POLLEN PREFERENCES AND FACTORS WHICH INFLUENCE POLLEN COLLECTION BY THE HONEY BEE *Apis mellifera* L.

by

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INTRODUCTION

The success of pollination of many crops is based primarily on the activity of honey bees, and many conditions probably govern the selection of pollen by bees. In relation to plants, flower odor, color, structure, time of blooming, amount and concentration of nectar, and amount and type of pollen may be factors. Other general factors including weather, brood conditions, and the distance from the hive to the feeding source may influence the activity of bees.

This study concerned some of the factors involved in the selection and recognition of pollen by honey bees when the characteristics of the pollen were separated from the characteristics normally attributed to the flowers of the food plants. Various treatments of the pollens were made to determine if preferences did exist.

It is important to determine which pollens are preferred by honey bees, if the factors causing preference can be isolated, and if these preferred qualities could be introduced to honey bees to cause more efficient crop pollination or to increase acceptability of pollen supplements.

LITERATURE REVIEW

The collection and utilization of pollens by honey bees has been noted by numerous workers. Parker (1926) studied the collection of pollen from various plants and thus determined the availability of certain pollens. The trapping of pollen and the factors involved in its collection were studied by Synge (1947), who found very marked differences in the relative amount of pollens of different species brought in by three colonies. He also indicated a possible difference between hand and bee-collected pollens by the presence of sugars which were incorporated with the pollen during the collection

process, and suggested results might be altered if these pollens were fed back to the bees. A further limitation is using bee-collected pollen, noted by Synge (1947), is that the choice is necessarily restricted to those pollens which the bees actually collect in some quantity. In view of this, he made further studies by offering a choice of red clover Trifolium pratense L. and white clover T. repens L. pollen to the bees within the hive. The results showed highly significant differences between the amount of pollen collected with white clover always being preferred. It was concluded that certain preferences did occur, but that they are common to bees in general and not the direct cause of the observed differences in pollen collection between the colonies.

According to Rashad and Parker (1958a), there were 354.45 grams of sorghum, 793.21 grams of yellow sweet clover, 1,517.56 grams of Indian corn, and 2,484.82 grams of smartweed collected by observed colonies during 1954. Rashad and Parker (1958a) also noted that 175.75 grams of alfalfa and 698.20 grams of sunflower pollen were collected during the summer of 1954. A total of 11,045.08 grams of pollen were collected from March 6 to October 12, 1954, indicating that sunflower and alfalfa pollens are not collected to any great extent in the Manhattan, Kansas area.

Levin and Bohart (1955) conducted experiments to determine preferences of different pollens when the honey bee's choice was not influenced by the structure, color pattern, or odor of the flower in which the pollen is normally found. These tested pollens included alfalfa, gumweed, greaseweed, black mustard, poverty-weed, and a mixture of yellow and white sweet clover. Pollen of black mustard Brassica nigra (L.) Kock collected in 1950 and 1953 was combined in one test to determine if age of pollen was a determining

factor in preference. They found that the mustard pollen collected in 1950 was at least as attractive as the same pollen collected in 1953. They concluded that moisture, sugar, and protein content appeared to have no influence on the attractiveness of the pollens. However, it was determined that the size of the pollen particle and the quantity of material in the feeding container did influence activity of honey bees. Final results indicated that some pollens are more attractive than others to honey bees when offered independantly of flower characteristics present during natural selection by the bees. Also by assuming that methyl salicilate, which was used in an attempt to mask the odors of the pollen, did not sufficiently mask the pollen odors indicated odor as a possible recognition factor in the selection of pollen by honey bees. It was anticipated also that these tests would show if the bees would have a preference for any of the pollens. Light is an important factor in the honey bee's search for a food source. Plants reflect and absorb light differently indicating a possible method for the study of pollen reflectance. Levin and Bohart (1955), using a Weston Master II light meter, concluded that reflectivity of light or color of pollens did not influence bee activity.

Many pollens have a yellow color, and Therese Oettingen-Spielberg (1949), found that bees searching in a room went to yellow more frequently than to blue (Ribbands, 1953). Lovell (1910), however, stated that since bees can be trained to various colors, experiments of this type are not easily interpreted (Ribbands, 1953). Frisch (1950) indicated, in a summary of previous work with various perception tests, the ability of the honey bee to perceive odors and distinguish colors. He found that bees depend on colors from a distance, but that upon close investigation, the food source is decided as the result of the odor sense of the bee. Hence, it was believed that there could be specific

materials present in pollens which might influence the activity of honey bees and which might be recognizable when pollens are tested independently of flower characteristics.

Chemical analysis of pollen indicates differences between various plant pollens collected by bees. It is commonly recognized that pollen is the only source of protein and is essential for successful breed rearing. Auclair and Jamieson (1948) studied the amino acids present in a number of pollens (Lunden, 1954). Lubliner and Mianowska (1955) investigated the pollen of 67 species to determine the presence of pigments, and suggested that bees prefer pollens containing carotenoids for breed rearing. Vivino and Palmer (1944) determined the vitamin content of various fresh bee-collected pollens (Lunden, 1954). These studies noted some of the more suitable pollens for brood rearing with respect no nutritive value of pollen. The literature dealing with the proteins and amino acids, carbohydrates, lipids, vitamins and hormones, pigments, inorganic materials, and other miscellaneous constituents of pollen has been reviewed by Lunden (1954).

Parker (1939) indicated that pollen substitutes or supplements have been divised for use when natural pollens were not available. In all situations in which these supplements were exposed to honey bees, there had not been enough adult bees reared to maintain good colony strength when bees have been reared by the aid of pollen supplements as food for the honey bee according to Parker (1939). Parker (1926) also determined that if natural pollen is available when substitutes are being fed, the pollen substitutes are immediately abandoned. Haydak and Tanquary (1943) found in their study of pollen and pollen substitutes in relation to honey bee nutrition, that beekeepers advise feeding pollen substitutes to bees only when natural pollen is not available. This

indicates that pollen substitutes are not usually preferred over natural pollen by honey bees, but are useful in cases where additional food substitutes are needed.

