

AGENTS CONCERNED WITH NATURAL CROSSING
OF COTTON IN OKLAHOMA

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By

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Bachelor of Science

Oklahoma Agricultural and Mechanical College

Stillwater, Oklahoma

1951

Submitted to the Department of Agronomy
Oklahoma Agricultural and Mechanical College
in Partial Fulfillment of the Requirements
for the Degree of
MASTER OF SCIENCE

1953

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ACKNOWLEDGMENT

The writer wishes to express his sincere appreciation to his major advisors, Drs. John M. Green, Melvin D. Jones, Ralph S. Matlock, and others for their advice and constructive criticism.

TABLE OF CONTENTS

	Page
INTRODUCTION	I
REVIEW OF LITERATURE	2
Extent of Cross Pollination	2
Agents Concerned	3
Isolation Limits	4
MATERIALS AND METHODS	6
Role of Wind in Cotton Pollen Dispersal	6
Role of Insects in Dispersal of Cotton Pollen	6
EXPERIMENTAL RESULTS	8
Wind-blown Pollen	8
Insect Dispersal	8
DISCUSSION	21
SUMMARY	24
LITERATURE CITED	26
APPENDIX	28

INTRODUCTION

The structure of the flower of upland cotton (Gossypium hirsutum L.) is such that self-fertilization appears highly probable. This is expected since numerous monodelphous stamens surround the pistil and the anthers dehisce soon after the flowers open allowing an abundance of pollen within each flower. Since flower opening precedes dehiscence there is opportunity for foreign pollen to affect pollination and produce some crossing. Numerous investigations have shown that natural crossing in cotton occurs with varying degrees. However there is little published information on the direct cause of this cross-fertilization.

Because of the occurrence of cross-pollination, breeders are faced with the difficulty of keeping commercial varieties and breeding stocks pure. However the possibility of utilizing natural crossing in the production of hybrid cotton seed may be of some value in cotton breeding. Loden and Richmond (II)^{1/} say, "Consideration of natural crossing as a possible method of hybrid seed production necessitates the accumulation of information relative to another problem, namely, what agents are most effective in cross-pollination."

The objectives of this study were to determine the role of wind and insects in cotton pollen dispersal, as well as some of the foraging habits of the different insect species.

^{1/} Figure in parentheses refer to "Literature Cited," page 26.

REVIEW OF LITERATURE

Extent of Cross Pollination

Published data on natural crossing in cotton can be divided according to planting arrangements into three major classes. These classes are: alternate rows of male and female plants, marker plants surrounded in the field, and fields either adjacent or at varying distances apart.

Allard (4) planted alternate rows of upland cotton in north Georgia and found that the progenies grown from at least 20 per cent of the bolls produced contained one or more hybrids. Webber (21) concluded from studies in the southeastern states that ordinarily 5 to 10 per cent of the seed are the result of cross pollination, while Balls (7) reported that 5 to 25 per cent of the plants resulted from cross fertilization in a field of Egyptian cotton. In Mississippi, Ricks and Brown (16) found that the percentage of natural crossing ranged from 4.9 to 11.1 per cent, but in Georgia only 1.0 per cent hybrids were reported (13). In India similar experiments resulted in two per cent natural crossing (1).

Plants of Triumph cotton were scattered through an "Okra-leafed" type upland strain by Shoemaker (17) in Texas so that each Triumph plant was completely surrounded by "Okra-leafed" plants. The proportion of off type plants that were produced the following year was approximately 10.9 per cent. Using similar planting arrangements in the Regional Crossing Test (18), the twelve cooperating stations throughout the Cotton Belt reported crossing percentages ranging from 3.2 per cent in

Texas, to a theoretical estimate of natural crossing of 80.4 per cent at Raleigh, North Carolina.

When different types of cotton were located in adjacent fields, Collings and Wallace (9) found only about 0.59 per cent crossing or hybrid plants. In India, Afzal and Khan (2) reported that no natural crossing occurred beyond 100 feet and very little beyond 25 feet. Planting small isolated blocks of red-leafed cotton at distances ranging from 700 to 4,200 feet, Pope, Simpson, and Duncan (15) established the occurrence of natural crossing up to 0.8 mile.

Agents Concerned

When glass plates smeared with vaseline were exposed in cotton fields in north Georgia by Allard (4) considerable quantities of cotton pollen were collected. In Egypt, Balls (7) using the same method as Allard, obtained negative results, while Kearney (11) concluded that the nature of the pollen grains of *Gossypium* was unfavorable to their being transported by currents of air.

In his investigations in Mississippi, Brown (8) concluded that cotton was apparently crossed only by insects as was also reported by Collings and Wallace (9) of South Carolina and Kearney (11) in Arizona. Afzal and Khan (3) found many types and species of insects affecting cross pollination, but three species of Hymenoptera, namely *Apis dorsata* Fab., *Anthophora confusa* Smith, and *Elis thoracica* Lepel. were found to be the most important. Allard (4) considered that in north Georgia a large wasp, *E. plumipes*, when present, was the most active insect in cross-pollination. He found the bee, *Melissodes bimaculata* and the honey bee to be the most abundant and most consistent of the flower visitors.

Of the various Hymenopteran species which might act as cotton pollinators, bumble bees seem to be the most important. Pope, Simpson, and Duncan (15) named the bumble bee as the most important agent of natural crossing in cotton in Tennessee as did Shoemaker (17) in Texas. Honey bees were frequent visitors of the cotton flower but were not regarded as important carriers except when they visited the flower for the express purpose of obtaining pollen.

Stephens and Finkner (19) and Ware (20) concluded that the differences in amounts of cross-pollination were due primarily to differences in size of the bee population. Because of the rather wide differences in the frequency of cross-pollination observed, Hayes and Garber (10) stated that it appeared that varietal differences are one probable cause for discrepancies.

