A METHOD OF QUANTITATIVE NECTAR DETERMINATION AND PRELIMINARY STUDIES OF THE EFFECT OF SOIL FERTILIZER TREATMENT ON NECTAR PRODUCTION OF ALFALFA

by

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INTRODUCTION

A striking contrast to the reliability of alfalfa as a hay crop is its great variability in seed production (2). This variability in production has led to considerable research upon the problem of alfalfa seed set. The effect of moisture on seed set was reported by Martin (9) and by Stewart (18). They found conditions for seed production generally more favorable in dry areas. Heat and light have been studied as to their possible effect upon seed set by Piper et al. (14). Heat was found to induce some tripping. Seamans (16) reported that thrips reduce seed yields. Sterile pollen was found by Clark and Pryer (3) to be a contributor to low seed yields in many alfalfa plants. Jones and Olson (6) found it necessary for the stigmatic surface of the alfalfa flower to be ruptured for fertilization. Grandfield (5) reported that increasing the root reserves increased seed yield. Phillips (13) obtained nonsignificant results in studies of field fertilizer treatments as they affect seed production.

The present interest in the alfalfa insect pollinators was stimulated by the reports of Armstrong and White (1), Brink and Cooper (2), and Tysdal (20, 21). These investigators found tripping essential for satisfactory seed set, although Brink and Cooper reported considerable seed set under field conditions without tripping.

It can be seen readily that if insect pollinators are responsible to a considerable extent for the alfalfa seed crop the nec-

tar supply of alfalfa flowers will probably be an important factor in determining the extent of pollination by insects. Bees are critical of nectar and select the richest sources (24).

The purpose of this study was to investigate methods for determining nectar quantitatively, to make preliminary studies of the influence of fertilizers on nectar production, and to observe possible differences in the apparent desirability to bees of the alfalfa plants on the different fertilizer plots.

REVIEW OF LITERATURE

Kenoyer (7) made an extensive study of environmental influences on nectar secretion. Several plants were studied, including alfalfa. He found a decreasing percentage of sugar in nectar as humidity increased, and that nectar secretion increased with temperature up to a certain optimum. The optimum conditions for nectar secretion were alternate high and low temperatures.

Tschudin (19) used the average daily honey production of 20,000 hives as a measurement of nectar flow and reported that nectar secretion apparently increases with altitude.

Sugar content of nectar was found by Ortel (10) to vary widely within the same species. Also a significant positive linear correlation between increasing temperature and the percent of sugar in the nectar of goldenrod (Solidago altissima) and white clover (Trifolium repens) was found (11). He reported that in some seasons there was a significant negative correlation between sugar concentration in the nectar and increasing humidity. The

latter study was in agreement with the earlier findings of Park (12).

Vansell (23) studied nectar from plants grown under different soil moisture levels. To minimize the effects of other factors, the stems of all of his plants were kept in a saturated atmosphere. The sugar concentration of the nectar of plants grown in dry soil was nearly twice as high as that of plants grown in wet soil. Vansell also reported that longer days stimulated earlier blooming of alfalfa and lengthened the seasonal nectar secretion period. He found that Turkestan produced more and richer nectar than the common alfalfa. The larger blossoms produced the most nectar. Continuous warmth was observed to produce maximum nectar flow in this experiment. This is not in agreement with the observations of Kenoyer (7) that alternate warm and cool temperatures were most conducive to maximum secretion.

The use of the micropipette and filter paper methods for obtaining a measurement of nectar content was mentioned by Kenoyer (7). The use of a calibrated micropipette to draw up nectar from the flower gave an approximate volumetric measurement of nectar production. The filter paper technique involved the absorption of nectar on pieces of filter paper of known weight, and reweighing after absorption.

Kenoyer determined the sugar content of the nectar by placing a weighed or counted number of flowers in a measured amount of water and agitating at frequent intervals for 30 minutes. The amount of sugar extracted was determined by Fehling's reduction

and computed on a single flower basis.

Ewert (4) used a centrifuge to extract nectar from flowers. The pedicles of the flowers were placed between the halves of a split cork, which were then inserted in a centrifuge tube so that the flowers hung downward in the tube. No centrifuge speeds were given. Pederson[1] used the centrifuge method with a speed of 2,400 r.p.m. for one minute. Swanson, in extracting clover nectar, used a speed of 1,750 r.p.m. for four minutes.[2] Swanson considered centrifuging to be the best of existing methods.

Ewert also showed that potassium increased the nectar yield of red clover. Stapel and Gotzsche (17) studied the effect of fertilizers upon nectar production under Danish conditions and were unable to corroborate Ewert's results. Their results showed that red clover grown on potassium-treated soils was decreased in nectar production during the seasons of 1939 and 1940.

It was found that potassium did increase the length of the corolla tube of clovers. They postulated that the increase in corolla tube and decrease in nectar would decrease the possibility of pollination by honeybees. They concluded, however, that potassium fertilization should not be overlooked, since heavier hay and seed yields were obtained by such treatments. The centrifuge method of extraction was used in this study. The amount of nectar was determined by weighing.

