F EXPERIMENTS FOR ESTIMATION OF ASED CROP-YIELDS DUE TO THEIR BEE-POLLINATION*

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For various reasons I was hesitant to accept this important assignment. It was due to my deficiencies in mathematics that I opted for biology during thirties when biology used to be highly descriptive or qualitative with little or no quantitative rigor. I therefore feel hesitant to face this gathering of eminent mathematicians and statisticians. However, I hold Dr. V. G. Panse in very high esteem and my deep regards for him outweighed all other considerations. I am really grateful to you for giving to me this opportunity to contribute, in my modest way, to these memorial lecture series in honour of the late Dr. V.G. Panse.

The topic that I have chosen for the occasion concerns with the designing of experiments to estimate increase in crop-yields due to their pollination by honey bees. Experimental designs in such cases have to take into account the relevant facts from:

(*a*) biology of bees, as also

(*b*) the floral biology of crops they pollinate.

Therefore, I should like to briefly indicate some of these basic biological features relevant to the problem of designing such experiments.

Crops are classified as:

(*a*) self-pollinated and

(*b*) cross pollinated, either by (*i*) wind or by (*ii*) pollinating insects like honey bees.

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Self-pollinated crops like rice, wheat and peas or [illegible] ted crops like maize or bajra do not depend on b[illegible] pollination and seed-set so that bees or other pollin[illegible] assume any relevance for influencing yields in such [illegible] most of the oilseed, vegetable, fruit and orchard crops, [illegible] pulses and particularly forage legumes exclusively depend [illegible] other insects to secure their cross-pollination leading to fruit o[illegible] In these crops any amount of fertilizers, irrigation or cultural ca[illegible] not lead to their optimal yields unless they receive insect pollinatio[illegible] Genetic systems of such crops carry variable degrees of self-sterility or self-incompatibility so that self-pollination leads to corresponding failure of fruit or seed set. For such crops, cross-pollination mediated by bees or other insects becomes obligatory and their yield responses by bee-pollination are directly proportional to the extent of self-sterility carried in their genetic systems. These plant species have evolved their floral-biology so as to make them more and more attractive to bees through gradual improvement of their floral forms, colours, scents, richer nectars with higher sugar concentrations or pollens with higher nutritive values etc., which appeal to visual, olfactory or gustatory instincts of bees and other pollinators. This sets into operation a process of inter-specific or even inter-varietal competition among plant communities to become more and more attractive to pollinating insects, so that bees have in fact functioned as natural plant-breeders on a very extensive scale ever since the origin of flowering plant from cretaceous to tertiary geological periods for millions of years (Deodikar, 1964).

There are several insect species which may mediate the pollination of certain crop plants. However, some of them may be associated with specific plants only and most other insects have a habit of drifting from one floral species to other. Thus for instance if during serial visits such insects drift from cotton to cucumber to clover, they assume little or no value as pollinators for any of these crops. In sharp contrast to this drifting behaviour, honey bees have evolved a highly developed instinctive behaviour which enhances their relative efficiency as crop-pollinators (Deodikar, 1961). The relevant features about their behavioural biology are as follows:

(*i*) A bee colony consists of a matriarchal society with just a single fertile female called queen laying thousands of eggs per day, a few hundred fertile males called drones restricted to breeding season only and thousands of sterile females called worker bees, together forming an integrated social unit.

(*ii*) There is a division of labour among these worker bees as nurses, scavengers, guards, field gatherers of nectar or pollen and scouts.

(*iii*) The function of the scout bees is to survey the plants flowering within the foraging range of over one kilometre around the colony, compare the relative merits of nectars and pollens of plant species flowering synchronously, select the plant species with highest sugar concentration in nectar or pollen with maximum nutritive values.

(*iv*) These scouts then return to their own hive and communicate their infomations about direction as well as distance of the preferred plant-species to inmates of their own hive in the form of certain dance-patterns which have the same utility as languages in our human communication as first shown by Prof. Karl von Frisch (1954).

(*v*) On the basis of this information thousands of worker bees from such conditioned colony forage on the preferred plant species and pollinate it incidentally, ignoring other plant species flowering synchronously.

(*vi*) Workers thus conditioned, restrict their serial visits mostly to the preferred plant species as long as their flowering lasts. After this they get de-conditioned and then get re-conditioned again to some other plant species in a similar way. This instinctive fixity of bees in favour of floral species preferred for the time being is called the '*floral fidelity*' of bees which in fact makes them the most efficient pollinators of our cultivated crops, as compared to other insects which have the habit of drifting from one floral species to another.

(*vii*) By artificially manipulating certain known traits in bee-behaviour, it is now possible to condition, de-condition and re-condition bees for pollinating a given crop, within certain limits.

