74	3.58	3.66	3.76	3.81	3.31	3.44	3.40	3.46	3.13	3.54	3.40	3.45	3.43	3.56
Redbine 60	3.79	3.50	3.76	3.33	3.21	3.18	3.70	3.27	3.19	3.43	3.11	3.26	3.46	3.33
Plainsman	3.39	3.48	3.02	3.52	3.47	3.40	3.30	3.37	3.04	3.38	2.95	3.35	3.19	3.42
Caprock	3.33	3.56	3.55	3.50	3.47	3.49	3.63	3.40	2.92	3.54	3.35	3.23	3.37	3.45
Means of hybrids	3.51	3.53	3.60	3.55	3.38	3.36	3.47	3.40	3.13	3.45	3.27	3.39	3.39	3.45

^{1/} $LSD_{.05}(F_1)$, individual hybrids = 0.45, means by female groupings = 0.18, means by male groupings = 0.15

$LSD_{.05}(F_2)$, individual hybrids = 0.11, means by female groupings = 0.04, means by male groupings = 0.04

Table 3. Mean protein percentage of F_1 and F_2 hybrid seed of grain sorghums.

Male parents	Female parents												Means of hybrids	
	Reliance		Martin		Kafir 60		Westland		Wheatland		Redlan			
	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2	F_1	F_2
Norghum	13.1	^{1/} 13.2	16.7	12.9	13.0	10.4	13.9	10.7	13.6	13.8	14.0	11.5	14.0	12.1
Texas 7078	14.8	11.0	14.0	11.8	12.5	11.2	13.6	13.5	14.6	11.1	13.4	13.5	13.8	12.0
Texas 04	15.3	13.2	13.9	13.6	11.2	12.2	14.1	14.2	13.9	13.7	9.4	13.9	13.0	13.5
Texas 07	16.8	12.3	16.5	13.1	12.7	12.8	14.2	12.8	14.0	13.5	14.1	13.7	14.7	13.1
Texas 74	17.5	16.1	16.7	14.2	10.6	14.3	13.6	13.6	14.9	12.3	7.9	14.6	13.5	14.2
Redbine 60	16.9	13.3	14.8	13.0	11.6	12.1	14.3	11.8	14.8	12.4	12.5	14.2	14.2	12.8
Plainsman	15.4	12.6	14.9	13.1	12.0	13.5	15.7	10.9	14.5	11.7	14.4	13.0	14.5	12.5
Caprock	18.0	11.4	14.7	14.1	14.0	13.4	11.4	12.6	14.7	10.7	14.7	13.7	14.6	12.7
Means of hybrids	16.0	12.9	15.3	13.2	12.2	12.5	13.9	12.5	14.4	12.4	12.6	13.5	14.0	12.8

^{1/} LSD_{.05} (F_1), individual hybrids = 2.1, means by female groupings = 0.87, means by male groupings = 0.75

LSD_{.05} (F_2), individual hybrids = 2.3, means by female groupings = 0.92, means by male groupings = 0.79

The parental and F_1 seeds were produced in the breeding nursery, but the F_2 seeds were obtained from an adjacent yield trial. Therefore, separate analyses of variance were calculated for the F_2 data and for the F_1 plus parental variety results. Significance at the 5% probability level was shown for the F_1 hybrids vs. parents comparison for oil percentage. Mean oil percentage for the F_1 's was 3.39, compared with 3.14 for the parents. The range among the hybrids in oil content of the F_1 seed was small (2.92 to 3.79). The mean and range in oil content of the F_2 seed were similar to those for the F_1 seed. Average mid-parent heterosis for oil content of the F_1 seed was 8%, and the F_2 seed averaged 10% higher than the parental mean.

Data from reciprocal crosses in soybeans by Singh and Hadley (1968) and in corn by Woodworth and Mumm (1935) show a strong maternal effect on oil percentage of the grain. Oil content of the F_1 seed in these studies was much closer to that of selfed seed from the female parent than to that of selfed seed from the male parent. Our study with sorghum did not include reciprocal crosses, but the data generally are not indicative of a strong maternal influence on oil content. Crosses with Redlan serve particularly well to support this conclusion. The Redlan female parent was lower in oil percentage than any of the male parents, but the F_1 seed of all crosses with Redlan exceeded the mid-parent value. Usually, the F_1 seed approached or exceeded the oil content of the male parent. Although less pronounced and consistent, this trend also was evident in many of the crosses involving other female parents. The reverse was true, however, in crosses with the Martin female parent. It was higher in oil content than any of the male parents, and the F_1 seeds of its crosses always exceeded the mid-parent oil percentage.

In the analyses of variance for both the F_1 and F_2 data, a partitioning of the variation among hybrids within each female parent was made. For oil percentage of the F_1 seed, the variation among the eight hybrids involving a given female parent was not significant in any instance. However, for the F_2 seed, the variation among hybrids involving a particular female parent always was significant (1% level). This contrast in variation within the two populations suggests that the oil content of sorghum seed is influenced by the genotype of the seed and is not strictly determined by the genotype of the plant producing the seed. Analyses of the oil content of single F_2 seeds would be necessary to categorize the nature and extent of the genotypic effects.

Protein percentages of the F_1 vs. parental seed differed significantly (1% level) and showed an average mid-parent heterosis of 15%. A few very low and high percentages extended the range from 7.9 to 18.0, but most F_1 hybrid seed had 11 to 14% protein. Variation among F_1 's was partitioned into variation among hybrids within each female parent, and variation was significant for all but the Combine Kafir 60 and Wheatland hybrids. For the F_2 seed, variation within the Westland, Combine Kafir 60, and Reliance hybrids was significant, but the hybrids within each of the other female parents did not differ significantly in protein content. Mean protein content of the F_2 seed (12.8) was about 8% less than that of the F_1 seed (14.0), but 5% above the parental mean.

Seed weights for the individual hybrids are not presented, but mean weights per 100 seeds for the parental, F_1 , and F_2 seed were 2.72, 3.23,

and 3.07 grams, respectively. Mid-parent heterosis for the F_1 seed averaged 19%. For the F_2 seed, mean seed weight was 13% greater than the average of the parental varieties. In the variance analysis of the F_1 data, only the hybrids involving the Martin female parent differed significantly for seed weight, but for the F_2 seed the variation among hybrids within each female parent was significant.

Correlation coefficients for oil vs. protein percentage, oil content vs. 100-seed weight, and protein content vs. 100-seed weight are presented in Table 4. The association of oil and protein content was positive among the parental varieties and among both the F_1 and F_2 seed of the hybrids. Only the coefficient for the F_2 seed was significant.

Table 4. Correlation coefficients for oil vs. protein percentage, oil content vs. 100-seed weight and protein content vs. 100-seed weight in three types of grain sorghum populations.

Population ²	Characters correlated ¹		
	Oil vs. protein	Oil vs. seed weight	Protein vs. seed weight
Parental varieties	0.288	-0.304	0.011
F_1 hybrid seed	0.184	0.048	-0.155
F_2 hybrid seed	0.299**	0.197	0.470**

¹ **, Coefficient exceeds the 1% level of probability.

² 26 df for parental varieties, 94 df for F_1 and F_2 hybrid populations.

Hybrids are planted on nearly all grain sorghum acres in the United States, and the F_2 seed is used for animal or human food and for processing by the milling industry. Therefore, the associations among attributes in the F_2 seed are especially pertinent. The positive correlations of protein and oil content indicate that selection by plant breeders for high protein grain should not preclude a simultaneous improvement in oil content. The correlation of 0.299 between protein and oil percentage in the F_2 seed, however, transposes to a coefficient of determination (r^2) of less than 0.1. This value would be even smaller for the parental and F_1 seed. Thus, selection for high protein content would not have been highly effective in also improving oil content, but at least, marked impediments to the simultaneous improvement of both attributes (as cited for soybeans) were not indicated within the populations of this study. Whether a similar relationship exists among sorghums more diverse for oil and protein content remains to be determined.

The correlations for both oil and protein content with 100-seed weight of the F_2 seed also were positive. For protein content vs. seed weight, the correlation is significant and fairly strong. Collectively, the correlations for the F_2 seed do not indicate any associations that would impede the development of hybrids that have large seeds with relatively high protein and oil content. The F_2 seed of several of the hybrids had relatively high values for all three attributes, particularly the Reliance x Texas 74, Martin x Texas 74, and Redlan x Texas 07 hybrids.

Correlations among the three attributes also were calculated for the

F₁ and F₂ seed of the hybrids within each female parent group. The degrees of freedom for testing the significance of the correlations within these groups are small (14 df), thus only the coefficients based on the entire F₁ and F₂ populations are presented. Moderately large coefficients were more frequently obtained for the correlations determined within individual female parents, and most were positive. A few non-significant negative associations were noted, but all significant coefficients were positive. The variations among individual group correlations indicate that the strength of the interrelationships among oil and protein content and seed weight may be affected by female parentage of the hybrids.

The F₂ data also were analyzed according to the Design II procedure of Comstock and Robinson (1948). The heritability of each attribute was calculated by determining the proportion of the total variance due to additive gene action. Heritabilities for oil content, protein content, and 100-seed weight were 65%, 32%, and 52%, respectively. These values may overestimate the true heritability of the characters, since the data are from a single experiment, and a separation of genotype x environment interactions from the genetic variance is not possible. Nevertheless, each character seems sufficiently heritable to indicate that selection in these populations should be reasonably effective for the improvement of oil and protein content and seed weight. Also, one should bear in mind that our populations were derived from crosses among a small group of highly selected parental varieties. The highly diverse lines of the world sorghum collection may well differ more widely in their genetic constitution for these characters. Crosses among these lines should provide populations with larger genotypic variances and higher heritabilities. Selection within populations of this type likely would be considerably more effective for improving the protein and oil content and seed size of grain sorghums.

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LIFE HISTORY OF MICROTETRAMERES CENTURI BARUS, 1966
(NEMATODA: TETRAMERIDAE). III. TAXONOMY

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ABSTRACT. The taxonomic status of the genus Microtetrameres is discussed. Arguments are presented for maintaining Tetrameres and Microtetrameres as separate genera and for substantiating their possible future placement in separate families.

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The validity of the nematode family Tetrameridae Travassos, 1914 has been challenged by some but accepted by many (Chabaud 1951, Cram 1927, La Page 1961, Oshmarin 1956, Oshmarin and Parukhin 1963, Petrov and Chertkova 1950, Rasheed 1960, Skrjabin 1916, Skrjabin and Soblev 1963, Sultana 1962, Travassos 1914, Yamaguti 1935, Yorke and Maplestone 1926). On the other hand, Tetrameridae has been listed as a synonym of Spiruridae (Chitwood and Wehr 1934). Still others maintained the latter designation (Lopez-Neyra 1947, Wehr 1934). Which ever familial designation was used, the two genera Microtetrameres and Tetrameres have been included within the same family.

Despite the fact that Yamaguti (1935) placed Tetrameres scolopacis in the family Tetrameridae, he (Yamaguti 1961) recently reverted to the use of Tropisurus instead of Tetrameres. The genus Tropisurus was erected by Diesing in 1835. He indicated in a footnote that this name was constructed from two Greek words, "tropis" (keel) and "ura" (tail). But, he erred (Stiles and Baker 1930, Wiegmann 1835) in constructing this generic name because the genitive case of "tropis" is "tropidos." Hence, the proper masculinized designation should be Tropidurus. This generic name, however, has been preempted in 1824 by Neuwied to name a reptile genus (Agassiz 1848). Therefore, in 1846, Creplin re-named the genus Tetrameres and general acceptance of this designation is indicated by its almost exclusive usage in nematological literature. Tetrameres, therefore, should be the valid name for this genus despite the acceptance of Tropisurus by others (Barus 1966, Ortlepp 1964, Yamaguti 1961).* Tetrameres is the type genus for the family Tetrameridae which includes Microtetrameres as the only other genus.

The genera Tetrameres and Microtetrameres parasitize birds only, except, doubtfully, Tetrameres bispinosa reported by Molin (1860) from Scincus sp. These genera are classified within Tetrameridae, but Lopez-Neyra (1958) proposed placing the genus Crassicauda in this

* The name Tetrameres Creplin 1846 has been validated by the International Commission on Nomenclature (Opinion 892, October 1969) and Tropisurus Diesing 1835 was suppressed, according to Chitwood (1970, J. Parasit. 56:374).

family also. This suggestion may be inappropriate as the latter genus is found in cetaceans (Baylis 1916, Baylis 1920, Baylis 1922, Joyeux and Baer 1951, Skrjabin and Andreeva 1934) and the other genera are found in birds.

Chabaud (1951) proposed including three subfamilies, Crassicaudinae, Geopetitiinae and Tetramerinae, within the family Tetrameridae, with Crassicauda and Geopetitia as the type genera, respectively, for the first two of the subfamilies. Tetrameris was considered the type genus for Tetramerinae which also included Microtetrameris.

These subfamilies, according to Chabaud (1951), should be grouped together because they are related morphologically to both spirurid and filarial nematodes. The remarkable anatomical resemblance between a first-stage juvenile Microtetrameris and a microfilaria has been reported (Ellis 1969a). Furthermore, Chabaud developed the idea that evolution would link these three genera within one family.

The maintaining of Microtetrameris and Tetrameris as two distinct genera is substantiated by anatomical differences between adults and juveniles of the two genera. For instance, adult female Tetrameris are globose or spindle-shaped; those of Microtetrameris are coiled. The buccal capsules of the females of the two genera are quite different, the latter being more complex. The eggs of the two genera differ in the presence of bosses on eggs of Microtetrameris and their absence in Tetrameris. Presumed and known males of Tetrameris possess longitudinal rows of spines; these are absent on presumed and known males of Microtetrameris (Ellis 1969b). First-stage juveniles of Tetrameris possess caudal spines or papillae (Chabaud 1954, Seurat 1918); third-stage Tetrameris americana, experimentally reared, also exhibit these (Cram 1931). Such structures are absent from third-stage M. centuri juveniles (Ellis 1969a).

Some members of the family Spiruridae, as well as juvenile Tetrameris, also possess spines or papillae on their posterior ends. Some of these spines are on a small terminal "bouton" (Seurat 1915-16); others adorn rounded tail ends (Chabaud 1954). Larger spines occur on the tails of presumed fourth-stage juvenile Tetrameris fissipina (Seurat 1918) and papillae have been seen on T. americana third-stage juveniles experimentally reared (Cram 1929, 1931). However, spines and papillae are absent on the posterior ends of laboratory-reared juveniles of M. centuri (Ellis 1969a). Spines were not reported on the same stage of M. helix (Cram 1934) nor from first-stage Tropidocerca (= Microtetrameris) inermis (Seurat 1913). Juveniles of M. helix, M. inermis, and M. centuri terminate posteriorly in a sharp tail. In third-stage juveniles the tail extends to a tiny, glabrous hemisphere.

Because of the anatomical differences and because known data show that members of the genus Tetrameris parasitize aquatic and semi-aquatic birds and those nematodes included in Microtetrameris are harbored by nonaquatic avian hosts, one might relegate the two genera to separate families, Chabaud (1951) notwithstanding. Such action seems premature, however, because of insufficient experimental and quantitative evidence. Therefore, until more information is available the two genera should be kept in Tetrameridae.

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COMPARATIVE MEASUREMENTS AND HOST AND GEOGRAPHICAL
DISTRIBUTION OF SPECIES OF MICROTETRAMERES
(NEMATODA: TETRAMERIDAE)

Charles J. Ellis

Females of the nematode genus Microtetrameres are parasites found exclusively in the proventricular glands of birds. Their presence has been reported by authors throughout the world whose investigations concerned only wild birds. However, three reports (Cram 1934, Ellis 1969a, 1969b) dealt with experimental rearing of these parasites. Adult males assumed to be of this genus have been mentioned but only one description of such a male raised from an egg is known (Ellis 1969b). The pathogenicity of this genus has been reported (Ellis 1970). However, this work does not indicate how wide-spread the pathological effects of this parasite might be within the Class Aves. Such information might have little practical value as no species of Microtetrameres has been found in birds of economic importance. Perhaps this fact contributes to the dearth of data concerning its pathology.

A large population of these nematodes in the proventriculus of any bird probably would be quite detrimental. Such a number of parasites probably would block the production of acid and in turn would disrupt the digestive processes of the host. Such a hypothesis has been offered (Ellis 1970). However, it remains to be verified in terms of numbers of nematodes per proventriculus.

Some doubt existed concerning the proper family to which this genus should be assigned. It has been classified variously within the Spiruridae, Tropisuridae, and Tetrameridae. However, arguments favoring the latter familial assignment have been presented (Ellis 1971). The taxonomy of the family Tetrameridae itself has been subjected to some question. With more study, perhaps it will be assigned certain other genera or subgenera.

Many investigators interested in the genus Microtetrameres must know its geographic and host distribution and the comparative anatomical measurements. These data can be determined accurately only by an extensive literature search.

Such a search has been completed and the data so accumulated are presented below. No attempt has been made to change the classificatory work of the various authors. All tabulated entries have been made from current literature or personal communication.

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Table 1. Species of Microtetrameres with their hosts and geographical distribution.

