FACTORS AFFECTING THE HYDROCYANIC ACID CONTENT OF WILD WHITE CLOVER.

Trifolium repens L.,

Thesis for the Degree of M. S.

MICHIGAN STATE COLLEGE

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THESIS

C O N T E N T S

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INTRODUCTION

It has been well established that a large number of plant species contain quantities of hydrocyanic acid which apparently is in the form of some non-toxic compound of cyanide, since it is well known that free cyanide is strongly toxic to plants. This compound may be one of several glucosides which under certain conditions, such as the crushing of the plant, break down under the action of associated enzymes into hydrocyanie acid and other products.

In the white clover breeding program carried on by the Division of Forage Plants, Central Experimental Farm, Ottawa, material collected from the vicinity of Nappan, N.S., where the species is thought to be indigenous or highly adapted, has been used extensively and has proved to be so promising that it has been studied from several angles. One of these lines of approach was to study some of the factors which affect the variability of the hydrocyanic acid content of this wild white clover and to consider the importance that the presence or absence of hydrocyanic acid content might have in the breeding program. The results of this study are presented in this paper and, while no very definite conclusions can be drawn from these results, they may be of value to the white clover breeder.

REVIEW OF LITERATURE

The review of literature set forth herein includes some of the articles on factors affecting the HCN content in grasses and wild white clover.

As early as 1906 Dunstan and Henry (8) stated that HCN was found in more than 100 different plants belonging to 22 different natural orders. In the same year Greshoff (12) published a very complete list of plants in which cyanogenetic glucosides were traced, but unfortunately little information is available in his papers concerning the amounts of the glucosides present in the plants. Except in the case of sorghum, Sudan, and Johnson grasses, there are very little data on the amounts of HCN in plants.

HCN Content of Various Crops

Numerous workers have used various quantitative methods in calculating the HCN content of different forage crops and they have expressed their results in different ways. For the purpose of comparing the results of these workers, their findings have been converted to parts per million (p.p.m.) on the green basis, as shown in Text Table I.

The data in Text Table I indicate that there is a wide variation between crops (compare the HCN content of Johnson grass and brown top). There is also a wide variation shown within crops (compare the different amounts of HCN found in sorghum or in wild white clover).

Text Table I Comparative Concentration of HCN in Various Crops (expressed in p.p.m. on the green basis)

Author	Crop	p.p.m.	Remarks
Dowell (7)	Johnson grass	5740	Maximum obtained
Franzke et al (11)	sorghum	2540	do (3 year average)
Willaman & West (38)	do	1140	Maximum obtained
Franzke et al (11)	do	410	do
do (11)	Sudan grass	420	As check plant, not min. obtained
Swanson (35)	do	150	Maximum obtained
Mirande (19)	wild w. clover	36-390	
Doak (6)	do	10-130	
Sullivan (34)	do	10-320	Greennouse test
do (34)	do	500	Very high content (one plant only)
Rigg et al (29)	do	16-124	Eleven samples from different countries
Finn	do	28	
do (29)	alfalfa	15	
do (29)	red clover	3	
do (29)	alsike	3	
do (29)	fescues	2	
do (29)	brown top	1	

* p.p.m. on the Green Basis	Relative Degree of Toxicity
0 - 50	Very low (safe to pasture)
50 - 100	Low (safe to pasture)
100 - 150	Medium (doubtful)
150 - 200	High (dangerous to pasture)
>200	Very high (very dangerous to pasture)

(After Boyd et al (3))

* Assuming that dry weight of Sudan grass is 20%

<u>Toxicity of Cyanide</u>

Leeman (14) indicates that various species of animals react differently when fed plants containing cyanophoric glucosides. These differences are caused by different anatomical structures and different detoxifying abilities of various animals. Cattle and sheep are ruminants and both are known to be subject to poisoning by cyanophoric glucosides. The paunch, or rumen, of these animals is neither strongly acid nor alkaline in reaction and contains a large flora of micro-organisms and considerable quantities of the enzyme emulsion which provide an excellent medium for the liberation of the toxic agent. On the other hand, horses and hogs, being non-ruminants, have only one stomach which is strongly acid due to the presence of HCL which inhibits the release of HCN.

<u>Lethal Dose</u>

There are comparatively few experiments in grasses which have been properly conducted with a view to estimating their toxicity. Hindmarsh (13) found, in administering Scheele's acid (HCN), that the lethal dose per 1 pound body weight of sheep or cattle was 1 mg. Seddon and King (33) confirmed Hindmarsh's determination of 1 mg. per pound body weight for sheep. Petrie (23) reported on an experiment made with <u>Cynodon incompletus</u> (a grass indigenous to Australia). This grass containing 160 p.p.m. of prussic acid was fed to sheep. It was estimated that the lethal dose per sheep of 150 pounds was two pounds of grass, capable of liberating 0.14 grams of prussic acid. This confirmed again the figure established by Hindmarsh (13) as, roughly, 1 mg. per pound body weight.

Leeman (14) points out that the important question is not how much HCN is introduced but how much of it can be liberated. One way of solving the question would be to determine the prussic acid content, first before it was fed, and then after the death of the animal. The grass found in the paunch could be retested. Another point to be considered is that of elimination from the animal body. Prussic acid is highly diffusible and will readily reach the blood stream, but it will by this same property be eliminated very quickly. The difference between the quantity of HCN eliminated and that contained in the ingested material will determine, to a large degree, the toxicity of the dose. The condition of the animal prior to eating the toxic plant must be taken into account as certain factors, such as hunger, overstrain, bad health, and thirst, exert considerable influence on the biological reactions. An animal that is in poor health or extremely humgry has been found to be more susceptible to the poison.

There have been occasional references where cattle were poisoned by substances other than HCN. As early as 1897 Pease (22) showed that mortality of cattle in India from Johnson grass could be imputed to nitrate poisoning. He was able to detect 20 per cent of potassium nitrate in stems of the grass. A very recent case occurred at South

Dakota State College, when Dr. Lipp, Staff Veterinarian, was called out to diagnose the case of 20 young cattle that had died after eating Sudan grass which had been stored in a stack. On analysis the Sudan forage was found to contain no appreciable quantity of HCN but tests revealed that it contained 8.47 per cent of nitrates.

HCN Content in White Clovers

Mirande (19) and Armstrong et al (1) observed that individual plants of white clover fall into two physiological groups : one in which the leaves produce minute quantities of prussic acid when they are killed, and the other in which this phenomenon does not occur. Armstrong et al (1) drew attention to the fact that, in their experience, the wild plant wherever tested always contained cyanide and that they had not succeeded in finding cyanide in white clover raised from "cultivated" seed at any stage of growth. From this it appears possible that the property of cyanogenesis might in some way be connected directly with the wild state and that its absence might be correlated with the cultivated condition of the plant. The results obtained by Pethybridge (25) showed however that, *inter alia*, seedlings from some samples of commercial "cultivated" seed gave a positive reaction when tested for HCN. He found that in testing 126 samples of commercial white clover 109 gave negative results and only 17 positive reactions. But of these 17 samples which were cyanophoric 15 were of United States and

-2 of Canadian origin. He also conducted numerous experiments with commercial English white clover, and it must be said that all his tests with these seedlings were invariably negative.

Factors Affecting the HCN Content

The literature on environmental factors is very extensive, including many conflicting statements and theories. Some of the various conditions which affect the prussic acid content in different crops are stages of maturity, rainfall, drought, frost, insect damage, moulds or fungi, fertilizers, temperature, and humidity. In this review of literature only a few of the factors which affect the HCN content in crops will be discussed.

