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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5. Miss Jeanne Hotchkiss' help was was most valuable in the development and performance of these experiments.

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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.

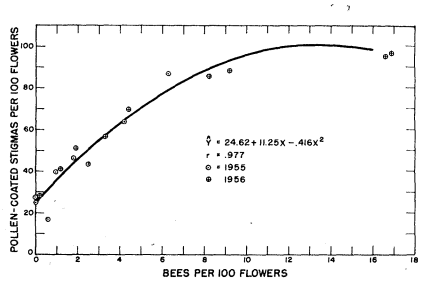

Fig. 1. Relationship between the number of honey bees in 100 flowers of Pima S-1 cotton and the number of stigmas of the flowers coated with pollen during the flowering seasons of 1955 and 1956 at Sahuarita, Ariz.

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-

mum activity of honey bees was seldom shown.

The number of colonies per acre necessary to maintain the desired floral visitation is not known and will doubtless vary according to the acres of cotton involved and the competition by other plants for the bees' attention. Ten colonies per acre may not be enough for small fields surrounded by competing plants, whereas one colony per acre may be sufficient in fields of several hundred acres. The extent of floral visitation by the bees is a good indicator of the number of colonies required (*3*).

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1. F. M. Eaton, *Science* 126, 1174 (1957).
2. The assistance of Henry Tucker, statistician, College of Agriculture, University of Arizona, is gratefully acknowledged.
3. This study was carried out in cooperation with the University of Arizona Agricultural Experiment Station, the U.S. Department of Agriculture Cotton Field Station (Sacaton), the Arizona Cotton Planting Seed Distributors, Inc., and the Arizona State Beekeepers' Association.

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Phospholipase-Induced Release of Cytochrome *c* from the Electron Transport Particle

Abstract. Digestion of the electron transport particle with phospholipase A results in the loss of its oxidative capacity. Evidence presented indicates that this is primarily due to the cleavage of the phospholipid-cytochrome *c* complex within the mitochondria.

In recent years there has been considerable discussion of the discrepancy between the activities of exogenous (free) and endogenous (bound) cytochrome *c* (*1–4*). It has been shown in this (*5*) and other (*2, 4*) laboratories that bound cytochrome *c* is only in small part extractable with salt solutions from particles derived from heart mitochondria. Thus, the bound cytochrome *c* of the electron transport particle is not extractable at all by 0.1*M* phosphate buffer, *p*H 7.4 (*6*), although the existence of a hemoprotein with an α band at 550 mμ can be confirmed by low-temperature spectroscopy (*7*). After the electron transport particle has been exposed to deoxycholate, an extract can be obtained which contains a hemoprotein with the α band characteristic of cytochrome *c*. When this extract is thoroughly dialyzed to remove bile salts, the hemoprotein precipitates out of solution and the precipitate can be redissolved only in deoxycholate solution. In an analogous fashion an insoluble hemoprotein with the spectral characteristics of cytochrome *c* can be extracted from the electron transport particle after treatment with isooctane. This particulate hemoprotein can also be dissolved in deoxycholate solutions (*6*).

We now find that cytochrome *c* is also extractable from the electron transport particle when the latter is exposed to the action of phospholipase A in the presence of calcium ions, but in this instance the hemoprotein is in the soluble, classical form. The hemoprotein is taken up on an IRC-50 column and eluted, under conditions described by Margoliash (*8*), for cytochrome *c*, and the spectral characteristics of the column-purified hemoprotein are indistinguishable from those of authentic cytochrome *c*.

The particulate forms of the hemoprotein obtained by deoxycholate and isooctane treatment of the electron transport particle are also converted to the soluble form of cytochrome *c* by the action of phospholipase A.

