Table 3. Enzymatic reduction of ketoproline to hydroxyproline. One milliliter of dialyzed supernatant fraction from a 1:2 KCl homogenate of rat kidney was used. All of the incubation beakers contained 0.5 ml of 0.5*M* phosphate buffer, *p*H 7.4; 1 mg of ketoproline; 5 μmole of nicotinamide, and 1 ml of rat-kidney preparation in a final volume of 3 ml. Where indicated, 0.5 μmole of DPN, 0.26 μmole of TPN, 200 μmole of glucose, and 250 units of glucose dehydrogenase were added. After 1.5 hours of incubation, hydroxyproline was assayed by a modification of the Wiss method (*2, 8*).

System	Hydroxyproline (μg)
DPN + glucose dehydrogenase system	42.7
TPN + glucose dehydrogenase system	52.5
DPN or TPN without glucose dehydrogenase	< 3.5

hydroxyproline. This reaction was found to require the presence of reduced pyridine nucleotides, either as such or generated in the incubation mixture by the glucose dehydrogenase system (*4*), as shown in Table 3. Reduced TPN (*5*) was found to be more active than reduced DPN. The rat-kidney preparation could not be replaced by purified commercial alcohol or lactic dehydrogenases. Neither reduced DPN nor reduced TPN was effective in the absence of the rat-kidney preparation.

The inhibitory effect of ketoproline on hydroxyproline metabolism is clearly established in these studies. The enzyme responsible for the reduction of ketoproline and the physiological significance of this reaction are under investigation.

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29 August 1958

Direct Observation of Evaporation from Quiescent Water

Abstract. The color change of a filter paper impregnated with cobaltous chloride and held just above the surface of water gives a good indication of the rate at which evaporation proceeds from individual regions of the surface. The marked effect of some monolayers on thermal convection currents within the liquid can be thus shown.

The usual technique of measuring the rate of evaporation of water from a quiescent surface and the effect of monolayers upon this is rather elaborate (*1*) yet does not provide any information about local conditions over small portions of the area studied. This report (*2*) presents a few observations based on a simple technique which gives qualitative but very direct visual information about the rate of evaporation and shows what happens over areas of the order of a few square millimeters. The technique is based on the color change produced by the vapor reaching a sheet of paper impregnated with cobaltous chloride and held very close to the surface.

Figure 1 shows the pattern—which was actually pink on blue—obtained when the indicator paper was placed above a square cell, 2 by 2 cm, filled with water whose surface was divided into two parts by a polyethylene barrier. To the left of the barrier the surface was clean, while some cetyl alcohol was sprinkled over the surface to the right of the barrier. In the photograph, taken 2 minutes after the paper was placed above the surface, the difference in the rates of evaporation from the two sides is strikingly apparent. Over the clean surface the paper is already pink, while over the monolayer it is still largely blue. In addition, the color change over the clean surface is uniform (it developed uniformly from the beginning), while over the protected part the change appears in spots, which gradually spread over the whole area.

Similar irregular development of the color, signifying uneven rate of evaporation in the presence of the monolayer, was observed with a variety of vessels. It is attributed to the presence of relatively large convection currents which rise warm, cause relatively rapid evaporation, and are thus cooled so that the rate of evaporation is reduced while they continue along the surface for a distance before finally sinking. On a clean surface the convection pattern is different, and local differences are much smaller. This interpretation is supported by observation of convection currents made visible by very slow injection of a very dilute solution of fluorescein into the surface. The convection currents, while irregular, seem to be more extended in the presence of the monolayer, and their general pattern corresponds to that of the spots on the indicator paper. Changes in the thermal resistance of the water, reported previously (*3*), are also in agreement with this observation.

This change in convection currents is connected with the well-known hysteretic resistance of a monolayer against extensions and contraction (*4*). When a cooled streamline of water detaches itself from the surface and sinks under the influence of gravity, the corresponding surface must shrink. Conversely, when a rising warm streamline reaches the surface it causes, necessarily, a local expansion of the surface. When the surface is clean, such expansions and contractions encounter no resistance, but when a monolayer is present they are impeded, and convection at the surface develops only over greater distances and when larger forces are present. The heat transport to the surface from the bulk of the water is thus necessarily affected and localized.