Climate and Soil:- Mirande (19) found as early as 1912 that the character of the soil influenced the HCN content in wild white clover.

In their study of sorghum, Willaman and West (39) concluded that climate was more influential than soil on the prussic acid content. They reported that an adequate water supply is usually accompanied by a low content and an inadequate supply by a high amount of prussic acid.

Franzke et al (11) demonstrated that increases in soil moisture showed increased growth and gradually decreased HCN in sorghum, as shown in Text Table II.

Text Table II HCN Content in Two Strains of Sorghum Grown in Greenhouse Cultures with Varying Moisture Contents (1938)

Moisture per cent	Low HCN Strain		High HCN Strain	
	Ave. Weekly Growth Inches	HCN p.p.m.	Ave. Weekly Growth Inches	HCN p.p.m.
15	1.417	880	1.417	3920
20	1.500	750	2.458	2120
25	2.583	510	2.417	2270
30	3.125	590	2.917	1570
35	3.167	380	3.125	1410

From the foregoing it appears that the amount of HCN decreased with the same regularity as the weekly growth in inches increased together with the regular increase of water added to the soil.

In studies of the same plant, Peters et al (24) stated that growth arrested by drought presented a very favourable condition for the elaboration of the poison, which observation was also confirmed by the findings of Francis (10). Vinall (36), after a critical survey of literature, concluded that injury to growing sorghum plants by drought increased the HCN content, but that stunted growth from lack of plant food in the soil diminished it. On the contrary, Rogers and Boyd (30) have shown in their investigations of Sudan grass that plants exposed to drought conditions contained less HCN than plants grown under normal conditions.

Effect of Fertilizers:- Several workers have studied the effect of nitrogen on the HCN content of sorghums. Maxwell (16) pointed out that soil rich in nitrogenous constitutents of plant food attended higher prussic acid. In the same year Brünnick (4) concluded that soils, to which sodium nitrate had been added, produced plants which contained slightly more HCN than the unfertilized plants. The same worker also applied sodium nitrate to millet and found that it behaved similarly.

Willaman and West (39) discovered that nitrogen added to a poor soil with abundant nitrogen will not show any effect after fertilization. On the other hand, Manges (15) of Kansas in 1936 stated: "Plants grown on fertilized soils, especially soils which have been fertilized with nitrates, contain less HCN than those grown on poor soils", indicating disagreement with previous workers.

Boyd et al (3), in their recent work on Sudan grass, observed that a high level of available nitrogen and a low level of available phosphorus in the soil tend to increase the poison content, while a low level of available nitrogen and a high level of available phosphorus have the opposite effect. They reported also that potash had no influence on the HCN content.

In the following year, Franzke and co-workers (11) made a similar study of fertilizer tests on the HCN content of sorghums. The kind of fertilizers regularly applied and the amount of HCN found in the samples from the four strains studied are given Text Table III.

Text Table III HCN Content (p.p.m.) in Plants of Four Selfed Strains Grown on Complete Fertilizer Plots in Brookings (1937)

Strain	None	N=350	P=200	K=200	N=350 P=200	N=350 K=200	P=200 K=200	N=350 P=200 K=200
1-30-s	3610	3860	4130	2720	4660	3380	2770	4430
15-30-s	5200	6220	5870	3810	6740	5890	3660	5580
18-30-s	4900	--	4110	3960	5690	4970	4310	5400
39-30-s	2230	2980	2030	1960	2090	2930	1380	2320
Average	3985	4353	4035	3113	4795	4293	3030	4433
Height	13.9	10.7	12.5	16.0	14.8	11.6	15.5	13.6

From the above table, the average amount of HCN was invariably higher in plants where nitrogen was regularly applied than where nothing was applied. It was likewise true that the HCN content was higher in all cases where nitrogen was used with superphosphate than where superphosphate was used alone. Moreover, the corresponding amounts of HCN were higher where nitrogen was added to both phosphorus and potassium, than where the latter were applied together without nitrogen.

From the foregoing it would appear that, for some reason, the regular use of nitrogen in the cropping system was correlated with an increase in the amount of prussic acid.

The HCN content of plants on potassium plots was invariably lower than that of unfertilized specimens.

Leeman (14) summarized the influence of soil on the HCN content as follows: "Although it is thus demonstrated that the soil has a marked effect on the prussic acid production, which is interesting from an academic point of view, from a practical point of view the increase is not such as to warrant any further investigations in that direction."

Diurnal and Seasonal Variations:- The production of prussic acid due to diurnal variations has frequently been reported. Ravenna (28) observed that there was an increase in HCN in sorghum from early morning to afternoon. Willaman and West (39) in studies of the same plant also noticed a gradual increase from morning to mid-day, at which time the HCN content was at its maximum.

In the case of *Sorghum vulgare*, Narasimha Acharya (20) found that the HCN increased from early morning to about 2 p.m., after which time there was a slight decline until 6 p.m., followed by a rapid decline at night. He was of the opinion that there was a correlation between HCN and photo-synthesis.

Marias and Rimington (17), in their study of *Dimorphotheca cuneata*, Less., found that the prussic acid increased from early morning until noon, whicn they thought "suggests a correlation with intense

photosynthetic activity".

The more recent work of Boyd et al (3) showed definite diurnal variations in the cyanide content of Sudan grass and sorghum. Samples were taken for analysis at 8.00 a.m., 1.00 p.m., and 7.00 p.m. In the case of Sudan grass, the HCN content at 1.00 p.m. was about 30% higher than the samples taken in the morning and evening. In the case of sorghums, the cyanide content at 1.00 p.m. was also considerably higher than in the morning and evening. It is to be noted that these results are in accordance with those reported by Narasimha Acharya (20). The data obtained in this experiment were subjected to statistical analysis, and the differences between the cyanide content at noon, morning and evening were found to be significant. In summarizing the diurnal effect, it may be concluded that the foregoing workers were in fairly close agreement.

In addition to diurnal changes, there is a pronounced seasonal variation in the HCN content of different crops. In 1933, Askew (2) in his studies of wild white clover discovered a seasonal variation. Again in 1937 Rogers and Frykolm (31) noted that the percentage of white clover plants containing no HCN decreased from 71.28 to 57.60 with the advance of season.

Ramsay and Henry (27), in their study of whitewood (<u>Heterodendron oleaefolium</u>) and native fuschsia (<u>Eremphila maculata</u>), reported that the HCN content reached a maximum during late summer.

<u>Influence of Frost</u>:- Frost, according to Peters, Slade, and Avery (24), did not influence the HCN content of sorghum except as a forerunner of a period of bright dry weather. In such a case, the bright warm weather was conducive to the growth of new tillers which are always higher in HCN content than the more mature parts of the plant. Manges (15) for the same reason, in the study of Sudan grass, stated that the HCN content increases after the first frost. In experimenting with the same crop Rogers and Boyd (30) observed an increase after frost, but they did not record any deaths imputable to this factor. Vinall (36) found that the prussic acid content of sorghum increased after frost, whereas Francis (10) stated merely that frosted sorghum plants were unsafe to pasture. The more recent work of Boyd et al (3) showed that in the case of Sudan grass the HCN content was no higher after frost than before it.

<u>Different Stages of Growth Affect the HCN Content</u>:- Narasimha Acharya (20) pointed out that in a normal crop of <u>Sorghum vulgare</u> the prussic acid decreased from the early stages progressively to the flowering stage. Previous workers, Peters et al (24), Willaman and West (39), Manaul and Dowell (18), also found a decrease from early to later stages of growth. Dunstan and Henry (8) observed that the stage at which maximum HCN prevailed varied considerably with different plants.