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[1] Marion W. Pederson, Utah Legume Seed Laboratory. From a letter dated Sept. 20, 1948.

[2] H. M. Tysdal, Head of Alfalfa Investigations, Forage Crops Division. From a letter dated Oct. 29, 1948.

MATERIALS AND METHODS

Method Studies and Comparisons with Existing Methods of Quantitative Analysis

The study of nectar variation of flowers from the different fertilizer treatments required a method of quantitative measurement. Several methods have been proposed and used by previous investigators. There is no agreement as to which method gives the best results, however, and it was necessary to devote considerable study to the selection of a satisfactory method for this investigation.

The determination of the percent of solids in nectar has been used by some investigators as a measurement of nectar variation between species of plants. This method makes use of the refractometer and has the advantage of speed. The sample for the reading is commonly taken from the honey sac of honeybees that are working a particular species. Bees tend to collect nectar from only one species of plant during any one flight from the hive.[1] The ease with which a reading may be taken with the refractometer is offset by the fact that there is no indication from this reading of the total volume or total sugar content of nectar.

Micropipetting was reviewed by Kenoyer (7) as a method of nectar measurement. Because of the obvious difficulty of obtaining a reading with alfalfa flowers, this method was not used in the present study.

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[1] J. E. Eckert. The First Air Freight. Country Gentleman. 112:1-19. Jan. 1942.

The use of weighed strips of filter paper to absorb the nectar of flowers and reweighing was also mentioned by Kenoyer. The method is slow and requires two delicate weighing operations for each reading. No tests were made using this method.

A method of quantitative measurement of nectar in flowers was reported by Kenoyer (7) in which a counted or weighed sample of flowers was placed in a known amount of water and agitated frequently for a period of 30 minutes. Sugar extracted by this method was then determined by Fehling's reduction.

It was observed early in the study of methods that some means of forcing water into the flower would be desirable. A hypodermic syringe was selected for this purpose. Preliminary washes on alfalfa flowers using a hypodermic syringe and varying amounts of water revealed that satisfactory results could be obtained by washing individual flowers with 4 cc of water. More efficient washing was obtained by dividing the water into two washes of equal volume. Two washes of 15 cc for each flower sample permitted the addition of hydrochloric acid for hydrolysis, and also allowed for rinsing the beaker during transfer without exceeding the total volume of 50 cc stipulated in the method of Fehling's reduction used (15).

Tests were made to determine the size of flower sample required to give a satisfactory nectar determination. A sample of 30 flowers was found adequate for sugar determination. This number also permitted a wide selection of flowers which tended to minimize the variation in nectar production between plants.

Flowers to be washed were placed in a holder designed to hold 30 flowers. The holder (Plates I and II) was clamped to a ring-stand so that the holder and flowers could be lowered into the beaker to prevent loss from splashing during the washing process.

The flowers were washed by placing the hypodermic needle between the standard petal and the sexual column and forcing approximately 2 cc of water into the floral cavity. Plate III is a photograph of the needle position. After all flowers had received the first wash, they were dried with a fine air jet and the washing and drying procedure was repeated, using the second 15 cc of water. Both washes were then transferred to a single 50 ml volumetric flask. Three cc of hydrochloric acid were added to the solution to hydrolyze all sugars to reducing sugars. After 12 hours hydrolysis the solutions were made to the 50 cc volume and the total sugars determined by the method of Quisumbing and Thomas (15).

Influence of Fertilizer Treatment on Seed Yield and Nectar Production

Several field fertiliser treatments were made for observation of seed set, for nectar production studies, and to provide areas for population counts of alfalfa pollinators. Three series of eight fertiliser treatments and two checks were made on Buffalo alfalfa at Manhattan, Garden City, and Hays. Plots in each treatment were 30 feet by 75 feet. Treatments were not replicated at the different stations, since it was desirable to keep the area

EXPLANATION OF PLATE I

A photograph of the equipment used in nectar extraction by the syringe method.


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2
C01761

EXPLANATION OF PLATE II

A close-up of the holder used in the syringe method of nectar extraction. A section of flowers has been removed to show the construction of the holder.

PLATE II

EXPLANATION OF PLATE III

The correct position of the hypodermic needle is shown.

PLATE III

in an individual treatment as large as possible for bee population studies.

The fertilizers were applied as a surface dressing in April, using a hand-operated fertilizer spreader with a curtain to keep the fertilizers from drifting. Nitrogen, phosphorus, and potassium were added to all treatments. In addition, calcium, magnesium, and boron were used in all possible combinations. The rates per acre for the different elements were: nitrogen, 75 pounds; phosphorus, 55 pounds; magnesium, 150 pounds; boron, 20 pounds; potassium, 30 pounds; and calcium, 250 pounds.

Seed yields were taken on the Hays and Garden City plots. No yields were obtained from the Manhattan plots, due to generally poor conditions for seed production.