<u>Microtetrameres</u> species	Avian species	Location	Investigator
<u>accipiter</u>	<u>Accipiter gentilis</u>	Idaho	Schell, 1953
<u>aquila</u>	<u>Aquila chrysaetos</u>	Montana	Schell, 1953
<u>asymmetricus</u>	<u>Lanius cristatus</u>	Russia	Oshmarin, 1956
<u>bubo</u>	<u>Asio flammeus</u>	Russia	Oshmarin, 1956
<u>bubo</u>	<u>Strix uralensis</u>	Russia	Oshmarin, 1956
<u>bubo</u>	<u>Otus bakkamoena</u>	Russia	Oshmarin, 1956
<u>bubo</u>	<u>Nyctea scandiaca</u>	Russia	Oshmarin, 1956
<u>bubo</u>	"long-tailed owl"	Russia	Oshmarin and Parukhin, 1963
<u>bubo</u>	<u>Bubo virginianus</u>	Oregon	Schell, 1953
<u>bucerotidi</u>	<u>Lophoceros flavirostris</u>	Transvaal	Ortlepp, 1964
<u>bucerotidi</u>	<u>Lophoceros erythrorhynchus</u>	Transvaal	Ortlepp, 1964
<u>calabocensis</u> ^a	<u>Buteo magnirostris insidiatrix</u>	Calabozo	Diaz-Ungria, 1965
<u>canadensis</u>	<u>Nyctea scandiaca</u>	Hudson Bay	Mawson, 1956a
<u>canadensis</u>	<u>Ardea herodias</u>	Quebec and Ontario	Mawson, 1956a
<u>centuri</u>	<u>Centurus superciliaris superciliaris</u>	Cuba	Barus, 1966
<u>cephalatus</u> ^a	<u>Turdus merula nigropileus</u>	Hyderabad	Sultana, 1962
<u>cloacitectus</u> ^a	<u>Buteo buteo</u>	Bohemia	Barus, 1966
<u>cloacitectus</u> ^a	<u>Buteo buteo</u>	Russia	Oshmarin, 1956
<u>contorta</u>	<u>Dichocerus bicornis</u>	Tonkin	Hsu, 1935
<u>contorta</u>	<u>Corvus frugilegus</u>	Russia	Skrjabin, Shikhobalova and Sobolev, 1949
<u>contorta</u>	"corvidae"	Gorkii	Spasskii and Oshmarin, 1939

<u>Microtetrameres</u> species	Avian species	Location	Investigator
<u>contorta</u>	<u>Corvus frugilegus</u>	Roumania	Stoican, 1960
<u>contorta</u>	<u>Dichocerus bicornis</u> ^b	Pennsylvania	Weidman, 1913
<u>corax</u>	<u>Corvus corone</u>	Egypt	Myers, Kuntz and Wells, 1962
<u>corax</u>	<u>Corvus corax</u>	Idaho	Schell, 1953
<u>corax</u>	<u>Pica pica hudsonia</u>	Montana	Todd and Worley, 1967
<u>creplini</u>	<u>Accipiter nisus</u>	Russia	Vavilova, 1926
<u>cruzi</u>	<u>Amospiza maritima macgillivraii</u>	North Carolina	Hunter and Quay, 1953
<u>cruzi</u>	<u>Bucco swainsoni</u>	Brazil	Travassos, 1914
<u>cruzi</u>	<u>Melanerpes flavifrons</u>	Brazil	Travassos, 1914
<u>egretes</u>	<u>Egretta garzetta</u>	Hyderabad	Rasheed, 1960
<u>egretes</u>	<u>Ardeola ibis ibis</u>	Cuba	Barus, 1966
<u>gubernaculiferens</u> ^{c,d}	"marsh harrier"	Tashkent	Sultanov, 1945
<u>gubernaculiferens</u> ^a	<u>Circus aeruginosus</u>	Central Asia	Sultanov, 1947
<u>helix</u>	<u>Corvus frugilegus</u>	Kirgizia	Ablasov and Chibichenko, 1962
<u>helix</u>	<u>Pica pica</u>	Kirgizia	Ablasov and Chibichenko, 1962
<u>hilix</u> (sic)	<u>Corvus leuillantii</u>	Primorsk	Bashkirova, 1960
<u>helix</u>	<u>Cyanopica cyana</u>	Primorsk	Bashkirova, 1960
<u>helix</u>	<u>Garrulus glandarius</u>	Primorsk	Bashkirova, 1960
<u>helix</u>	<u>Sturnus vulgaris</u>	New England	Boyd, 1951
<u>helix</u>	<u>Corvus americanus</u>	Washington, D.C.	Cram, 1927
<u>helix</u>	<u>Corvus brachyrhynchos brachyrhynchos</u>	Manitoba	Hodasi, 1963

<u>Microtetrameres</u> species	Avian species	Location	Investigator
<u>helix</u>	<u>Corvus brachyrhynchos</u> <u>brachyrhynchos</u>	Quebec	Mawson, 1956b
<u>helix</u>	<u>Corvus brachyrhynchos</u> <u>brachyrhynchos</u>	Iowa - Wisconsin	Morgan and Waller, 1941
<u>helix asiaticus</u>	<u>Pica pica</u>	Russia	Oshmarin, 1956
<u>helix asiaticus</u>	<u>Garrulus glandarius</u>	Russia	Oshmarin, 1956
<u>helix asiaticus</u>	<u>Cyanopica cyana</u>	Russia	Oshmarin, 1956
<u>helix asiaticus</u>	<u>Corvus corone</u>	Russia	Oshmarin, 1956
<u>helix</u>	<u>Corvus frugilegus</u>	Russia	Skrjabin, Shikobalova and Sobolev, 1949
<u>helix</u>	"corvidae"	Gorkii	Spasskii and Oshmarin, 1939
<u>helix</u>	<u>Corvus frugilegus</u>	Roumania	Stoican, 1960
<u>helix</u>	<u>Corvus corone cornix</u>	Bulgaria	Stoimenov, 1963
<u>helix</u>	<u>Tockus birostris</u>	Hyderabad	Sultana, 1962
<u>inermis</u> ^e	<u>Oenanthe isabelina</u>	Kirgizia	Ablasov and Chibichenko, 1962
<u>inermis</u> ^e	<u>Sturnus vulgaris</u>	Kirgizia	Ablasov and Chibichenko, 1962
<u>inermis</u> ^e	"sparrow-hawk"	Algeria-Russia- Turkestan	Cram, 1927
<u>inermis</u> ^e	<u>Passer domesticus</u>	Algeria-Russia- Turkestan	Cram, 1927
<u>inermis</u> ^e	<u>Astur nisus</u>	Algeria-Russia- Turkestan	Cram, 1927
<u>inermis</u> ^e	<u>Lanius</u> sp.	Algeria-Russia- Turkestan	Cram, 1927

<u>Microtetrameres</u> species	Avian species	Location	Investigator
<u>inermis</u> ^e	<u>Alauda arvensis dulcinx</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Anthus spinoletta blakistani</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Corvus cornix sharpei</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Corvus frugilegus</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Emberiza leucocephalus</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Emberiza schoenichus pallidior</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Parus cinereus</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Parus major</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Phoenicurus erythronotus</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Scotocerca inquietta platyvra</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Turdus atrogularis</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Troglodytes troglodytes</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Sturnus vulgaris poltoratskyi</u>	Tadzhikistan	Dubinina and Serkova, 1951
<u>inermis</u> ^e	<u>Corvus coroneae</u> (sic)	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957

<u>Microtetrameres</u> species	Avian species	Location	Investigator
<u>inermis</u> ^e	<u>Falco subbuteo</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957
<u>inermis</u> ^e	<u>Sturnus vulgaris</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957
<u>inermis</u> ^e	<u>Astur palumbarius</u>	Algeria-Russia-Turkestan	von Linstow, 1879
<u>inermis</u> ^e	<u>Hypotriorchis subbuteo</u> ^f	Kirgizia	Petrov and Chertkova, 1950b
<u>inermis</u> ^e	<u>Corvus frugilegus</u>	Zelenec u Prahy	Rysavy, 1957
<u>inermis</u> ^e	<u>Corvus corax tingitanus</u>	Algeria	Seurat, 1913
<u>inermis</u> ^e	"moineau domestique"	Algeria	Seurat, 1913
<u>inermis</u> ^e	<u>Temenuchus pagodarum</u>	Hyderabad	Singh, 1949
<u>inermis</u> ^e	<u>Brachypternus bengalensis</u> ^g	Hyderabad	Singh, 1949
<u>inermis</u> ^e	<u>Corvus corone</u>	Algeria-Russia-Turkestan	Skrjabin, 1916
<u>inermis</u> ^e	<u>Corvus frugilegus</u>	Algeria-Russia-Turkestan	Skrjabin, 1916
<u>inervis</u> (sic) ^e	<u>Corvus frugilegus</u>	Roumania	Stoican, 1960
<u>inermis</u> ^e	<u>Leiopicus mahrattensis blanfordi</u>	Hyderabad	Sultana, 1962
<u>inermis</u> ^e	<u>Passer domesticus</u>	Maryland	Wilson, 1956
<u>inermis</u> ^e	<u>Corvus frugilegus</u>	Russia	Zekhnov, 1949
<u>longiovatus</u>	<u>Glaucidium siju vittatum</u>	Cuba	Barus, 1969
<u>minima</u>	<u>Tachyphonus cristatus bruneus</u>	Brazil	Travassos, 1914
<u>mirzai</u>	<u>Cerchneis tinnunculus interstinctus</u> ^h	India	Rasheed, 1960
<u>oriolus</u>	<u>Oriolus oriolus</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957

<u>Microtetrameres species</u>	<u>Avian species</u>	<u>Location</u>	<u>Investigator</u>
<u>oriolus oriolus</u>	<u>Oriolus oriolus turkestanicus</u>	Kirgizia	Petrov and Chertkova, 1950a
<u>oriolus orientalis</u> ^a	<u>Oriolus chinensis</u>	Russia	Oshmarin, 1956
<u>oriolus</u>	<u>Turdus merula</u>	Kirgizia	Gagarin, Abblasov and Chibichenko, 1957
<u>oriolus</u>	<u>Turdus ruficollis</u>	Kirgizia	Gagarin, Abblasov and Chibichenko, 1957
<u>oriolus rasheedi</u>	<u>Oriolus oriolus</u>	Russia	Skrjabin and Sobolev, 1964
<u>oshmarini</u>	<u>Asio flammeus</u>	Russia	Skrjabin and Sobolev, 1963
<u>oshmarini</u>	<u>Nyctea scandiaca</u>	Russia	Skrjabin and Sobolev, 1963
<u>oshmarini</u>	<u>Otus bakkamoena</u>	Russia	Skrjabin and Sobolev, 1963
<u>oshmarini</u>	<u>Strix uralensis</u>	Russia	Skrjabin and Sobolev, 1963
<u>osmaniae</u>	<u>Accipiter nisus nisosimilis</u>	Hyderabad	Rasheed, 1960
<u>papillocephala</u> ^a	<u>Butastur indicus</u>	Russia	Oshmarin, 1956
<u>pelecani</u>	<u>Pelecanus onocrotalus</u>	Middle Asia ⁱ	
<u>pokrowskyi</u>	<u>Ardea cinerea</u>	Mongolia	Petrov and Chertkova, 1950a
<u>pusilla</u>	"motley thrush"	Primorsk	Bashkirova, 1960
<u>pusilla</u>	<u>Turdus dauma</u>	Primorsk	Bashkirova, 1960
<u>pusilla</u>	<u>Turdus dauma</u>	Russia	Oshmarin, 1956
<u>pusilla</u>	<u>Turdus naumanni</u>	Primorsk	Bashkirova, 1960
<u>pusilla</u>	<u>Turdus naumanni</u>	Russia	Oshmarin, 1956

<u>Microtetrameres</u> species	Avian species	Location	Investigator
<u>pusilla</u>	<u>Turdus rufiventris</u>	Brazil	Travassos, 1915
<u>pusilla</u>	<u>Turdus viscivorus</u>	Russia	Skrjabin, Shikhobalova and Sobolev, 1949 ^J
<u>pusilla</u>	<u>Platycichla flavipes</u>	Brazil	Travassos, 1915
<u>rasheedae</u> ^a	<u>Hierococcyx varius</u>	Hyderabad	Sultana, 1962
<u>saguei</u>	<u>Myadestes elisabeth elisabeth</u>	Cuba	Barus, 1966
<u>saguei</u>	<u>Myadestes elisabeth elisabeth</u>	Cuba	Barus and Garrido, 1968
<u>singhi</u> ^a	<u>Falco jugger</u>	Hyderabad	Sultana, 1962
<u>spiculata</u>	<u>Cyanocitta cristata</u>	New England	Boyd, Diminno and Nesslering, 1956
<u>spiralis</u>	<u>Ardeola ibis ibis</u>	Egypt	Myers, Kuntz and Wells, 1962
<u>spiralis</u>	<u>Bubulcus coromandus</u>	India	Maplestone, 1931
<u>spiralis</u>	<u>Bubulcus ibis</u>	Alabama	Dismukes and Stuart 1970
<u>spiralis</u>	<u>Lanner falcon</u>	North Wales	Threlfall, 1965
<u>spiralis</u>	<u>Bubulcus lucidus</u>	Algeria	Seurat, 1915
<u>spiralis</u>	<u>Falco feldegi</u>	Wales	Threlfall, 1965
<u>travassosi</u>	<u>Gracula religiosa</u> ^k	Hyderabad	Rasheed, 1960
<u>tubocloacis</u> ^{a, l}	<u>Aquila clanga</u>	Russia	Oshmarin, 1956
<u>xiphidiopicl</u>	<u>Xiphidiopicus percussus percussus</u>	Cuba	Barus, 1966
sp.	<u>Falco tinnunculus</u>	Slovakia	Barus, 1966
sp.	<u>Mimocichla plumbea rubripes</u>	Cuba	Barus and Garrido, 1968
sp.	<u>Motacilla alba</u>	Primorsk	Bashkirova, 1960
sp.	<u>Motacilla flava</u>	Primorsk	Bashkirova, 1960

<u>Microtetrameres</u> species	Avian species	Location	Investigator
sp.	<u>Pica plca hudsonia</u>	Montana	Carney, 1967 ^m
sp.	<u>Coccyzus americanus</u>	Iowa	Daniell ^m
sp.	<u>Cyanocitta cristata</u>	Iowa	Daniell ^m
sp.	<u>Quiscalus quiscula</u>	Iowa	Daniell ^m
sp.	<u>Sturnella magna</u>	Iowa	Ellis, 1961
sp.	<u>Quiscalus versicolor</u>	Iowa	Ellis, 1961
sp.	<u>Colaptes auratus</u>	Iowa	Ellis, unpublished
sp.	<u>Cyanocitta cristata</u>	Iowa	Ellis, unpublished
sp.	<u>Melanerpes erythrocephalus</u>	Iowa	Ellis, unpublished
sp.	<u>Melospiza georgiana</u>	Iowa	Ellis, unpublished
sp.	<u>Myiarchus crinitus</u>	Iowa	Ellis, unpublished
sp.	<u>Passerella iliaca</u>	Iowa	Ellis, unpublished
sp.	<u>Progne subis</u>	Iowa	Ellis, unpublished
sp.	<u>Quiscalus quiscula</u>	Iowa	Ellis, unpublished
sp.	<u>Riparia riparia</u>	Iowa	Ellis, unpublished
sp.	<u>Stelgidopteryx ruficollis</u>	Iowa	Ellis, unpublished
sp.	<u>Toxostoma rufum</u>	Iowa	Ellis, unpublished
sp.	<u>Xanthocephalus xanthocephalus</u>	Iowa	Ellis, unpublished
sp.	<u>Emberiza bruniceps</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957
sp.	<u>Oriolus oriolus</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957
sp.	<u>Pastor roseus</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957

<u>Microtetrameres</u> species	Avian species	Location	Investigator
sp.	<u>Pica pica</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957
sp.	<u>Pyrhocorax graculus</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957
sp.	<u>Turdus merula</u>	Kirgizia	Gagarin, Ablasov and Chibichenko, 1957
sp.	<u>Quiscalus quiscula versicolor</u>	Manitoba	Hodasi, 1963
sp.	<u>Accipiter nisus</u>	Primorskii	Oshmarin, 1956
sp.	<u>Coccothraustes coccothraustes</u>	Russia	Oshmarin, 1956
sp.	"goshawk"	Russia	Oshmarin and Parukhin, 1963
sp.	<u>Turdus merula</u>	Kirgizia	Petrov and Chertkova, 1950b
sp.	<u>Oriolus oriolus</u>	Hyderabad	Rasheed, 1960
sp.	<u>Athene noctua</u>	Turkmenistan	Rizhikov and Kozlov, 1959
sp.	"brown owl"	Turkmenistan	Rizhikov and Kozlov, 1959
sp.	<u>Falco tinnunculus</u>	Turkmenistan	Rizhikov and Kozlov, 1959
sp.	<u>Galerida cristata</u>	Turkmenistan	Rizhikov and Kozlov, 1959
sp.	<u>Lanius cristatus</u>	Turkmenistan	Rizhikov and Kozlov, 1959
sp.	<u>Motacilla alba</u>	Turkmenistan	Rizhikov and Kozlov, 1959
sp.	<u>Turdus ruficollis</u>	Turkmenistan	Rizhikov and Kozlov, 1959

<u>Microtetrameres</u> species	Avian species	Location	Investigator
sp.	<u>Passer domesticus</u>	Moldavia	Sciumilo, 1963
sp.	<u>Turdus</u> <u>mg.</u>	Colorado	Slater, 1967
sp.	<u>Corvus frugilegus</u>	Roumania	Stoican, 1960
sp.	<u>Accipiter cirrohocephalus</u>	Australia	Thomas, 1960 ^m
sp.	<u>Anthochoera chrysoptera</u>	Australia	Thomas, 1960 ^m
sp.	<u>Cladorhynchus leucocephalus</u>	Australia	Thomas, 1960 ^m
sp.	<u>Erolia acuminata</u>	Australia	Thomas, 1960 ^m
sp.	<u>Falco berigora</u>	Australia	Thomas, 1960 ^m
sp.	<u>Falco peregrinus</u>	Australia	Thomas, 1960 ^m
sp.	<u>Gymnorhina hypoleuca</u>	Australia	Thomas, 1960 ^m
sp.	<u>Lobibyx novae hollandiae</u>	Australia	Thomas, 1960 ^m
sp.	<u>Myzantha melanocephalus</u>	Australia	Thomas, 1960 ^m
sp.	<u>Ninox novae zeelandiae</u>	Australia	Thomas, 1960 ^m
sp.	<u>Psephotus haemonotus</u>	Australia	Thomas, 1960 ^m
sp.	<u>Agelaius phoeniceus</u>	Iowa	Ulmer, 1966 ^m

^a Males only reported.

^b From a zoon.

^c No description of new species given, hence, a nomen nudum.

^d See Sultanov, 1947.

^e Status of this species is questionable because of variation in descriptions by Seurat (1913) and von Linstow (1879).

^f This genus is a synonym for Falco (Ripley, 1961).

^g This genus is a synonym for Dinoplum (Peters, 1948).

^h Genus is Falco according to Ripley (1961).

ⁱ See Skrjabin, Shikhobalova and Sobolev (1949, p. 72).

No other information given.

^k Genus emended to Mainatus by Sharpe (1890).

^l Transferred to Gubernaculomeres by Oshmarin and Parukhin (1963).

^m Private communication.

Table 2. Comparative measurements (in millimeters) of Microtetrameres spp. originally described from the western hemisphere.