In the experiments under discussion (*5*), the filter paper was firmly attached to a glass plate, both to insure an even surface and to prevent access of vapor from the back. The plate was first covered with a thin layer of "rubber cement for pasting paper," and the filter paper was firmly pressed onto it. The whole was then submerged in a moderately concentrated solution of $CoCl_2$ (prepared without heating), excess liquid was pressed out between filter papers, and the assembly was dried in a vacuum desiccator. The rims of the vessels had to be coated with paraffin to prevent creeping of the water. After the rim had been adjusted to the horizontal, the vessel was filled with water to within about 1 mm of the top. The water surface was protected, when protection was desired, by manual sprinkling of a few specks of commercial cetyl alcohol upon it. A wait of a few minutes allowed the convection currents to develop and stabilize. The glass-backed indicator paper was then

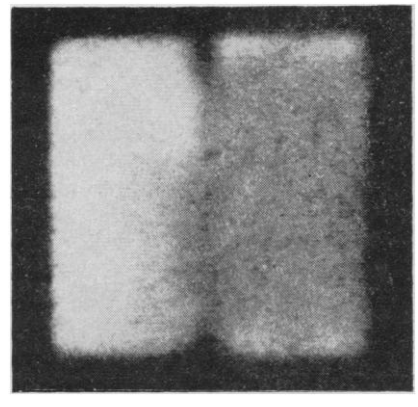

Fig. 1. The local pattern of evaporation from a water surface 2 cm square; the left side is clean, the right side is protected by cetyl alcohol. This photograph of cobaltous chloride indicator paper was taken 2 minutes after the paper was placed above the water surface.

placed on the rim and observed. A photographic record could be obtained on color film or on panchromatic film; a very deep red filter was used with the latter.

For visualization of convection currents, a solution of fluorescein sufficiently dilute (about 50 mg/lit.) to make any density difference negligible was injected from a mechanized syringe at a rate slow enough (about 10^{-3} ml per 3 minutes) to reduce any disturbance of the natural convection to a minimum. The path of the dye was observed with side illumination against a black background.

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15 September 1958

Cotton-Flower Visitation and Pollen Distribution by Honey Bees

Abstract. A rapid method of estimating the pollinating efficiency of honey bees in cotton is described. A prevailing average of 10 honey bees in each 100 flowers was found to be sufficient to cause practically all the stigmas to become coated with pollen.

The recent discovery by Eaton (*1*) that cotton can be rendered male-sterile by spraying with a chemical has opened the way to production of hybrid cotton. The possible use of pollinating insects in the transfer of pollen to these male-sterile plants has aroused interest in the activity of honey bees (*Apis mellifera* L.) in the cotton flower. In normal flowers not visited by pollinating insects the part of the stigma not in contact with the anthers is usually free of pollen. When there is extensive honey-bee visitation the stigma becomes well coated with pollen.

In 1955 and 1956 experiments were conducted at Sahuarita, Ariz., to determine the number of honey-bee visitors to normal cotton flowers in relation to distribution of pollen over the stigma. In 1955 the test field contained about 40 acres of Pima cotton (*Gossypium barbadense*), primarily the variety Pima S-1. There was no other cotton within a mile, but flowering desert plants were abundant during the cotton-flowering period. More than 200 colonies of honey bees were placed on the borders of this field, but because of the competition by the desert flora, the rate of five colonies per acre of cotton is misleading. In 1956 the field contained almost 80 acres of Pima cotton, and again slightly more than 200 colonies were supplied, but there was less competition by desert flora.

In both seasons at weekly intervals throughout the flowering periods counts were made, between 10 A.M. and noon, of (i) the number of Pima S-1 flowers in designated plots throughout the field; (ii) the number of bees seen in them as the observer walked along the row; and (iii) the number of stigmas, viewed under × 3 magnification, which appeared well coated with pollen above the anthers. The Pima S-1 flower is well suited for this observation, as its stigma may extend as much as 20 mm above the uppermost anther. The presence of pollen on this area is usually evidence that insects have been in the flower. These counts are summarized in Table 1.