Nectar determinations on flowers from the Manhattan field plots were made for five days. The samples were obtained by taking one flower from each of the 30 racemes selected at random within a treatment. The racemes were selected while walking the length of a plot and returning along a different route. The location of the line-of-sampling was changed each day.

Effect of Fertilizer Treatment on the Nectar Production of Alfalfa Grown in the Greenhouse

Sixteen fertilizer treatments, replicated three times, were used on potted alfalfa plants in the greenhouse. These plantings were made during the fall semester by Phillips (13) to study the effect of fertilizer treatment on alfalfa seed set. During the

spring semester these plants were used for investigating methods of nectar extraction and for determining the effect of fertilizers on nectar production. Data for the latter are shown in Table 8.

The sixteen treatments were B, Ca, Mg, B + Ca, B + Mg, Ca + Mg, B + Ca + Mg, NPK, NPK + B, NPK + Ca, NPK + Mg, NPK + B + Ca, NPK + B + Mg, NPK + Ca + Mg, NPK + B + Ca + Mg, and a check involving no treatment. Soil from near the Thayer Experiment Field was used. Table 1 shows the rate of application of the fertilizers.

Table 1. Rate of fertilizer application per pot.

Element	Form	grams/pot
Nitrogen	$NH_4 NO_3$	0.68
Phosphorus	45% P_2O_5	0.50
Potassium	60% K_2O	0.27
Boron	$Na_2 B_4O_7$	0.09
Calcium	$Ca SO_4$	2.26
Magnesium	$Mg SO_4$	1.36

The greenhouse fertilizer treatments were repeated during the fall semester of 1943 using all the treatments involving NPK listed above, and a no-treatment check. The eight fertilizer treatments and check were replicated four times. Flowers from these plants were used during the fall semester for studying methods of nectar extraction.

Influence of Fertilizer Treatment on the Population of Pollinating Insects

Studies were made at Manhattan and Hays to determine differences in desirability of the nectars of the eight fertilizer treatments and two checks as reflected in the population of pollinating insects on each plot. Readings were taken for five days at five times during the day. The times were 9 and 11 a.m. and 1, 3, and 5 p.m.

Six colonies of honeybees were placed approximately one-fourth mile to the west of the fertilized plots on the agronomy farm at Manhattan to provide bees for general pollination as well as for the population readings. Six colonies were also located at the Hays station, but the location was rather remote from the fertilizer plots.

Counts of bees were based upon one-square-yard areas selected at random within each plot. The species and number of bees in each square-yard area were recorded. The position of the area observed was changed for each reading.

EXPERIMENTAL RESULTS

Method Studies and Comparisons with Existing Methods of Quantitative Analysis

The micropipette and filter paper techniques reviewed by Kenoyer (7) were not tested in this study. The micropipette method was believed to be unadaptable to the alfalfa flower because of the small amount of nectar. The filter paper technique in-

volves the absorption of nectar with weighed strips of filter paper and subsequent reweighing. Because of the two delicate weighing operations and the tedious process of absorbing the nectar from individual flowers, this method was not used.

A hypodermic syringe was used to force distilled water into the floral cavity of alfalfa flowers. In washing, the hypodermic needle was placed between the sexual column and the standard petal. The flowers were placed in holders designed to hold 30 flowers; the holders could be lowered below the rim of a 600 cc beaker for the washing process.

Test washes were made to determine the number of rinses and the total volume of water required to obtain efficient nectar extraction. Results of the trial washes revealed that two rinses of 2 cc each were sufficient for an individual flower. Two 15 cc volumes or 30 cc of distilled water per 30-flower sample extracted the nectar as efficiently as larger amounts. Each flower in the sample received 2 cc at each of the two washes. Sugar determinations were made on the nectar solutions using the Quisumbing-Thomas method of Fehling's reduction (15).

Tests were made to compare the syringe method of extraction with the washing technique outlined by Kenoyer (7). By Kenoyer's method a weighed or counted sample of flowers was placed in a beaker containing a known amount of distilled water. The sample was then agitated frequently for a period of 30 minutes. The extracted sugar was then determined by Fehling's reduction. In this study the method of Fehling's reduction reported by Kenoyer

was replaced with the Quisenberry-Thomas method (15). The latter method requires more time and equipment, but is more easily controlled.

Samples of 30 flowers each selected at random from racemes of plants having the same fertilizer treatment were used in all determinations. Flowers to be extracted by the Kenoyer method were placed in a beaker containing 30 cc of distilled water and swirled vigorously at approximately two-minute intervals for 30 minutes.

The results obtained by the two methods are shown in Table 2. The syringe method was more efficient in all of the trials. The best comparative result obtained by the Kenoyer method was 0.32 mg of invert sugar from the 30-flower sample as compared with 2.24 mg for the same number of flowers washed by the syringe method. The value of 0.32 is 14.2 percent of the higher figure. Because of the low values obtained, the Kenoyer method was dismissed from further consideration.