MATERIALS AND METHODS

Pollen Collection

Five colonies were used in the collection of pollen for these preference studies. The same pollen traps used by Rashad and Parker (1958a) were used for the pollen collection. These traps were altered to eliminate their removal from the colonies when they were not in use. A 5 mesh screen scrapped the pollen pellets from the legs of the bees. However, this screen was not removable unless the entire trap was removed. To prevent disturbing colonies at intervals during pollen collection, the screen was soldered on metal strips one inch wide by 18 inches long. The strips were set in grooves which allowed the screen to be removed in order to allow the colony to replenish it's pollen supply when necessary. Sufficient amounts of yellow sweet clover Melilotis officinalis Lam., indian corn Zea mays L., and smartweed Polygonum Spp. pollen were obtained using these traps. It was necessary to collect sorghum Sorghum vulgare Pens. pollen by hand. For future reference, those pollens referred to as "natural pollens" were the pollens that were bee-collected, using the traps; and the sorghum which was hand collected and which had no treatment other than separation.

Collection of sorghum pollen was done by shaking sorghum flowers into large, (#20) brown Kraft paper bags where both pollen and anthers were accumulated. Considerable amounts of both pollen and anthers were collected in this manner. After separation of the anthers from the pollen using a Rotomatic

Experimental Sifter equipped with a 20 mesh screen, the anthers were dried at 132°F. in a Thelco model 18 oven and then directed onto the fan of a squirrel cage fan and the broken anthers and pollen from them were blown into a paper bag. After this proceedure the pollen was again separated using the Rotomatic Sifter.

Pollen was collected from June 1 until September 6, 1960. Daily visits were usually made to the Kansas State University apairy to remove the pollen. The pollens were separated immediately from foreign material and were stored in a freezer at -4°F. The pollen grains were examined microscopically to identify each species, and then separation with an aspirator was made on the basis of the color of the pollen pellets. The pollen of sunflower Helianthus spp. and alfalfa Medicago sativa L. were originally planned for these studies, but the bees did not collect sufficient amounts and no satisfactory hand collection technique was discovered. It is assumed that competing plants were present in quantities which eliminated any search for sunflower and alfalfa pollen.

Preparation of Pollen

Immediately before each test, the pollen to be tested was prepared.

In the primary preparation of these pollens, measured amounts of solvents were added to a determined volume of pollen. This measurement was used for overall accuracy because different pollens do not weigh the same. The solvents used were absolute ethyl alcohol, anhydrous diethyl ether, and water. Each solvent was used at a rate of 10 milliliters per one heaping teaspoon of natural pollen. Weights were determined for each teaspoon of pollen used in each experiment. Yellow sweet clover and smartweed weighed 4.65 grams, Indian

corn 4.25 grams and sorghum 3.90 grams per teaspoon. After the addition of the solvent, the mixture was shaken vigorously for five minutes and then allowed to set for one hour. This process did not necessarily insure complete extraction of all soluble material from the pollen, but should have been sufficient to remove most of the soluble components. This step in the preparation of the pollens, as well as other phases of preparation, was conducted at room temperature. For future reference, "extracted pollen" was that pollen which remains after it had been washed by a solvent. "Pollen extract" was the liquid material containing the soluble pollen components. After the pollen extract had been added to cellulose or returned to pollen, the material which could not be evaporated is called the "residue". The pollen extract was separated from the pollen by use of a Buchner funnel. At this time, the pollen extract was placed in an airtight glass jar and stored in the refrigerator at 37°F. The extracted pollen was placed in an evaporation hood for preliminary drying and then dried at 132°F. in the Thelco model 18 oven. Pollen taken from the oven was ground to a standard particle size using a Wiley Mill equipped with a 40 mesh screen.

The pollen extracts were added to a cellulose carrier after the tests with the extracted pollen had been completed. Whatman cellolose powder was used as the carrier. Ten milliliters of extract were absorbed by 1.75 grams ofcellulose. This proportion was used throughout the tests with all extracts mixed with cellulose. Cellulose is biologically inert and of no significant value to the honey bee. It was assumed that if that is this material was collected, it would be due to an attractive substance incorporated on the cellulose.

Smartweed pollen was handled somewhat differently than the other pollens

since it clogged the Wiley Mill to such an extent that it could not be ground. The natural bee-collected pollen was dried with a portable Aminco lyophilizer and then subjected to the solvents as described above. After drying in the hood and oven, the pollen was crushed in a mortar to the approximate particle size of the other pollens. The treatment of smartweed pollen was carried out in the same manner as the other pollens as far as washing with the solvents was concerned.

Experimental Arrangements

Pollen was made available to foraging bees about 3 feet in front of the hive. The pollen was placed in petri dishes for the first experiment with yellow sweet clover, but these dishes proved unsatisfactory because they were too shallow and much of the pollen was blown out of the dishes as the bees collected and packed the pollen. As the pollen accumulated outside the dish, the bees attempted to collect it and observations were difficult to make. The petri dishes were replaced in later tests with stendor dishes 2 inches in diameter and 13/16 inches deep. This type of dish limited the number of bees that could feed at a dish, eliminated much of the loss by blowing, and allowed the amount of pollen to be regulated accurately.

The position of the dishes were recorded in relation to the hive and rotated at intervals of ten minutes. Counts of the number of bees feeding at these dishes were taken every 2 minutes or at intervals of 1 minute depending on the number of foragers present for a particular test. Observations were recorded for each material as they were rotated to allow counts to be made in each position. This provided a latin square design of treatments X position replicated by time. Further arrangements and procedures, specific for a

particular test, will be explained in detail under individual experiments. Snedecor's (1959) text was followed for statistical analysis.

Preference studies were conducted inside the entomology greenhouse. The area provided was 15 feet by 30 feet with a peaked roof of light grey plastic which did not appear to affect the transmitted light to any great extent. The greenhouse was heated by steam in the fall. Temperatures could not be regulated closely since the only air conditioning available was fresh air pulled through the greenhouse by a large fan.

A three comb nucleus hive of Italian honey bees served as a source of foragers. A comb and one half of brood with the bees adhering, plus an equal amount of stores, made up the nucleus orininally. A small amount of brood was maintained throughout the tests, however, this decreased in the first part of November about the time the tests were completed. A source of 2:1 sugar water and a constant supply of water were provided. The bees were allowed free flight in the greenhouse. There was a fairly rapid loss of adult bees and there is little doubt that the tests involved only worker bees which had never foraged for pollen. Brood of all ages and food stores were supplied the colony when they were needed.