Williams (40) found that the HCN content of wild white clover decreased rapidly with the age of the leaves as shown in Text Table IV.

Text Table IV HCN Content of Wild White Clover Leaves at Different Ages

Plant No.	Young folded leaves	Few days old	Few weeks old
1	Very strong	Medium	Very weak
2	Strong	Medium	Weak
3	Very strong	Very weak	None
4	Very strong	Strong	Weak

Inheritance of Cyanogenesis

The recent work of Williams (40) relating to the genetics of cyanogenesis in white clover (*Trifolium repens*) showed that the extreme variations in the HCN content of young leaves of different plants were due less to differences in environmental condition than to some intrinsic property of the individual. The cyanogenetic reactions of the plants were tested by Guignard's picrate paper method described by Armstrong et al (1).

Crosses made between homozygous HCN plants and homozygous free HCN plants gave evidence of dominance of positive plants to negative plants. Segregation for cyanogenesis in backcrosses resulted in a 1 : 1 ratio expected on the basis of simple Mendelian segregation. The segregating generations resulting from heterozygous crosses showed a

3 : 1 ratio of positive HCN plants to negative HCN plants.

Sullivan and co-workers (34) conducted similar experiments on the inheritance of cyanogenesis in wild white clover. These American workers confirmed the findings of Williams (40), namely, that cyanophoric plants were dominant to acyanophoric plants.

Recently Franske et al (11) investigated the inheritance of prussic acid in sorghum. They reported that acyanophoric plants appeared to be partially dominant to cyanophoric plants.

According to Coleman and Robertson (5) of Colorado State College, the differential ability of inbred lines of Sudan grass to produce HCN may be inherited. In their tests, high HCN production appeared to be more closely associated with non-glossy leaves than with glossy leaves. Similarly, high HCN content was concomitant with purple-tinged seedling leaves, but to a lesser degree.

STUDIES ON THE INFLUENCE OF CERTAIN FACTORS ON THE HCN CONTENT OF WHITE CLOVER

The Influence of Soil Moisture on the HCN Content of White Clover (Experiment 1)

Experimental Procedure

This experiment was set up in the greenhouse during the summer of 1939 in an effort to ascertain the effect of the quantity of soil moisture on the HCN content of white clover.

Material used:- After a preliminary test of a fairly large number of plants, three groups of 5 plants each were selected on the basis of their HCN content. One group was classified as having high HCN content, another medium HCN content and the other low HCN content. Each individual of the three groups was then divided vegetatively into nine separate units. These were started in 3-inch clay pots containing good loam soil which was thoroughly mixed to ensure uniformity of growth. This made it possible to have available, for each of the three treatments used, 3 units of each of the five plants in all three groups.

Treatments:- The treatments meant that a certain percentage of soil moisture had to be maintained in the pots, as follows:

Treatment 1	- 34	per	cent	soil	moisture
2	- 22	"	"	"	"
3	- 15	"	"	"	"

This was accomplished by weighing the pots daily and bringing them up, by watering, to the predetermined weight required for such a percentage moisture.

<u>Method of Testing</u>:- A review of literature shows that numerous quantitative methods have been used in estimating the HCN content of various crops. These methods require a considerable amount of time and the use of fairly large quantities of material. Since numerous individual units of wild white clover had to be studied in this experiment, a rapid method adaptable to small quantities was required.

The method used is that of Nowosad and MacVicar (21) which is an adaptation of the picric-acid method previously used by such workers as Pethybridge (25), Sampson (32), Foy and Hyde (9) in studying the HCN content of white clover.

This method is based on the evolution of hydrocyanic acid and the reaction of this acid when it comes in contact with filter paper treated with an alkaline picrate solution. The details of the preparation of filter paper and standards for colorimetric readings were carried out as outlined by Nowosad and MacVicar (21). By using a Duboscq colorimeter, exact quantitative readings were made which were later calculated and expressed in Mg. HCN per c.c. of solution.

In sampling, two samples of five compound leaves were taken from each unit, from which the average HCN content was calculated. The sizes of the leaves were found to vary slightly under different treatments. To offset any error due to a difference in leaf size, precaution was taken to sample only those leaves which were of average size. Young folded leaves were avoided in sampling as they have been found by Williams (40) to be higher in HCN content than mature leaves. Diseased and injured leaves were also avoided. All samples were made at approximately the same time of day (early afternoon). This precaution was taken because of the possibility of diurnal influence as mentioned in (28), (39), (20), (17), (3), (2), (31). Sampling of all units was carried out at 10-day intervals over a period of 70 days.

For the purpose of comparing the HCN content of wild white clover with the findings of other workers, the quantitative method of Boyd et al (3) was used to test one plant of moderate HCN content. It was found to contain 28 p.p.m. of HCN on the green basis (see Text Table I).

<u>Results</u>:- While, as was expected, considerable variation was found when the data were analyzed, it was possible to subject the findings to statistical analysis and to draw conclusions on the basis of such analyses.

The following tables indicate the influence of the soil moisture treatments on the HCN content of each of the three plant groups under study.

Text Table V   Analysis of Variance for Treatments of Plants "High" in HCN Content at the End of 40 Days. (For complete data see Appendix Table I.)

Due to	D.F.	M.S.	F Value	F Value for	
				5 p.c.	1 p.c.
Treatments	2	.00123060	49.98	3.26	5.25
Plants	4	.00003080	1.23	2.63	3.89
Replicates	2	.00001560			
Error	36	.00002460			

Treatment		Means Mg. HCN/c.c.
No.	Moisture Level	
1	34 p.c. soil moisture	.0449
2	22 p.c. soil moisture	.0386
3	15 p.c. soil moisture	.0270
Necessary difference for significance		
	(P = .05)	.0035
	(P = .01)	.0047

For (P=.05) treatment 1 is significant to treatments 2 and 3,
and " 2 " " " treatment 3.

For (P=.01) treatment 1 is highly significant to treatments 2
and 3, and " 2 " " " " treatment 3.

It will be observed that the "high" HCN plants reacted significantly to the different moisture levels. Treatment No. 1, in which the moisture level was maintained at 34 per cent, produced a mean quantity of .0449 mg.

per c.c., which was significantly higher than the .0386 mg. of treatment 2 (22% moisture) and the .0270 mg. of treatment 3 (15% moisture). Furthermore, treatment 2 gave significant results over treatment 3.

Text Table VI Analysis of Variance for Treatments of Plants "Medium" in HCN Content at the End of 40 Days. (For complete date see Appendix Table II.)

Due to	D.F.	M.S.	F Value	F Value for 5 p.c.	F Value for 1 p.c.
Treatments	2	.000125	125.00	3.26	5.25
Plants	4	.000678	678.00	2.63	3.89
Replicates	2	.000004	4.00	3.26	5.25
Error	36	.000001			

Treatment No.	Treatment Moisture Level	Means Mg. HCN/c.c.
1	34 p.c. soil moisture	.0299
2	22 p.c. " "	.0277
3	15 p.c. " "	.0241
Necessary difference for significance (P = .05)		.0007
(P = .01)		.0010

For (P = .05) treatment 1 is significant to treatments 2 and 3, and " 2 " " " treatment 3.

For (P = .01) treatment 1 is highly significant to treatments 2 and 3, and " 2 " " " " treatment 3.