Isolation Limits

In order to prevent crossing in cotton, isolation limits have been suggested by several authors. Collings and Wallace (9) suggested a distance of 40 feet as sufficient to prevent crossing. Webber (21) stated that it was practically impossible to secure absolute isolation as this would require a distance of five to ten miles from any other cotton. He recommended a practical isolation of a quarter to half mile from any other cotton, particularly if this patch could be placed so that it was surrounded by woods. Ballard (5) of Georgia asserted that Sea Island cotton should not be planted within one mile of short staple cotton if the seed is to be used for planting purposes.

Corn barriers up to nine rows in width did not provide sufficient isolation in Tennessee to prevent crossing in the experiments conducted by Pope, Simpson, and Duncan (15) while Afzal and Khan (2) found an open space superior to both sorghums and corn in reducing outcrossing in cotton.

MATERIALS AND METHODS

Role of Wind in Cotton Pollen Dispersal

For the studies of cotton pollen dispersal by wind, a field of upland cotton approximately three acres in size was selected. The field was located in an open area on relatively high ground to avoid interference from natural barriers.

Between the hours of 8:30 A. M. and 2:30 P. M. microscope slides were exposed at varying heights in and around the field. The slides contained an area coated with a thin film of vaseline which was exposed to the air. These slides were attached at a 45 degree angle to six weather vanes which pivoted to keep the slides facing the wind. In this way pollen grains were trapped as they were carried by the wind. The slides were exposed for 30 minute intervals during the day and counts were made to determine the amount and time of pollen dispersal by the wind.

Role of Insects in Dispersal of Cotton Pollen

The role which insects play in the dispersal of cotton pollen was studied primarily in a large field of upland cotton where artificial pest control was not practiced.

Species of insects that are important in crossing cotton were caught with a hand net and identified. These species were noted when any plant was studied.

After establishing the time of day when pollen was shed, the foraging habits of the insect pollinators were observed during this period.

Individual pollinators were spotted as the cotton flowers began to open and followed until they left the field. The length of time an insect spent in foraging as well as the number of plants and flowers visited was noted. Several individuals of each species were observed and their movements during foraging were plotted.

The kind and number of pollinators visiting each flower were observed in a designated area of the above field by observing ten flowers tagged early in the morning on the day they opened.

Further studies concerning the time and frequency of insect visitation were conducted by selecting a 10-row plot of cotton and dusting the 21 opened flowers of the first row with methylene blue. These flowers were dusted at 9:00 A. M. At 30 minute intervals during the remaining morning hours counts were made of the flowers of the remainder of the rows. The flowers were examined and any flower containing a trace of methylene blue was noted. After the first count was made flowers containing traces of methylene blue were dusted with the chemical.

EXPERIMENTAL RESULTS

Wind-blown Pollen

A total of six pollen grains was caught on slides exposed at 30 minute intervals for three successive days (Table 1). Slides were observed at half hour intervals from 8:30 A. M. to 2:30 P. m. However, cotton pollen grains were caught only during the half-hour periods ending at 11:30 A. M., 12:00 noon, and 12:30 P. M.

Table 1--Number of pollen grains caught on slides during half-hour intervals for three consecutive days

Time of day slides were collected	Number of pollen grains caught		
	1st day	2nd day	3rd day
11:00	0	0	0
11:30	1	1	0
12:00	0	1	1
12:30	0	2	0

Insect Dispersal

Three species of bumble bees were found to be the only carriers of cotton pollen. These three species were Bombus americanorum (Fabr.), B. fraternus (F. Sm.), and B. auricomus (Robt.). Of the three species, B. fraternus was the smallest while the other two species were larger and more darkly colored.

A species of honey bees, Apis mellifica L., was observed collecting the nectar on various plants but it was not considered important in

the dispersal of cotton pollen.

The courses followed by 20 bumble bees were plotted during the morning hours. The flight patterns for two bees of each of the three different species of Bombus are shown in Figures 1 to 6. Except for an occasional flight from flower to flower down the row, there seemed to be no apparent trend in their flight pattern in regard to wind direction.

Data on foraging habits of the 20 bees observed are summarized in Table 2. Of the three species of bumble bees, B. americanorum proved to be the most prolific pollinator. This species visited on the average of 5.15 flowers per minute. Each visit would include at least one complete circuit of the staminal column. Of the other two species B. fraternus visited an average of 4.26 flowers per minute, while B. aureocomus averaged a little more than three flowers per minute. Different bees were noted to have stopped at from 13 to 193 flowers before returning to their nest. While it was not determined if they had done any foraging before the observations were begun, it is safe to say that they bees would at times visit up to 200 flowers before returning to their nest.