An enclosed box was constructed to evaluate the effects of odor and sight in relation to activity of honey bees and their selection of pollen. The box was 13.5 inches by 12 inches and 4 inches deep, with a hole $\frac{1}{2}$ inch in diameter drilled in the center of each of four compartments which measured 5 inches by 5.75 inches individually.

In order to determine the color differences between pollens, the light reflectance of natural and treated pollen was measured using a Beckman DU Spectrophotometer equipped with a reflectance attachment. Measurements were

made of the reflectance of wave-lengths of light between 5400 and 3200 Angstroms. The reflectance from a magnesium carbonate standard which reflected 98% of the total light directed on it was compared to the reflectance of light from each pollen sample.

RESULTS AND DISCUSSION

Light Reflectance From Pollen Samples

Reflectance studies were conducted to determine the differences in color among the pollens used. Results, condensed in figures 1, 2, 3 and 4, show very little difference in pollen colors either natural or treated, at any given wavelength.

Yellow Sweet Clover

In this experiment, natural yellow sweet clover pollen was washed with anhydrous diethyl ether, absolute ethyl alcohol, and water. After obtaining the three extracted pollens, natural yellow sweet clover was used as a check. A fifth material, powdered cellulose, was included in this test to determine if cellulose could be used as a carrier for extracts of these pollens in future preference studies. The test materials were added to petri dishes and the number of foraging bees gathering pollen was observed. The position of the dishes in relation to the hive was rotated every ten minutes as observations recorded at 5 different locations of the dishes. Five observations were recorded at 2 minute intervals at each position.

Significant differences were found between pollens treated with certain solvents. The water extracted pollen was significantly more attractive than

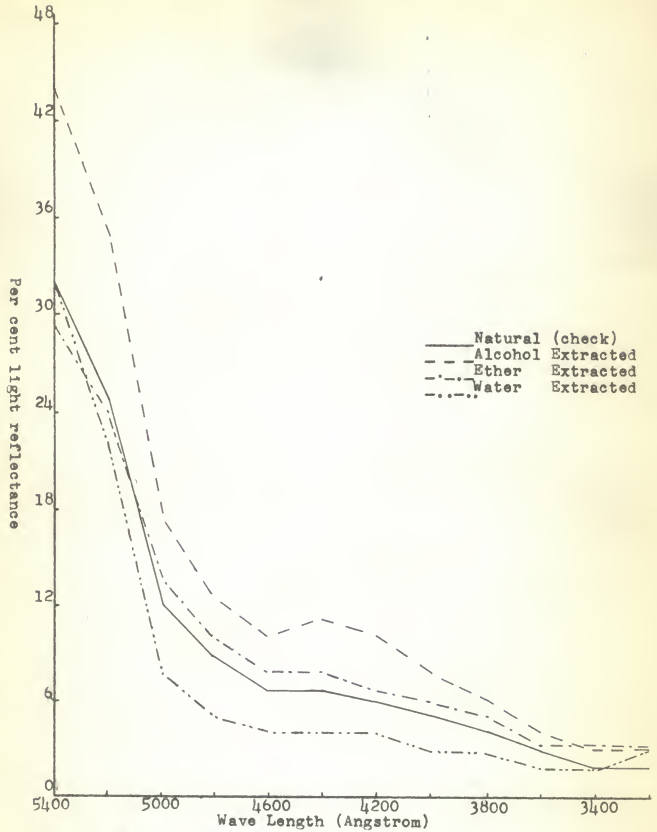

Fig. 1 Light reflectance from yellow sweet clover pollen extracted with solvents.

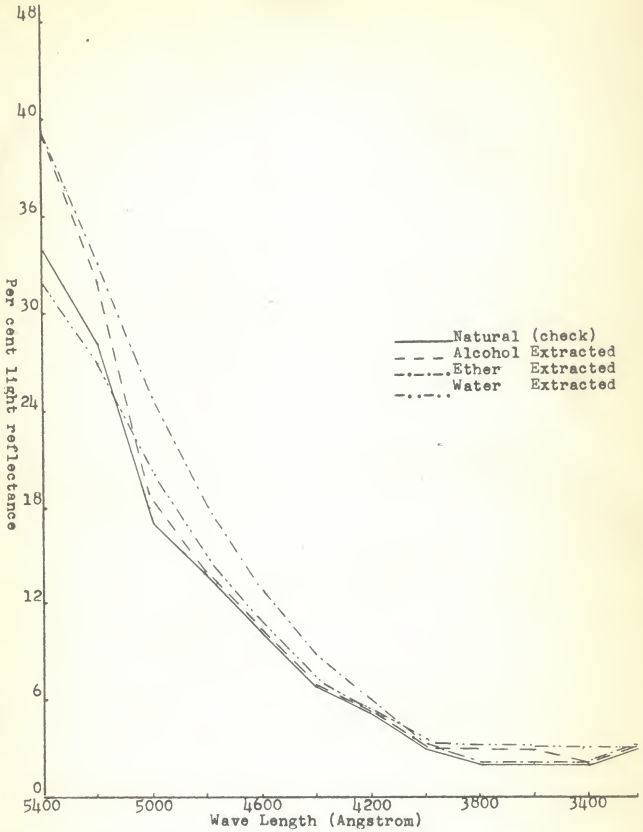

Fig. 2 Light reflectance from Indian corn pollen extracted with solvents.