A perusal of this table shows the reaction of the group "medium" HCN to the moisture levels, indicating results very similar to those obtained with the "high" group. While the mean differences are much smaller, ranging from .0299 mg. for treatment 1 to .0241 mg. for treatment 3, the necessary difference for significance is also much lower.

Text Table VII Analysis of Variance for Treatments of Plants "low" in HCN Content at the End of 40 Days. (For complete date see Appendix Table III.)

Due to	D.F.	M.S.	F Value	F Value for 5 p.c.	F Value for 1 p.c.
Treatments	2	.00004808	6.74	3.26	5.25
Plants	4	.00057125	80.12	2.63	3.89
Replicates	2	.00000109			
Error	36	.00000713			

Treatment No.	Treatment Moisture Level	Means Mg. HCN/c.c.
1	34 p.c. soil moisture	.0163
2	22 p.c. " "	.0158
3	15 p.c. " "	.0130
Necessary difference for significance (P = .05)		.0019
(P = .01)		.0025

For (P = .05) treatments 1 and 2 are significant to treatment 3.
For (P = .01) treatments 1 " 2 " highly significant to treatment 3.

While the reaction of the "low" HCN group was along the same lines as that of the "high" and "medium" groups with HCN content ranging from .0163 mg. to .0130 mg., it will be observed that treatment 1 did not show significance over treatment 2. It is probable that the "low" HCN content of this group accounted for variations so small that they could not be detected.

Text Table VIII Analysis of Variance for Soil Moisture Treatments Applied to Plants of three HCN Levels, i.e. "high", "medium", and "low" HCN Content. (For complete data see Appendix Table IV.)

Due to	D.F.	M.S.	F Value	F Value for	
				5 p.c.	1 p.c.
Treatments	2	.00093902	192.42	3.13	4.92
HCN Content	2	.00538142	1102.75	3.13	4.92
Replicates	18	.00000724	1.48	1.75	2.21
Plants	12	.00042687	87.47	1.89	2.45
Treatments x Plants	24	.00003092	6.34	1.67	2.07
Treatments x HCN Content	4	.00023271	47.69	2.50	3.60
Error	72	.00000488			

(Continued)

Treatment		Means
No.	Moisture Level	Mg. HCN/c.c.
1	34 p.c. soil moisture	.0304
2	22 p.c. " "	.0273
3	15 p.c. " "	.0214
Necessary difference for significance		
	(P = .05)	.0009
	(P = .01)	.0012

For (P = .05) treatment 1 is significant to treatments 2 and 3, and " 2 " " " treatment 3.

For (P = .01) treatment 1 is highly significant to treatments 2 and 3, and " 2 " " " " treatment 3.

A combined analysis of tne data for the three groups snowed that treatment 1 with .0304 mg. HCN was significantly higher than treatments2 and 3 with .0273 mg. and .0241 mg. respectively. Treatment 2 was also significantly higher than treatment 3.

The data obtained have been plotted graphically in Fig. 1. This graph shows the HCN content over a 70-day period for each of tne three treatments.

Discussion:- The obvious conclusion that must be drawn from the results of this experiment is that an adequate supply of soil moisture is followed by a high concentration of HCN, while a restricted moisture supply is associated with a

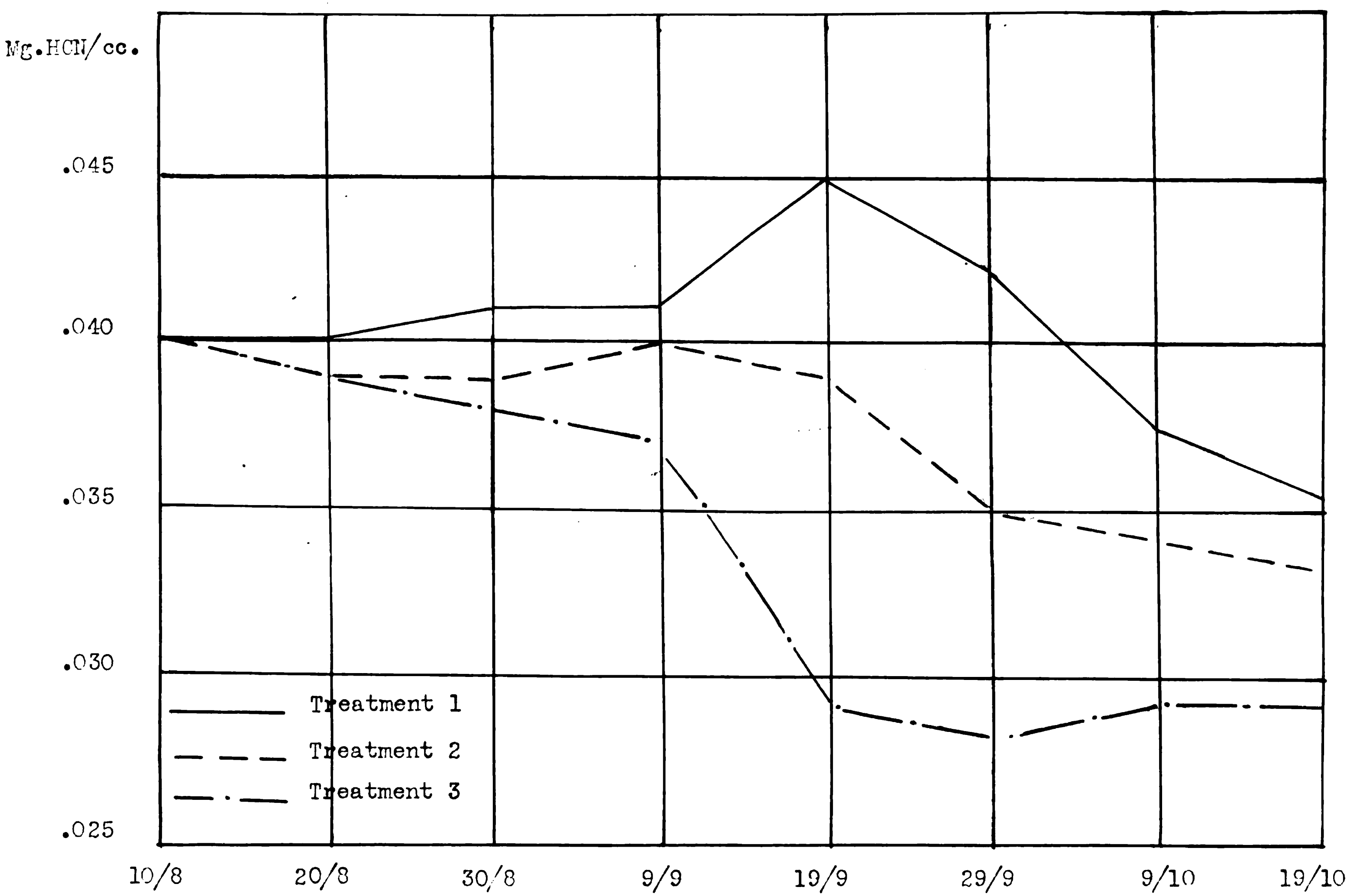

Fig. 1. The influence of three soil moisture treatments on the HCN content over a 70-day test.