Species	Lgth.	Width	Buccal capsule lgth.	Buccal capsule width	Spicule length long.	Spicule short.	Pharynx length musc. gland.	Nerve ring to end ^d	Cerv. pap. to end ^d	Exc. pore to end ^d	Cloaca-tail dist.	Cloacal papillae ^a	
<u>accipiter</u>													
Schell, 1953	3.6-4.4	.079-.104	.023	--	2.03-2.4	.100-.105	.212-.234	.650-.708	.144-.151	.162-.183	--	.165-.178	4 pre-4 post-
<u>aquila</u>													
Schell, 1953	3.6-4.3	.090-.100	.025	--	1.48-1.8	.210-.234	.324-.340	.840-.960	.190-.205	.298-.300	.240-.252	.178-.195	4 pre-4 post-
<u>bubo</u>													
Schell, 1953	3.5-3.9	.079-.081	.023-.025	--	1.8-1.9	.228-.230	.306-.370	.740-.790	.180-.190	.230-.240	.190-.220	.210-.220	4 pre-6 post-
<u>centuri</u>													
Barus, 1966	3.04-3.78	.064-.076	.015-.019	.010-.012	2.06-2.23	.095-.118	.190-.228	.460-.499	.107-.140	--	.114-.156	.087-.110	4 pre-4 post-
<u>corax</u>													
Schell, 1953	3.7-4.7	.086-.090	.021-.025	--	3.2-3.8	.120-.140	.244-.266	.620-.780	.151-.187	.194-.237	.154-.194	.160-.207	4 pre-4 post-
<u>cruzi</u>													
Travassos, 1914	1.17-1.4	.086	.021	.004-.005	.651-.787	.082	.093	.290	--	--	--	.132	2 pre-6 post-
<u>helix</u>													
Cram, 1927	4.9 (3.9) ^b	.100	.021	--	(3.12) ^b	(.120) ^b	.274	.541	.191	--	--	.183	4 pre-4 post-
<u>minima</u>													
Travassos, 1914	1.4	--	--	--	.990	.100	--	--	--	--	--	--	--

Table 2. Continued

MALES													
Species	Lgth.	Width	Buccal capsule		Spicule length		Pharynx length		Nerve length	Cerv. pap.	Exc. pore	Cloaca-	Cloacal papillae ^a
			lgth.	width	long.	short.	musc.	gland.	to end ^d	to end ^d	to end ^d	tail dist.	
<u>pusilla^c</u>													
Travassos, 1915, 1919	3.5- 4.0	.100- .120	.017	.007	1.32	.085	.300	.042	--	--	--	.170	4 pre- 1 ad- 4 post-
<u>saguei</u>													
Barus, 1966	1.90- 2.35	.076- .087	.015	.010	1.60 2.02	.095 1.030	.181- .219	.380- .460	.129- .152	--	.174- .190	.095 .118	2 pre- 3 post-
<u>spiculata</u>													
Boyd, 1956	2.02- 2.242	.079- .095	.020- .024	--	2.312- 2.576	.103- .147	.135- .164	.455- .540	.086- .100	.162- .165	.100	.127- .147	4 pre- 4 post-
<u>xiphidiopicl</u>													
Barus, 1966	2.69	.095	.019	.008	1.90	.099	--	.530	.125	--	.144	.114	0 pre- 4 post-

^a Indicated as pre-cloacal, ad-cloacal and post-cloacal papillae

^b Dimensions in parentheses from experimental work including immature males (Cram, 1927).

^c Dimensions taken from the two reports indicated

^d Anterior end.

Table 3. Comparative measurements (in millimeters) of Microtetrameres spp. originally described from the western hemisphere.

<u>FEMALES</u>											
Species	Overall size	Buccal capsule lgth.	width	Egg lgth. width		Pharynx length musc. gland.		Nerve ring to end ^d	Exc. pore to end ^d	Distance Anus-vulva Anus-tail	
<u>accipiter^a</u>											
Schell, 1953	1.3-1.5 1.0	.018	--	.043	.025	.207- .215	1.56- 2.24	.103	--	.108- .129	.100- .140
<u>aquila</u>											
Schell, 1953	1.3-1.5 1.0	.027 .028	--	.044- .050	.023- .026	.306- .330	1.34- 1.39	.169- .172	.169- .187	.082- .090	.115- .133
<u>bubo</u>											
Schell, 1953	1-1.2 1.0	.021 .022	--	.046	.028	.277- .280	1.43- 1.47	.162- .165	.180- .190	.108- .162	.172- .178
<u>corax</u>											
Schell, 1953	1.2-1.3 1.0-1.2	.024- .025	--	.047	.032	.241- .284	1.36- 1.40	.126- .169	.165- .190	.082- .130	.129- .187
<u>centuri</u>											
Barus, 1966	.83-1.14	.019	.015	.049- .053	.033- .038	.198- .220	.662- .895	.128- .145	.165		.129
<u>cruzi^c</u>											
Travassos, 1914	1.5- 2.0	.016- .020	.008	.050 .060	.024- .028	.160	.620	--	--	.200- .226	.074 .100
<u>helix</u>											
Cram, 1927	1.2-1.3 1.0-1.3	.0225	--	.042	.033	.225 .230	--	--	--	.075	.141
<u>minima</u>											
Travassos, 1914	.78- .64	.012	.007	.045	.024	.073	.490	--	--	--	.068

Table 3. Continued

<u>FEMALES</u>											
Species	Overall size	Buccal capsule		Egg		Pharynx length		Nerve ring to end ^d	Exc. pore to end ^d	Distance	
		lgth.	width	lgth.	width	musc.	gland.			Anus-vulva	Anus-tail
<u>pusilla</u> ^c											
Travassos, 1915, 1919	2.0- ^b 1.5	.010- .016	.009- .012	.042- .049	.028- .035	.210- .273	.920 .974	--	--	--	.140
<u>saguei</u>											
Barus, 1966	--	.015	.011	--	--	.132- .173	.760- .910	.132	--	--	.103- .132
<u>spiculata</u>											
Boyd, 1956	1.2- 1.0	.022- .023	--	.048- .050	.031	.224- .236	1.05- 1.08	.109	--	.090 .120	.096 .103
<u>xiphidiopici</u>											
Barus, 1966	--	.021	.011- .012	.049- .055	.033- .038	.152- .185	.870- .901	.102- .125	--	--	.107- .152

^a Longitudinal flanges present.

^b Dimensions taken from the two reports indicated.

^c Longitudinal furrows present.

^d Anterior end.

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RUMEN LACTIC ACID LEVELS IN CATTLE¹

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ABSTRACT. Sixteen yearling Holstein steers and heifers were used in three factorial experiments to determine the effect of the following variables on ruminal lactic acid: 1) sex, steers vs. heifers; 2) grain level, low vs. high; 3) antibiotic feeding, none vs. antibiotic mixture; 4) dietary change, low grain to high and vice versa; 5) forage, hay vs. green-chopped alfalfa.

Heifers and steers did not differ in ruminal lactate concentration. Low-grain diets produced significantly ($P < 0.05$) higher lactate concentration than did high-grain diets in Experiment 1, but not in Experiments 2 and 3. Antibiotic feeding caused a slightly depressed appetite in animals receiving the high-grain diet but had no appreciable effect on ruminal lactate concentration. Abrupt increases in dietary grain level caused depressed appetite. Either an abrupt increase or decrease in dietary grain level resulted in a small but significant ($P < 0.05$) decrease in lactate concentration. Substituting green-chopped alfalfa for alfalfa or grass hay significantly ($P < 0.01$) decreased ruminal lactate. Most of the lactate concentrations were very low (1.0 mg or less/100 ml), regardless of treatment.

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High-grain rations are widely used for ruminants to supply sufficient energy for high levels of milk production or rapid feedlot gains. Sometimes, however, animals do not adapt readily to such rations. Transient depressed appetite, diarrhea and general listlessness are reactions commonly observed in ruminants that have ingested large amounts of readily available carbohydrates (Krogh 1959, 1961; Ryan 1964b; Scarisbrick 1954; Uhart and Carroll 1967). Similar reactions have been observed in studies on the prevention of pasture bloat when a combination of antibiotics (streptomycin, tylosin, erythromycin and penicillin) was fed to dairy cattle receiving high-grain rations (Shellenberger 1964; Van Horn et al. 1963).

Reid, Hogan and Briggs (1957) associated high levels of ruminal lactic acid with "indigestion" due to consumption of large amounts of readily available carbohydrates. Uhart and Carroll (1967) associated ruminal lactic acid with an "off-feed" reaction when steers were abruptly shifted from an alfalfa hay ration to a high-grain ration. Further, Mangan, Johns and Bailey (1959) reported an increase in ruminal lactate concentration after administration of penicillin.

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The present experiments were conducted to study the relationship between ruminal lactic acid concentration and the following dietary factors: amount of grain, abrupt changes in amount of grain, antibiotics and green-chopped alfalfa.

EXPERIMENTAL PROCEDURE

Ruminal lactic acid was measured, using 8 Holstein steers and 8 Holstein heifers, under various dietary regimes in three factorial experiments. Five variables and their respective comparisons were used: 1) sex, steers vs. heifers; 2) grain level, 0.91 kg grain/animal daily plus forage ad libitum vs. as near the ad libitum level of grain feeding as could be maintained without feed refusals, plus limited forage; 3) antibiotic feeding, none vs. 206 mg/animal daily of a combination of streptomycin, tylosin, erythromycin and penicillin in the ratio of 7:7:7:4; 4) dietary change, none vs. changing from 0.91 kg grain/head daily to near ad libitum and vice versa; 5) forage, hay vs. green-chopped alfalfa. Six heifers and six steers were housed and fed in pairs in box stalls but were fed individually. All animals had access to fresh water in individual water bowls.

Experiment 1.

Variables 1, 2 and 3 were used in a 2 x 2 x 2 factorial arrangement replicated twice; the four older heifers and four older steers composed one replication, and the eight younger animals composed the second.

All animals were fed their respective diets (high-grain or low-grain) approximately 6 months before antibiotic feeding. The grain mixture consisted of 53.9% ground corn, 32.4% crushed oats, 10.7% soybean meal, 2.0% dicalcium phosphate and 1.0% salt. Each animal receiving the high-grain diet also was fed 0.91 kg alfalfa or grass hay daily, while animals receiving the low-grain diet were fed alfalfa or grass hay ad libitum. The antibiotic mixture was fed with the morning feeding of grain for 28 days.

Rumen fluid was sampled according to the schedule shown in Table 1. Water to the stanchions and stalls was shut off approximately 30 minutes before sampling. Samples were taken with a suction strainer (Raun and Burroughs 1962) and 50-ml syringe connected by a length of Tygon tubing (8 mm inside diameter). The tubing was passed through a 61 cm section of 19 mm inside diameter steam hose used to push the strainer over the animal's tongue and past the epiglottis. After the animal had swallowed the strainer, 20-30 ml of fluid were drawn to flush the line and were discarded. Then a 50-ml sample was drawn and placed in a bottle containing 1.0 ml of a saturated HgCl₂ solution. The equipment was thoroughly rinsed after each animal was sampled. Samples from the 16 animals were transported to the laboratory and centrifuged in a Servall Super-speed centrifuge (Model SS-1) for 30 minutes at 18,800 x g. The supernatant fluid from this preparation was frozen and stored in a sample bottle until analyzed for lactic acid by the colorimetric procedure of Barker and Summerson (1941) with the following modifications: The oxidation and color development reactions were conducted in 25 x 150 mm screwcap culture tubes fitted with Teflon-lined caps. The sulfuric acid

TABLE 1. SAMPLING SCHEDULE FOR EXPERIMENTS 1, 2, AND 3.

Experiment 1		Experiment 2		Experiment 3	
Days ^a	Hours ^b	Days ^c	Hours ^b	Days ^d	Hours ^b
-7	3	-1	3	-4	3
-1	3	0	0, 3, 6	0	0, 3, 6
0	0, 3, 6	1	0, 3, 6	1	0, 3, 6
1	0, 3, 6	2	3	2	3
2	3	3	0, 3, 6	3	0, 3, 6
3	0, 3, 6	4	3	4	3
4	3	5	3	5	3
5	3	6	3	6	3
6	3	7	0, 3, 6	7	0, 3, 6
7	0, 3, 6	14	3	14	3
14	0, 3, 6			21	3
27	0, 3, 6				
28	0, 3, 6				
42	0, 3, 6				

^aDays relative to initiation of antibiotic feeding. Antibiotics were fed first with the morning feeding on day 0 and fed last on the morning of day 27. A minus sign means before antibiotic feeding was begun.

^bHours relative to the morning feeding. Zero hour refers to sampling just before feeding, while 3 and 6 refer to that number of hours after feeding.

^cDays relative to initiation of dietary change. Diets were changed with the morning feeding of day 0 and continued throughout the experiment. A minus sign means before dietary change.

^dDays relative to the initiation of green-chopped alfalfa feeding. Green material was first fed with the morning feeding of day 0 and continued throughout the experiment. A minus sign means before green-chopped alfalfa was fed.

was placed in the tubes and chilled in an ice bath, after which the sample was overlaid on the acid. The tubes were immediately capped, and the contents were mixed. Protein was precipitated with $\text{Ba}(\text{OH})_2$ and ZnSO_4 . Optical densities of the solutions were determined at a wavelength of 560 $\text{m}\mu$ as in the original method.

Experiment 2.

Factors 1, 2, 3 and 4 were used in a $2 \times 2 \times 2 \times 2$ factorial arrangement. No antibiotics were fed, but the antibiotic factor was retained in the statistical analysis to detect any residual antibiotic effect. This resulted in 16 treatment combinations, one represented by each animal. Replication was not possible; therefore, the higher-order interactions were used to estimate the error terms. Animals changed to the high-grain diet first received grain at the rate of 4.5 kg/animal daily, and the rate was increased as rapidly as the animals would continue to consume all the grain offered.

Rumen fluid was sampled as shown in Table 1. The samples were handled as in Experiment 1 except that meta-phosphoric acid was used to precipitate protein, and optical densities were determined at a wavelength of 565 $\text{m}\mu$.

Experiment 3.

Factors 1, 2, 4 and 5 were used in a $2 \times 2 \times 2 \times 2$ factorial arrangement of treatments. The animals designated to receive antibiotics in Experiment 1 received green-chopped alfalfa *ad libitum* in this experiment. Alfalfa was cut with a flail-type forage harvester and fed twice daily. The animals changed to the high-grain diet or to the low-grain diet in Experiment 2 were continued on these diets throughout Experiment 3. Samples were handled similarly to those in Experiment 2. The 16 treatment combinations again necessitated using higher-order interactions to estimate experimental error.

Statistical analysis.

Data from each of the three experiments were analyzed in two groups; one consisted of all data obtained on those days in which 0-, 3- and 6-hr samples were taken, and the second was composed of all data from samples taken 3 hours after feeding. The first group in each experiment was treated statistically as a split-split-plot design in which whole plots were individual animals, subplots were days within the animal's life and sub-subplots were hours within those days. The second group in each experiment was treated as a split-plot design in which whole plots were individual animals and subplots were days within that animal's life. The various responses were compared by the F test as outlined by Steel and Torrie (1960).

RESULTS AND DISCUSSION

Experiment 1.

Grain consumption patterns were erratic before and during antibiotic feeding. During the first 5 days of antibiotic feeding, transient depressed appetite appeared in all animals receiving antibiotic and the high-grain diet. By the sixth day, grain consumption had returned to normal. The animals receiving the high-grain diet with no antibiotic consumed feed at a relatively constant rate through the first week; thereafter, grain con-

sumption was variable in all animals receiving the high-grain diet. One animal receiving the low-grain diet and antibiotic refused a small portion of grain one day after antibiotic was first fed; no other indications of depressed appetite were observed in animals on this diet. This supports the observation that adverse reaction to antibiotic feeding occurs primarily in animals receiving a high-grain diet (Van Horn *et al.* 1963) but, in this experiment, depressed appetite was the only reaction observed. There was no increase in ruminal lactic acid associated with depressed appetite, indicating that the loss of appetite in this study was not caused by an accumulation of lactic acid in the rumen.

Table 2 shows the analysis of variance of the data of Experiment 1 treated as a split-split-plot design. Of the main effects, only grain level was significant ($P < 0.01$). Animals receiving the low-grain diet averaged 0.89 mg lactic acid/100 ml rumen fluid, while those receiving the high-grain diet averaged 0.44 mg/100 ml. The hours x days interaction is significant at $P = 0.05$ hours x treatments at $P = 0.01$, but the range of the lactic acid values is small. Males receiving antibiotics had higher lactate values than those receiving none, whereas the reverse was true for the females. Moreover, with the low-grain diet, antibiotics increased lactate values for the males and decreased the values for the females. Contrary to expectations (Van Horn *et al.* 1963), there was little response to antibiotics by either sex when fed the high-grain diets. The data treated as a split-plot design reveal patterns rather similar to those described for the split-split-plot data.

Experiment 2.

Average daily grain consumption of animals abruptly changed from the low-grain diet to the high-grain diet increased rapidly during the first 2 days of dietary change and then continued to increase more gradually to a maximum of 8.2 kg per animal on the sixth day. On the seventh day of dietary change, average consumption dropped to 6.5 kg, after which it gradually returned to near the maximum. Again, consumption was variable in all animals receiving the high-grain diet.

The lactic acid data of this experiment did not greatly differ from those in Experiment 1. Statistical analysis of the split-split-plot data showed significance ($P < 0.05$) only for the main effect of dietary change. Ruminal lactate concentrations in animals changed from the low-grain diet to the high-grain diet were similar to those of animals changed from high grain to low grain. Either change in the dietary grain level, however, caused average ruminal lactic acid concentration to decrease. Conversely, analysis of the split-plot data showed significance ($P < 0.05$) only for the grain level x dietary-change interaction. Changing from high-grain to low-grain diets caused the average ruminal lactic acid concentration to increase from 0.36 mg/100 ml to 0.59 mg/100 ml, but changing from low-grain to high-grain diets brought about a decrease from 1.20 mg/100 ml to 0.43 mg/100 ml. Even though the latter change is nearly three-fold, it represents a range in average lactate concentration of less than 1.0 mg/100 ml.

These results are contrary to those of Ryan (1964a) and Uhart and Carroll (1967) who reported increased ruminal lactic acid when sheep or steers were changed from an all-hay diet to a hay-grain diet. The results

TABLE 2. ANALYSIS OF VARIANCE OF SPLIT-SPLIT-PLOT DATA - EXPERIMENT 1^a

Source of Variation	Degrees of freedom	Mean square	F
Whole - plot analysis			
Replications	1	0.40	0.44
Treatments	7	6.57	7.30**
A (sex)	(1)	0.89	0.99
B (grain)	(1)	19.99	22.21**
C (antibiotic)	(1)	0.39	0.43
A x B	(1)	0.00	0.00
A x C	(1)	10.59	11.77**
B x C	(1)	0.36	0.40
A x B x C	(1)	13.80	15.33**
Error (a)	7	0.90	
Total	15		
Sub-plot analysis			
Days	7	0.97	1.29
Days x treatments	49	0.77	1.03
Error (b)	56	0.75	
Total	112		
Sub-sub-plot analysis			
Hours	2	0.97	1.24
Hr. x days	14	1.51	1.94*
Hr. x treatments	14	2.02	2.59**
Hr. x A	(2)	2.70	3.46*
Hr. x B	(2)	4.98	6.38**
Hr. x C	(2)	1.83	2.35
Hr. x A x B	(2)	1.94	2.49
Hr. x A x C	(2)	0.31	0.40
Hr. x B x C	(2)	1.24	1.59
Hr. x A x B x C	(2)	1.18	1.51
Hr. x days x treatments	98	0.59	0.76
Error (c)	128	0.78	
Total	256		
Grand Total	383		

^aIncludes all lactic acid data obtained on those days on which samples were taken 0, 3, 6 hr. after feeding.