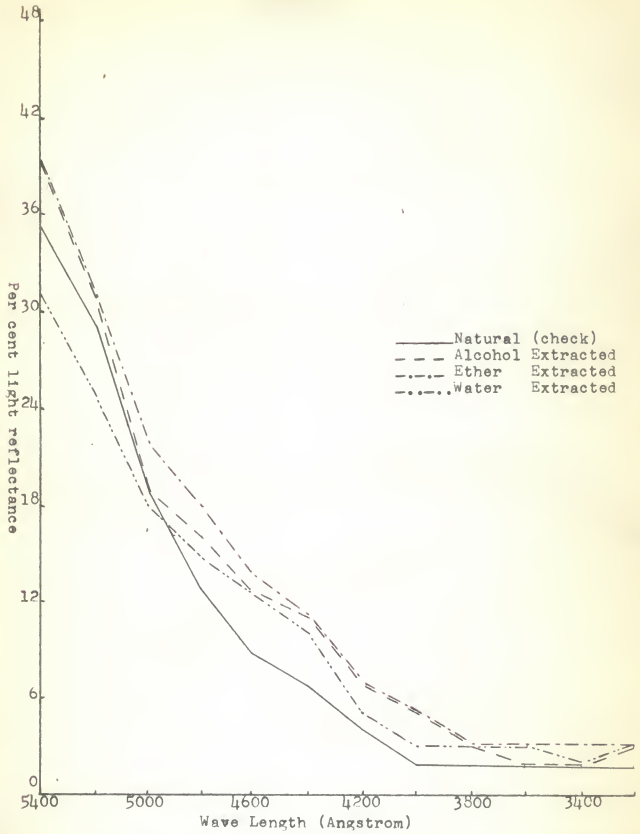

Fig. 3 Light reflectance from sorghum pollen extracted with solvents.

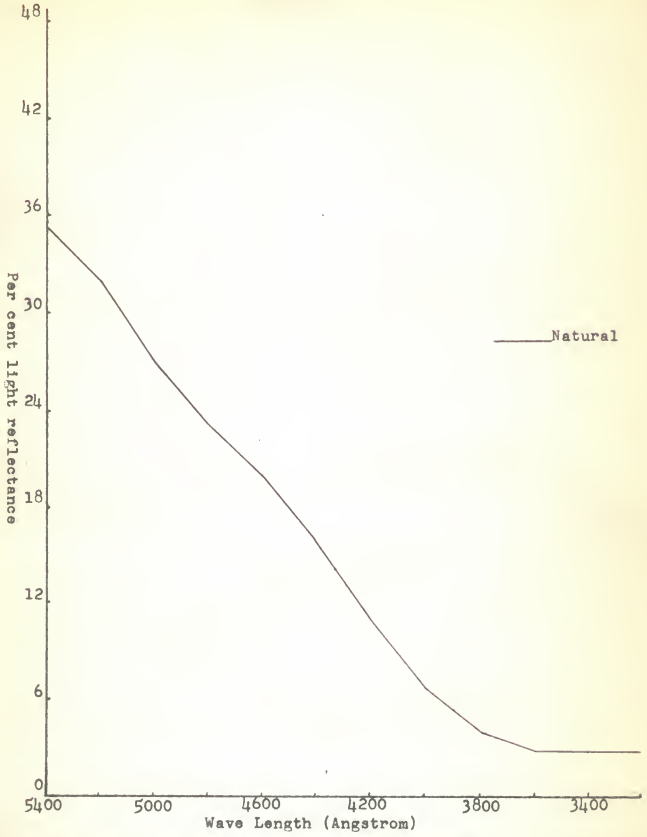

Fig. 4 Light reflectance from smartweed pollen.

Table 2. Counts of the number of honey bees foraging on treated yellow sweet clover pollen and untreated cellulose at 2 minute intervals at each of five dish locations.

Dish location	Cellulose (natural)	Ether extracted pollen	Alcohol extracted pollen	Natural pollen	Water extracted pollen
	0	1	0	4	1
	0	0	0	1	3
A	0	0	0	2	5
	0	0	0	2	5
	0	1	1	3	6
	0	0	1	4	4
	0	1	2	6	5
B	0	0	0	3	10
	0	1	1	2	8
	0	0	0	2	6
	0	0	0	2	9
	0	0	2	3	10
C	0	0	3	1	9
	0	0	4	4	8
	0	0	1	2	6
	0	1	1	3	4
	0	0	1	3	9
D	0	0	3	5	5
	0	0	1	1	8
	0	0	1	2	4
	0	0	1	0	6
	0	1	2	1	6
E	0	1	2	2	6
	0	0	1	1	8
	0	1	2	2	9
Total Visits	0	8	30	61	146
Ave. Visits Observ.	.0	.32	1.20	2.44	5.84

Chemical LSD=1.07

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	4	6.3104	1.5776	.36	ns
Chemicals	4	134.9504	33.7376	77.60	***
Time	4	3.5904	.8976	.21	ns
Error	12	5.2191	.4349		
Total	24				

***Significant at .001 level

alcohol extracted, ether extracted, or the natural pollen. Natural pollen was preferred over alcohol extracted and ether extracted pollen, but there was no significant difference between alcohol extracted and ether extracted pollen. The high amount of activity on water extracted pollen compared to the natural pollen was not expected and is unexplainable. It would appear that a repellant material is removed by water.

The lack of activity on cellulose indicated that cellulose could be used as a carrier for pollen extracts since it was not collected in the natural from. Also shown is the nonsignificance of time between counts, and the nonsignificance of the position of the pollens.

It can be concluded from this first examination of yellow sweet clover pollen that some bee-preferred characteristics were soluble in ether and in alcohol and that a recognizable amount of these characteristics was removed by the solvents.

Yellow Sweet Clover-Cellulose

This test using yellow sweet clover pollen was to determine if the alcohol and ether extracts taken from sweet clover pollen could be incorporated on cellulose, and if the bees would collect the impregnated cellulose.

Table 3, which is not a latin square, again indicates that a considerable amount of a bee-preferred pollen constituent was removed by the ether extraction and some was removed by the alcohol extraction. These constituents, as measured by bee activity, were then found to be present as residues on cellulose. Alcohol extracted pollen was significantly more attractive than the other materials, and both the ether residue and alcohol residue were significantly more attractive than the ether extracted pollen. It appears that

Table 3. Counts of the number of honey bees foraging on treated yellow sweet clover pollen, and on cellulose impregnated with these extracts at 2 minute intervals at each of five dish locations.