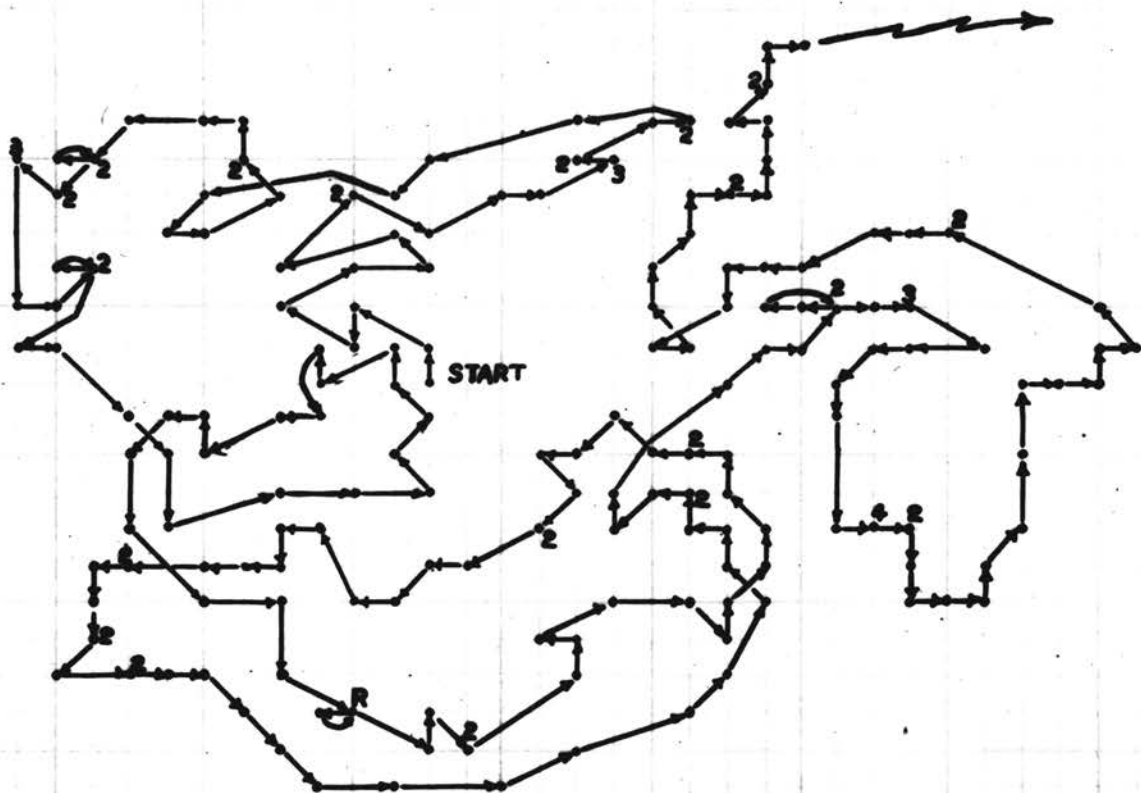


Figure 1--Flight path of *B. americanorum* visiting
166 plants and 193 flowers during a period
of 31 minutes

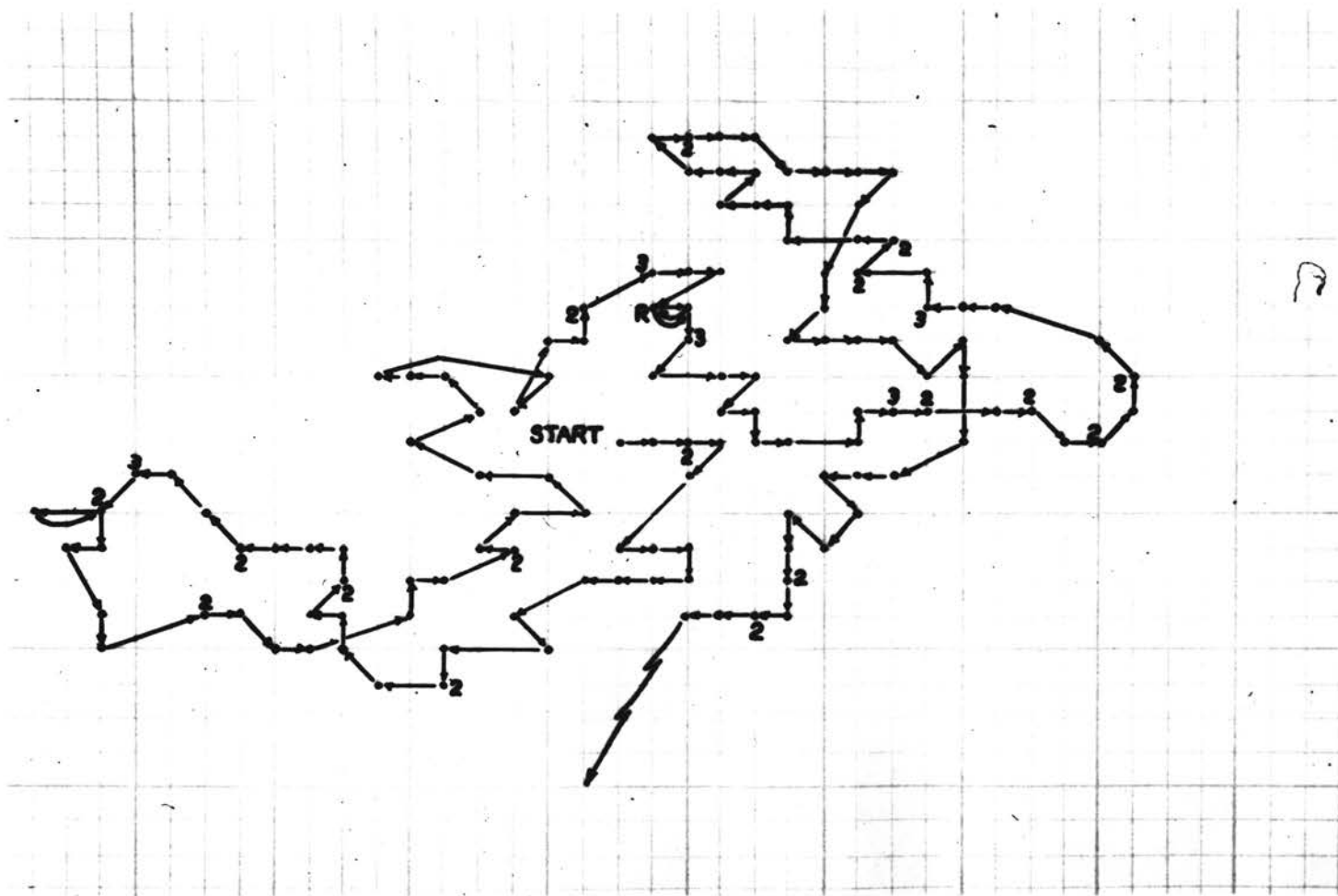


Figure 2--Flight path of *B. americanorum* visiting
 126 plants and 159 flowers during a period
 of 30 minutes