Table 2. Comparison of sugar extraction by Kenoyer and syringe methods.[1]

Sample	Kenoyer	Syringe
1	0.32	2.24
2	0.32	2.63
3	0.89	3.18
4	0.16	8.20

[1]Values indicate mg invert sugar per 30-flower sample.

The syringe method of nectar determination was used in the greenhouse study of nectar production during the spring semester of 1948, and the following summer the method was used for nectar determinations from field material.

Method studies were resumed during the fall semester of 1948. Comparisons were made between the syringe method and the centrifuge method reported by Ewert (4).

Corollas of flowers extracted by the centrifuge method were clipped away and the entire raceme suspended in an inverted position in a centrifuge tube. The raceme was held in position by pinching the peduncle in a split cork. A picture of a sample of flowers ready for centrifuging is shown in Plate IV.

In the present study, centrifuge speeds of 1700 and 2400 r.p.m. for four minutes were used. The centrifugate was transferred to a 50 ml volumetric flask, and sugar determinations were made by Fehling's reduction. Flowers from one plant, all picked at the same time, were used for each comparison. This permitted direct comparison of the centrifuge method with the syringe method. Due to the small number of flowers available it was necessary in some instances to divide the flowers equally for the different determinations and adjust the results to a 30-flower basis.

Table 3 shows the results obtained by the centrifuge method as compared with the results obtained by washing with the hypodermic syringe. The results vary considerably, but in general the syringe method appears to be superior to the centrifuge method at 1700 r.p.m. The average for 10 paired analyses was 12.48 mg

EXPLANATION OF PLATE IV

A sample ready for extraction by the centrifuge method. Corollas of flowers have been clipped.

PLATE IV

invert sugar for the syringe method as compared with 9.57 mg for corresponding number of centrifuge method at 1700 r.p.m. The centrifuge method at 2400 r.p.m. gave higher values than either the syringe method or the 1700 r.p.m. centrifuge extraction in four comparisons. The average value for the centrifuge extraction at 2400 r.p.m. was 17.36 mg invert sugar per 30-flowers as compared with a value of 11.11 mg for the samples extracted by the syringe method.

Table 3. Comparison of the syringe and centrifuge methods of extraction.[1]

Syringe	Centrifuge	
	1700 r.p.m.	2400 r.p.m.
3.18	1.68	
4.48	1.14	
25.12	11.82	
20.78	16.60	
11.78	19.27	
15.04	7.52	
14.40	11.18	20.58
20.00	4.48	13.40
5.60	10.40	11.84
4.44	20.55	18.65

[1] Values indicate the milligrams of invert sugar per 30-flower sample.

Influence of Fertilizer Treatment on Seed Yield and Nectar Production

Field fertilizer treatments were made at the Manhattan, Hays, and Garden City stations for the purpose of obtaining data on seed yield, population of pollinating insects, and nectar

production.

The fertilizer applications were: NPK, NPK + B, NPK + Mg, NPK + Ca, NPK + Mg + B, NPK + Mg + Ca, NPK + Mg + Ca + B. The rates at which the different elements were applied are shown in Table 4. These are the same elements and rates used by Phillips (13) in studying the effect of fertilizer treatment on seed production of alfalfa in 1947. The fertilizers were applied in April with the hand spreader.

Table 4. Rate of nutrient application in field fertilizer treatment.

Element	Form	Rate of application in lbs. per acre
Nitrogen	NH_4NO_3	75
Phosphorus	45% P_2O_5	55
Potassium	60% K_2O	30
Calcium	$CaSO_4$	250
Magnesium	$MgSO_4$	150
Boron	$Na_2B_4O_7$	20

Seed Yields. Seed yields were obtained at the Hays[1] and Garden City stations,[2] and, although the 1948 season was conducive to very low yields, significant differences at the 5% level were

[1] Seed data from the Hays plot were collected by Friedrich Mennen, Forage Crops specialist at the Hays station.

[2] Sample cuttings of plots at Garden City were made by Alvin Love, Associate Agronomist at the Garden City station.

Table 5. Seed yields from alfalfa plots at Garden City and Hays.[1]

Plot	Treatment	Garden City		Hays	
		Grams/plot sample	lbs./A	Grams/plot sample	lbs./A
1	NPK	71.0	21.9	9.6	2.5
2	Check	114.4	35.3	20.0	5.1
3	NPK+B	151.5	46.7	36.9	9.4
4	NPK+Mg+B	109.8	33.9	35.6	9.1
5	NPK+Mg	127.4	39.3	40.0	10.2
6	NPK+Ca	129.5	39.9	50.0	12.8
7	NPK+Mg+Ca+B	147.0	45.3	41.6	10.7
8	Check	94.1	29.0	47.6	12.2
9	NPK+Ca+B	87.3	26.9	47.6	12.2
10	NPK+Mg+Ca	158.0	48.7	88.7	22.7

[1]Data collected during summer of 1948.

obtained between yields on the different fertilizer treatments. The analysis of variance is shown in Table 5a. Table 5 indicates the yield in grams per plot and pounds per acre at each of the stations.