*Significant at the 5% level of probability.

**Significant at the 1% level of probability.

agree, however, with those of Haskins *et al.* (1969) who found lactic acid concentration in rumen fluid quite low on all treatments, but higher when steers were fed some hay than when an all-grain diet was fed. Furthermore, Eadie, Hobson and Mann (1967) fed barley to steers and young calves and noted that the lactic acid concentration in all samples was low, about 1 mg/100 ml. Mackenzie (1967) noted that several authors reported low or undetectable levels of ruminal lactic acid in animals fed roughage rations, but a range of values from 9 mg/100 ml to over 1350 mg/100 ml was noted in animals fed low-roughage rations. The animals in the present experiment were accustomed to some grain in the diet. Perhaps the microbial population of the rumen had already adjusted to the presence of grain in the diet and could rapidly adapt to the abrupt increase in grain level.

Experiment 3.

In contrast to Experiments 1 and 2, an occasional high ruminal lactic acid value was observed in this experiment. One day after the first feeding of green-chopped alfalfa, one steer receiving the high-grain diet and no green-chopped alfalfa suddenly exhibited ruminal lactic acid concentrations of 223.0 mg/100 ml in the prefeeding sample and 10.4 mg/100 ml in the sample taken 3 hours after feeding. The animal had been on this same diet throughout all three experiments. A heifer receiving the high-grain diet and no green-chopped alfalfa, and whose diet had not been changed, had ruminal lactic acid concentrations of 15.0 mg/100 ml 6 hours after feeding on day three and 12.0 mg/100 ml 3 hours after feeding on day 21. All other values were comparable to those of Experiments 1 and 2. This large range in values caused the higher order interactions (experimental error) to be quite large, resulting in no significant responses in the analysis of the split-split-plot data. Since the two highest values are not among the split-plot data, the interaction terms in the analysis of these data are not so large (Table 3). The main effect of dietary change is similar to that described for Experiment 2. Changing the forage from hay to green-chopped alfalfa caused a decline in average ruminal lactic acid concentration. The mean lactic acid in rumen fluid declined from 1.29 to 0.53 mg/100 ml for diets wherein grain intake was not changed and 0.59 to 0.43 for diets involving a marked change in grain intake (either an increase or a decrease). This agrees with the results of Forenbacher and Srebočan (1963) who found that feeding fresh fodder to cows resulted in lower ruminal lactate concentrations.

In Experiments 1 and 2, there was no increase in ruminal lactic acid associated with depressed appetite; in Experiment 3, there was no depressed appetite when high lactic acid concentrations appeared. Dowden and Jacobson (1960) found lactic acid to have no significant effect on appetite when infused intravenously. This suggests that lactic acid, as a metabolic intermediate, may, at least in some instances, not be closely related to depressed appetite. Tremere, Merrill and Loosli (1968) found that ground wheat, as the only ingredient of concentrate fed at a high level, produced high ruminal lactate concentrations but noted no similar increase when the concentrate mixture was composed of only 50% ground wheat. Thus, the carbohydrate components of wheat may be more conducive to lactate production than those of corn. However, a

TABLE 3. ANALYSIS OF VARIANCE OF SPLIT-PLOT DATA - EXPERIMENT 3^a

Source of Variation	Degrees of freedom	Mean square	F
Whole-plot analysis			
A (sex)	1	0.31	0.63
B (grain)	1	0.16	0.33
C (dietary change)	1	6.92	14.12*
D (green alfalfa)	1	9.41	19.20**
A x B	1	1.06	1.98
A x C	1	0.75	1.53
A x D	1	0.33	0.67
B x C	1	1.82	3.71
B x D	1	0.38	0.78
C x D	1	4.00	8.16*
"Error (a)" ^b	5	0.49	
Total	15		
Sub-plot analysis			
Days	10	1.95	0.78
Days x A	10	0.95	0.38
Days x B	10	1.02	0.41
Days x C	10	2.18	0.88
Days x D	10	2.37	0.95
Days x A x B	10	2.52	1.01
Days x A x C	10	0.86	0.35
Days x A x D	10	1.36	0.55
Days x B x C	10	1.22	0.49
Days x B x D	10	0.77	0.31
Days x C x D	10	2.56	1.03
"Error (b)" ^c	50	2.49	
Total	160		
Grand Total	175		

^aIncludes all 3 hr. samples of Experiment 3.

^b"Error (a)" includes the A x B x C, A x B x D, A x C x D, B x C x D and A x B x C x D interactions.

^c"Error (b)" includes all four-, and five-factor interactions.

*Significant at the 5% level of probability.

**Significant at the 1% level of probability.

ruminal lactic acid concentration of 223.0 mg/100 ml was obtained in one animal in Experiment 3 of the present study when corn was the major concentrate.

Considerable variability in ruminal lactic acid concentration was observed in the present experiments. This also has been noted by previous workers (Chow and Walker 1964a, 1964b; Ndumbe, Runcie and McDonald 1964; Otterby and Rust 1965; Reid *et al.* 1957). Reported concentrations of lactic acid have varied from traces (less than 1.0 mg/100 ml) to more than 1800 mg/100 ml (Tremmer *et al.* 1968). Furthermore, high ruminal lactic acid concentrations are not always reproducible (Reid *et al.* 1957; Scarisbrick 1954).

Of the 1072 observations in the present studies, 98% were less than or equal to 5.0 mg/100 ml, and 89% were less than or equal to 1.0 mg/100 ml. Since most of the values obtained were quite low and since the high values that did appear could not be associated with treatment, conditions other than those in these experiments must be responsible for high concentrations of ruminal lactate.

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MEASUREMENT OF AMBIENT AIR TEMPERATURE WITH ASPIRATED AND UNASPIRATED THERMOCOUPLES IN THE FIELD^{1, 2}Harry C. Vaughan and Clarence Sakamoto³

ABSTRACT. A series of measurements was made by using both aspirated and shielded and unshielded copper-constantan thermocouples of various physical sizes. The purpose of this study was to establish the magnitude of the error involved in using unshielded, unspirated couples. The standard for comparison consisted of a number-16 Brown and Sharp gauge copper-constantan wire welded and then silver-soldered into a 9.5-mm diameter copper cylinder 3.26 cm long and gold-plated. This, in turn, was housed in a coaxial plastic radiation shield covered with gold leaf and painted flat black on the inside. In addition, the couple was aspirated with a 7.3-m-sec⁻¹ flow of air. The data show that, depending on the size of the thermocouple, differences of as much as 7°C can occur if the couples are not shielded and unspirated.

INTRODUCTION

It has long been recognized that a measurement of true ambient air temperature is difficult, if not impossible, to obtain (Brunt 1952). It is important that researchers interested in the measurement of ambient air temperature become aware of the potential magnitude of errors present in this aspect of micrometeorological field observations. The magnitude of temperature errors encountered during daytime periods are examined in this paper. These measurements were in the free air over short grass and over a soybean crop, using unshielded thermocouples of various physical sizes. A shielded, aspirated thermocouple served as a reference standard.

Middleton *et al.* (1938) have shown the relationship between the mass of a thermal transducer and its response. A thermal transducer is defined to be any resistance or emf. instrument that responds to temperature changes. In this paper, only thermocouples were considered; the results, however, also are applicable to other thermal transducers. In general, the smaller the wire size, the more nearly it will come to representing the ambient temperature. It has been postulated that, if the wire were sufficiently small, no error from incoming radiation would

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occur. But this approach is not always practical, because the wire may be too frail to withstand exposure to the free air (Thorntwaite 1949).

If the transducer is unshielded and unspirated, the data may be erroneous for the following reasons. When the mass of the sensor is increased, its thermal capacity also will increase, and the lag in the response becomes more pronounced; this lag, in itself, is not undesirable, but such a sensor exposed to direct solar radiation will give a positively biased reading. If the free air flow is increased, the transducer will respond as a heat source. Its thermal energy, consequently, will be transferred more effectively with the increased flow of air, and thus, the sensor will be cooled. Unfortunately, the observer has no way of knowing whether the air sample is cooler or if the flow past the transducer has accelerated. The relationship between the mass of the transducer and its response is further influenced in that the instantaneous temperature, as observed by a low-lag thermal transducer, experiences great excursions, which may be of such short duration that most recording systems will be unable to follow them. Figure 1 represents a short record of such a transducer in conjunction with a fast-response recording system.

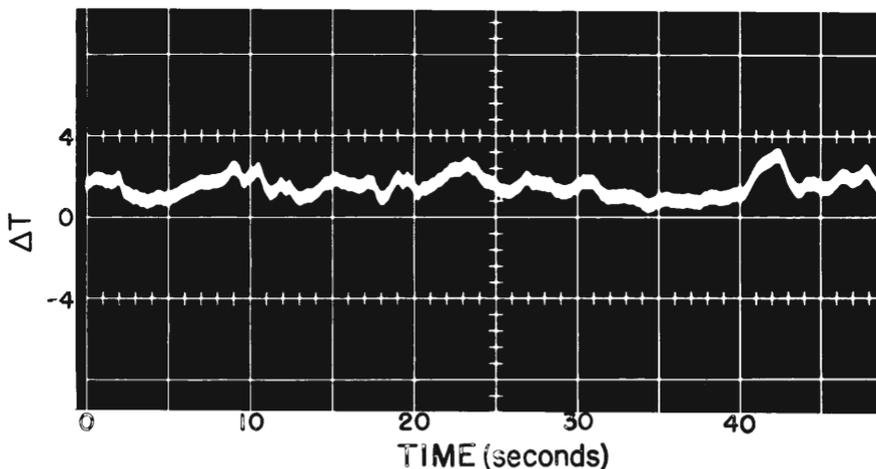


Figure 1. Temperature fluctuations in the free air using an unshielded thermocouple. The abscissa represents time. Each small division equals one second. The ordinate represents a temperature scale with each small division equal to eight-tenths of a centigrade degree.

The purpose of this study is to show the magnitude of some temperature anomalies that may be encountered under normal conditions when using unshielded-unspirated thermal transducers of various physical size exposed to sunlight.

Theory of radiation error:

The degree of accuracy with which the ambient air temperature may be measured at any given time, is determined by the incremental difference between the temperature of the transducer and that of the medium in which it is immersed. If one assumes a transducer of zero thermal lag and a recording system with infinitesimal inertia, then the only error would be that introduced by radiation impinging on the transducer. Ideally, a polished metal coating would reflect all radiation impinging on the transducer, thus resulting in a zero radiation error. Several metals have a reflectivity approaching one in portions of the spectrum; no single coating, however, seems to possess the attribute of an ideal white body (Kleinschmidt 1935).

An alternative procedure would be to surround the transducer with a reflector. Because the reflecting surface is less than ideal, however, some energy would be absorbed and reradiated at longer wavelengths. Angstrom (1885) has shown this process follows Lambert's law with good approximation. By increasing the number of reflecting surfaces, each with a good radiator on its inner side, the initial error may be reduced to any desired amount. The disadvantage of this method is that the transducer is no longer exposed to the free air and must be aspirated to assure true fidelity in light winds. It can be further argued that the aspiration results in a gross air sample in place of the discrete sample obtained with an unshielded transducer. If the flow of air past the transducer is constant, an equilibrium condition between its temperature and that of the free air would be maintained. The temperature measured would be between the "true" temperature and the elevated temperature resulting from the remaining radiation.

Geiss (1950) measured differences of 0.5 °C for shaded and unshaded platinum thermocouples with 0.002-cm diameters for average winds of about 1 m-sec. Thornthwaite (1949) used copper-constantan thermocouples of 0.024-cm diameter and observed radiation errors as large as 2 °C under unspecified conditions in March. The experimental results indicate substantial radiation errors. McDonald (1951) calculated values about an order of magnitude smaller and attributed the discrepancy in the theoretical and experimental results to poorly understood dissipation data for transducers.

Description of instrumentation:

Six copper-constantan thermocouples, ranging from number -40 to number -16 Brown and Sharp wire-gauge size, were used in these comparisons. The couples were made by overlapping about 2 mm of wire and then silver-soldering. This resulted in a slight enlargement of the wire at the junction. Table 1 gives a number of physical characteristics of the individual couples. The number -16 gauge wire junction was soft-soldered into a gold-plated copper cylinder 9.5 mm in diameter by 32.6 mm long. A rectangular aluminum rack, 10.16 cm by 30.48 cm was used to support the couples. The thermocouple rack was supported, unshielded, over the grass or soybean crop. The ends of the individual test thermocouples passed through porcelain insulators imbedded in the aluminum support rack. At this point, they were soft-soldered to individual number -14 copper wire on the one end and to number -16 constantan wire on the other (Figure 2).

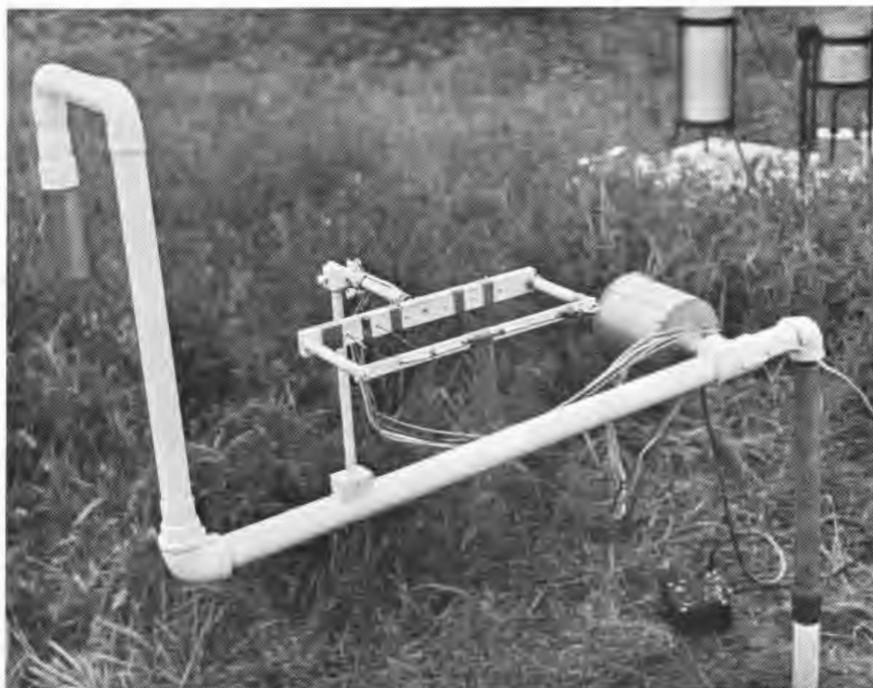


Figure 2. Thermocouple rack and shielded aspirated thermocouple mounted in inverted "J" tube with blower at right hand end of support stand.

To minimize the reference junction error, a common constantan wire was used with the six test couples. This terminated in a silver-soldered junction, which was then soft-soldered into a copper cylinder with identical dimensions to those used with the number-16 wire in the test rack. An independent cold junction of the same size was provided for use with the aspirated, shielded thermocouple. Both cold junctions were painted with General Electric Glyptal enamel to reduce the possibility of extraneous galvanic action between the couples in the ice bath. Distilled water ice was used throughout the work.

The aspirated reference thermojunction was made of number-16 copper-constantan wire silver-soldered into a gold-plated copper cylinder identical to that mounted on the test rack. In this instance, the couple was housed in a coaxial plastic radiation shield covered with gold leaf and painted flat black on the inside (Hewson 1951).

The radiation shield was mounted 1 m above ground on a 1.25-inch pipe in the form of an inverted "J." Air was drawn through the radiation shield and associated pipe at the rate of $7.3 \text{ m}\cdot\text{sec}^{-1}$ by a small blower which, in turn, exhausted the air downward approximately 1.5 m from the sampling point. Mounted on the horizontal portion of the pipe was an additional support for the six unshielded test thermocouples (Figure 2).

Table 1. Physical properties of thermocouples.

Property	Couple Number					
	1	2	3	4	5	6
B. and S. Size	16	20	28	30	38	40
Wire Diameter (mm)	1.24	0.80	0.30	0.27	0.10	0.07
Couple Diameter (mm)	9.47	1.65	0.75	0.62	0.35*	0.46*
Calibration Error ($^{\circ}\text{C}$) [†]	-	-0.005	-0.004	-0.003	-0.008	-0.007
Lag (Seconds)	114 ^a	7.25	3.16	1.52	.34	.72

* Spherical

[†] Average departure from standard

^a For non-aspirated thermocouple

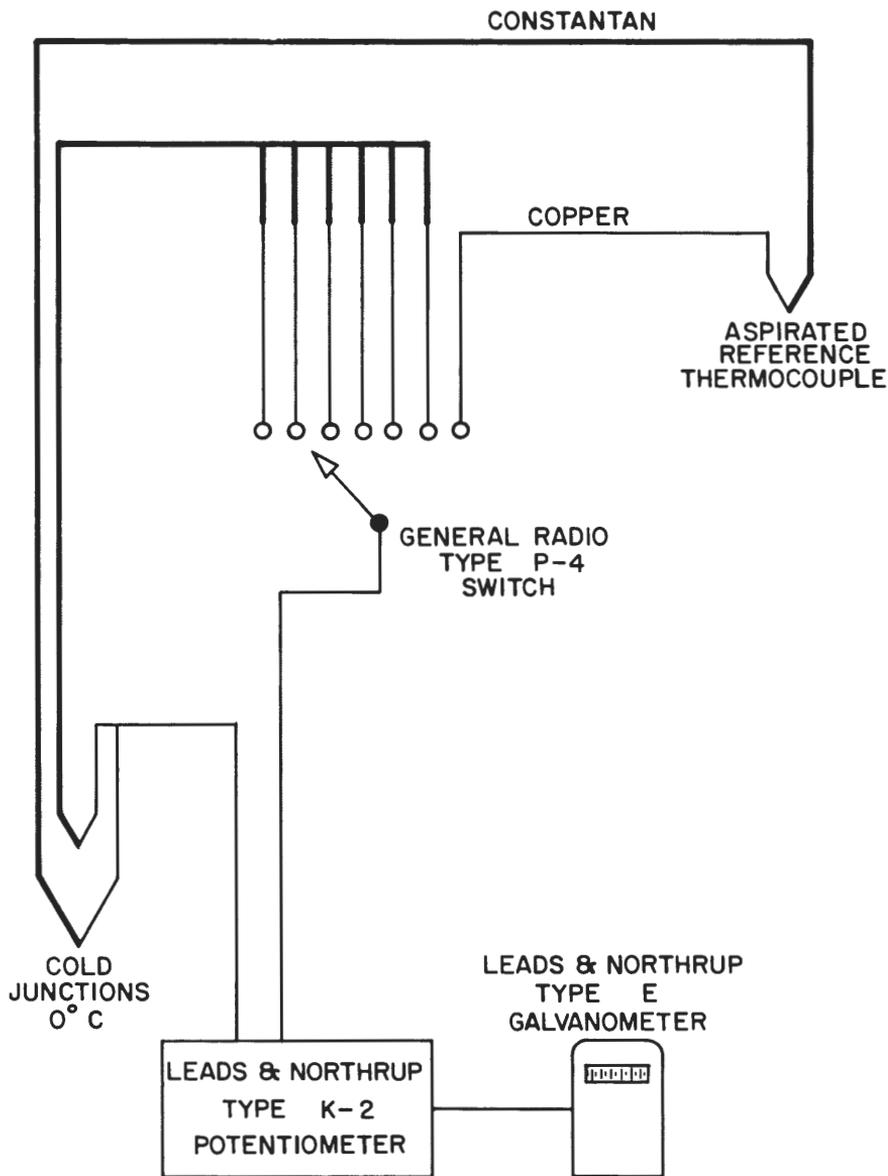
The thermocouple cable extended about 9 m from the test area to a General Radio P-4, low-noise switch and the cold junction. From this point, the thermocouple output was carried over an additional 10 m of shielded cable to the building where the electrical measurements were made. A K-2, Leeds and Northrup potentiometer and an E-2 galvanometer provided the measuring circuit. A schematic diagram is shown in Figure 3. The galvanometer has a natural period of 3.0 sec and was critically damped with a 50-ohm resistor.