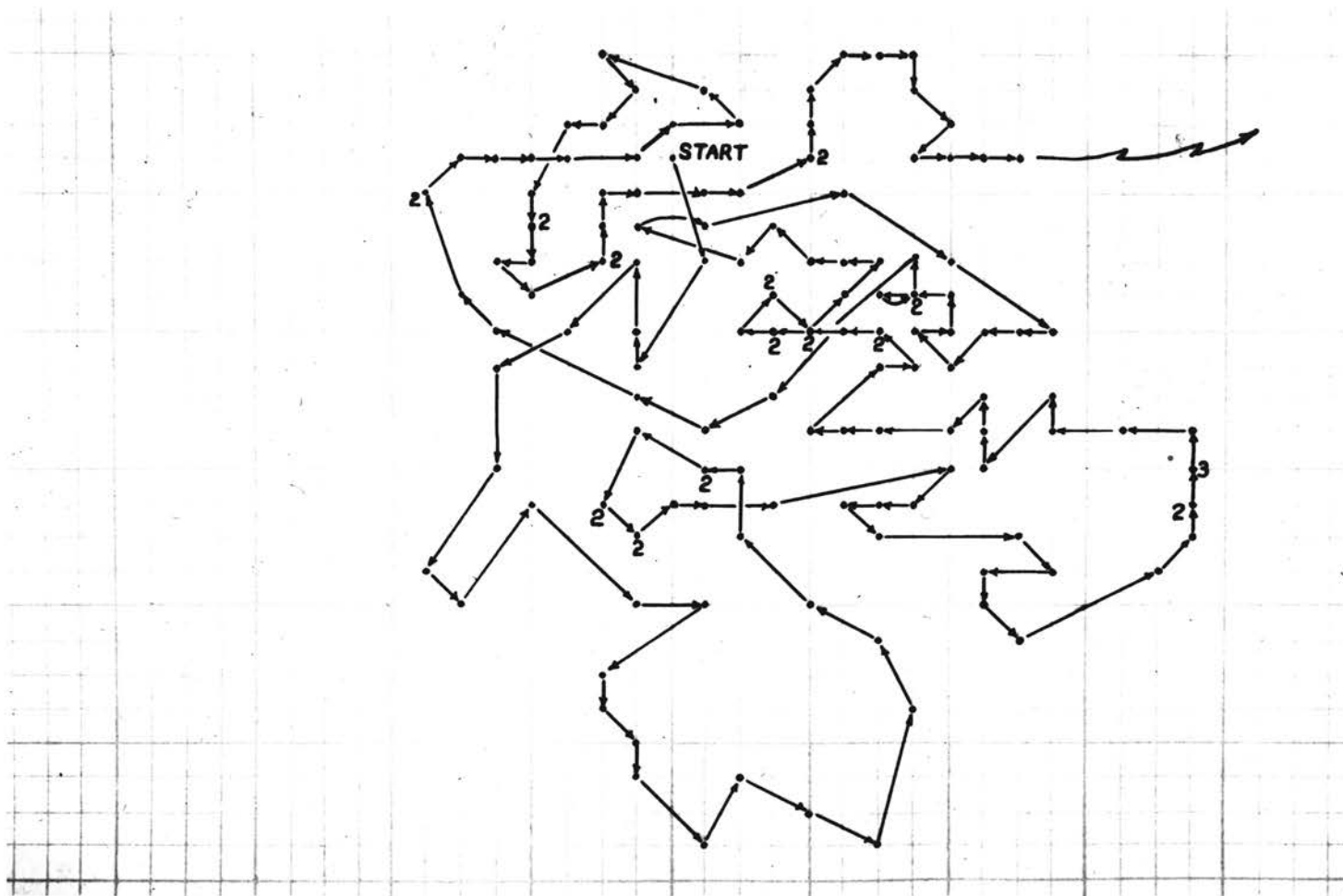


Figure 3--Flight path of *B. aureocomus* visiting 127 plants and 146 flowers during a period of 43 minutes