Dish location	Ether extracted pollen	Alcohol residue	Ether residue	Alcohol extracted pollen
	4	1	1	3
	1	2	0	2
A	1	1	3	2
	0	2	1	1
	1	3	2	1
	1	1	3	2
	0	0	3	2
B	0	3	3	3
	0	1	2	3
	0	3	2	3
	0	1	2	4
	0	0	1	4
C	0	3	2	5
	0	2	3	2
	1	2	3	2
	0	2	2	3
	0	2	2	3
D	0	2	2	3
	0	3	3	3
	0	3	2	4
	0	1	2	1
	0	1	1	4
E	0	2	2	3
	1	2	3	4
	0	2	1	3
Total Visits	10	45	51	70
Ave. Visits/ Observ.	.40	1.80	2.00	2.80

Chemical LSD=.69

Source of Variation	D/F	Ss	Ms	F	Sig.
Chemicals	3	15.058	5.019	20.83	***
Time	3	.330	.110	.46	ns
Error	16	3.862	.241		
Total	22	19.250			

*** Significant at .001 level

different preference causing materials were removed by the alcohol and ether extractions since there was no significant difference between ether and alcohol residues on cellulose.

Indian Corn

Beginning with this series of experiments, stendor dishes replaced petri dishes. Indian corn pollen, the extracted pollen and pollen residue on cellulose were examined in the same test to determine the relative attractiveness of natural pollen, extracted pollen, and the extract-impregnated cellulose.

As shown in Table 4, alcohol extracted pollen was significantly more preferred than all other materials tested. There was no difference between natural untreated pollen and water extracted pollen, both of which were significantly preferred over ether extracted pollen. Ether extracted pollen was preferred over all extracts on cellulose. When a choice was made between natural or extracted pollen and the impregnated cellulose, the impregnated cellulose was highly non-preferred.

Indian Corn-Cellulose

The second experiment using Indian corn pollen involved the extracts on cellulose to determine bee activity when extracted or natural pollen offered no competition. As shown in Table 5, there was not a significant difference found between these extracts although the alcohol residue showed highest activity. Each solvent removed some factor for attractiveness since all sources were worked by the bees.

The final experiment with Indian corn pollen (Table 6) was initiated to determine if the various solvents that were used previously served as a repelling factor to foraging bees. In the preparation of these pollens, the

Table 4. Counts of the number of honey bees foraging on treated Indian corn pollen, and on cellulose impregnated with these extracts at 2 minute intervals at each of seven dish locations.

Dish location	Water residue	Ether residue	Alcohol residue	Ether ext'ed pollen	Water ext'ed pollen	Natural pollen	Alcohol ext'd pollen
	0	0	0	1	2	1	3
A	0	0	0	1	4	3	3
	0	0	0	1	3	3	2
	0	0	0	0	1	3	1
	0	0	0	1	2	2	3
B	0	0	0	0	3	2	3
	0	0	0	1	3	3	4
	0	0	0	1	3	3	3
	0	0	0	1	3	1	3
C	0	0	1	1	2	5	2
	0	0	1	2	4	3	3
	0	0	0	0	3	3	2
	0	0	0	0	3	2	3
D	0	0	0	1	3	3	2
	0	0	0	1	3	2	3
	0	0	0	0	3	2	3
	0	0	0	1	1	2	3
E	0	0	0	1	1	3	3
	0	0	0	0	2	2	3
	0	0	0	1	1	4	5
	0	0	0	0	1	4	4
F	0	0	0	1	3	3	4
	0	0	0	1	1	3	3
	0	0	0	1	4	4	2
	0	0	0	1	3	4	3
G	0	0	0	0	2	2	4
	0	0	0	1	3	0	3
	0	0	0	2	1	1	5
Total Visits	0	0	2	22	69	73	85
Ave. Visits/ Observ.	0	0	.07	.79	2.43	2.61	3.04

Chemical LSD=.42

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	6	1.4592	.2432	1.69	ns
Chemicals	6	78.0127	13.0021	90.4179	***
Time	6	.4949	.0825	.5737	
Error	30	4.3138	.1438		
Total	48				

***Significant at .001 level

Table 5. Counts of the number of honey bees foraging on cellulose impregnated with various Indian corn pollen extracts at 2 minute intervals at each of three dish locations.

Dish location	Ether residue	Water residue	Alcohol residue
	0	2	2
	0	1	2
A	1	2	3
	1	2	1
	1	1	4
	2	2	5
	1	2	3
	1	4	2
B	1	3	2
	1	2	2
	1	2	2
	2	1	2
	1	2	2
	1	0	2
C	2	0	2
	3	2	2
	2	2	2
	3	2	2
Total Visits	24	32	42
Ave Visits/ Observ.	1.33	1.78	2.33

Chemical LSD=3.07

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	2	.0817	.0408	.05	ns
Chemicals	2	1.5057	.7528	.99	ns
Time	2	.0228	.0114	.01	ns
Error	2	1.5282	.7641		
Total	8				

Table 6. Counts of the number of honey bees foraging on treated Indian corn pollen and on extracts added to the extracted pollen at 2 minute intervals at each of seven dish locations.

Dish location	Ether ext'ed pollen	Water extract on ext'ed pollen	Ether extract on ext'ed pollen	Water ext'ed pollen	Alcohol extract on ext'ed pollen	Natural pollen	Alc. ext. pol.
	1	1	1	1	2	2	2
A	0	2	2	2	2	3	1
	0	3	2	1	2	5	3
	1	1	2	2	1	2	1
B	0	2	1	2	2	3	3
	0	1	2	2	2	4	2
	1	1	2	2	3	1	2
C	1	1	1	2	2	3	2
	1	2	2	3	2	2	3
	2	2	3	1	1	3	3
D	1	2	1	1	3	3	3
	2	2	1	2	4	4	3
	1	1	3	3	3	3	4
E	1	2	3	3	2	2	3
	1	4	2	3	2	3	4
	1	2	1	3	2	1	1
F	3	1	2	3	2	1	2
	1	2	2	4	2	4	4
	2	2	1	2	2	3	2
G	0	1	3	3	4	1	3
	2	3	2	3	4	0	3
Total Visits	22	38	39	48	49	53	54
Ave. Visits/ Observ.	1.05	1.80	1.86	2.28	2.33	2.52	2.57

Chemical LSD=.61

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	6	2.0547	.3424	1.11	ns
Chemicals	6	12.1074	2.0179	6.55	***
Time	6	3.4305	.5718	1.86	ns
Error	30	9.2386	.3080		
Total	48				

usual proceedure was used for preparing the extracted pollen. Preparation of the pollen to be compared was undertaken somewhat differently. The solvents were added to the pollen and then the mixture was placed in a hood to evaporate the solvents. The pollen constituents which had been dissolved in the solvent should have been retained on the pollen when the solvent was evaporated. After drying the pollens were ground to the appropriate size in the Wiley Mill. There were no significant differences between alcohol extracted pollen, natural pollen, alcohol extracts evaporated on alcohol extracted pollen, and water extracted pollen. However, ether extract evaporated on ether extracted pollen and water extract evaporated on water extracted pollen were significantly less preferred than the above four pollens. Ether extracted pollen was significantly less preferred than any other pollen in this test. Since both ether and water addition and subsequent evaporation reduced bee activity significantly, they would both have to be considered as repellants. No reason is known to explain the reduced activity caused by either of the treatments.