This equipment was suitable for measuring voltages of 0.5×10^{-6} volts. In terms of temperature, this represents approximately $1 \times 10^{-3}^{\circ}\text{C}$. No such claim is made for the over-all precision of the system; from the calibrations, however, it was evident that no systematic errors were present. The system was calibrated by submerging the thermocouple rack in a distilled-water bath, which was continuously agitated, and measuring the water temperature with a previously calibrated mercury-in-glass thermometer graduated to 0.1°C .

To change the water-bath temperature, ice was introduced into the bath. When the water bath came to equilibrium, the thermocouple outputs were measured. During both the measurements and calibrations, the cold junctions were gently agitated. Table 1 shows the mean departure of individual couples from the standard during these calibrations.

Experimental procedure:

Data were collected over pasture land every 15 min. for a number of daylight hours. A similar series of observations was made over a large field of soybeans for 9.5 daylight hours. These observations were made under a variety of sky conditions. During the observations, sky conditions were noted each hour; in addition, solar radiation data were available from an Epply Pyranometer mounted on the roof of the Agronomy Building. This instrument was located approximately 1 mile NNW of the soybean field and 1.5 miles ESE of the pasture site.



Schematic Diagram of Thermocouple Measuring Circuit

Figure 3.

Additional meteorological data were available from a 32-m tower located 25-m south of the pasture site. Wind velocity and temperature data were available 12 min. out of the hour from five levels: 2, 4, 8, 16, and 32 m. In addition, a continuous record of wind direction was available from the 16- and 32-m levels.

The procedure used in the collection of data was: first, the potentiometer was standardized; then, as quickly as possible, the output from each thermocouple was noted. A telephone was used to communicate with the operator who controlled the selector switch and agitated the cold junction bath. The observational procedure was the same for all five observations, starting with the thermocouple number 1 and ending with the aspirated reference junction. A field check was made, much like the calibration described, which assured that no systematic error was present when the system was in the field.

Table 2. Maximum temperature departure ($^{\circ}\text{C}$) of text thermocouples from aspirated-shielded thermocouple.

Date (Aug.)	Couple Number						8 ^a
	1	2	3	4	5	6	
11	1.10	0.71	0.51	0.46	0.46	-0.25	-
13	4.21	3.53	2.06	1.79	1.15	1.36	-
15	3.10	1.42	-3.93	-6.10	-2.18	-0.84	-
19	6.57	5.57	3.77	2.98	2.21	2.68	3.8
26 (unshaded)	2.25	2.16	1.47	1.44	-1.49	1.07	-
26 (shaded) ^b	1.14	1.47	1.62	-1.77	-1.90	1.72	-

^a Unaspirated, shielded B and S gauge 22 thermocouples.

^b The term shaded relates to a blocking of direct sunlight by an object placed 1 to 2 m distant from the unshielded thermal transducers. The aspirated, shielded reference-standard is described elsewhere in the text.

Experimental results:

In an effort to show the erratic nature of the midday temperature when observed with a low-lag transducer and recording system, a short series of observations was made by using thermocouple number 5 and a Tektronix 502 oscilloscope. This instrument is well adapted to this type of observation, having a sensitivity of $200 \mu\text{v cm}^{-1}$ ($5.0^{\circ}\text{C cm}^{-1}$) and a calibrated sweep of 5 sec cm^{-1} . This provided the single, 50-sec observation shown in Figure 1. Some difficulty was experienced in making these observations because of the superimposed carrier frequency of the university radio station operating at 640 MHz. This, along with some other extraneous noise, was removed by a simple RC filter, which did not affect the relatively low frequency of the thermocouple fluctuations.

TABLE 3

DATE	OBSERVATIONS		SITE	RANGE OF CLOUD COVER ⁺	TIME OF MAXIMUM ERROR (LOCAL STANDARD TIME)					
	Start	End			Thermocouple					
					1	2	3	4	5	6
August 11	1400	1545	Short grass	10/10 Sc	1445	1445	1445	1445	1445	1400
August 13	0900	1515	Short grass	3/10 - 9/10 Cs	1030	1230	1045	1000	0930	1115
August 15	1000	1115	Short grass		1000	1000	1115	1115	1100	1100
August 19	0815	1745	Soybean	1/10 Cs 2/10 Cu	1230	1230	1230	1215	1145	1145
August 26	0800	1600	Short grass	1/10 - 3/10 Ci 3/10 Ac	0900	0945	0845	1015	1015	1015
August 26*			Short grass		0907	1322	1322	1152	1052	1322

⁺ Cloud type: Sc = stratocumulus, Cu = cumulus, Cs = cirrostratus, Ac = altocumulus, Ci = cirrus

* Observations made with thermocouples shaded from direct sunlight.

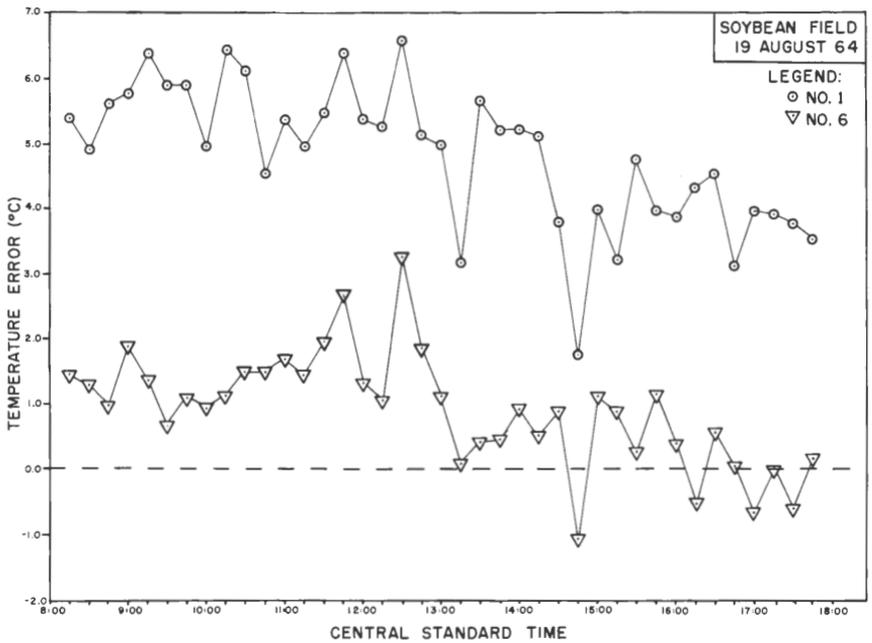


Figure 4. March of the temperature departure of the unshielded B&S 16 (No. 1) and B&S 40 (No. 6) thermocouples from the standard aspirated and shielded thermocouple on August 19, 1964.

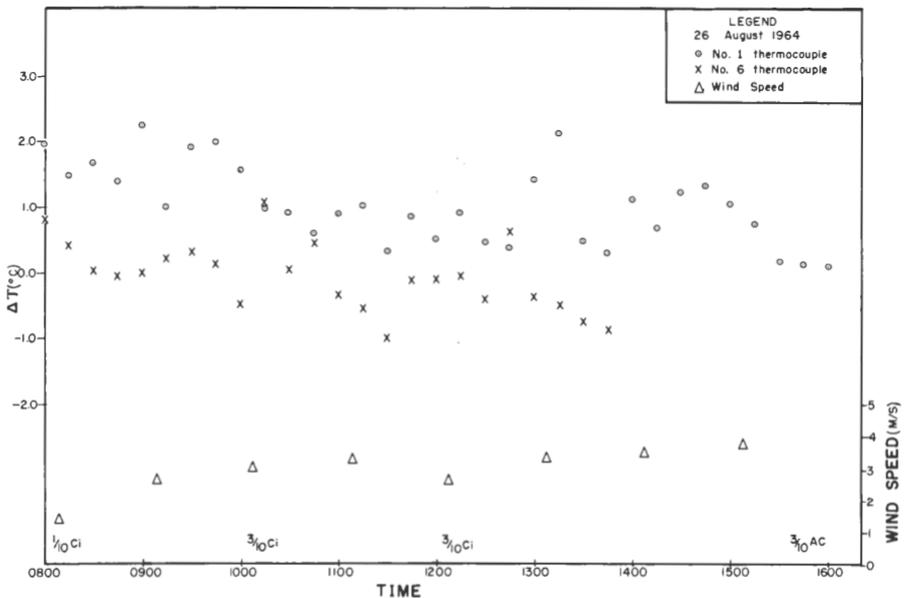


Figure 5. March of the temperature departure of the unshielded B&S 16 (No. 1) and B&S 40 (No. 6) thermocouples from the standard aspirated and shielded thermocouple with wind speed on August 26, 1964.

The five observational periods described in this paper range from 1 hr, 15 min, to 9 hr, 30 min. The maximum temperature anomalies, with reference to the shielded ventilated reference standard, are given in Table 2. A detailed plot of the temperature error associated with the largest (1) and the smallest (6) of the six thermocouples is given in Figure 4. These data were collected at a height of approximately 50 cm over a soybean field on Aug. 19, 1964. There seems to be a slow rise in error readings, which reach a maximum near noon. About this same time, the amplitude of the oscillations increase. After the noon maximum error, there is a tendency toward smaller errors. Whether these violent oscillations and subsequent decrease in the error were related to the passage of fair-weather cumulus clouds during the early afternoon could not be determined from available radiation data. Neither wind speed nor cloud cover suggested a direct relationship to the marked decline in error, particularly with regard to the small couple. These data are presented in Figure 5. Examination of the remaining data suggest that this trend is not unique to the data presented in Figure 4. The times of maximum anomalies are given in Table 2. Two of the observational periods are too short to substantiate this trend, but data taken over short grass also suggest that the maximum error occurs before the time of maximum insolation (Table 3, Aug. 13). Table 3 presents the range of cloud cover during the observational period.

CONCLUSIONS

The foregoing data suggest that temperature errors as great as 6 or 7°C (i. e., 12°F) are realistic in unshielded, unspirated transducers during midday, summer observations. The error is directly related to the physical size of the transducer, as suggested by Middleton *et al.* (1938). It also seems that highly reflective surfaces without adequate ventilation will not appreciably reduce the potential error. If the observer is unable to provide facilities for an aspirated, shielded instrument, then, the smaller the transducer, the smaller will be the error. Unfortunately, this approach may result in a fast-response transducer being matched with a slow recording instrument, which, to an extent, mechanically integrates the rapidly fluctuating input signal.

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SELECTION OF SUITABLE TISSUES FOR USE IN THE
RNA-DNA RATIO TECHNIQUE OF ASSESSING
RECENT GROWTH RATE OF A FISH¹

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ABSTRACT. Feeding experiments with bluegills (*Lepomis macrochirus*) indicated that growth rate, as indicated by changes in length and weight, was reflected in RNA-DNA ratios of liver, stomach, intestine, anterior muscle, and posterior muscle tissue samples. RNA-DNA ratios in bluegill liver were most sensitive to changes in body weight, followed by muscle tissue, stomach, and intestine, respectively.

INTRODUCTION

Using controlled laboratory studies, Bulow (1970) demonstrated that quantitative determinations of ribonucleic acid (RNA) and deoxyribonucleic acid (DNA) can be used as biochemical indicators of recent growth of fishes. Although DNA concentrations remained fairly constant, RNA concentrations varied and were highest in those undergoing fastest growth and protein synthesis. For example, golden shiners (*Notemigonus crysoleucas*) deprived of food for 14 days had a mean RNA-DNA ratio of 2.30. When feeding was resumed, ratios averaged 5.73 after 4 days. In other experiments, daily feeding of golden shiners at 0, 2, and 6% body weight for 15 days produced mean weight changes of -16.1, 2.2, and 21.3%. These growth rate differences were reflected by mean RNA-DNA ratios of 2.19, 2.83, and 4.32, respectively. The RNA-DNA ratio thus provides an indication of how well a fish is feeding, synthesizing protein, and presumably growing at the time of sampling.

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Whole, eviscerated fish were used in the golden shiner experiments. For larger species of fishes, it would be necessary to take selected organ or tissue samples rather than to use the entire fish. The present experiment was designed to gain information regarding organs or tissues that best exhibit RNA-DNA differences at various growth rates.

METHODS

Bluegills (Lepomis macrochirus) were held for 10 and 20 days and fed pelleted fish feed at rates of 0, 0.5, and 4.0% of the total body weight of fish per day. Twenty-four 15-gallon tanks, with one fish per tank were used. Thus, there were four fish for each treatment. Fish were fed once daily, with excess feed and waste materials being siphoned from the tanks between feedings. An overflow system provided an entire change of water approximately every 8 hr. Water temperature was held between 22.0 and 24.0°C.

The entire liver, stomach, intestine, anal fin, and pelvic fins were removed from each fish at the termination of each feeding run. Stomach and intestine samples were flushed out to remove possible contaminating materials. In addition, muscle tissue samples were removed from the left side of each fish anteriorly, between the longitudinal axis and dorsal fin, and posteriorly, in the caudal peduncle region.

To inhibit ribonuclease action, samples were immediately quick frozen by immersion in a preparation of dry ice and methanol. Samples were kept in capped vials and stored in a freezer for 4 to 5 days before preparation for RNA and DNA analyses.

Preparation of fish tissue samples, standard solutions, and reagents as well as extraction of nucleic acids and determination of RNA and DNA content were the same as those described by Bulow (1970), with the several exceptions. Total nucleic acid was extracted from dry, fat-free tissue by the methods of Webb and Levy (1955). Twenty-mg samples were used for liver, stomach, and intestine and 50-mg samples for muscle samples. Fin samples proved unsuitable since they contained extremely low nucleic-acid contents. Analysis of RNA-P content followed the orcinol method described by Schneider (1957), with a modification by Lusena (1951). Nucleic-acid aliquots of 0.5 ml were used for stomach, intestine, and muscle extracts, and 0.1 ml was used for liver. DNA-P was again determined by using the Burton modification of the diphenylamine reaction (Burton 1956).

Since orcinol reacts with DNA as well as with RNA, a correction must be made for the fraction of optical density resulting from DNA present in each sample. This was done through use of the DNA-P standards in the orcinol reaction. Calculations were made as described by Schneider (1957).

Results were reported as concentrations of RNA-P and DNA-P. Concentrations of RNA and DNA can be estimated by assuming that the phosphorus content of both RNA and DNA is approximately 10%.

Because unusually high concentrations were found in some bluegill tissues, particularly liver, some interference was suspected by chromogenic substances in the orcinol reaction. Many substances particularly concentrated in liver tissue interfere with the orcinol reaction (Munro

and Fleck 1966b). To determine if such interference may have occurred and influenced results, remaining samples of liver tissue powder from each set of four fish were pooled and extracted twice with cold 5% trichloroacetic acid (TCA) before normal nucleic acid extraction. This was done by adding 3 ml of cold 5% TCA to each tube containing 20 mg of tissue powder, refrigerating for 12 hr, mixing, centrifuging, and drawing off the supernatant fluid. An additional 3 ml of cold TCA was then added, the mixture centrifuged, and the supernatant again discarded. This procedure removed interfering low-molecular-weight compounds (Munro and Fleck 1966b). RNA-P and DNA-P were then extracted and measured in the usual way. Results were compared with samples run simultaneously, but with no cold TCA extraction.

RESULTS

Bluegills held for 10 days at feeding rates of 0, 0.5, and 4.0% of the body weight per day responded with a slight loss, maintenance, and gain in standard length, respectively (Table 1). Corresponding mean weight changes were -3.0, -0.9, and 2.9 gm. Liver samples yielded highest RNA-P values, followed by stomach and intestine, posterior muscle, and anterior muscle samples, respectively. RNA-P increased with higher feeding rate in all tissues except stomach, in which there was a decline at the 0.5% level. DNA-P values were highest in liver and intestine samples, followed by stomach, posterior muscle, and anterior muscle samples. Liver DNA-P declined with higher feeding rate. DNA-P levels for most other tissues were highest in fish not fed and similar at the 0.5 and 4.0% feeding rates. RNA-DNA ratios were highest in liver, followed by anterior muscle, posterior muscle, stomach, and intestine.

Percentage change in body weight was reflected by RNA-DNA ratios in all tissues sampled (Table 1).

Bluegills held for 20 days responded with a loss, gain, and larger gain in standard length and weight at the three feeding rates (Table 2). Liver RNA-P values were very high, followed by intestine, stomach, posterior muscle, and anterior muscle. RNA-P increased with higher feeding rate in all tissues except anterior and posterior muscle, which declined at the 0.5% level. DNA-P values were highest in intestine samples, followed by liver, stomach, posterior muscle, and anterior muscle samples, respectively. Liver DNA-P declined sharply with higher feeding rates. Stomach and posterior muscle DNA-P also declined with feeding rates, but less sharply. DNA-P levels for all tissues were highest in fish not fed. RNA-DNA ratios were highest in liver, followed by anterior muscle, posterior muscle, stomach, and intestine.