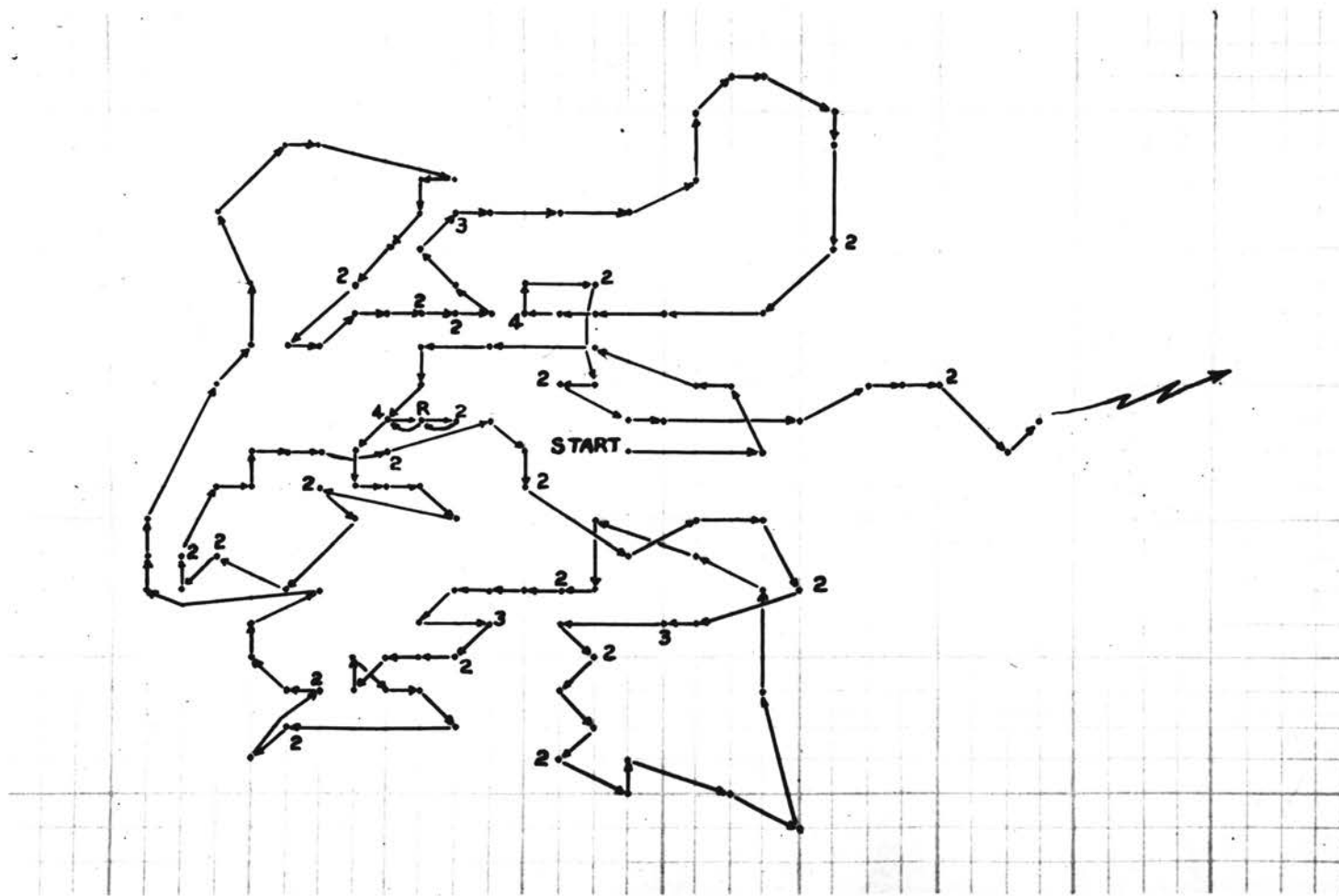


Figure 5--Flight path of B. fraternus visiting
123 plants and 156 flowers during a
period of 31 minutes

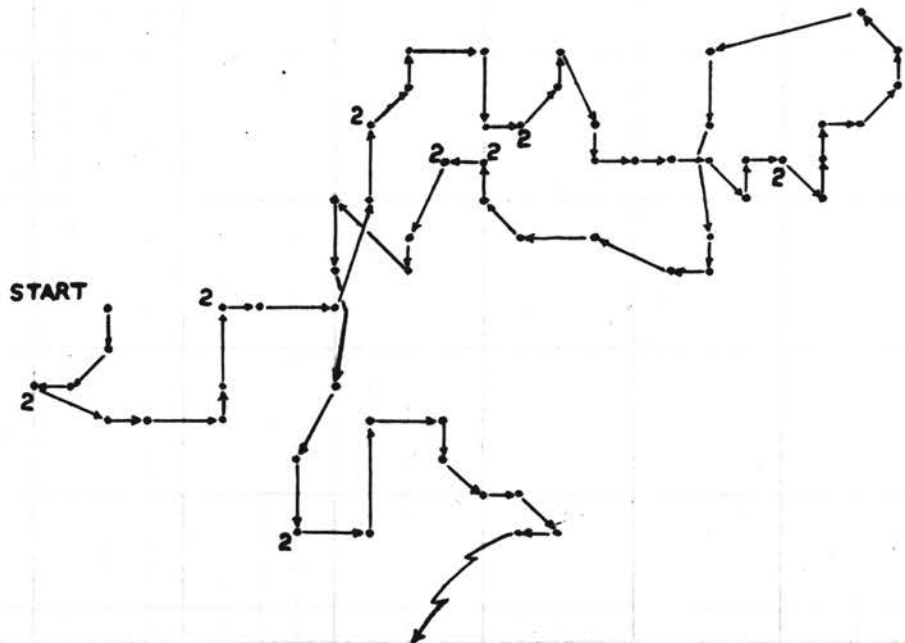


Figure 6--Flight path of B. fraternus visiting
61 plants and 70 flowers during a
period of 17 minutes

Certain flowers were watched throughout the period of insect inactivity and the frequency of insect visitation was recorded on charts for seven consecutive days. Average results for five days are given in Table 3 and the daily counts for those five days are given in Appendix Tables I to 5. Showers of rain on the other two days caused a large and almost complete migration of the insects from the cotton field. Insect visitations ran as high as 80 to 85 per flower per day. The flowers appearing in solid stands and on the upper branches of the stalk were observed to be visited with higher frequency than those appearing on sparsely spaced plants and on the lower branches.

Several factors were noted which influenced the time of flower opening and the frequency and time of bumble bee visitation. On a clear morning, the flowers usually opened at approximately 6:30 A. M. Time of opening was delayed by haziness or cloudiness. While it was not uncommon to see a bee force its way into a partially opened flower, the peak of insect activity occurred between the hours of 9:00 and 10:30 A. M. (Figure 7) varying somewhat with the amount of cloudiness. By 1:00 P. M. the field was almost devoid of bees.

The dispersal of methylene blue from 2I treated flowers in the first row of a 10-row block to all flowers in the remaining rows was complete in 2 hours (Table 4).

The greatest number of flowers were visited during the first half-hour of observation (9:00 to 9:30). The number of visits gradually decreased with very little visitation after 11:00 A. M. There was no evidence of a higher frequency of visits to flowers located nearest the treated row than to those 30 to 35 feet away.

Table 2--Number of flowers visited per minute by three species
of bumble bees (Bombus spp.)