(Each treatment is represented by the mean of 5 plants, replicated three times and tested in duplicate.)

lower concentration. Rogers and Boyd (30) report similar findings in their study of Sudan grass when they observed that plants exposed to drought conditions contained less HCN than those grown under normal conditions. On the other hand, Willaman and West (39), while working with sorghum, concluded than an adequate supply of soil moisture is usually accompanied by a low concentration of HCN and that an inadequate supply of soil moisture is usually accompanied by a high concentration of HCN. Likewise, in studies of the same crop, Franske et al (11) demonstrated that increases in soil moisture showed increased growth attended with a reduction in the HCN content.

The Influence of Season on the HCN Content of White Clover (Experiment 2)

Experimental Procedure

The area selected for this experiment is located on the Central Experimental Farm, Ottawa. The soil is a good clay loam, well drained and reasonably uniform.

Material used:- Plants grown in 3-inch clay pots from selected Nappan wild white clover seed and which were transplanted to the field in early June were used in this experiment.

Method of Testing:- The method used in this experiment was the same as was delineated in experiment 1 except that the samples for HCN tests were taken every 10 days on 96 plants over a period of 90 days, viz., July 10th to October 8th.

Climate:- The data contained in Text Table IX were furnished by the Dominion of Canada Meteorological Service from the station at Ottawa. In addition to reporting the weather conditions for the period that the plants were tested, figures are also included for the month of June, as these had a direct bearing on soil conditions at the time of the experiment.

Text Table IX Meteorological Data

1939	Mean Temperature	Average Maximum Temp.	Average Minimum Temp.	Total Precipitation	Total hours of bright sunshine
	oF	oF	oF	Inches	Hours
June	63.5	74.5	52.6	3.61	241.6
July	67.9	79.3	56.6	6.32	294.3
August	68.5	79.8	57.3	3.24	303.5
September	56.1	66.3	45.9	2.89	176.4
October	44.0	53.1	34.9	3.02	112.8

Results:- The following table indicates the variation in the HCN content due to climatic conditions of plants tested in the field.

Text Table X Average HCN Content of 42 Plants Tested at 10-Day Intervals, over a Period of 90 Days - July 10 to October 8, 1939.
(For complete data see Appendix Table V.)

Dates tested	Mg. HCN/c.c.
July 10	.024
July 20	.024
July 30	.028
August 9	.037
August 19	.036
August 29	.034
September 8	.031
September 18	.029
September 28	.028
October 8	.027
Necessary difference for significance (P = .05)	.005

N.B. Actually 96 plants were tested of which 54 gave negative HCN readings throughout the test.

Using the calculated necessary difference for significance (P =.05), the HCN readings taken on August 9, August 19, August 29, and September 8, were significant to all other dates tested.

The foregoing is also expressed graphically (see Fig. 2). The graph shows that there was a slight increase in the HCN content throughout the month of July up until August 9 when a seasonal maximum was reached, and that from then on the HCN content gradually decreased until the end of the experiment (October 8).

<u>Discussion</u>:- Numerous workers have discovered a pronounced seasonal variation in HCN content of different crops. In the case of wild white clover, Askew (2) merely stated that there was a marked seasonal variation. Rogers and Frykolm (31), while working with the same crop, showed an increase in the cyanogenetic power of plants with an increase in the size of plants and with progress of season. The same workers observed that the percentage of plants reacting negatively decreased from 71.28 per cent to 57.60 per cent with the advance of season, and they did not mention any particular time during the season when a maximum HCN level was reached.

As mentioned above the highest HCN content was registered on August 9. This was likely due to an abnormally heavy precipitation of 5.60 inches during the last four days of July when the average HCN level for the 42 plants tested was .028 mg. HCN per c.c. because approximately 10 days later the level for the same 42 plants was found to be .037 mg. HCN per c.c. During no other period under test was a similarly rapid rise detected. If the heavy precipitation influenced the rapid rise in HCN content, there is agreement with the results of experiment 1, when under greenhouse

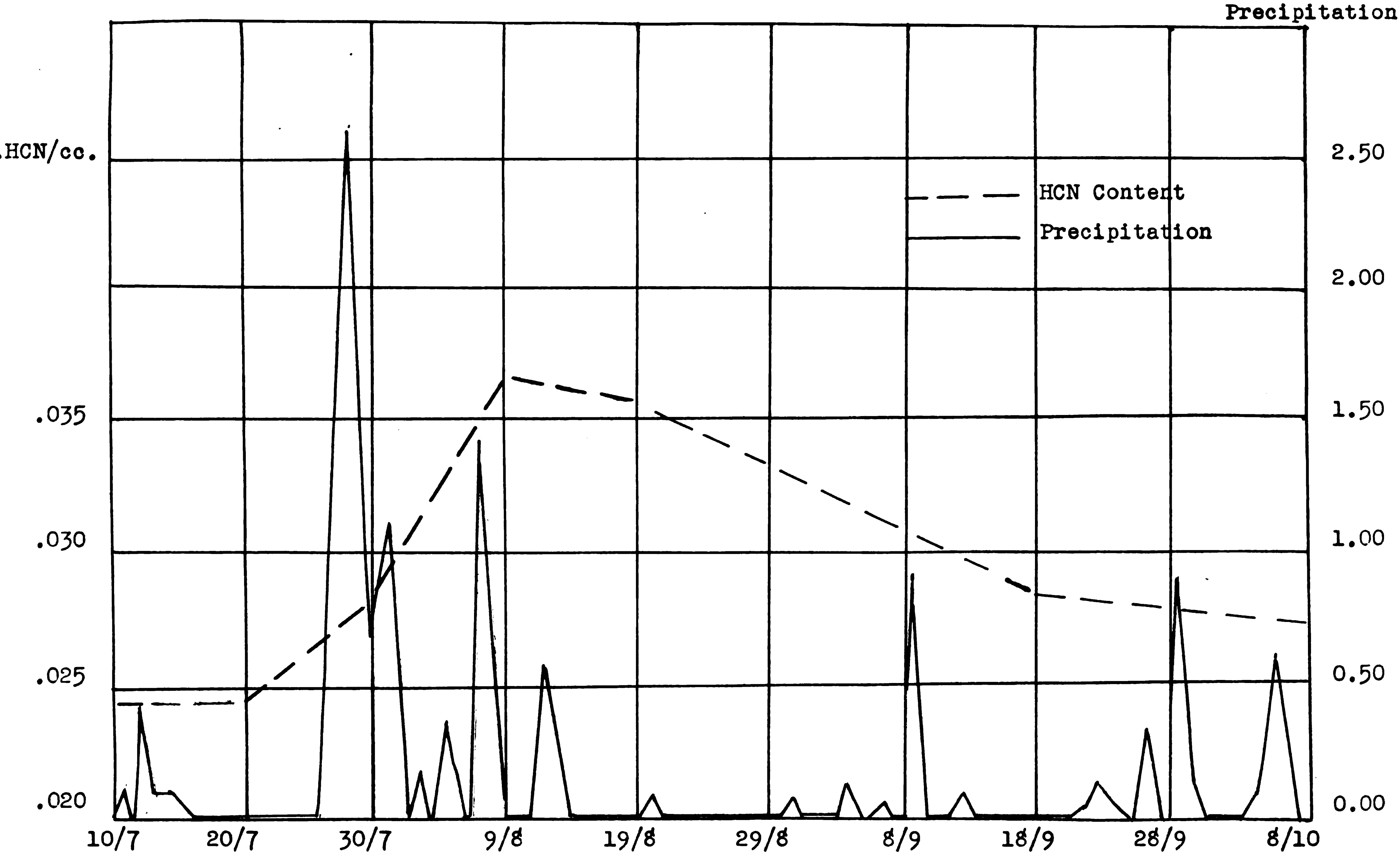

Fig. 2. Average HCN content of 96 plants over a 90-day test and daily precipitation in inches.

conditions adequate soil moisture caused an increase in HCN content.