Sorghum

Since sorghum pollen was hand collected, it contained no bee-added component. Again the effectiveness of ether in decreasing the bee activity on the extracted pollen was shown as this pollen had significantly less bee activity than did the other pollens shown in Table 7. There was no significant differences between natural pollen, alcohol extracted pollen, and water extracted pollen.

The effect of both chemicals and time were significant as shown in the analysis of variance. This significance of time was expected because only

Table 7. Counts of the number of honey bees foraging on treated Sorghum pollen, at 1 minute intervals at each of four dish locations.

Dish location	Ether extracted pollen	Water extracted pollen	Alcohol extracted pollen	Natural pollen
	0	1	0	1
	0	1	0	1
	0	1	0	0
A	0	2	1	1
	1	2	1	1
	1	1	0	1
	0	1	0	1
	0	2	1	1
	0	0	1	2
	1	1	1	2
	1	1	2	3
B	0	2	2	5
	0	2	2	2
	1	2	1	5
	0	1	3	4
	0	1	1	3
	1	0	3	0
	1	1	2	1
	0	3	2	3
C	1	3	2	4
	1	1	2	1
	1	1	1	1
	0	2	1	0
	0	2	2	2
	1	1	2	1
	1	2	2	2
	2	1	2	3
D	2	1	1	3
	1	2	1	1
	2	2	3	2
	2	2	4	2
	2	3	4	4
Total Visits	23	48	50	63
Ave. Visits/ Observ.	.72	1.50	1.57	1.97

Chemical LSD=.77

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	3	1.9550	.6500	4.76	ns
Chemicals	3	3.2635	1.0878	5.43	*
Time	3	3.4691	1.1564	5.77	*
Error	6	1.2025	.2004		
Total	15				

*Significant at .05 level

40.7% of the total bees were observed during the first half of this test.

Sorghum-Cellulose

In the second test with sorghum pollen, the pollen extracts were added to the cellulose. As shown in Table 8, the activity on alochol and ether extracts on cellulose did not differe significantly even though the alcohol extracted pollen was significantly preferred in the previous tests. Water extract on cellulose was significantly less preferred than alcohol and ether extract on cellulose. This supports the theory that a considerable amount of some preference factor was removed by both alcohol and ether, but the two factors may not be the same. The alcohol extract appeared to be more preferred than the ether extract.

Sorghum-Colored Cellulose

In another examination of sorghum, cellulose was colored yellow by mixing with a non-odorous (Wel*Cote)[1] tusk ivory colored pigment. The extracts were added and the material dried and run through the Wiley Mill. Colors of the cellulose extract not artificially colored (Table 8) were white, tan and light yellow. This test indicated activity was not based on color since the activity per sample remained relatively the same between the two tests (Tables 8 and 9). Results of sorghum pollen extracted with water showed that water was an ineffective solvent in the attempt to extract a preferred material from this particular pollen. It removed some material but hardly comparable to the amounts removed by alcohol and ether. There was no significant difference between the activityeon natural pollen, alcohol residue, and ether residue.

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1 Wel*Cote is made by the Welco MFG. Co., Kansas City, Mo.

Table 8. Counts of the number of honey bees foraging on cellulose impregnated with various sorghum pollen extracts at 1 minute intervals at each of three dish locations rotated twice.

Dish location	Water residue	Ether residue	Alcohol residue
	0	1	1
A	1	2	2
	0	2	1
	0	1	1
	1	0	0
B	0	2	2
	0	1	2
	0	1	2
	0	1	1
C	0	1	3
	0	2	3
	0	2	2
	0	1	2
A	0	1	2
	0	2	3
	0	2	2
	0	2	2
B	0	1	3
	0	2	3
	0	3	2
	0	1	1
C	2	1	2
	1	2	3
	1	2	4
Total Visits	6	36	49
Ave. Visits/ Observ.	.50	2.92	4.08

Chemical LSD=1.34

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	2	.0417	.0208	.14	ns
Chemicals	2	20.0417	10.0208	68.73	*
Time	2	.8750	.4375	3.00	ns
Error	2	.2916	.1458		
Total	8				

*Significant at .05 level

Table 9. Counts of the number of honey bees foraging on colored cellulose impregnated with various sorghum pollen extracts at 1 minute intervals at each of three dish locations rotated twice.

Dish location	Water residue (colored)	Ether residue (colored)	Natural pollen	Alcohol residue (colored)
	0	1	2	1
A	0	1	1	1
	0	1	2	0
	0	1	3	1
	0	0	2	1
B	0	1	3	2
	0	1	1	2
	0	1	1	2
	0	1	0	1
C	0	2	0	1
	0	2	1	1
	0	2	0	2
	0	1	1	1
D	0	1	2	2
	0	2	2	2
	0	1	2	2
	0	1	2	1
A	0	2	1	3
	0	1	4	2
	0	0	2	2
	0	1	2	1
B	0	2	1	2
	0	3	1	2
	0	1	1	1
	0	1	0	3
C	0	1	1	2
	0	2	2	3
	0	2	3	3
	0	3	2	3
D	0	3	3	2
	0	2	2	4
	1	1	3	2
Total Visits	1	45	53	58
Ave. Visits/ Observ.	.06	2.81	3.31	3.63

Chemical LSD=1.48

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	3	.7617	.2539	.35	ns
Chemicals	3	31.8242	10.6081	14.47	**
Time	3	2.4179	.8060	1.10	ns
Error	6	4.3985	.7331		
Total	15				

**Significant at .05 level

Smartweed

After the prescribed preparation of the pollen with the different solvents, it was offered to the bees in the same manner as in previous tests with other pollens. Activity could not be recorded because no bees collected this pollen; the reason is unknown.