Species	No. of flowers	Time spent (minutes)	Av. No. flowers per min.
B. americanorum	40	15	2.6
	193	31	6.3
	159	30	5.3
	51	8	6.4
Total	443	84	5.15
B. fraternus	42	10	4.2
	156	31	4.0
	70	17	4.1
	27	7	3.9
	22	6	3.7
	42	12	3.5
	83	20	4.1
	105	22	4.8
	36	6	6.0
	32	8	4.0
65	14	4.6	
Total	680	153	4.26
B. aureocomus	123	33	3.0
	13	5	2.6
	37	12	3.0
	146	43	3.3
	49	13	3.7
Total	368	106	3.12

Table 3--Average number of insects visiting individual cotton flowers during half-hour intervals covering the period from flower opening to early afternoon

Time of day	Flower number										Av.
	1	2	3	4	5	6	7	8	9	10	
6:30	.4	.2	.0	.6	.2	.0	1.4	.0	.2	.4	.3
7:00	1.4	.8	.6	.8	.8	.8	1.0	1.2	1.6	.6	1.0
7:30	1.8	3.2	2.4	3.2	2.4	2.6	2.8	1.8	3.0	2.6	2.6
8:00	4.4	2.6	4.0	3.6	3.8	3.0	3.6	3.2	4.0	4.2	3.6
8:30	3.6	1.6	2.4	4.2	3.2	3.4	3.6	3.2	4.2	5.0	3.4
9:00	6.4	4.8	5.8	5.4	5.2	4.8	3.8	5.0	3.8	4.0	4.9
9:30	7.0	5.4	5.8	8.0	7.0	8.8	5.2	5.6	5.0	8.2	6.6
10:00	5.8	6.2	7.0	5.6	6.2	7.8	7.2	5.6	6.6	6.0	6.4
10:30	6.0	3.6	6.0	6.4	6.4	5.0	5.8	5.2	6.6	6.8	5.8
11:00	5.2	3.8	4.4	4.6	5.4	6.0	3.0	3.0	4.6	4.0	4.4
11:30	3.8	3.2	4.0	4.8	3.8	3.0	2.4	2.2	4.0	2.6	3.4
12:00	1.6	.8	2.6	2.0	2.4	1.6	2.0	1.4	1.4	1.6	1.7
12:30	1.0	1.2	.4	1.0	1.2	.4	.6	1.2	.6	.4	.8
Total	48.4		45.4		48.0		42.4		45.6		44.9
		37.4		50.2		47.2		38.6		46.4	

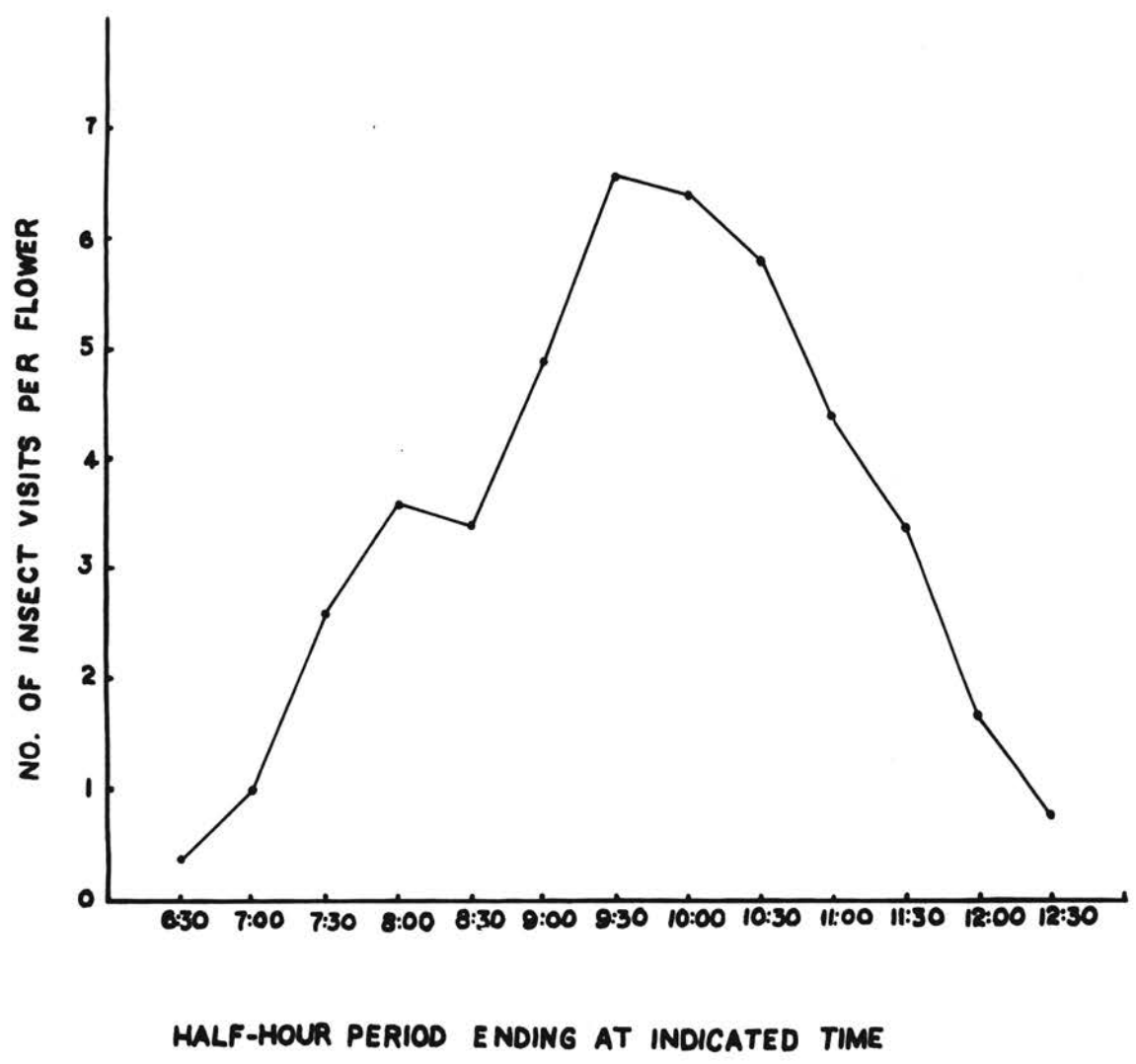


Figure 7--Graphic presentation of insect visitations per flower during time indicated on chart.