Percentage change in body weight was reflected in RNA-DNA ratios for all tissues sampled (Table 2). In all tissues, there was an increase in ratio with higher feeding rate and faster growth. Changes in ratios were greatest in liver, followed by posterior muscle, anterior muscle, stomach, and intestine, respectively.

RNA-P and DNA-P were recorded in $\mu\text{g}/100$ mg dry, fat-free tissue. Since literature values are often reported in terms of fresh weight, both fresh weight and dry, fat-free weight of the various organ and tissue

Table 1. Changes in standard length (S.L.) and fresh weight (wt.), and nucleic acid response of various organs and tissues of bluegills held for 10 days at three feeding rates^a

	Days - Feeding rate ^b					
	10 - 0.0		10 - 0.5		10 - 4.0	
Number of fish	4		4		4	
Initial S.L. (mm)	100.7 ±	2.5	98.5 ±	2.7	97.7 ±	3.7
S.L. change (mm)	-0.5 ±	0.3	0.0 ±	0.0	2.7 ±	0.4
Initial wt. (g)	35.9 ±	2.6	33.1 ±	3.2	33.5 ±	3.8
Wt. change (g)	-3.0 ±	0.3	-0.9 ±	0.6	2.9 ±	0.3
Wt. change (%)	-8.3 ±	0.7	-2.3 ±	2.2	8.6 ±	0.1
Liver RNA-P ^c	3,358.9 ±	142.7	4,256.3 ±	216.2	4,946.5 ±	37.2
Liver DNA-P	225.7 ±	30.8	156.3 ±	11.4	107.9 ±	10.0
RNA-P/DNA-P	15.9 ±	2.4	27.9 ±	3.0	47.8 ±	7.5
Stomach RNA-P	454.4 ±	11.7	431.7 ±	17.8	506.9 ±	9.0
Stomach DNA-P	130.6 ±	5.6	108.0 ±	7.2	111.4 ±	2.9
RNA-P/DNA-P	3.5 ±	0.1	4.0 ±	0.1	4.6 ±	0.1
Intestine RNA-P	439.9 ±	92.0	495.5 ±	63.5	553.7 ±	80.8
Intestine DNA-P	182.8 ±	23.4	197.3 ±	11.7	186.1 ±	17.9
RNA-P/DNA-P	2.4 ±	0.5	2.5 ±	0.2	3.0 ±	0.1
Anterior muscle RNA-P	248.4 ±	13.9	254.3 ±	12.1	302.9 ±	18.3
Anterior muscle DNA-P	15.5 ±	2.7	11.9 ±	1.6	12.9 ±	0.5
RNA-P/DNA-P	16.3 ±	1.8	22.3 ±	2.5	23.5 ±	1.2
Posterior muscle RNA-P	303.1 ±	8.9	301.7 ±	29.3	357.4 ±	31.0
Posterior muscle DNA-P	20.7 ±	1.5	16.7 ±	2.0	17.5 ±	3.4
RNA-P/DNA-P	14.8 ±	0.9	18.8 ±	2.9	21.0 ±	2.6

^aValues given are mean ± standard deviation of the mean.

^bFeeding rate in percentage of body weight of fish per day.

^cRNA-P and DNA-P in ug per 100 mg dry, fat-free tissue.

samples are given in Table 3. Conversion to ug/mg fresh tissue can be made with the formula:

$$\frac{\text{ug/100 mg DFFT} \times \frac{\text{DFFT weight (mg)}}{100}}{\text{Fresh weight (mg)}}$$

Extraction with cold 5% TCA before nucleic-acid extraction had a definite effect in reducing RNA-P values and therefore indicated that there were high concentrations of interfering chromogenic substances in the liver tissue (Table 4). DNA-P values, however, showed considerably less change. The result was a decrease in RNA-DNA ratios; however, the rank of these ratios did not change. Ratios still increased

Table 2. Changes in standard length (S.L.) and fresh weight (wt.), and nucleic acid response of various organs and tissues of bluegills held for 20 days at three feeding rates^a

	Days - Feeding rate ^b					
	20 - 0.0		20 - 0.5		20 - 4.0	
Number of fish	4		4		4	
Initial S.L. (mm)	104.5 ±	4.8	106.0 ±	4.7	98.0 ±	1.6
S.L. change (mm)	-0.3 ±	0.2	2.0 ±	0.4	7.5 ±	0.8
Initial wt. (g)	36.1 ±	4.0	45.7 ±	5.0	37.3 ±	4.0
Wt. change (g)	-3.3 ±	0.4	0.5 ±	0.3	7.9 ±	1.1
Wt. change (%)	-9.4 ±	1.1	1.2 ±	0.7	21.1 ±	4.6
Liver RNA-P ^c	3,242.3 ±	256.2	3,493.0 ±	444.8	4,434.5 ±	208.4
Liver DNA-P	210.8 ±	27.7	134.9 ±	19.6	98.0 ±	8.8
RNA-P/DNA-P	16.9 ±	4.1	27.9 ±	6.0	47.1 ±	6.8
Stomach RNA-P	428.7 ±	23.2	467.2 ±	15.2	518.5 ±	17.3
Stomach DNA-P	121.2 ±	4.5	112.5 ±	1.8	102.5 ±	5.9
RNA-P/DNA-P	3.5 ±	0.1	4.2 ±	0.1	5.1 ±	0.3
Intestine RNA-P	502.7 ±	15.9	507.9 ±	24.8	601.1 ±	10.7
Intestine DNA-P	223.4 ±	26.6	191.2 ±	15.3	221.1 ±	9.2
RNA-P/DNA-P	2.4 ±	0.4	2.6 ±	0.3	2.7 ±	0.1
Anterior muscle RNA-P	275.7 ±	22.0	250.0 ±	5.9	314.9 ±	30.2
Anterior muscle DNA-P	15.6 ±	1.9	12.9 ±	0.5	13.5 ±	0.5
RNA-P/DNA-P	18.0 ±	1.1	19.4 ±	0.5	23.5 ±	2.4
Posterior muscle RNA-P	349.2 ±	16.2	325.9 ±	2.3	380.1 ±	7.2
Posterior muscle DNA-P	22.4 ±	1.9	20.9 ±	3.0	18.3 ±	1.2
RNA-P/DNA-P	16.0 ±	1.7	17.0 ±	3.3	23.2 ±	1.7

^aValues given are mean ± standard deviation of the mean.

^bFeeding rate in percentage of body weight of fish per day.

^cRNA-P and DNA-P in ug per 100 mg dry, fat-free tissue.

with higher feeding rate and faster growth rate. For greater accuracy, however, initial cold TCA extraction should be included in the standard procedures.

DISCUSSION

By using values obtained with cold, TCA-extracted liver tissue and converting to similar units of measure, it is possible to make comparisons with values obtained in other studies. RNA concentrations of bluegill liver were slightly lower than those found in mammalian liver (Leslie 1955), but fell within the range of values reported for sockeye salmon liver (Creelman and Tomlinson 1959). Liver DNA concentrations of bluegills fell within ranges reported for mammalian liver by Leslie (1955) and sockeye salmon liver by Creelman and Tomlinson (1959).

Table 3. Fresh weight and dry, fat-free weight of various organs and tissues of bluegills held for 10 and 20 days at three feeding rates

Days - rate ^a	Liver ^b	Stomach	Intestine	Anterior muscle	Posterior muscle
10 - 0.0	337.5 ± 43.9 ^c 59.4 ± 7.7 ^d	255.0 ± 9.6 34.7 ± 1.3	310.0 ± 23.9 44.0 ± 4.8	1,165.0 ± 80.0 180.6 ± 12.4	575.0 ± 52.1 79.3 ± 7.2
10 - 0.5	427.5 ± 22.9 75.3 ± 4.0	255.0 ± 20.2 34.7 ± 2.8	377.5 ± 63.5 53.6 ± 9.0	1,195.0 ± 47.7 181.3 ± 6.9	612.5 ± 47.5 84.5 ± 6.6
10 - 4.0	632.5 ± 123.7 111.3 ± 21.8	302.5 ± 29.0 41.1 ± 4.0	387.5 ± 38.5 55.0 ± 8.0	1,455.0 ± 115.1 225.5 ± 17.9	685.0 ± 83.9 94.5 ± 11.6
20 - 0.0	280.0 ± 28.0 49.3 ± 4.9	275.0 ± 24.0 37.4 ± 3.3	330.0 ± 41.3 46.9 ± 5.9	870.0 ± 40.6 134.9 ± 6.2	445.0 ± 89.4 61.4 ± 7.2
20 - 0.5	795.0 ± 244.6 139.9 ± 43.1	360.0 ± 27.1 49.0 ± 3.7	420.0 ± 17.8 59.6 ± 2.5	1,177.5 ± 64.2 182.5 ± 10.0	640.0 ± 78.8 88.3 ± 10.9
20 - 4.0	817.5 ± 156.3 143.9 ± 27.5	405.0 ± 23.1 55.1 ± 4.7	482.5 ± 37.5 68.5 ± 5.4	1,307.5 ± 82.6 202.7 ± 12.7	600.0 ± 62.9 82.8 ± 8.7

^aFeeding rate in percentage of body weight of fish per day.

^bMean ± standard deviation of the mean of four samples.

^cFresh weight of sample in mg.

^dDry, fat-free (DFFT) weight of sample in mg.

Table 4. Comparison of nucleic acid determinations with and without prior extraction of interfering compounds with cold trichloroacetic acid.

	Days - Feeding rate ^a					
	10 - 0	10 - 0.5	10 - 4.0	20 - 0	20 - 0.5	20 - 4.0
	<u>Without Cold TCA Extraction</u>					
RNA-P ^b	1,833.6	2,354.4	2,401.8	2,262.9	2,182.8	2,332.8
DNA-P	180.7	144.4	108.2	273.5	106.7	101.7
RNA-P/DNA-P	10.2	16.3	22.2	8.3	20.5	22.9
	<u>With Cold TCA Extraction</u>					
RNA-P	750.0	1,299.6	1,103.1	445.2	1,050.9	923.4
DNA-P	244.6	170.2	102.6	265.1	124.9	91.4
RNA-P/DNA-P	3.1	7.6	10.7	1.7	8.4	10.1
	<u>Magnitude of Difference^c</u>					
RNA-P	2.4	1.8	2.1	5.1	2.1	2.5
DNA-P	0.7	0.8	1.1	1.0	0.9	1.1
RNA-P/DNA-P	3.3	2.1	2.1	4.9	2.4	2.3

^aFeeding rate in percentage of body weight of fish per day.

^bRNA-P and DNA-P in ug per 100 mg dry, fat-free tissue.

^cMagnitude of difference between measurements with and without prior cold TCA extraction.

In making such comparisons for other bluegill tissues tested, it is necessary to use values obtained without initial cold TCA extraction.

RNA concentrations of bluegill stomach and intestine are similar to those reported for mammalian small intestine (Leslie 1955), but slightly higher than RNA concentrations found for the alimentary tract of sockeye salmon (Creelman and Tomlinson 1959). DNA concentrations of bluegill stomach and intestine fell within the range reported for sockeye salmon alimentary tract (Creelman and Tomlinson 1959), but were lower than values reported for mammalian small intestine (Leslie 1955).

RNA concentrations of bluegill muscle tissue were similar to those reported for mammalian muscle (Leslie 1955), but considerably higher than concentrations found in sockeye salmon (Creelman and Tomlinson 1959; Bluhm and Tarr 1957). DNA concentrations of bluegill muscle were similar to values reported for sockeye salmon muscle by Creelman and Tomlinson (1959), but higher than levels reported by Bluhm and Tarr (1957). DNA concentrations in bluegill muscle fell within the ranges reported for mammalian skeletal muscle (Leslie 1955).

The validity of such comparisons is questionable since, in each study, different species and different analytical procedures were used. In this area of research, there is a need for standardization of techniques and

methods of reporting results. At present, there seems little agreement among researchers on methods of extraction, estimation of RNA and DNA, and reporting of data. Munro and Fleck (1966a, 1966b) should be consulted for recent reviews on the methods of measuring nucleic acids in biological materials. Even though it is difficult to compare absolute values between studies, comparisons between trends and changes found within various studies should be reliable.

This study substantiated the findings of Bulow (1970), that RNA-DNA ratios can be used as indicators of recent growth rate of fishes. It also demonstrated that large fish can be sampled by removing selected organ or tissue samples. RNA-DNA ratios of bluegill liver were most sensitive to changes in body length and weight. In addition, initial removal of interfering compounds with cold TCA extraction would be a desirable addition to previously described techniques.

ACKNOWLEDGMENTS

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EVALUATION OF STRESS INDICES FOR CORN IN IOWA¹

Walter C. Corsi and Robert H. Shaw²

ABSTRACT. Four methods of computing moisture stress indices were correlated with corn yield data to determine the best index. The indices computed for each day were summed over the 66-day period from June 27 through Aug. 31. An index that assumed the yield reduction was the same as the reduction in actual evapotranspiration from potential evapotranspiration expressed as a percentage of potential, gave the highest correlations.

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In many areas, particularly in the western part of the Corn Belt, plant water stress causes significant reductions in crop yields. Over the years, many studies have been conducted on the relationship between weather and yield. No attempt will be made to review this literature in detail.

Rainfall was one of the first meteorological parameters used in studying weather-yield relationships. Soil moisture represents a step closer to the water supply of the plant, but only very limited data are available (Stanhill 1957; Baier 1965; Baier and Robertson 1966, 1967, 1968). It is now generally accepted that water stress in the plant is not necessarily produced by either of these factors alone, but is the result of an imbalance between the available water in the soil profile and the atmospheric demand for water (Philip 1957; Denmead and Shaw 1963). A parameter measuring the internal water status of the plant, which is the result of this imbalance, would be the most desirable parameter to study. Of the indices reported, only one involves computing the internal water status of the plant directly; the others use it only indirectly.

Daily soil moisture was calculated for a selected group of stations in Iowa. An April, gravimetric soil-moisture sample was used as the starting point, and daily rainfall and Class A, evaporation-pan data were used as inputs. Four daily stress indices were computed from these data. These indices were an attempt to evaluate the supply versus demand for soil water for each day and to measure the effect on crop yields. This paper summarizes the results of a study where four moisture-stress indices were related to corn yields at 10 locations in Iowa.

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STRESS INDICES

The use of drought or stress indices is not new. The work reported here is a further development of that reported by Dale and Shaw (1965a, b) in which days without moisture stress per season were correlated with corn yields from experimental plots near Ames. Dale (1968) presented results on the climatology of days without moisture stress in Iowa for four locations.

In the original determination of nonstress days, each day was considered either as a stress or nonstress day, with no intermediate degrees involved. In the present study, several different indices were examined, which assigned a severity of stress for each day considered. The indices compared are:

Index I.

This is the original nonstress day (moisture) as used by Dale (1968) and reported on by Dale and Shaw (1965a, b). When the soil moisture cannot meet the atmospheric demand for water, the plant is considered under stress. Denmead and Shaw (1962) expressed the amount of soil moisture in the corn root zone at the estimated turgor loss (stress) point as a function of the transpiration at field capacity. Dale (1964)³ defined a stress day as any day in which the combination of evapotranspiration demand and available soil moisture did not permit potential water loss.

Two estimates are necessary to identify the stress condition on each day: (a) the soil moisture available in the root zone, or in the top foot of the profile, expressed as a percentage of field capacity (PAV or PAVI) and (b) the atmospheric evaporative demand expressed as the percentage of available field capacity in the root zone required by existing atmospheric conditions to prevent the corn plant from losing turgor (TH).

In the computer program for estimating soil moisture (Dale and Hartley 1963), the stress condition is given by

$$\text{Ratio} = \text{PAV or PAVI/TH}$$

where the greater of the two PAV values is used. If the ratio is ≥ 1.00 , the day has no moisture stress; if less than 1.00, it is a moisture-stress day; i. e., the index is either 1 (no stress) or 0 (stress).

Index II.

This index uses the Ratio as just computed but the degree of stress on a stress day is given by $1.00 - \text{Ratio}$. Stress on a given day can have any value between 0 and 1, with greater stress occurring with the greater index; opposite of how it was used in Index I.

Index III.

Another way to evaluate the degree of stress is to examine the relationship between the actual evapotranspiration (ET), as computed by the soil-moisture program, and the potential evapotranspiration (PET). When ET is occurring at the PET rate, no stress is assumed. The

³Robert F. Dale. 1964. Weather effects on experimental plot corn yields: Climatology of selected favorable and unfavorable conditions. Ph.D. Thesis. University Microfilm Order No. 64-9258. Iowa State University, Ames.

assumption is made that, for any reduction in actual ET from the PET, a similar yield reduction will occur. The computer program computes the ratio of ET/PET. This is called STRS. If STRS is 1.00, the available moisture is \geq that needed to meet the atmospheric demand, and no stress occurs. If the value is less than 1.00, then 1.00 - STRS gives the stress index for that day. Values can range from 0 to 1, with greater stress occurring with the greater value.

In computing STRS, only the percentage of available soil moisture in the root zone has been considered. In computing soil moisture, adjustments are made for evaporation from the top foot of soil, but these do not affect the value of STRS. Although the effect is expected to be small, subsequent work will examine the effect of considering PAV or PAVI, whichever is greatest, on this index.

Index V.

This index is a modification of what was originally called Index IV. Laing (1966)⁴ developed a relationship that predicted the relative water content of the crop canopy at 1400 hours each day from the daily soil moisture in the root zone and Class A, evaporation-pan data. This relative water-content value is then used to estimate a relative photosynthetic index (yield) for each day. This index is thus a function of the relative water content, percentage available soil moisture, and the Class A, evaporation-pan loss. For each day, the index can range from 0 to 1, with the lesser values associated with lesser stress.

Each index was summed over the 66-day period from June 27 through Aug. 31. This period was used in place of Dale's (1968) period of 6 weeks before silking to 3 weeks after silking, more as a matter of convenience than for any other reason. Dates before and after this period were not considered in computing stress.

YIELD DATA USED

When the soil moisture survey was started in 1954, the idea was to select a representative area for the soil-moisture sample. At many locations, an area was not available to set up a specific site for soil moisture sampling; therefore an available "representative" area on the farm was used. Because of this, the soil moisture sites at most of the locations have changed position several times. Yields from the soil-moisture sites have often resulted from a range of management conditions. Of the locations used, nine are on experimental farms that had yield data from crop-rotation experiments. The other location, on a private farm, had field yield data available. Data were used where the starting soil moisture was measured on an area where meadow was grown in the previous year (MC) and where corn was grown in the previous year (CC). The yield data and soil-moisture site descriptions for each location are given in the following text because of their importance in interpreting the results obtained.

⁴ Douglas R. Laing. 1966. The water environment of soybeans. Ph.D. Thesis. University Microfilm Order No. 66-6988. Iowa State University, Ames.