It is interesting to observe that the results obtained in this experiment do not support those of Rogers and Frykolm (31) in that, in spite of seasonal development and increase in plant size, the HCN content of September 28 and October 8 was not significantly higher than that of July 10, the time of the first test.

The Influence of Temperature on the HCN Content of White Clover (Experiment 3)

Experimental Procedure

This experiment was conducted in the greenhouse with a view to ascertaining the influence of temperature on the HCN content of white clover.

Material used:- Three plants of varying HCN content were chosen for this experiment. Each of the three plants was then divided vegetatively into nine separate units, and the procedure outlined in Experiment 1 was used. These plants were started in 3-inch clay pots containing good clay loam which had been thoroughly mixed to ensure uniformity.

Treatments:- The population of 27 plants was divided into three representative groups and each group subjected to a different temperature. Temperatures were recorded twice daily at 7.00 a.m. and 5 p.m. for both soil and atmosphere. Text table XI summarizes the temperature data for a 55 day period.

Text Table XI Summary of Temperature Data during 55 day Test.

Treatment	Greenhouse Temperature (Average for 55 days)			Soil Temperature (Average for 55 days)		
	7 a.m.	5 p.m.	Mean	7 a.m.	5 p.m.	Mean
1	65° F	68° F	66.5° F	59° F	62° F	60.5° F
2	58° F	60° F	59.0° F	55° F	57° F	56.0° F
3	55° F	57° F	56.0° F	53° F	55° F	54.0° F

Method of Testing:- The method for testing in this experiment was similar to that used in Experiment 1.

<u>Results</u>:- The following table indicates the influence of both greenhouse and soil temperatures on the HCN content of tne plants tested. The data analyzed statistically are presented below.

Text Table XII Analysis of Variance for Temperature Treatments of plants at the End of 55 Days. (For complete date see Appendix Table VI.)

Due to	D.F.	M.S.	F Value	F Value for 5 p.c.	F Value for 1 p.c.
Treatments	2	.00000048			
Plants	2	.00136403	284.17	4.49	8.53
Replicates	2	.00001158	3.55	4.49	8.53
Plants x Treatments	4	.00000104			
Error	16	.00000326			

Treatment No.	Treatment Temperature Level	Means Mg. HCN/ c.c.
1	Greenhouse 66.5° F: Soil 60.5° F	.0232
2	" 59.0° F: " 56.0° F	.0237
3	" 56.0° F: " 54.0° F	.0233
	Necessary difference for significance (P = .05)	.0018
	(P = .01)	.0025

For (P = .05) there are no significant differences between

treatments.

For ($P = .01$) there are no significant differences between treatments.

It is evident that there are no significant differences due to the temperature ranges to which these plant units were submitted.

<u>Discussion</u>:- The mean daily temperature range in the different sections of the greenhouse was from 56° F to 66.5° F and for the respective soils the range was from 54° F to 60.5° F.

At Ottawa, plants grown under ordinary field conditions are sometimes exposed to a wide range of temperature during a 24 hour period, even during the summer season. Temperature changes of as much as 40° F to 50° F are relatively common during a full day. With this in mind, a small experiment was conducted in which a typical plant was tested for HCN content daily over a 20 day period. During this time the average daily temperature varied from 51° F to 72° F but the variation in HCN was very slight, ranging from .026 to .030 mg. HCN per c.c. of solution. The total precipitation during the period was only 0.15 inches.

From the foregoing it appears that normal seasonal fluctuations in temperature have little or no effect on the concentration of HCN in white clover plants. It may be that very great extremes would have some influence since they might affect the growth processes of the plants.

In the review of literature only one reference was found on the influence of temperature on the HCN content of plants. This reference by Franske et al (11) related to a greenhouse experiment on sorghum in which was studied the influence of a combination of two factors on the HCN content, viz., light and temperature. It was found that reduced light combined with reduced temperature resulted in slightly lower HCN content.

The Influence of Light on the HCN Content of White Clover (Experiment 4)

Experimental Procedure

This small experiment was set up in the greenhouse during the winter of 1939-40 in order to study the influence of direct light as opposed to diffused light on the HCN content of wild white clover.

Material used:- Twelve plants with varying HCN content were selected for this test. Each plant was divided vegetatively into four separate units and planted in 3-inch clay pots containing good loam soil which was thoroughly mixed to ensure uniformity. The purpose of cloning in quadruplicate was to provide replicates for two treatments.

Treatments:- The plants were divided into two similar groups. One group was exposed to normal sunlight conditions in the greenhouse, while the other was enclosed in a white cotton cage which was arranged so as to permit adequate air circulation but at the same time diffuse the light which the plants received. Plants were grown under these conditions for 55 days.

Method of Testing:- The procedure for testing in this experiment was the same as for experiment 1. Duplicate samples were taken from each plant at three different dates during the course of the experiment and tested for HCN content.

Results:- The following table indicates the variation in the HCN content due to the influence of light. The data were treated statistically.

Text Table XIII Analysis of Variance for light Treatments on the HCN Content of plants Tested on Three Different Dates over a Period of 55 Days. (For complete date see Appendix Table VII.)

Due to	D.F.	M.S.	F Value	F Value for 5 p.c.	F Value for 1 p.c.
Plants	11	.00121896	375.06	1.93	2.51
Treatments	1	.00007803	24.01	3.98	3.98
Dates	2	.00004655	14.32	3.13	3.18
Replicates	1	.00001225	3.77	3.98	3.98
Plants x Treatments	11	.00000560	1.72	1.93	1.93
Plants x Dates	22	.00000296			
Treatments x Dates	2	.00003213	9.89	3.13	3.13
Plants x Treatments x Dates	22	.00000588	1.81	1.69	2.11
Error	71	.00000325			

No.	Treatment	Means Mg. HCN/c.c.
1	Ordinary sunlight	.0279
2	Reduced sunlight	.0294
	Necessary difference for significance	
	(P = .05)	.00058
	(P = .01)	.00077

For (P = .05) treatment 2 is significant to treatment 1.

For (P = .01) " 2 " highly significant to treatment 1.

No.	Dates	Means Mg. HCN/c.c.
1st	November 13, 1939	.0280
2nd	December 11, 1939	.0282
3rd	January 6, 1940	.0298
Necessary difference for significance		
	(P = .05)	.00072
	(P = .01)	.00094

For (P = .05) 3rd date is significant to 1st and 2nd dates.
For (P = .01) " " " highly significant to 1st and 2nd dates.

It will be observed that significant differences were obtained in this test i.e., after a 55 day period the amount of HCN in plants grown in controlled light was .0015 mg. per c.c. higher than that of plants grown in ordinary light.

From Appendix Table VII it may be seen that the significance between dates can be attributed chiefly to the influence of treatment 2, as there were only slight differences between dates under treatment 1.

Discussion:- The data presented above reveal that reduced light produced an increase in the HCN content, which is contrary to the findings of Franske et al (11) who found that reduced light combined with reduced temperature resulted in slightly lower HCN content. It must be noted, however, that the above experiment was conducted over a 55 day period and that, during the first 29 days of the test, no significant differences were found but that during the latter part of the period, when the days were becoming a little longer, significant readings were obtained. Consideration must also be given to the fact that the temperature under diffused light was often 10° F higher than in ordinary light.