Table 4--Rate of dispersal by insects of methylene blue powder from flowers on the first row to flowers on the remaining nine rows of a block of ten 50-foot rows of cotton

Row No.	No. of flowers per row	No. of flowers visited during the indicated half-hour period			
		9:30	10:00	10:30	11:00
1.	21				
2.	13	3	10	--	--
3.	31	13	11	4	3
4.	17	7	6	1	2
5.	21	6	8	5	2
6.	23	11	6	5	1
7.	12	10	1	1	--
8.	22	10	7	5	--
9.	27	7	8	8	4
10.	8	4	4	--	--

DISCUSSION

The role of wind in affecting cross pollination appeared to be of no importance. The results of three days exposure of vaseline coated slides netted only six pollen grains which were caught between the hours of 11:30 A.M. and 12:30 P.M. With the flowers for the most part opening early in the morning, it seems likely that the pollen grains were dried and infertile at this time. These results agree with Balls (7) and Brown (8) who stated that the pollen grains were blown about too late in the day to affect cross pollination.

Three species of bumble bees, B. americanorum (Fabr.), B. fraternus (F. Sm.), and B. auricomus (Robt.) were identified as the most important pollinators of the cotton plant. These findings confirm those of Stephens and Finkner (19), Shoemaker (17), and Pope et. al. (15), who had previously named the bumble bee as the chief vector of cotton pollen.

As soon as the flowers began to open in the morning, insects were in the field. This is in agreement with Allard (4) who stated that the bees began to visit the flowers soon after sunrise just as the flowers started opening.

The beginning of flower opening varied with the climatic factors of the day. Cloudiness or extreme haziness would delay flower opening somewhat. Kearney (11) in Arizona, concluded that as a rule many of the flowers are entered by pollen conveying insects soon after the expansion of the corolla has begun, but at the same locality it was evident that there was considerable variation on different days in the earliness of

the arrival of insect conveyed pollen in the flowers of Pima cotton. This was doubtless to be accounted for by the variation in the weather, and in the kind, number and habits of the pollinating insects.

By 1:00 P.M. very few pollinators were observed foraging in the cotton field. This was thought to be due not to the extreme heat or lack of humidity, but rather to the dried quality of the pollen grains.

There was no apparent trend in the flight pattern of the 20 bees, as evidenced in Figures 1 to 6, other than a tendency for a particular bee to follow a given row for a short distance proceeding from flower to flower. These findings coincide with those of Stephens and Finkner (19). They found that bees generally worked from flower to flower in the immediate neighborhood of a focal center.

With the insect visitation running to such large numbers per flower, it is evident that a high amount of cross fertilization can be expected. This amount will vary with the population of the bees in the cotton field. Stephens and Finkner (19) concluded that the higher the bee ratio, or the greater the number of bees in relation to the number of flowers, the higher was the expected amount of natural crossing.

In the study of insect visitation on the small isolated block of cotton, the results were very striking. At the end of the first 30 minute interval four flowers of the tenth row as well as a few on each of the rest of the rows were noted to have received methylene blue from flowers of the first row. With the 42 inch row spacing the distance from the first to the tenth row was 35 feet. This was less than half the distance mentioned by Stephens and Finkner (19) who found that the dye particles were carried to flowers at a distance of at least 80 feet from the dusted flower. It is not necessarily true that pollen from

the first row would effect pollination in all rows as is suggested by the dispersal of the dye. The dye particles are smaller and dryer than pollen grains and difference in gathering and disseminating the two substances by bees could be expected. Nevertheless, the thoroughness of the bees in flower visitation is demonstrated by the results obtained.

SUMMARY

The agents concerned with dispersal of cotton pollen were studied at Stillwater, Oklahoma during the growing season of 1951. The results are summarized below.

1. Wind was found to be of no importance as a possible means of cross-pollination of cotton in Oklahoma.

2. Three species of bumble bees, Bombus americanorum (Fabr.), B. fraternus (F. Sm.), and B. auricomus (Robt.) were found to be the important agents of cotton pollination.

3. The peak of the bumble bee population in the cotton field was reached between the hours of 9:30 and 11:00 A.M., although bees were visiting the cotton flowers continuously from sunrise until approximately 1:00 P.M.

4. Foraging habits of the three species of bumble bees varied to some extent, but it was not uncommon to see individual bees of any of the species visit from 150 to 200 flowers before returning to their nest.

5. Individual cotton flowers were visited by as many as 90 to 95 bumble bees during a single day, but the average for a five day period was 44.9 visits per flower per day.

6. Bumble bees carried particles of dye for at least 35 feet from flowers which were dusted with methylene blue. A small isolated block of cotton containing 194 open flowers was completely covered by the bees within a period of two hours.

7. It seems reasonable to conclude that the 25 to 30 per cent cross-pollination previously observed near Stillwater is not as high as might be expected on the basis of observed insect activity.