Ames (Agronomy Farm). During the period of record, the Agronomy Farm was relocated. The yield data were from a well-fertilized, corn-corn-oats-meadow (CCOM) on Nicollet loam or Webster silty clay loam. These soils are somewhat poorly drained, but have drainage tile installed. Under wet conditions, they will have free water in the subsoil. The soil-moisture data are from similar soils, except for a 5-year period when data were collected from a Colo silty clay loam at a nearby irrigation-research area. This soil is a poorly drained, alluvial soil.

Beaconsfield (Shelby-Grundy Farm). Yield data used were from the soil moisture sites and from a CCOM rotation with good fertilization on a Grundy silty clay loam with a slope of 2-4%. Some plots are imperfectly drained. The soil-moisture sites were in bulk corn areas with widely varying previous management, but having soil characteristics very similar to those of the rotation experiment. In some years the soil moisture site yield was quite different from the rotation yields.

Bloomfield (Southern Iowa Farm). The soil of this farm is an Edina silt loam with high clay content in part of the subsoil and with poor drainage. Moisture samples were taken in a tile-drainage experiment with high fertility and a COM rotation. Yield data are from this experiment for meadow-corn (MC) and from a nearby continuous-corn area for corn-corn (CC), also in the drainage experiment.

Castana (Western Iowa Farm). This area is hilly. The plots were located on an Ida silt loam soil with slopes of up to 15%. This is a deep loess soil, low in organic matter and available phosphorus. The rotation yield data are from an experiment receiving adequate fertilization. The soil moisture site has changed frequently, with individual years having quite different management from that of the rotation experiment.

Doon (Northwest Iowa Moody Farm). The Moody soil is a well drained, silty clay loam with slopes of 2-5%. Yield data come from COMM and CCO rotations in which the soil-moisture samples were taken.

Independence (Carrington-Clyde Farm). The soil at this site in northeastern Iowa has been renamed Kenyon. It is a loam soil, moderately well drained with many sand pockets in the subsoil. Yield data are from an adjacent CCOM rotation.

Kanawha (Northern Iowa Farm). The soil at this site is a Webster silty clay loam, which is naturally poorly drained, but which has tile drainage. The yield data come from a CCOM rotation from which the soil-moisture samples were taken in about half the years. The samples for the remaining years were taken in available areas. In several of these years, meadow areas with poor stands were the only meadow sites available.

Marshalltown. This is the only site on a private farm. The soil is a Muscatine silty clay loam with very little slope. This soil is somewhat poorly drained. Yield data are field values for the field within which the soil-moisture samples were taken. Although labeled CC, this has been a Sb-C sequence in some years.

Norwich (Soil Conservation Farm). The soil at this site is a Marshall silty clay loam, moderate to well drained, with slopes of 2-4%. Yield data are from a well-fertilized CCOM rotation. Soil moisture samples were taken from a number of bulk corn areas with widely different management.

Sutherland (Galva-Primghar Farm). The soil of this site is a Galva silty clay loam, well drained, with 2-4% slope. Yield data come from a CCOM rotation and from the nearby soil-moisture-site area.

When different varieties were used during the period of the data and when records of these varieties were available, the yields were adjusted to the last hybrid grown by using data from the Iowa Corn Yield Test (1958-1967). Adjustments were made for Ames, Bloomfield, Doon, Independence, and Sutherland. There were only small ranges in the population levels used at each location; therefore, no stand adjustments were made.

RESULTS AND DISCUSSION

The correlation coefficients between yield and the different indices at each location are given in Table 1. In comparing the indices, index III

Table 1: Correlation coefficient (r) between corn yield and four different indices.

Location and Experiment	Index			
	1	2	3	5
Ames CC	0.79	-0.90	-0.92	0.82
Ames MC	0.67	-0.86	-0.84	0.72
Beaconsfield CC	0.30	-0.36	-0.47	0.28
Beaconsfield SMS	0.73	-0.81	-0.79	0.78
Bloomfield CC	0.31	-0.28	-0.42	0.25
Bloomfield MC	0.49	-0.45	-0.51	0.40
Castana CC	0.63	-0.75	-0.77	0.70
Castana MC	0.70	-0.80	-0.82	0.73
Castana SMS	0.67	-0.79	-0.92	0.74
Doon CC	0.73	-0.90	-0.92	0.84
Doon MC	0.77	-0.90	-0.93	0.92
Independence CC	0.06	-0.08	-0.13	0.10
Independence MC	0.51	-0.62	-0.61	0.55
Kanawha CC	0.62	-0.79	-0.81	0.71
Kanawha MC	0.53	-0.67	-0.60	0.60
Marshalltown CC	0.64	-0.71	-0.63	0.65
Norwich CC	0.57	-0.74	-0.87	0.68
Norwich MC	0.58	-0.68	-0.90	0.62
Norwich SMS	0.51	-0.60	-0.74	0.53
Sutherland CC	0.62	-0.78	-0.84	0.73
Sutherland MC	0.74	-0.86	-0.89	0.82
Sutherland SMS	0.64	-0.78	-0.85	0.73

$$r^{05} (14 \text{ df}) = .50; \quad r^{01} (14 \text{ df}) = .62$$

had the highest correlation coefficient 17 of 22 times and, index II, 5 of 22 times. Index III was statistically different (1 or 5% level) from index I 9 times and near the 10% significance level 3 times. Index III was significantly different from index II only once, with 2 values near the 10% level of significance. Index III was significantly greater than index V for

5 values. There seems little to choose between indices II and III, although index III consistently gave the highest correlation. Index III was accepted as the best index, and further discussion will be limited to that index. In interpreting the correlations with yield, the site information given previously should be considered. Also, the general level of stress for different areas needs to be considered because lower correlations were generally found in the wetter parts of the state. The average values of the index for the period used ranged from 7 at Independence to 25 at Doon for MC. Individual yearly values have ranged from 0.1 to 56.

At Ames, the correlations were surprisingly high since the moisture samples for almost a third of the years came from a different soil type than did the yield data. The correlation for the CC site at Beaconsfield was not significant. Management and soil moisture conditions at the moisture site were believed enough different from the rotation experiment to cause this. A good correlation with yield was found for the soil moisture site. Correlations at Bloomfield were low, although significant for the MC site. This soil is characterized more as being too wet rather than too dry. The highest index value for MC was 37, with the next highest 26, indicating no severe stress has occurred. The highest index value for CC was only 28.

At Castana, the soil moisture site showed the highest correlation. The differences in management between this site and the rotation sites are believed to cause this. Both Doon and Sutherland have what are considered excellent data. This, combined with their drier locations, resulted in high correlations.

The correlation for CC at Independence was not significant, although that for MC was significant. No high stress values were measured. Lack of significance for the CC site was due to a small range in the corn yields for most years, with the highest and lowest yields measured under wet conditions. This was not true for MC. Independence had the lowest index of the stations used.

Kanawha had a good correlation for CC, but was considerably lower for MC. The lower correlation for MC was believed due to several years with poor stands of meadow at the soil moisture site. The soil moisture site did not adequately represent the corn-yield site.

Marshalltown had a relatively low correlation. At that location, only 2 years have had an index value greater than 20. This station did show an increase in the yield variation explained by using a quadratic relationship. At Norwich, the soil moisture site had extremely variable management, which affected the yield level; yet, the index calculated was well correlated with the rotation yields.

As far as Iowa is concerned, index III explained a considerable amount of the yield variation measured in the western half of the state, with a much poorer relationship in the wetter, eastern part. Because the period of record has been generally a below-normal stress period (Dale 1968) and because the data used were not from experiments specifically designed to evaluate stress-yield relationships, the results seem very satisfactory.

Future work will evaluate the effect of weighting the stress index at different phenological periods and the effect of consecutive days of stress on the relationship with yield.

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A KEY TO THE SPECIES OF BOLTERIA UHLER WITH
 DESCRIPTIONS OF SIX NEW SPECIES (HEMIPTERA, MIRIDAE)

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ABSTRACT. A key to 14 species of Bolteria Uhler is provided. New species of Bolteria are B. arizonae from Arizona; B. dakotae from South Dakota; B. nevadensis from Nevada; B. omani from California; B. siouxan from Wyoming; and B. schaffneri from Colorado.

The genus Bolteria Uhler, type Bolteria amicta Uhler, was described in 1887 from New Mexico. The genus went unrecognized by other workers for many years, or until the writer found type material in the Luger collection at the University of Minnesota in 1919. In the same year Knight (1919) published concerning the characters of the genus, its position in family Miridae, and noted that Dichrooscytus speciosus Van Duzee really belongs in genus Bolteria. It was also pointed out that Dichrooscytus speciosus var. rubropallidus Knight (1918) is a good species in genus Bolteria. Other species have since been described and are now included in a key which follows. The male genital segment does not offer useful external characters for separating species, hence the lack of illustrations for such purpose. It now appears that species of Bolteria breed only on conifers, particularly the genus Juniperus, where several species have been found.

Key to the Species of Bolteria

- 1. Antennal segment I not equal to width of vertex. 2
 Antennal segment I in length, greater than width of vertex.
 semipicta Blat.
- 2. Frons pale, transversely marked with red lines. 3
 Frons without distinct transverse lines. 7
- 3. Rostrum reaching beyond hind coxae. 4
 Rostrum only reaching upon hind coxae. 6
- 4. Corium chiefly pallid or opaque white. 5
 Corium rather uniformly dark red to fuscous; pronotum reddish to fuscous brown, disk and calli flecked with red; femora dark red, hind pair white on anterior aspect and with two rows of red spots; small ovate form, length 3.7 mm.
 nicholi Kngt.

5. Clavus with a pale stripe running lengthwise through the middle; pronotum with blackish spot or ray beginning at outer margin of callus and curving around behind and toward middle of disk; length, 5.1 mm. amicta Uhler
Clavus dark reddish to black, margin bordering claval suture only, pallid; pronotal disk white, without a blackish ray extending around behind callus, although the basal margin of the disk is black; length 4.3 mm. rubropallida Kngt.
6. Pronotum opaque white, calli black, a small black ray extending posteriorly from lateral margin of each callus; cuneus white, narrow apex red; length (♀) 4.8 mm. omani n. sp.
Pronotal disk with a broad submarginal basal band of reddish brown color; calli brownish black; cuneus pallid, central area with a triangular red brown spot, and apex with a small black spot; length (♂) 3.8 mm. arizonae n. sp.
- 7(2). Hemelytra with red, sometimes only dusky red, but the cuneus red on outer margin. 8
Hemelytra testaceous, inner half of clavus and apical area of corium darkened with fuscous; outer margin of cuneus fuscous; length (♂) 4.2 mm, (♀) 3.9 mm. luteifrons Kngt.
8. Scutellum white, usually with a dark mark on median line at base. 9
Scutellum and pronotum reddish, although finely reticulate with paler; propleura and epimera of mesothorax white; clavus uniformly reddish like the corium; length (♀) 4.6 mm. balli Kngt.
9. Pronotum pallid to opaque white, basal margin of disk darker. . 10
Pronotal disk and corium chiefly red; apical fourth of corium, inner half of clavus bordering scutellum, blackish; length 4.8 mm. speciosa Van D.
10. Corium and clavus with opaque white areas. 11
Corium and clavus rather uniformly light fuscous, subtranslucent, the hypodermis filled with granules of red pigment; length 4.0 mm. nevadensis n. sp.
11. Cuneus white, outer margin and apical one-third red, or reddish black. 12
Cuneus dark, fuscous to reddish, the inner basal angle opaque white, but with flecks of dark color; length (♀) 3.8 mm. dakotae n. sp.
12. Epimeron of mesothorax fuscous; legs pallid to dusky. 13
Epimeron of mesothorax white; legs white; length 4.7 mm. juniperi Kngt.
13. Membrane fuscous, with an opaque spot bordering apex of larger areole; epimeron of the mesothorax fuscous; length (♀) 3.2 mm. siouxan n. sp.
Membrane without an opaque callus spot bordering apex of larger areole; length (♂) 3.0 mm, (♀) 3.1 mm. schaffneri n. sp.

Bolteria omani new species

In the key this species runs in the couplet with arizonae, but size is larger; pronotal disk without a basal submarginal band; cuneus white, apex only reddish black.

Female. Length 4.8 mm, width 2.2 mm. Head; width 1.2 mm, vertex .50 mm; vertex rather broadly convex, carina not elevated as in most species; white, frons with six transverse red lines each side of middle, in part fused; vertex with a red spot each side set in contact with the eye; clypeus with red each side of middle on basal half, apex fuscous; juga with lower half red. Rostrum, length 1.6 mm, reaching upon apex of hind coxae, yellowish to reddish. Antennae; segment I, length .48 mm, red, dorsal aspect white; II, 1.8 mm, cylindrical, slender, slightly thicker on apical half, yellowish; III, broken. Pronotum, length 1.0 mm, width at base 1.9 mm; disk rugose punctate; white, calli black, with a fuscous ray extending posteriorly from outer margin of each callus. Scutellum convex, smooth and white; mesonotum exposed, black but reddish on median line.

Hemelytra opaque white; clavus narrowly fuscous along inner margin and at base; apical edge of corium and two incomplete marks on middle, reddish to black; apex of embolium with black; cuneus white, narrow apex with reddish black; membrane fuscous, darker within areoles, veins white. Venter white, irregularly marked with reddish. Sternum fuscous to red. Legs pallid to white, femora irregularly marked with red.

Holotype: ♀ June 4, 1935, San Jacinto Mts., California (P. Oman), on Pinon pine. U.S.N.M. Collection. Paratypes: 2 ♀, taken with the type.

Bolteria arizonae new species

In the key this species runs in the couplet with omani, but differs in having a broad, submarginal basal band of reddish brown on disk of pronotum.

Male. Length 3.7 mm, width 1.5 mm. Head; width .98 mm, vertex .35 mm; carina slightly arcuate as viewed from above, vertex with a prominent reddish spot each side next to the eye; frons with a patch of transverse red lines each side, some lines so close they appear to merge; clypeus, bucculae, and lora except basal half, deep red. Rostrum, length 1.4 mm, reaching upon apex of hind coxae, reddish. Antennae; segment I, length .40 mm, pale yellowish; II, 1.9 mm, brownish yellow, cylindrical, slightly more slender near base; III, .67 mm, slender, brownish; IV, broken. Pronotum, length .68 mm, width at base 1.28 mm; propleura and central area of disk, pallid to white; transversely across calli, lateral margins of disk, and a broad, transverse subbasal band reddish brown, the narrow basal edge and central area of disk, opaque white. Scutellum moderately convex, white; mesonotum dark brownish black. Hemelytra, lateral margins only slightly arcuate; clavus white, with two longitudinal dark reddish brown lines, the inner line wider on middle and reaching edge by scutellum; corium white but with dark brown along inner side of radius, widening to take in much of apical half of corium; embolium dark brown, paler just beyond middle, but broadly reddish brown on apical area and confluent with apex of corium. Cuneus reddish brown, the margins white except narrow apex which is brownish black. Membrane fuscous, veins white.

Sternum dark brown, epimera with brown but margins white. Legs white and in part brown; femora dark brown, apical half more or less white, hind pair with a row of red dots along median line; tibia white, spines dark brown; tarsi fuscous. Venter white, marked laterally with brown; basal half of genital segment dark reddish brown. Male claspers typical of the genus.

Female. Length 3.7 mm, width 1.7 mm. Head: width 1.02 mm, vertex .49 mm; color and markings similar to the male. Rostrum reaching upon apex of hind coxae. Antennae: segment I, length .38 mm; segment II, 1.6 mm, color and form similar to that of the male. Pronotum, length .72 mm, width at base 1.3 mm; coloration similar to that of the male.

Holotype: ♂ August 18, 1927, Coconino County, Arizona (R.H. Beamer); Kansas University Collection. Allotype: same data as the holotype. Paratypes: 2♂, 2♀, taken with the types (R.H. Beamer). 7♂♀ (P.A. Readio); 4 ♂♀ (L.D. Anderson), taken with the types.

Bolteria nevadensis new species

Allied to speciosa Van D., but differs in color of the hemelytra; corium and clavus shaded with light fuscous, subtranslucent, the hypodermis filled with granules of red pigment.

Male. Length 4.0 mm, width 1.5 mm. Head: width 1.21 mm, vertex .60 mm; yellowish to pale fuscous, frons uniformly dusky yellow. Rostrum, length 2.1 mm, reaching upon base of genital segment, dark red. Antennae: segment I, length .26 mm, pallid to pale fuscous; II, broken. Pronotum, length .69 mm, width at base 1.3 mm; reddish yellow, collar black, calli pale fuscous, basal margin of disk fuscous; propleura pallid to dusky yellow. Scutellum white, disk moderately convex; mesonotum yellowish to fuscous. Hemelytra except cuneus, dusky, subtranslucent, hypodermis filled with light red granules of pigment. Cuneus opaque white, lateral margin and apical half dark red. Membrane light fuscous, veins red. Venter brownish black, genital segment dusky brown. Sternum brownish black, epimera white. Legs white, no marks, tips of tarsi fuscous.

Female. Length 4.1 mm, width 1.6 mm. Head: width 1.31 mm, vertex .64 mm; form similar to the male; frons yellow, clypeus fuscous, lora and juga light fuscous. Rostrum, length 2 mm, reaching upon ovipositor to just short of middle. Antennae broken. Form and coloration very similar to the male.

Holotype: ♂ June 24, 1966, White Pine County, Nevada, 6 miles southwest of Ely, altitude 6500 ft. (W. Gagné), taken on Juniperus. Knight Collection. Allotype: ♀, taken with the type.

Bolteria dakotae new species

In the key to species dakotae runs nearest to juniperi Kngt., but differs in having the cuneus fuscous to reddish, also by other color distinctions.

Female. Length 3.8 mm, width 1.4 mm. Head: width 1.0 mm, vertex .56 mm; pale to yellowish without marks, clypeus fuscous, juga reddish yellow. Rostrum, length 1.7 mm, reaching upon middle of venter, reddish. Antennae: segment I, length .40 mm, pallid; II, 1.4 mm, nearly cylindrical, but more slender on basal one-fourth, yellowish

to pale fuscous, with short, fine pale pubescence; III, broken. Pronotum, length .56 mm, width at base 1.2 mm; pale yellowish brown, collar and calli dark brown, propleura pale to yellowish. Scutellum moderately convex, with transverse striae evident, pallid to white, shaded brownish at middle of base; mesonotum dark brown. Hemelytra clothed with fine, recumbent, pale to yellowish pubescence; clavus dark brown, outer margin pallid; corium brown to dark brown, pallid bordering clavus; embolium brown to dark brown. Cuneus brown to dark brown, inner basal angle somewhat pallid. Membrane pale fuscous, veins dusky to reddish. Venter dark brown, genital segment paler on apical half. Sternum reddish brown; epimera dusky to fuscous. Legs pallid to pale fuscous; femora pale fuscous, tibiae dusky, tarsi fuscous.