Correlation of HCN Content with Habit of Growth in White Clover (Experiment 5)

Experimental Procedure

The area used for this experiment was adjacent to the area used for experiment 2.

Material used:- In the summer of 1938 a breeding block of 4248 plants was established. These plants were started in 3-inch clay pots in the greenhouse in early spring, and later transplanted in the field.

Method of Study:- By making HCN readings on a large random sample of the nursery plants and by utilizing the notes relating to growth type, leafiness, and leafhopper injury, which were made by the white clover plant breeder, it was possible to get some measure of how growth factors might govern the presence or absence of HCN.

Method of Testing:- The methods of sampling and testing for HCN content were the same as outlined in experiment 1, with the exception that three compound leaves instead of five were taken in sampling.

The plant characters were scored in the following manner:

Growth type 1 very close growing - wild type
2 semi-upright - intermediate type
3 upright - approaching common type
4 approaching mammoth type

Leafiness -- scored 1 to 5

Leafhopper injury -- scored 0 to 5.

Results:- Three correlations were run, namely, growth type and HCN content, leafiness and HCN content, and leafhopper injury and HCN content. Text table XIV summarizes the results.

Text Table XIV Correlations of Plant Characters and the HCN Content.

Character	R	Level of Significance for P = .05 Fishers V.A. Table
Growth type and HCN content	- .0065	.1946
Leafiness " " "	+ .0146	.1946
Leafhopper injury and HCN content	- .0135	.1946

Discussion:- It is evident that the correlation coefficients obtained are so small that they are below tne level of significance. It is rather surprising that a higher correlation figure was not obtained between growth type and HCN content since other workers, such as Doak (6), have found that persistency and high production, which are governed to some extent by growth type, were correlated with high HCN content.

GENERAL DISCUSSION

In a study of this kind in which many contributing factors are not under control, wide variations and discrepancies in the data must be expected and allowed for in drawing conclusions. However, it has been possible in this study, by the use of a satisfactory number of replicates and by statistical analysis, to show that some factors had a marked influence on the HCN content of white clover while others had indeterminate effects.

The data presented indicate that under greenhouse conditions adequate soil moisture produces a high concentration of HCN, and that restricted soil moisture results in a relatively lower concentration. This problem was also studied under field conditions by obversation of the influence of precipitation on the HCN content, and it was noted that heavy precipitation was followed by an abrupt rise in the HCN content. It is realized that precipitation measurements do not give so accurate an estimation of available soil moisture as soil moisture determinations taken under greenhouse conditions. The findings of some other workers have been in agreement, while others have drawn opposite conclusions, but it must be noted that crops such as Sudan grass and sorghum, and not white clover, were studied and that, therefore, water requirements and habit of growth were much different.

Experiments have been reported where a pronounced seasonal variation in HCN content of wild white clover was found. This is in general agreement with the results of this study which, in addition, have revealed that there is a period during the growing season when a maximum amount of HCN is present.

A study of temperature effects showed a relatively unaltered HCN content over a range of temperature. It is readily seen that there were so many uncontrollable factors present in this part of the study that the findings must be considered inconclusive. It is evident that moderate changes in temperature, especially if the changes were constant and plants exposed to them for fairly long periods, would definitely affect the growth processes of the plants and provide a condition in which changes in HCN content might be expected.

In considering the influence of light on the HCN content, the difficulty of securing satisfactory control must be emphasized. It was found that diffused light augmented the HCN content significantly. This finding is qualified, however, since a 55 day period, during which the daily duration of light increased, was necessary to obtain significant results. Franske et al (11), as stated previously, found that reduced light combined with reduced temperature caused a slight reduction in HCN content in Sudan grass.

Perhaps of most interest is the failure to obtain significant correlations between different plant characters and HCN content. Other investigators have demonstrated that in white clover the HCN content is directly correlated with persistence and productivity, but in this study where a fairly large sample was taken no correlation was established.

On the whole, this study must be considered to be of an exploratory nature, inasmuch as many unpredictable factors showed up to condition the results. The findings may nevertheless serve as a useful guide to more detailed work.

SUMMARY

1. Adequate soil moisture produced a high concentration of HCN in wild white clover, and restricted soil moisture resulted in a relatively lower concentration.
2. There were a pronounced seasonal variation in the HCN content of wild white clover and also an indication of a period of maximum HCN content.
3. The influence of temperature on the HCN content was found to be statistically insignificant.
4. It was found that diffused light augmented the HCN content significantly in comparison to ordinary light.
5. There were no significant correlations found between the plant characters studied and the HCN content.

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App. Table 1. Summary of data from Experiment 1.
Mg. HCN per cc. for group "high" in HCN content.

Plant	Treatment 1			Treatment 2			Treatment 3		
	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3
1	.060	.053	.059	.042	.041	.037	.032	.016	.018
2	.042	.041	.040	.040	.041	.040	.027	.028	.027
3	.043	.044	.045	.039	.039	.034	.031	.027	.029
4	.037	.038	.046	.035	.036	.037	.030	.026	.027
5	.041	.043	.042	.040	.038	.040	.031	.029	.028

App. Table 2. Summary of data from Experiment 1.
Mg. HCN per cc. for group "medium" HCN content.

Plant	Treatment 1			Treatment 2			Treatment 3		
	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3
1	.035	.034	.036	.031	.030	.032	.028	.027	.028
2	.044	.042	.044	.041	.042	.040	.038	.036	.037
3	.027	.025	.025	.024	.023	.023	.020	.021	.018
4	.026	.023	.025	.024	.024	.023	.022	.020	.019
5	.022	.019	.021	.019	.020	.019	.016	.015	.017

App. Table 3. Summary of data from Experiment 1.
Mg. HCN per cc. for group "low" HCN content.

Plant	Treatment 1			Treatment 2			Treatment 3		
	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3
1	.016	.012	.011	.014	.013	.014	.015	.014	.012
2	.012	.010	.011	.010	.009	.009	.009	.000	.000
3	.022	.022	.023	.021	.021	.022	.020	.022	.022
4	.026	.025	.025	.024	.026	.026	.022	.024	.026
5	.009	.009	.012	.010	.009	.009	.000	.009	.000

App. Table 4. Summary of data from experiment 1. The influence of soil moisture on the HCN content at end of 40 days for plants "high", "medium" and "low" groups. (Mg. HCN per cc.)

Plants		Treatment 1			Treatment 2			Treatment 3			Totals by Treatments			Totals
		Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3	Treat. 1	Treat. 2	Treat. 3	
High HCN Group	1	.060	.053	.059	.042	.041	.037	.032	.018	.016	.172	.120	.066	.358
	2	.042	.041	.040	.040	.041	.040	.027	.028	.027	.123	.121	.082	.326
	3	.043	.044	.045	.039	.039	.034	.031	.027	.029	.132	.112	.087	.331
	4	.037	.038	.046	.035	.036	.037	.030	.026	.027	.121	.108	.083	.312
	5	.041	.043	.042	.040	.038	.040	.031	.029	.028	.126	.118	.088	.332
Totals		.223	.219	.232	.196	.195	.188	.151	.128	.129	.674	.579	.406	1.659
Medium HCN Group	1	.035	.034	.036	.031	.030	.032	.028	.027	.028	.105	.093	.083	.281
	2	.044	.042	.044	.041	.042	.040	.038	.036	.037	.130	.123	.111	.364
	3	.027	.025	.025	.024	.023	.023	.020	.021	.018	.077	.070	.059	.206
	4	.026	.023	.025	.024	.024	.023	.022	.020	.019	.074	.071	.061	.206
	5	.022	.019	.021	.019	.020	.019	.016	.015	.017	.062	.058	.048	.168
Totals		.154	.143	.151	.139	.139	.137	.124	.119	.119	.448	.415	.362	1.225
Low HCN Group	1	.016	.012	.011	.014	.013	.014	.015	.014	.012	.039	.041	.041	.121
	2	.012	.010	.011	.010	.009	.009	.009	.000	.000	.033	.028	.009	.070
	3	.022	.022	.023	.021	.021	.022	.020	.022	.022	.067	.064	.064	.195
	4	.026	.025	.025	.024	.026	.026	.022	.024	.026	.076	.076	.072	.224
	5	.009	.009	.012	.010	.009	.009	.000	.009	.000	.030	.028	.009	.067
Totals		.085	.078	.082	.079	.078	.080	.066	.069	.060	.245	.237	.195	.677
Totals 3 Groups		.462	.440	.465	.414	.412	.405	.341	.314	.308	1.367	1.231	.963	3.561