Yields were lowest on the NPK treatments at both Hays and Garden City. The highest yields at both stations were obtained from the plots treated with NPK + Mg + Ca. Yields for the other treatments did not rank the same at the two places, although NPK + Mg ranked fifth at Garden City and sixth at Hays. NPK + Mg + B ranked seventh and eighth at the two stations, respectively.

No differences in vegetative growth or color were observed between the various treatments. The number and size of flowers appeared to be uniform throughout the plots.

Table 5a. Analysis of variance of alfalfa seed yields on fertilizer treatments at Hays and Garden City.

Factors	Degrees of freedom	Sums of squares	Variance	Calculated: F	Table readings of F (P=.05)	(P=.01)
Total	19	4,355.14	229.22			
Between fertilizer treatments	9	731.42	81.27	5.47*	3.39	5.92
Between Hays and Garden City	1	3,480.00	480.00	234.34**	5.12	10.56
Interaction (treatment location in state)	9	133.72	14.85			

*Significant at 5% level but approaching 1%.
**Highly significant at the 1% level.

Field Nectar Production Studies. Preliminary nectar determinations using the syringe method of extraction were made on the field plots at Manhattan. Variations which approached significance were obtained between plants within a treatment. This difference between the plants in a treatment increases the sampling variation somewhat. To minimize the effect of the plant variations one flower from each of 30 racemes was used in the 30-flower samples for the regular nectar determinations. Table 6 shows the values obtained during five days of nectar investigation on the field material. The analysis of variance is shown in Table 6a.

Nectar determinations were made on the eight-field fertilizer treatments and two check areas for five days. A highly significant daily variation in production was observed; nonsignificant values between treatments were obtained. The values obtained from the field material were much lower than those obtained from the greenhouse material during the spring semester of 1947. This can be partially accounted for by the fact that racemes were selected at random at the time of the nectar determination and were not bagged beforehand.

A significant variation in nectar production was observed between check plots and NPK treatments in a separate investigation. Repeated determinations revealed lower sugar production in flowers from the NPK treatments than from flowers on the check areas, as is shown in Table 6. These results were not substantiated in later determinations.

Table 6. Nectar production of alfalfa flowers from plants on field fertilizer plots.[1]

Treatment	July 15	July 16	July 27	Aug. 5	Aug. 10
NPK	3.20	2.24	2.40	1.44	3.04
NPK+Mg	4.48	2.24	2.40	2.40	3.04
NPK+B	3.20	3.36	2.72	1.60	2.24
NPK+Ca	4.16	2.40	3.20	0.64	2.40
NPK+Mg+B	4.00	2.24	2.56	1.44	2.72
NPK+Mg+Ca	3.20	2.72	3.20	0.80	2.42
NPK+Ca+B	3.68	2.40	2.40	2.24	2.40
NPK+Ca+B+Mg	3.20	2.08	2.24	2.24	2.88
No treatment	1.60	2.88	2.40	0.80	2.08
No treatment	3.36	2.40	2.24	0.80	1.44

[1]Values indicate sugar production of nectar mg per 30-flower sample.

Table 6a. Analysis of variance of nectar production of alfalfa flowers from plants grown under different field fertilizer treatments.

Factors	Degrees of freedom	Sums of squares	Variance	Calculated: F	Table readings of F (P=.05)	(P=.01)
Total	49	34.57				
Between soil fertilizer treatments	9	3.53	0.39	1.31	2.16	
Between days	4	20.16	5.04	16.80**	2.64	3.90
Interaction	36	10.88	0.30			

**Highly significant at 1% level.

Table 7. Nectar production of flowers from plots treated with NPK compared with untreated plots.[1]

Operator A		Operator B	
Mg invert sugar in nectar of 30-flower sample			
NPK	No treatment	NPK	No treatment
3.36	3.84	2.4	2.88
3.57	3.84	2.24	2.72
3.46	3.04	2.4	3.57
Av. 3.46	3.57	2.34	3.06

[1] A comparison of washing technique between operators was obtained incident to investigation.
Average nectar production on fertilizer treatments 2.09 mg and on no treatment plots 3.31 mg.

Table 7a. Analysis of variance between NPK treatments and no treatment.

Factors	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F (P=.05)	(P=.01)
Total	11	3.40				
Between treatments	1	.50	.50	6.42*	5.32	11.26
Between operators	1	2.00	2.00	25.45**	5.32	11.26
Interaction (treatment x operator)	1	.27	.27	3.43		
Error	8	.63	.079			

* Significant variation between treatments at 5% level.
**Highly significant error between extraction of individual operators at 1% level.

Effect of Fertilizer Treatment upon the Nectar Production of Alfalfa Grown in the Greenhouse

Nectar determinations were made on flowers from greenhouse plants. Samples of 30 flowers were selected from a composite of all the racemes of a treatment in full bloom at the time of the determination. Care was taken to select untripped flowers from the upper part of the raceme, although tests had revealed very little variation in nectar production from the base to the tip of raceme if the flowers showed no signs of deterioration.