Natural Pollen Preference

Each of the natural pollens (Table 10) of yellow sweet clover, Indian corn, sorghum and smartweed were incorporated into one test to determine which natural pollens were preferred.

Indian corn was significantly most preferred, sorghum and yellow sweet clover were approximately equally attractive, and significantly higher than smartweed, upon which no activity was observed.

Natural Water Extracted Pollen Preference

A separate test using the natural pollens washed with water was indicated due to the reaction of yellow sweet clover pollen shown in Table 2. In that initial test, yellow sweet clover pollen extracted in water was highly preferred over the pollen in the natural form. The results shown in Table 11 confirm the previous work as yellow sweet clover extracted pollen was again preferred. Basically, it appears that the water removed a repellant material found in yellow sweet clover pollen. There was no difference between the Indian corn and sorghum extracted pollen. Smartweed extracted pollen was not collected.

Table 10. Counts of the number of honey bees foraging on natural pollen at 1 minute intervals at each of four dish locations repeated twice.

Dish location	Smartweed	Yellow sweet clover	Sorghum	Indian corn
	0	2	1	2
A	0	1	0	1
	0	1	1	2
	0	0	1	0
	0	1	0	2
B	0	0	0	2
	0	0	0	3
	0	1	0	1
	0	2	0	1
C	0	1	0	2
	0	1	0	1
	0	0	0	1
	0	1	1	1
D	0	1	3	3
	0	1	1	1
	0	1	1	3
	0	2	1	3
A	0	0	1	4
	0	1	1	2
	0	0	1	2
	0	0	1	3
B	0	0	2	3
	0	0	1	3
	0	1	1	4
	0	1	2	3
C	0	1	2	4
	0	1	2	3
	0	2	1	2
	0	1	1	3
D	0	1	0	2
	0	1	1	3
	0	1	2	2
Total Visits	0	27	29	72
Ave. Visits/ Observ.	0	1.64	1.81	4.50

Pollen LSD=.87

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	3	.9688	.3229	1.27	ns
Pollens	3	41.5313	13.8437	5.42	*
Time	3	.4688	.1563	.61	ns
Error	6	1.5311	.2552		
Total	15				

*Significant at .05 level

Table 11. Counts of the number of honey bees foraging on natural water extracted pollen at 1 minute intervals at each of four dish locations repeated twice.

Dish location	Smartweed extracted pollen	Sorghum extracted pollen	Indian corn extracted pollen	Yellow sweet clover extracted pollen
	0	0	2	3
A	0	0	2	2
	0	0	2	3
	0	0	1	4
	0	1	0	1
B	0	1	0	1
	0	0	2	3
	0	0	2	4
	0	1	1	3
C	0	1	2	2
	0	0	1	3
	0	0	0	4
	0	0	1	3
D	0	2	0	3
	0	3	2	3
	0	2	2	4
	0	2	1	4
E	0	1	1	3
	0	0	1	3
	0	0	0	3
	0	1	0	1
F	0	0	1	3
	0	0	1	4
	0	0	2	5
	0	2	1	3
G	0	1	1	2
	0	1	0	2
	0	1	0	1
	0	1	0	2
H	0	2	1	2
	0	2	0	4
	0	1	2	4
Total Visits	0	26	32	92
Ave. Visits/ Observ.	0	.81	1.00	2.88

Pollens LSD=1.15

Source of Variation	D/F	Ss	Ms	F	Sig.
Positions	3	1.7657	.5886	1.33	ns
Pollens	3	70.9219	23.6406	5.34	**
Time	3	1.3907	.4636	1.05	ns
Error	6	2.6561	.4427		
Total	15				

*Significant at .05 level

Activity of Observed Bees Under Varying Conditions

The question of what specific factors might influence the foraging workers in their selection of a preferred pollen remained unanswered during these tests. Because of the semi-controlled condition of these experiments, many natural characteristics are absent. In order to evaluate the effects of sight and odor, a box was constructed as was earlier described. Stendor dishes were filled with the four natural pollens and were placed inside the box, one in each compartment 2 inches below the entrance hole.

Table 12. Counts of bee activity on pollen when pollen was visible, when it was enclosed in a box, and when it was placed in a box with foreign odor added.

OUTSIDE BOX (Visible)

October 24, 90°F.

Pollen	Visits
Smartweed	0
Yellow Sweet Clover	27
Sorghum	29
Indian Corn	72

(Data summarized from Table 10)

WITHIN BOX (Hidden from View)

October 26, 85°F.

Pollen	Visits
Smartweed	0
Yellow Sweet Clover	8
Sorghum	21
Indian Corn	32

(unscented)

November 2, 78°F.

Pollen	Visits
Smartweed	3
Yellow Sweet Clover	28
Sorghum	29
Indian Corn	35

(Scented with natural peppermint)

The box was allowed to remain stationary for 20 minutes and at the end of this time, the box was rotated 90° and the results were observed. The box again was turned 90° and observations made. This was continued until each pollen

had been observed in each position at intervals of 10 minutes.

Results of these tests (Table 12) shows the activity observed on pollens which were visible and those which were not visible to the foraging bee. In the test of November 2, attempts were made to mask the odors of the pollens by placing a 5 milliliter vial of pure peppermint oil extract inside each compartment beside the pollen sample. The results did not basically change from the tests carried out on October 26 when no masking odor was present, and the test of October 24 when the pollens were visible to the bees. Initially, the bees appeared to be somewhat confused by the added odor, but after a short time the bee activity indicated that the previously preferred pollen could be detected even though a foreign odor was present.

In further tests on the behavior of the foraging bees, tests of November 7 and 8 were conducted. Conditions present in these tests were different, and more activity was directed to smartweed pollen which had not been preferred previously (Table 13).

Table 13. Total bee visits to four natural pollens.