Holotype: ♂ July 20, 1928, Englewood, South Dakota (H. C. Severin); Knight Collection.

Bolteria siouxan new species

Allied to schaffneri, but distinguished by having a callus spot in membrane bordering apex of larger areole.

Female. Length 4.2 mm, width 1.5 mm. Head: width 1.2 mm, vertex .59 mm; yellowish brown, lower half of head medium brown color. Rostrum, length 1.9 mm, reaching upon first genital segment, color reddish brown. Antennae: segment I, length .38 mm, pallid; II, 1.40 mm, cylindrical, slender, thicker on apical half, dusky yellow; III, .70 mm, slender, dusky; IV, .48 mm, fuscous. Pronotum, length .68 mm, width at base 1.34 mm, yellow brown over opaque white hypodermis, calli more brown, collar brownish black, basal margin of disk dark fuscous. Mesonotum dark brown. Scutellum moderately convex, yellowish white. Corium and embolium reddish brown, inner margin of corium opaque yellowish white and flecked with brown; clavus dark brown, margin bordering claval suture opaque yellowish white but basal one-third all brown. Cuneus opaque dull white, narrow outer margin red, apex dark brownish red. Membrane light fuscous, veins red, a callus spot bordering apex of larger areole. Clavus and corium clothed with appressed, fine short pubescence, color matching that of the subsurface. Ventral surface light brown, or dusky brown; venter medium brown to reddish brown. Legs pallid to opaque white, tibial spines pale to light brown, tips of tarsi infuscated.

Holotype: ♀ July 29, 1931. Medicine Bow Mts., Wyoming (H. H. Knight); Knight Collection.

Bolteria schaffneri new species

Allied to siouxan but smaller and more slender; without the callus spots in wing membrane.

Male. Length 3.0 mm, width 1.5 mm. Head: width 1.14 mm, vertex .56 mm; medium brown, without marks on frons, the clypeus only slightly darker brown; eyes a different shade of brown. Rostrum, length 1.92 mm, reaching to base of genital segment, brown, apex black. Antennae: segment I, length .35 mm, pallid to light brown; II, 1.5 mm, cylindrical, slender, somewhat thicker on apical half; III, .75 mm, slender, pale to dusky; IV, .38 mm, fuscous. Pronotum, length .67 mm, width at base 1.28 mm; collar black, calli brown; central area of disk opaque white in the hypodermis, chitin surface shaded with light brown,

basal margin fuscous to black, but not sharply defined. Mesonotum dark brown. Scutellum moderately convex, white with a yellow tinge on surface. Clavus brownish black, opaque yellowish along suture margin; embolium reddish brown, corium reddish brown to brownish black, opaque yellowish along margin by clavus. Clothed with short, appressed pubescence, but more sparsely set than in siouxan. Membrane rather evenly shaded with fuscous, veins red, without a callus spot by apex of larger areole. Ventral surface fuscous brown, genital segment somewhat paler. Legs opaque white, tips of tarsi fuscous; tibial spines yellowish to brown.

Female. Length 3.1 mm, width 1.5 mm. Head: width 1.23 mm, vertex .64 mm; the eyes curve slightly to posterior position. Rostrum reaches back to middle of first genital segment. Antennae: segment I, length .40 mm; II, 1.14 mm; III, .75 mm, pale; IV, .48 mm, fuscous. Pronotum, length .70 mm, width at base 1.31 mm. Color and pubescence very similar to the male.

Holotype: ♂ August 11-14, 1969, Gould, Jackson County, Colorado (J. C. Schaffner); U. S. N. M. Collection. Allotype: same data as the type. Paratypes: 1♂, 3♀, taken with the types. 1♀ August 19, 1969, North Pass, Saguache County, Colorado (J. C. Schaffner). Named in honor of the collector, Dr. Joe C. Schaffner, Texas A and M University, who has been very active with the Miridae, and of great help to the author.

Bolteria amicta Uhler

Bolteria amicta Uhler, 1887, p. 33

Bolteria amicta Knight, 1919, p. 127

The original specimens were collected in New Mexico by Dr. Bolter.

Records: NEW MEXICO: ♀ June 1913, Silver City (J. B. Wallis).

ARIZONA: ♂♀ June 22, 1929 (A. A. Nichol); ♂ May 5, 1933, Chiricahua Mts. (E. D. Ball). UTAH: ♀ May 21, 1909, West Wats (E. D. Ball).

Bolteria speciosa (Van Duzee)

Dichroscytus speciosus Van Duzee, 1916, p. 236

Bolteria speciosa: Knight, 1920, p. 127 (1919)

Bolteria speciosa: Knight, 1928, p. 131, key.

Bolteria speciosa: Knight, 1968, p. 203

The types came from G. Alpine Creek, Tahoe, California (E. P. Van Duzee), taken on Juniperus. NEVADA: ♂♀ June 23, 1965, Nevada Test Site (H. H. Knight and Joe Merino), taken on Juniperus.

Bolteria juniperi Knight

Bolteria juniperi Knight, 1968, p. 202, fig. 252

Several specimens were collected on Juniperus osteosperma, which is the host plant. All specimens of this species were taken only on young trees, five to seven feet in height. NEVADA: ♂♀ June 23, Nevada Test Site (H. H. Knight and Joe Merino). ARIZONA: ♂ June 16, altitude 8000 ft., Grand Canyon. UTAH: 1♂, 6♀ June 29, 1965, Scipio (H. H. Knight).

Bolteria rubropallidus Knight

Dichroscytus speciosa var. rubropallidus Knight, 1918, p. 11

Bolteria rubropallidus Knight, 1928, p. 132

Described from NEW MEXICO: ♂♀ June 7-17, 1916, Jemez Spring,

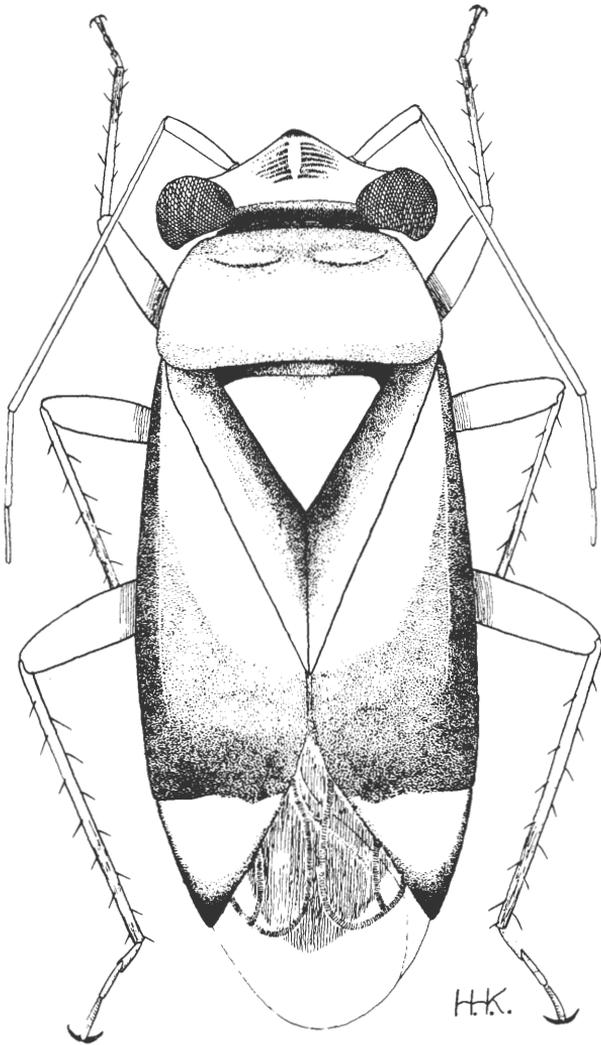


Figure 1. Bolteria juniperi, ♂.

Bolteria nicholi Knight

Bolteria nicholi Knight, 1928, p. 129.

Described from Williams, Arizona, June 18, 1925, where 10 ♂♀ were collected on Pinus edulis (A. A. Nichol). NEVADA: ♂♀ June 24, 1966, Pine County, Ely, alt. 6500 ft. (W. Gagné)

Bolteria luteifrons Knight

Bolteria luteifrons Knight, 1921, p. 73

This species was described from a single specimen, ♂ April 17, 1908, Raleigh, North Carolina (E. P. Van Duzee). The next specimens came from MAINE: ♂♀ July 8, 1938, Bar Harbor (A. E. Brower). MISSOURI: ♂♀ April, 1939, Roaring River State Park (R. C. Froeschner); ♂♀ May 24, 1942, Gray Summit (R. C. Froeschner). Next, the species was found in IOWA: ♂♀ June 3, 1953, Ames (H. H. Knight) where it was found breeding on Juniperus virginiana. Specimens were taken each year up to 1969, but it disappeared from the Juniper trees after that year. It has failed to show since and we believe the species was exterminated around Ames by the mosquito control program which used a DDT spray for a few years. ARKANSAS: ♂♀ May 13, 1963, Washington County; received for identification.

Bolteria balli Knight

Bolteria balli Knight, 1928, 130.

Described from a single specimen, ♀ June 23, 1913, Kanab, UTAH (E. D. Ball), a specimen received in an exchange many years ago.

Bolteria semipicta Blatchley

Bolteria semipicta Blatchley, 1926, p. 743

This species was described from Dunedin, Florida; 1♂ April 23, taken "at porch electric light." The very long first antennal segment distinguishes this species from all others, and may indicate another genus.

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- DROST, JIM L.
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- DURAND, BERNICE BLACK
A point and local position operator
High Energy Physics
- DUVEN, DENNIS JAMES
Convergence of the difference equation for the error covariance matrix arising in Kalman filter theory
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- EL-LAKANY, MOHAMED ALI
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- EL-TABLAWEY, TALAAT AHMED
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- ELTZE, ERVIN MARVIN
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- EMIOLA, LATEEF OYEBAMIJI
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- ENGARDT, RALPH DUANE
Nuclear magnetic resonance determination of the activation volume for self-diffusion in aluminum
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Some physical and physiological aspects of evapotranspiration from a soybean canopy
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- FIGHT, ROGER DEAN
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- FINCH, ROBERT BRUCE
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- FIORINO, JOHN A.
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- FISCHER, DARYL ROBERT
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- FISHER, TOM LYONS
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- FLASH, PATRICK JOHN
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- FLEMING, RICHARD WILLIAM
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- FODA, ESAM A.
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- FRENCH, HOWARD LINCOLN, JR.
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- FRIEDMAN, ROBERT MARK
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- FRIEDRICH, DOUGLAS DUANE
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- FUGATE, ROBERT Q.
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- GALLANT, A. RONALD
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- GANFIELD, DAVID JUDD
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- GANFIELD, MONG CHING WONG
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- GEADELMANN, JON LEE
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- GIROLO, JACK EMILE
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- GOOD, CARL MUNGER, III
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- GUINAN, JOSEPH FRANCIS
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A study of the Fermi surfaces of antiferromagnetic chromium-rich alloys using the de Haas-van Alphen effect
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- HACKERT, MARVIN LEROY
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- HAMILTON, DAVID ALEXANDER, JR.
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- HAMMOND, HOWARD RAY
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- HANDY, CHARLES B.
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- HANSEN, HERBERT EUGENE
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- HARTER, GARY DEAN
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- HASKELL, BRENT ALAN
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- HEIN, WARREN WALTER
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- HEINER, TERRY CHARLES
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- HENDERSON, DAVID COURTLAND
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- HEROLD, EDWARD STEPHEN
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- HEXEM, ROGER WAYNE
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- HILLS, THOMAS M.
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- HIRNING, HARVEY JAMES
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- HOCHHAUS, LARRY
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- HOLDEN, PALMER JOSEPH
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- HULETT, JOE RICHARD
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- HYBERTSON, RONALD LEE
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- ISAKI, CARY TSUGUO
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- JAMLANG, EVAMONICA MERCEDITA
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- JENSEN, LOUIS
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- JENSON, EARL ANGUS
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- JOHNSON, GARY DEAN
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- JOHNSTONE, JAMES KEITH
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- JOLLY, FRANK H.
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- JUNGK, ROBERT ARTHUR
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- KAMALU, THEODORE NKIRE
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- KAMINSKI, EDWARD EUGENE, JR.
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- KELLER, DAVID ANTHONY
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- KESKE, ROBERT GUSTAV
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- KOLLMAN, GERALD EUGENE
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- KNOWLTON, TED MERRILL
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- KRAMER, MAURICE STEPHEN
Relationship of marital status to selected characteristics and activities of male undergraduate students at Iowa State University
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- KROHN, HOWARD EMIL
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- LACASA-GOMAR, JAIME
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- LASSAHN, GORDON DENNIS
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- LAUER, GEORGE NICHOLAS, II
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- LAWSON, DAVID FRANCIS
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Organic Chemistry
- LAZO, EVARISTO
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- LEVY, LARRY JAMES
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- LINDVALL, RONALD NEAL
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- LOUGHNER, GEORGE EMMANUEL
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- LOUP, ROLAND JOSEPH
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- LOWE, REX LOREN
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- LURA, RICHARD DEAN
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- LYNDE, RICHARD ARTHUR
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- MAC EACHERN, ALEXANDER
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- MANNING, WILLIAM ROGER
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- MARANGU, JOHN PAUL
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- MARTIN, LESLIE LEON
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- MARTINEZ-GARZA, ANGEL
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- MASTERTSON, PAUL BRUCE
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- MAZE, ROBERT CRAIG
An ultrahigh vacuum study of wetting in liquid metal -- solid metal systems
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- MC ATEE, JOHN WAYNE
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- MC ATEE, JOYCE WOOD
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- MC CASLIN, NORVAL L.
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Preparation of uranium metal and dense UO₂ shapes by the carbon reduction of U₃O₈
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- MC DOWELL, TERENCE LEE
Mechanism of the photoconversion of 5,5-dimethylbicyclo (4.1.0)-hept-3-en-2-one to 2,3,5-trimethylphenol and 3,4,5-trimethylphenol
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- MC ELHONE, DONALD HUGHES
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- MC GEE, DUBOIS PHILLIP
Construct validation of a measure of alienation in Black adolescents
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- MC VEY, GARY C.
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- MELLON, DONALD WILLIAMS
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- MORAGHAN, BRIAN JOSEPH
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- MORRIS, FLOYD EVERETT
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- MYERS, RICHARD W.
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- NAITO, HERBERT KUNIO
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- NANDA, BHUPINDER SINGH
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- NEARHOOF, EDWARD ORRIN
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- NORD, DENNIS LYNN
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- NOURI, ESMAT MOUSTAFA
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- OBENG, HENRY BENJAMIN
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- O'BRIEN, PETER CHARLES
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- OLESON, GARY K.
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H⁺ and D⁻ impurities (U-centers) in
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- OWENS, JOHN CHARLES
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- PANLASIGUI, ROGELIO ANDRES
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- PANOUSIS, NICHOLAS THEODORE
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- PARSONS, GERALD E.
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- PATZWALD, JOHN B.
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Even-tempered atomic orbitals in quantum chemical ab initio calculations: Light atoms, heavy atoms, triatomic alkali ions
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An experimental and theoretical evaluation of the nitrous oxide-acetylene flame as an atomization cell for flame spectroscopy
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- REIF, LICENCIADO, ISAAC
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- RHOADES, KEITH RAY
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- RICHEY, JACK ATHA
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- RICHARDS, KENNETH EDGAR, JR.
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- ROBINSON, WILLIAM H.
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A proposal for improving the U.S. Department of Agriculture's cost and return studies
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- ROST, DUANE FOSTER
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- ROST, THOMAS LOWELL
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- RUDOLPH, FREDERICK BYRON
Purification, properties, and kinetics of adenylosuccinate synthetase from Escherichia coli
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- RULF, DONALD CLARENCE
Heat capacities of four rare earth trichloride hexahydrates from 5 to 300°K
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- SAEGROVE, MARCUS JOHN
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- SALMON, LYDIA SUSAN
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- SALMON, WILLIAM IRWIN
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- SANFORD, JEANNE JOEHLMANN
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- SAWYERS, BETTY A.
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- SCHMITT, DONALD PETER
Vertical distribution of Xiphinema americanum in minimal and medial developed loess soil in southwest Iowa
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The preparation and characterization of some alkanethiolatoosmium compounds
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The effect of soil moisture and day temperature on photosynthesis, growth and needle anatomy of Scotch pine seedlings
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The development of encoding processes in memory
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Strength and behavior of cold-rolled steel-deck-reinforced concrete floor slabs
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Computer simulation of pattern recognition using statistical decision functions
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- SEAY, EDMOND EGGLESTON, JR.
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Transient and steady two-dimensional flow of water in unsaturated soils
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- SHEN, I-MING
Acceleration of the projection method for solving systems of linear equations
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Ecological effects on F_1 progenies from reciprocal matings of three biotypes of the European corn borer, Ostrinia nubilalis (Hübner)
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Gas chromatography of mixed-ligand complexes of the lanthanides and related elements
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The common law of schools in Iowa
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- SLABAUGH, MICHAEL RAY
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- SMITH, DAVID WILLIAM
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- SMITH, FRED BAXTER, JR.
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- STACY, DENZIL WAYNE
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- STAMP, DAVID LEE
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- STRACHAN, DENIS MICHAEL
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- STOCK, WILLIAM ALBERT
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- STOECKER, BARBARA SYMNS
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- STONE, RANDOLPH
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- STRISSEL, JERRY FRED
Bacteria-free soybean plants
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- TANG, VICTOR KUANG-TAO
Allocation in stratified sampling based on preliminary tests of significance
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- THAXTON, CHARLES BURTON
A technique for cooling single crystals below 90°K for X-ray diffraction, and the crystal structures of $H_2T_{a6}Cl_{18}H_{20}$ and the photo dimer of 1-1-dimethyl-2, 5-diphenyl-1-silacyclopentadiene
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- TONNIES, JAMES JOHN
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- TREADWELL, GEORGE EDWARD, JR.
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- TRENN, HUGH LESLIE
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- TU, CHANG-CHU LIN
Biosynthesis of a benzoxazinone in maize
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- TUCKER, ROBERT HENRY
A quantum electrodynamics for vector mesons
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- TURNER, CHARLES EDWARD
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- TURPIN, FRANK THOMAS
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- VAUGHT, RUSSELL SCOTT
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- VILLEGAS, CESAR TOLENTINO
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- VINT, LARRY FRANCIS
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- VOSS, DAVID ALBERT
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- WAMPLER, GENE LEROY
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- WANGSNES, PAUL JEROME
Evaluation of a direct method for measuring absorption from the gut
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- WARD, RONALD W.
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