Statistical analysis established no significance between treatments. There was, however, a very highly significant variation between days. No explanation is offered for the daily variation since it probably is due to several interacting factors.

Table 8 shows the data obtained during the three days in which this study was conducted. The missing data are due to insufficient numbers of flowers at the time of sampling. Analysis of variance is shown in Table 8a.

Influence of Fertilizer Treatment on the Population of Pollinating Insects

Linsley and MacSwain (8) and Vansell and Todd (24) postulated a relationship between the variations in nectar production or sugar concentration and the preference of bees for the nectar of flowers from certain fields and plants.

Studies were made to determine possible preferences by bees for the alfalfa nectar from different fertilizer treatments. Bee

Table 8. Nectar production of alfalfa grown in the greenhouse.[1]

Treatment	March 18	March 25	April 9
Magnesium	31.2	22.1	26.7
Boron	—	—	—
Calcium	31.2	14.6	23.1
Mg+B	—	—	—
Mg+Ca	31.2	18.7	19.8
Ca+B	31.8	18.3	23.4
Ca+B+Mg	22.3	21.1	20.6
No treatment	23.9	20.7	26.9
NPK	29.4	—	27.0
NPK+Mg	23.2	19.8	29.0
NPK+B	19.7	15.0	21.8
NPK+Ca	19.8	21.1	29.4
NPK+Mg+B	21.6	19.8	23.1
NPK+Mg+Ca	26.8	22.6	26.9
NPK+Ca+B	23.2	19.7	28.0
NPK+Ca+B+Mg	—	—	—

[1]Values indicate nectar production in mg invert sugar/30-flower sample

Table 8a. Analysis of variance of nectar determinations made from alfalfa grown in the greenhouse.

Factors	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F .05	Table readings of F .01
Total	49	34.98				
Between fertilizer treatments	9	3.52	.391	1.24	2.16	3.03
Between days	4	20.15	5.038	16.04**	2.64	3.90
Interaction treatment x days	36	11.31	.314			

**Highly significant variation between days.

population counts were made at the field plots at Hays and Manhattan. Counts were made at 9 a.m., 11 a.m., 1 p.m., 3 p.m., and 5 p.m. for a five-day period. The species and number of bees on a square-yard area were recorded for each fertilizer plot. The location of the square yard sample area was changed for each reading.

The bee population readings were taken at Manhattan on August 3, 4, 5, 7, and 8 after all plots were in full bloom. No differences were observed in size or number of flowers on any of the plots. All plots came into bloom at approximately the same time. Data were taken on the Hays plots August 5, 9, 10, 13, and 16. The plots were in full bloom; no differences could be observed in time of blooming or in size or number of flowers on any of the plots.

Tables 8 and 9 show the hourly number and the total number of insects for each treatment at Manhattan and Hays. Insect numbers on the plots were very low. There was, however, statistical significance at the 1% level in numbers of pollinating insects between the treatment plots at Manhattan, as is shown in analysis of variance Table 9a. It is of interest that the counts made at Manhattan included only honeybees, while the readings at Hays included only wild bees, principally Megachile species.

Table 9. Population counts of pollinating insects on plots at Manhattan, 1948.[1]

Plot	Treatment	9 a.m.	10 a.m.	1 p.m.	3 p.m.	5 p.m.	Treatment total
1	NPK	0	0	0	0	0	0
2	Check	1	2	1	3	1	8
3	NPK+B	0	1	0	1	1	3
4	NPK+Mg+B	0	0	0	0	0	0
5	NPK+Mg	0	2	0	1	2	5
6	NPK+Ca	0	1	2	0	0	3
7	NPK+Ca+B+Mg	0	3	1	2	1	7
8	Check	0	2	4	0	0	6
9	NPK+Ca+B	1	1	2	2	1	7
10	NPK+Mg+Ca	0	5	3	0	1	9
	Total	2	17	13	9	7	48

[1]Hays data were obtained through the cooperation of W. W. Franklin, Alfalfa Insect Investigations, Department of Entomology, Kansas State College.

Table 9a. Analysis of variance of data from population counts of pollinating insects at Manhattan.

Factors	Degrees of freedom	Sums of squares	Variance	Calculated F	Table readings of F (P=.05)	(P=.01)
Total	49	67.92				
Between times during the day	4	29.12	7.28	12.77**	2.65	3.93
Between fertilizer treatments	9	18.32	2.035	3.57**	2.14	2.92
Interaction (treatment x days)	36	20.48	.57			

**Highly significant at the 1% level for variation between treatments and between times of day.

Table 10. Population counts of pollinating insects on alfalfa plots at the Hays station, 1948.[1]

Plot	Treatment	Time of reading: 9 a.m.	11 a.m.	1 p.m.	3 p.m.	5 p.m.	Treatment total
1	NPK	0	0	0	1	0	1
2	Check	0	1	0	1	0	2
3	NPK+B	1	1	0	0	0	2
4	NPK+Mg+B	0	2	1	2	1	4
5	NPK+Mg	0	1	0	1	0	0
6	NPK+Ca	0	2	1	1	0	4
7	NPK+Ca+Bolg	0	0	1	1	1	3
8	Check	0	1	0	1	1	3
9	NPK+Ca+B	0	2	1	0	0	3
10	NPK+Mg+Ca	0	0	0	2	2	4
	Total	1	10	4	8	5	28

[1]Insect counts made by W. W. Franklin, Alfalfa Insect Investigations, Department of Entomology, Kansas State College.