When bound lipid cytochrome *c* is released from the electron transport particle by deoxycholate or isooctane, a requirement for cytochrome *c* in the oxidation of both reduced diphosphopyridine nucleotide (DPNH) and succinate by molecular oxygen emerges (*3, 6*). The same relationship has now been observed for the phospholipase-treated electron transport particle. Coincident with the release of cytochrome *c*, the succinic and DPNH activities of the electron transport particle decline, and these activities are in part restored by the addition of externally derived cytochrome *c*. There are significant differences, however, in the respective decay curves for DPNH and succinic activity. The former activity is lost almost immediately in the phospholipase-treated electron transport particle, whereas the latter activity declines more gradually (see Table 1) and reaches a maximum coincident with the maximal release of bound cytochrome *c*. Furthermore, restoration of DPNH oxidase activity by addition of cytochrome *c* is not as complete as the restoration of succinoxidase activity. A similar pattern of decline and restoration of the two activities has also been observed with the isooctane-treated electron transport particle. These observations suggest (i) that phospholipase may release components other than bound cytochrome *c* which are required in the DPNH oxidase chain exclusively; (ii) that more than one form of bound cytochrome *c* is present in the electron transport particle; or (iii) that the bound cytochrome *c* of the succinic and DPNH chains may be different.

As Edwards and Ball (*9*) have shown, the addition of fatty acids such as oleate, which would be released by phospholipase action, to the mitochondrial electron transport system causes inhibition of succinoxidase. We find that the addition of beef heart mitochondrial phospholipid or of beef serum albumin will completely reverse the inhibition of both succinic and DPNH oxidase activities caused by the addition of fatty acids to the untreated electron transport particle. Cytochrome *c*, however, does not help reverse this inhibition. After long-term digestion of the electron transport particle with phospholipase, addition of beef serum albumin or phospholipid along with cytochrome *c* becomes necessary for the maximum restoration of oxidase activities. This phospholipid or beef serum albumin requirement can thus be related

Table 1. Effect of phospholipase treatment on DPNH and succinate oxidation by the electron transport particle. PL, phospholipid, Cyt. *c*, cytochrome *c*.

Phospholipase* treatment (min)	Succinic oxidase† (μmole/min × mg)			DPNH oxidase† (μmole/min × mg)			Cytochrome *c*‡ released (μmole × 10^{-3}/mg of protein)
	+ PL	+ Cyt. *c*	+ Cyt. *c* + PL	+ PL	+ Cyt. *c*	+ Cyt. *c* + PL	
0	1.24	1.2	1.2	1.8	1.9	2.0	
7				0.6	1.1	1.2	0.04
15	0.8	0.9	0.9	0.1	0.3	0.4	0.08
60	0.4	1.0	0.9	0.0	0.0	0.1	0.12
120	0.1	0.4	0.7	0.0	0.0	0.0	0.15
120 (control)	1.1	1.2	1.1	1.6	1.7	1.8	0.02

* Each tube contained 60 mg of electron transport particle protein; $3 \times 10^{-4}M$ potassium phosphate buffer at *p*H 7.4, $5 \times 10^{-5}M$ calcium chloride, and 0.5 mg of venom in a total volume of 5 ml. The tubes were incubated in a water bath at 38°C. The control was incubated under the same conditions, without venom. At the indicated intervals of time, the mixtures were chilled to 0°C, mixed with cold sucrose containing $5 \times 10^{-5}M$ Versene, and immediately centrifuged at 40,000 rev/min for 30 min. to separate the particles. The enzyme was further washed with cold sucrose and used for activity determination. The phospholipase A used in these experiments was purified from *Crotalus adamanteus* venom by heat treatment (*9*). Similar results are obtained by using untreated venom.

† The assay procedure was the same as that described previously (*6*): 50 μg of beef heart mitochondrial phospholipid (PL) and 1.0 mg of cytochrome *c* were used in the succinoxidase assay, whereas 5 μg of PL and 0.1 mg of cytochrome *c* were used in DPNH oxidase as a supplement.

‡ Cytochrome *c* was assayed spectrophotometrically by following the change in absorbance at 550 mμ with dithionite on the supernatant from the incubated mixtures; it was also assayed after adsorption on and elution from the IRC-50 column.