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APPENDIX

Appendix Table I. Number of insects visiting individual cotton flowers during half-hour intervals covering the period from flower opening to early afternoon on August 8, 1951

Time of day	Flower number										Total	Av.
	1	2	3	4	5	6	7	8	9	10		
6:30	0	0	0	0	0	0	0	0	0	0	0	0
7:00	2	1	2	1	1	2	1	4	3	2	19	1.9
7:30	2	3	4	3	2	4	3	2	3	4	30	3.0
8:00	4	4	2	6	3	5	1	4	1	2	32	3.2
8:30	2	2	1	0	1	2	2	2	2	4	18	1.8
9:00	4	4	2	4	3	3	3	3	1	1	28	2.8
9:30	3	1	0	7	5	5	0	2	1	4	28	2.8
10:00	3	1	2	5	8	5	6	3	4	4	41	4.1
10:30	4	2	3	6	6	4	4	3	2	5	39	3.9
11:00	6	3	3	5	5	8	2	3	4	6	45	4.5
11:30	1	2	1	2	2	1	1	0	0	0	10	1.0
12:00	0	0	1	0	1	0	0	0	0	0	2	0.2
12:30	0	0	0	0	0	0	0	0	0	0	0	0.0
Total	31	23	21	39	37	39	23	26	21	32	292	29.2

Appendix Table 2. Number of insects visiting individual cotton flowers during half-hour intervals covering the period from flower opening to early afternoon on August 9, 1951

Time of day	Flower number										Total	Av.
	1	2	3	4	5	6	7	8	9	10		
6:30	0	0	0	1	1	0	1	0	0	0	3	0.3
7:00	1	2	0	1	0	0	3	0	1	0	8	0.8
7:30	2	4	1	5	2	1	2	4	2	1	24	2.4
8:00	7	5	6	2	6	4	8	4	3	4	49	4.9
8:30	4	3	3	5	4	2	4	6	2	2	35	3.5
9:00	6	7	6	6	3	2	4	4	3	2	43	4.3
9:30	3	13	7	6	11	10	10	7	4	6	77	7.7
10:00	10	8	11	1	6	6	1	7	8	4	62	6.2
10:30	6	5	3	4	2	2	5	9	10	3	49	4.9
11:00	6	4	3	2	2	1	2	2	4	1	27	2.7
11:30	5	4	3	2	1	2	4	1	2	2	26	2.6
12:00	1	2	2	3	1	1	2	2	1	1	16	1.6
12:30	0	4	0	2	3	0	0	1	2	0	12	1.2
Total	51	61	45	40	42	31	46	47	42	26	441	44.1

Appendix Table 3. Number of insects visiting individual cotton flowers during half-hour intervals covering the period from flower opening to early afternoon on August 10, 1951

Time of day	Flower number										Total	Av.
	1	2	3	4	5	6	7	8	9	10		
6:30	1	0	0	2	0	0	0	0	1	0	4	0.4
7:00	3	1	1	2	3	1	0	2	1	1	15	1.5
7:30	1	2	3	4	4	2	2	1	4	3	26	2.6
8:00	1	1	6	1	2	1	4	5	5	2	28	2.8
8:30	4	1	2	8	7	4	3	3	2	4	38	3.8
9:00	7	5	6	6	5	6	4	8	1	6	54	5.4
9:30	6	4	6	12	4	8	7	4	6	8	65	6.5
10:00	6	7	10	3	7	9	11	4	6	5	68	6.8
10:30	4	4	8	7	9	4	6	5	5	7	59	5.9
11:00	4	6	5	5	4	6	2	3	5	4	44	4.4
11:30	2	3	7	6	4	2	3	4	4	1	36	3.6
12:00	1	1	2	2	3	0	1	4	1	2	17	1.7
12:30	0	1	0	0	0	1	0	1	1	0	4	0.4
Total	40	36	56	58	52	44	43	44	42	43	458	45.8

Appendix Table 4. Number of insects visiting individual cotton flowers during half-hour intervals covering the period from flower opening to early afternoon on August 12, 1951

Time of day	I	2	3	4	5	6	7	8	9	10	Total	Av.
6:30	0	0	0	0	0	0	0	0	0	0	0	0.0
7:00	I	0	0	0	0	I	0	0	3	0	5	0.5
7:30	I	I	0	0	I	2	I	0	I	2	9	0.9
8:00	4	2	3	4	3	2	4	2	5	7	36	3.6
8:30	3	2	3	4	3	2	7	3	4	7	38	3.8
9:00	9	4	9	6	10	8	5	4	7	4	66	6.6
9:30	12	5	8	5	11	14	6	7	3	8	80	8.0
10:00	6	4	6	8	2	9	4	2	6	6	53	5.3
10:30	8	5	9	7	4	6	8	4	4	9	64	6.4
11:00	7	5	4	2	6	4	3	2	I	4	38	3.8
11:30	3	2	4	7	5	4	I	I	4	5	36	3.6
12:00	3	0	4	3	4	4	2	0	I	2	23	2.3
12:30	I	I	2	0	I	0	2	0	0	2	9	0.9
Total	58	31	52	46	50	56	43	25	39	56	456	45.6

Appendix Table 5. Number of insects visiting individual cotton flowers during half-hour intervals covering the period from flower opening to early afternoon on August 13, 1951

Time of day	Flower number										Total	Av.
	I	2	3	4	5	6	7	8	9	10		
6:30	I	I	0	0	0	0	0	0	0	2	4	0.4
7:00	0	0	0	0	0	0	I	0	0	0	I	0.1
7:30	3	6	4	4	3	4	6	2	5	3	40	4.0
8:00	6	I	3	5	5	3	I	I	6	6	37	3.7
8:30	5	0	3	4	I	7	2	2	II	8	43	4.3
9:00	6	4	6	5	5	5	3	6	7	7	54	5.4
9:30	II	4	8	10	5	7	3	8	II	15	82	8.2
10:00	4	II	6	II	8	10	14	12	9	II	96	9.6
10:30	8	2	7	8	II	9	6	5	12	10	78	7.8
11:00	3	I	7	9	10	II	6	5	9	5	66	6.6
11:30	8	5	5	7	7	8	3	5	10	5	61	6.1
12:00	3	I	4	2	3	3	5	I	4	3	29	2.9
12:30	4	0	0	3	2	I	I	4	0	0	15	1.5
Total	62	36	53	68	60	66	51	51	84	75	606	60.6

VITA

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The content and form have been checked and approved by the author and thesis adviser. The Graduate School Office assumes no responsibility for errors either in form or content. The copies are sent to the bindery just as they are approved by the author and faculty adviser.

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