Table 11. Correlation of the data from seed yields, nectar production, and insect pollination investigations.

	Seed Yields: Hays	Garden City	Population of Insect Pollinators: Hays	Manhattan
Manhattan field nectar	+.53	+.26	-.02	-.22
Hays seed yield		+.57	+.67*	+.52
Garden City seed yields			+.22	+.37
Hays insect counts				+.25

*Significant at 5% level.

CORRELATION OF RESULTS

A significant correlation, +.67, is shown in Table 11 between the seed yields and the bee population of the plots at Hays.

Correlation between the seed yields at Hays and Garden City was fairly high, +.57, but was still short of significance, which for this study was .632 at the 5% level. The correlation between the Hays seed yields and the population of honeybees at Manhattan was +.52. This does not approach significance but it does indicate a reasonably close relationship between the seed yields on the various treatments at Hays and the preference of honeybees for the plants on the different fertilizer treatments at Manhattan.

Only a few observations were made in this preliminary study. It is possible that significance would be established in future experiments for some of the factors that exhibit only a moderate amount of correlation in the present study. This might be accomplished by continuing the experiment to permit a larger number of observations and for more than one year.

DISCUSSION

In the investigation which was made to determine a satisfactory method of quantitative nectar determination for alfalfa flowers, the syringe method of extraction developed in this study was superior to the method used by Kenoyer (7) in 1917. In some instances the syringe method was also superior to the centrifuge

method reported by Ewart (4). At centrifuge speeds of 1700 r.p.m. extraction averaged 9.57 mg of invert sugar per 30-flower sample compared with a value of 12.48 mg obtained by the syringe method in 10 paired extractions.

At centrifuge speeds of 2400 r.p.m. for a period of four minutes the centrifuge method of extraction is apparently superior to the syringe method. Only four comparisons are presented in Table 3, but in these the average value for the four centrifuge extractions was 17.36 mg of invert sugar per 30-flower sample as compared to 11.11 mg for the four comparable syringe extractions. Since the centrifuge extractions can be accomplished with greater ease than the syringe extractions, larger numbers of extractions can be handled. The centrifuge method has less opportunity for operator error, but a greater possibility of sampling error than the syringe method, since an entire raceme is used.

Nonsignificant results were obtained from sugar determinations of the nectar of alfalfa plants growing under different fertilizer conditions during the spring semester of 1948. These plants were clones which had been transplanted from the field to the greenhouse by Phillips (13) for seed set studies during the fall semester of 1947. They were carried over during the spring semester without additional fertilizer treatment. Whether the failure to show consistent differences was due to environmental factors, to small nutrient differences that may have remained at the time of this study, or a lack of refinement in technique is not known.

Table 8 shows the sugar values obtained by analysis of the flowers from the greenhouse material. It is of interest to compare the generally high values obtained from all plants in the greenhouse study with the low values prevailing under field conditions, as shown in Table 7. Although there normally may be somewhat lower production of nectar in the field than in the greenhouse, a large part of the decrease probably is due to the presence of nectar-consuming insects in the field.

Nectar determinations were made on flowers from field fertilizer plots on July 15, 16, 27, and August 5 and 10. Nonsignificant readings were obtained between the treatments in this study, but variation between days was highly significant.

Significance between treatments was established in repeated nectar determinations of flowers from NPK and check plots, although this variation was not shown in the previously discussed determinations. Nectar from flowers from NPK treatments was lower in sugar production than was the nectar from the no treatment plots. This is of interest, since the NPK plots were also lower than the checks in the population studies of insect pollinators at Manhattan and Hays. This evidence is made more conclusive by the seed yields from Hays and Garden City, in which the NPK treatments were lower in seed production than the checks.

Seed yields from fertilized plots at Hays and Garden City revealed significant variation between treatments. The seed yields were very low, however; Phillips (13) obtained nonsignificant differences in seed yields during the summer of 1947 from an ex-

periment which included the same treatments and locations used in the present study.

Significant differences were observed in insect populations between fertilizer treatments at Manhattan. In correlating the results of the data obtained from seed yields, insect pollination counts and nectar production, a significant correlation was established between the pollinating insect population at Hays and the Hays seed yields. It is also shown that a considerable degree of correlation but somewhat below the level of significance existed between the Hays seed yields and the population of pollinators on the plots at Manhattan.

The limited amount of data collected in the present study perhaps precludes the establishment of significant differences. Continuation of the investigations would provide more observations which would make averages more accurate and increase the degrees of freedom so that significance might be established for differences and relationships which are now supported by moderate but nonsignificant probabilities.