WITHIN BOX

November 7, 74°F.		November 8, 87°F.	
Pollen	Visits	Pollen	Visits
Smartweed	18	Yellow Sweet Clover	16
Yellow Sweet Clover	30	Indian Corn	29
Indian Corn	35	Smartweed	30
Sorghum	36	Sorghum	38

The amount of brood present had decreased after November 2, and on November 8 brood was absent in the nucleus. This is the usual condition of the hive during the latter part of the period when smartweed is collected in the field. Even though smartweed pollen collected in the fall may not be used

for brood rearing, it is useful. Haydak and Tanquary (1943) noted that even in winter, pollen is utilized by the bees, and the amount of the available pollen present in the hive influences wintering and the subsequent spring development of the colony.

Since the temperatures on November 2 and 7 had been relatively low, it was theorized that a repellant factor which seemed to be active at high temperatures might not be released at low temperatures. The test on November 8 was run at a higher temperature to determine if activity on the smartweed would decrease. It was concluded that higher temperature did not release a repellant material.

It was necessary to mark a number of bees to observe individual behavior in foraging from the box. A toothpick was used to apply artist's oil pigment paint dissolved in ethly acetate to the thorax of each of several foraging bees. The marked bees returning from the hive would fly without hesitation to the original hole used in the collection of the first pollen load. Immediately, however, the bee discovered a change if the box had been turned, and she then attemptedtto find the preferred pollen by investigation of each hole. Upon finding the correct location, the bee would go inside the box to collect the pollen. It is assumed that an odor difference was the differentiating factor.

Forty-seven observations of one bee foraging on natural pollen, were made, and during this time the forager left 8 times to investigate other pollens before continuing to collect the preferred pollen. The pollen dishes were shifted at 10 minute intervals to eliminate a training to the location of a particular food source. The honey bees were found to be specific to a species once foraging had begun. When one of the pollen sources was removed,

bees specific for that particular pollen investigated the other pollens still available, but then stopped collecting pollen and returned to the hive.

Another experiment was conducted, in an abbreviated form, using extracts of the four natural pollens on filter paper. Small strips of Whatman No. 1 filter paper, one inch in width, was placed in dishes filled with the pollen extracts. After the papers had absorbed the extracts, they were dried in an evaporating hood. The strips of paper were then placed in petri dishes and bee activity was observed. Figure 6 pictures honey bees investigating impregnated filter paper strips placed in petri dishes and bee activity was observed. Figure 6 pictures honey bees investigating impregnated filter paper strips placed in petri dishes. These foraging bees had previously collected pollen from dishes, but since they could not collect any material from the papers, individual bees made only one or two investigations of the papers. Although bees would not continue to attempt to forage on the papers, this attempted foraging indicates the presence of some pollen recognition factor which was incorporated on filter paper by the pollen extracts.

EXPLANATION OF PLATE I

Fig. 5. A honey bee investigating impregnated filter paper.

Fig. 6. Honey bees investigating impregnated filter paper.

PLATE I

Fig. 5.

Fig. 6.

GENERAL DISCUSSION

The bees which were used in these tests had no previous experience collecting pollen and should not have had a previously developed preference for a specific pollen.

Treatment of different pollens with various solvents indicated that all pollens contained some attractive materials which were soluble in either alcohol or ether. Of the three solvents used, ether was responsible for removing the greatest amount of some unknown preference factor as measured by bee activity on extracted pollen. Alcohol measured in this same manner was not particularly effective in removing preference causing materials. When the alcohol extracts were impregnated on cellulose, however, the moisture was usually the most attractive of the residues tested. Extraction of pollen with water did not reduce bee activity appreciably, however, some components of pollen which were recognizable to the bee were removed from Indian corn and sorghum pollens.

Natural yellow sweet clover pollen, extracted with water, was observed in two tests to be highly preferred over the identical pollen not washed with water. This reaction was not shown by any other water extracted pollen. Apparently there was some unknown water soluble bee-repelling material present in natural yellow sweet clover pollen.

Odor was believed to be the primary factor responsible for the recognition of the pollens used in these tests. It should be noted that odor may not be the result of the preference causing material. The substance causing preference may have been assioclated with some secondary odor-causing material. When pollen extracts were incorporated on a cellulose carrier, bees were able to recognize and show preferences for extracts. These bees were probably

only recognizing the residue which most nearly approximated the preferred materials of the previous tests in which natural and extracted pollens had been available.

Major disadvantages in the use of cellulose appeared to be the fact that it was extremely light, it was blown out of the dishes when bees hovered over it, and it did not appear to pack well on the legs of the bees.

It was apparent that honey bees could select pollen on the basis of characteristics of the pollen without regard to the other floral characteristics or other factors present under natural conditions. Whether this occurs under field conditions probably involves many factors which are difficult, if not impossible, to test.

It is evident that additional studies are needed in the isolation and identification of the preferred properties found in pollen. In the future, it may be found that the use of synthetic or natural components of pollens may be helpful in increasing bee activity on certain crops or in increasing the attractiveness of pollen supplements or substitutes. This study has not been sufficient to determine whether or not plants of the same species differ in pollen characteristics, or if there might be a possibility of breeding plants to obtain a pollen more acceptable to honey bees.

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POLLEN PREFERENCES AND FACTORS WHICH INFLUENCE POLLEN COLLECTION BY THE HONEY BEE *Apis mellifera* L.

by

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AN ABSTRACT OF A THESIS

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Studies of the pollen preferences of foraging bees were studied at Kansas State University between July 5, and November 8, 1960. Pollens collected and used in these preference studies included yellow sweet clover Melilotis officinalis Lam., Indian corn Zea mays L., sorghum Sorghum vulgare Pens., and smartweed Polygonum spp.

In an attempt to remove attractive features from pollen, solvents of anhydrous diethyl ether, absolute ethyl alcohol and water were added to samples of each pollen. After the separation of extracted pollen from the extract, the extracted pollens were dried, ground, and offered to a three comb nucleus hive of Italian bees in a greenhouse. Results indicated that materials attractive to the bee could be removed from pollen. Organic solvents were more effective than water in removing these materials.

Pollen extracts were incorporated on powdered cellulose and results showed that these extracts contained soluble components of pollen since the bees collected the impregnated cellulose.

Pollen odor was assumed to be the factor by which honey bees recognized the differences in pollens after preferred pollens were selected. Color was not an apparent factor in the selection of pollen. Honey bees became collectors of a specific species of pollen when a choice of pollens was given. Hive conditions may cause preference of pollens.