App. Table 5. Summary of data from experiment 2. The HCN content of 96 plants tested at 10-day intervals over a period of 90 days.(Mg. HCN per cc.)

Clonal Row No.	July 10	July 20	July 29	Aug. 9	Aug. 19	Aug. 29	Sept. 8	Sept. 18	Sept. 28	Oct. 8
1	.038	.034	.036	.048	.044	.044	.043	.043	.042	.040
2	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
3	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
4	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
5	.022	.020	.024	.033	.030	.028	.026	.024	.024	.024
6	.024	.025	.026	.036	.032	.030	.030	.028	.026	.027
7	.045	.047	.050	.057	*	.054	.052	.052	.050	.050
8	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
9	.012	.015	.018	.030	.030	.024	.022	.020	.018	.018
10	.050	.051	.052	.059	.058	.054	.054	.052	.052	.054
11	.024	.028	.028	.035	.032	.032	.028	.028	.027	.025
12	.030	*	.034	.042	.042	.040	.038	.034	.034	.030
13	.009	.008	.009	.016	.014	.010	.010	.010	.010	.010
14	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
15	.038	.040	.045	.045	.047	.044	.042	.042	.040	.040
16	.039	.042	.042	.046	.044	.044	.042	.040	.042	.040
17	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
18	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
19	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
20	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
21	.015	.015	.020	.024	.022	.020	.017	.017	.017	.017
22	*	.026	.028	.034	.032	.030	.028	.028	.026	.026
23	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
24	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
25	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
26	.034	.036	.038	.040	.038	.036	.034	.036	.034	.034
27	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
28	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
29	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
30	.014	.013	.016	.033	.030	.026	.031	.024	.020	.018
31	.017	.017	.024	.028	.030	.026	.024	.020	.020	.018
32	.034	.032	.034	.044	.044	.040	.038	.036	.036	.032
33	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
34	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
35	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
36	.014	.018	.016	.022	.020	.020	.014	.016	.012	.012
37	.016	.017	.023	.028	.034	.032	.028	.019	.018	.008
38	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
39	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
40	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
41	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
42	.022	.020	.024	.045	.039	.038	.037	.032	.030	.030
43	.032	.033	.041	.043	.041	.043	*	.038	.033	.032
44	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
45	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
46	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
47	.020	.021	.028	.048	.046	.040	.025	.027	.027	.026
48	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000

(cont'd)

49	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
50	.012	.013	.022	.032	.028	.026	.022	.020	.018	.014
51	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
52	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
53	.013	.013	.024	.036	.032	.028	.026	.024	.022	.020
54	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
55	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
56	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
57	.017	.018	.024	.032	.030	.028	.026	.022	.020	.022
58	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
59	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
60	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
61	.008	.009	.012	.022	.022	.018	.016	.010	.010	.010
62	.042	.045	.044	.055	.056	.054	.054	.052	.050	.050
63	.014	.014	.013	.027	.026	.024	.024	.018	.020	.020
64	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
65.	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
66.	.012	.013	.027	.027	.027	.024	.022	.022	.020	.016
67	.040	*	.043	.058	.056	.055	.050	.052	.046	.044
68	.017	.015	.020	.028	.036	.028	.028	.024	.022	.020
69	.016	.014	.020	.032	.033	.030	.033	.023	.023	.022
70	.009	*	.008	.019	.014	.014	.012	.010	.009	.009
71	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
72	.010	.012	.011	.022	.020	.018	.014	.016	.016	.014
73	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
74	.028	.026	.037	.044	.042	.040	.038	.038	.038	.034
75	.036	.034	.036	.052	.050	.048	.045	.044	.042	.042
76	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
77	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
78	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
79	.015	.015	.017	.033	.032	.026	.020	.020	.018	.018
80	.040	.037	.042	.054	.054	.044	.046	.042	.040	.036
81	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
82	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
83	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
84	.015	.013	.022	.032	.038	.034	.033	.022	.020	.020
85	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
86	.034	.036	.042	.052	.048	.044	.040	.036	.036	.036
87	.034	.036	.034	.042	.044	.042	.040	.040	.036	.038
88	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
89	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
90	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
91	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
92	.024	.026	.032	.039	.038	.034	.032	.030	.028	.030
93	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
94	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
95	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
96	.000	.000	.000	.000	.000	.000	.000	.000	.000	.000
Mean	.024	.024	.028	.037	.036	.034	.031	.029	.028	.027
" of	(41)	(39)	(42)	(42)	(41)	(42)	(41)	(42)	(42)	(42)

* No sample available

App. Table 6. Summary of data from experiment 3. The influence of temperature on the HCN content at the end of a 55-day test. (Mg. HCN per cc.)

	Temperature 1			Temperature 2			Temperature 3		
Plants	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3	Rep.1	Rep.2	Rep.3
1	.010	.011	.008	.011	.012	.011	.010	.013	.007
2	.035	.037	.033	.035	.034	.035	.038	.034	.032
3	.025	,023	.027	.025	.027	.023	.026	.027	.023

App. Table 7. Summary of data from experiment 4. The influence of light on the HCN content of plants tested on three different dates over a period of 55 days. (Mg. HCN per cc.)

	November 13 - 1939				December 11 - 1939				January 6 - 1940			
	Treat.1		Treat.2		Treat.1		Treat.2		Treat.1		Treat.2	
Plants	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2	Rep.1	Rep.2
1	.012	.015	.012	.016	.014	.015	.013	.018	.018	.017	.016	.020
2	.029	.027	.029	.028	.029	.028	.029	.028	.025	.028	.028	.032
3	.041	.043	.040	.041	.042	.040	.040	.042	.038	.041	.042	.044
4	.035	.030	.034	.032	.032	.028	.033	.030	.031	.034	.034	.037
5	.034	.034	.033	.035	.036	.032	.034	.036	.036	.035	.036	.038
6	.028	.024	.028	.027	.028	.026	.028	.028	.029	.024	,030	.028
7	.036	.039	.034	.038	.036	.040	.038	.039	.041	.036	.042	.041
8	.044	.045	.046	.042	.042	.042	.044	.042	.046	.042	.048	.042
9	.032	.027	.030	.030	.029	.027	.033	.032	.030	.026	.037	.036
10	.016	.019	.019	.015	.016	.018	.019	.015	.020	.014	.022	.016
11	.017	.015	.015	.016	.014	.019	.018	.017	.018	.018	.021	.018
12	.016	.014	.015	.016	.015	.013	.017	.018	.017	.012	.024	.021