SUMMARY

An investigation of methods of quantitative determination of nectar from alfalfa flowers was made. Comparisons were made between the method used by Kenoyer (7) and the syringe method developed in the present study. The syringe method was the more efficient of the two. The centrifuge method at 2400 r.p.m. for four minutes may also be satisfactory.

Nectar determinations were made during the spring semester and summer of 1948 on flowers from plants grown under different fertilizer treatments. The results between treatments, with one exception, were nonsignificant. NPK in repeated determinations produced less nectar than the flowers from the no treatment plots.

Seed yields were taken at Hays and Garden City on plots which had been fertilized in April, 1948. Significant differences, approaching the 1% level, were obtained between treatments.

Population counts of pollinating insects on the different fertilizer treatments were made at Hays and Manhattan. It is of interest that only honeybees were found on the Manhattan plots, while at Hays only wild bees, principally Melachila specie were present. The variation of honeybees among the fertilizer plots at Manhattan was highly significant, as was the variation between days on which observations were made.

A significant correlation between the seed yields and the population of bees was found on the fertilizer plots at Hays. Considerable correlation was shown between Hays and Garden City seed yields from fertilizer treatments and between the Hays seed yields and pollinators at Manhattan. It is conceivable that a significant correlation could be obtained for some of the factors which show only a fairly high degree of correlation in this study by modifying the design of the present investigation and collecting more data.

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REFERENCES

1. Armstrong, J. M., and W. J. White.
Factors influencing seed setting in alfalfa. Jour. Agr. Sci. 25:161-179. 1935.

2. Brink, R. A., and D. C. Cooper.
Mechanism of pollination in alfalfa. Amer. Jour. Bot. 23:678-683. 1936

3. Clark, A. H., and J. R. Fryer.
Seed setting in alfalfa. Sci. Agr. 11:38-43. 1930.

4. Ewert.
(Method of extracting nectar with centrifuge). Leipziger Bienen Zeitung. 54:173-176. 1939.

5. Grandfield, C. O.
Alfalfa seed production as affected by organic reserves, air temperature, humidity, and soil moisture. Jour. of Agr. Res. 70:123-132. 1945.

6. Jones, L. H., and P. J. Olson.
Role of insects, weather conditions, and character in seed setting of alfalfa. Sci. Agr. 23:315-321. 1943.

7. Kenoyer, Leslie A.
Environmental influences on nectar secretion. Bot. Gaz. 63:4:249-265. 1917.

8. Linsley, E. G., and J. W. MacSwain.
Factors influencing the effectiveness of insect pollinators of alfalfa in California. Jour. Econ. Ent. 40:349. 1947.

9. Martin, J. H.
Relationship of moisture to seed production in alfalfa. Iowa Agr. Expt. Sta. Res. Bul. 23. 1915.

10. Ortel, Everett.
Variation in the sugar concentration of some southern nectars. Jour. Econ. Ent. 37:525-527. 1944.

11. Ortel, Everett.
Effect of temperature and relative humidity upon the concentration of nectar. Jour. Econ. Ent. 39:513-515. 1946.

12. Park, O. W.
The influence of humidity on sugar concentration in the nectar of various plants. Jour. Econ. Ent. 22:534-544. 1929.

13. Phillips, W. M.
Alfalfa seed production as affected by various fertilizers and by soil moisture levels. Master's thesis, Kansas State College. 1948.

14. Piper, C. V., M. W. Evans, R. McKee, and W. J. Morse.
Alfalfa seed production: pollination studies. U. S. D. A. Bul. 75. 1914.

15. Quisumbing, P. A., and A. W. Thomas.
Method of sugar determinations by Fehling's reduction. Methods of analysis of official agricultural chemists. p. 133. Association of Official Agricultural Chemists. 1945.

16. Seamans, H. L.
The alfalfa thrip and its effect on alfalfa seed production. Canadian Entomologist. 55:101-105. 1923.

17. Stapel, Chr., and Olaf Gotzsche.
On Nektarsekretion og Krumreslaemade m. m. hos Rodklovern under forskellige Godskningsforhold.

18. Stewart, G.
Alfalfa growing in the United States and Canada. New York: Macmillan Co. 1926.

19. Tschudin, Ernest.
Nectar secretion affected by altitude. Gleanings in Bee Culture. 49:100. 1921.

20. Tysdal, H. M.
Is tripping necessary for seed setting in alfalfa? Jour. Amer. Soc. Agron. 32:570-585. 1940.

21. Tysdal, H. M.
Influence of tripping, soil moisture, plant spacing, and lodging on alfalfa seed production. 38:515-535. 1946.

22. Vansell, G. H.
Nectar secretion in poinsettia blossoms. Jour. Econ. Ent. 33:409-410. 1940.

23. Vansell, G. H.
Alfalfa nectar and the honey bee. Jour. Amer. Bot. 83:106-107. 1943.

24. Vansell, G. H., and F. C. Todd.
Alfalfa tripping by insects. Jour. Amer. Soc. Agron. 38:470-488. 1946.