They show that in both years there was a high correlation between honey bees observed in the flowers and pollen-coated stigmas. Ordinarily honey bees show preference for extrafloral nectar of cotton over nectar from within the flower, but when enough bees are present, both kinds are collected. The bees seldom collect cotton pollen for storage within the hive. Whether pollen observed on the stigma was from the same flower or from other flowers was not determined. However, honey bees usually emerged from cotton flowers thoroughly dusted with pollen and often entered the next flower without cleansing themselves. The coated stigmas, therefore, would be indicative of exposure to cross-pollination, and the correlation between honey bees and coated stigmas would indicate effectiveness in relation to floral visitation.

There was no significant correlation between the small number of wild bees seen in these flowers and stigma coverage, but the highest wild-bee count obtained was only 1.3 per 100 flowers early in the season, when only eight flowers were present per 100 feet of row.

Table 1. Weekly counts of flowers of Pima S-1 cotton, bee visitors, and pollen-coated stigmas in Sahuarita, Ariz., 1955 and 1956.

Date	Flowers/ 100 ft of row	Bees/100 flowers		Coated stigmas/ 100 flowers
		Honey bees	Wild bees*	
		1955		
11–16/7	36	0.1		25.5
18–23/7	65	4.2		63.6
25–30/7	37	6.3		86.3
1–6/8	66	2.5		43.1
8–13/8	64	1.0		39.4
15–20/8	78	0.6		16.9
22–27/8	63	0		27.9
29/8–3/9	49	1.8		46.1
		1956		
25–30/6	8	9.2	1.3	88.0
2–7/7	16	16.6	1.0	95.1
9–14/7	28	16.9	0.3	96.4
16–21/7	44	4.4	0.3	69.6
30/7-3/8†	108	0.2	0	28.1
6–11/8	71	1.2	0.1	41.1
13–18/8	74	1.9	0.2	50.9
20–25/8	123	3.3	0.2	56.4
27–31/8	89	8.2	0.3	85.8

* No counts were made in 1955.
† No data were collected for the week of 23–28 July.

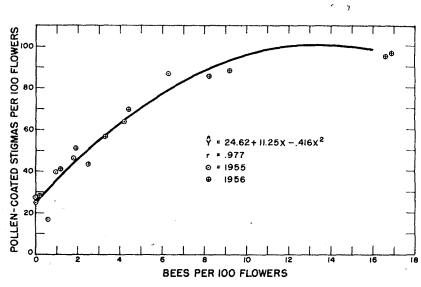

Fig. 1. Relationship between the number of honey bees in 100 flowers of Pima S-1 cotton and the number of stigmas of the flowers coated with pollen during the flowering seasons of 1955 and 1956 at Sahuarita, Ariz.

Figure 1 shows the relationship between floral honey-bee visitors and stigmas coated with pollen. It shows that with increased numbers of floral visitors there was repetition of visits to individual flowers, so that increase in the number of bees to more than about 10 per 100 flowers did not increase their effectiveness. When less than this number visited the flowers not all stigmas became well coated.

In calculating the curve, the relationship $\hat{Y} = 24.62 + 11.25\ X - .416\ X^2$ ($r = .977$) was found to be superior to the straight-line function $\hat{Y} = 36.33 + 4.37\ X$ ($r = .888$), where $\hat{Y}$ is the predicted number of coated stigmas per 100 flowers and X is the number of bees per 100 flowers, by testing in an analysis of variance the additional reduction of deviation from regression due to fitting a curve (*2*).

The same method could be used to determine the population and value of wild bees in other areas. The number of such bees necessary to achieve the desired stigma coverage would probably vary with the species.

Bees did not show equal attention to all flowers. Occasionally a Pima S-1 flower failed to open perfectly or was not favorably exposed to pollinating insects. This may be another reason why stigma coverage failed to increase in direct ratio to bee-population increase. As all flowers were examined, the maxi-