This brief outline of relevant biological facts about bees and the crops they pollinate is essential to provide the necessary context for our main-problem of designing pollination experiments for estimation of enhanced crop yields. Conventional design for conducting such pollination experiments consists of :

(*a*) enclosing small plots of known area under a given crop just before its flowering under textile or wire cage of appropriate mesh size so as to exclude bees and other pollinators and then ascertain yields per plot due to self-pollination;

(*b*) introduce a certain number of bees or a small bee-hive inside similar enclosures so that the confined bees may be forced to pollinate the crop blooming within such enclossures and then ascertain such yields due to bee-pollination.

Comparisons of yields per unit area under (*a*) and (*b*) indicates the enhancement of yield due to bee-pollination. This basic procedure may have different variants as reviewed earlier (Deodikar, Thakar and Phadke, 1970).

These comparisons have considerable indicative value and a voluminous published data from various countries has accumulated based on this procedure or their minor variants (Deodikar and Suryanarayana, 1973). However, this conventional design has several limitations. The patch of the crop enclosed under nets develops its own micro-climate due to exclusion of light and air-currents leading to local changes in humidity and temperature during the entire flowering duration which may be considerably prolonged for certain crops. In spite of appropriate controls, observed differences in yields may not necessarily be attributable to absence or presence of bees in such enclosures. Changes in micro-climate adversely affect nectar secretion and bee-preferences. Confinement of bees in such enclosures leads to abnormal bee-behaviour and the annoyed bees may ignore the flowers which they normally prefer in the open fields. It is too expensive to construct such nets of optimum sizes in sufficient numbers for replicated trials. Further, it is impossible to follow this conventional procedure in the case of perennial arboreal species as it is unpractical to enclose an entire fruit tree under wire cage.

In order to overcome these problems, a modified design for such pollination experiments has been reported by our group working at the Central Bee Research Institute at Poona (Deodikar, Thakar and Phadke, 1970). The essential features of this design may be summarised as follows :

(*a*) Select at random about 100 mature flower buds of a given crop in a field. Enclose each flower bud, just before blooming, in a small colourless transparent cellophane bag of appropriate size with a few pin-hole perforations for aeration. The perforations should be small enough to exclude any insect pollinator. The open rim of the bag is tied around the sturdy stalks below the flower with cotton plug and marked (SP) by a tag. Remove the bags after 2 to 3 days, when the corollas wither. The tag (SP) is kept intact. Except for this short duration of enclosure for an individual flower, the fruit develops well under normal exposure to sunshine and air. This treatment is called "Self-Pollinated", and abbreviated as 'SP'.

(*b*) On the same day, observe fresh blooming flowers for bee visits. If a particular flower under observation has received atleast three such visits by bees, the flower is marked with a light cotton thread carrying the tag (BP) and allowed to develop in normal course, exposed to light and air as usual. Atleast 100 flowers are thus noted and tagged on the same day. This treatment is designated as 'Bee-Pollinated' and is abbreviated as 'BP'.

(*c*) On the same day, tag about 100 flowers at random irrespestive of whether they are visited or not by bees. These are left to local chance visit of bees or other pollinators depending on their local population densities or to any possibility of self-pollination or even wind-pollination or no pollination at all, as may be the case with an individual crop as influenced by local ecological or phenological conditions. These flowers are marked (OP) and are allowed to develop into fruits in natural course with normal exposure to sunshine and air. This treatment is called 'Open-Pollinated' and abbreviated as 'OP'.

Observations are also made on the flowering time and duration, time of anthesis, nectar secretion, estimation of sugar concentration of nectar, other cultivated plants, orchard crops, forest species or even the weeds flowering simultaneously, within a normal foraging range and competing among themselves to attract bees for pollination, competition from extra-floral nectaries, if any, on the crop, etc.

At the appropriate stage of plant maturity tagged fruits under *a*, *b* and *c* are separately harvested along with their respective labels (SP), (BP) and (OP). Observations are then recorded on percentage of fruit set, individual fruit size in terms of length and breadth, fruit-shape in terms of length to breadth ratio, fruit-weight, fruit colour, percentage of dissolved solids in the fruit juice, mean seed number per fruit, seed size, seed shape, chemical composition of fruit or seed, etc., under all the three treatments. Even quantitative as well as qualitative observations of fruit colour, mesocarp or pericarp colour, seed-coat colour or seed-coat appendages are recorded to infer cases of xenia or meta-xenia effects, if any, due to cross-pollination by bees. Such xenia or meta-xenia effects may have special economic importance in some cases.

Tabulation of such quantitative and qualitative data provides a comparison of fruit set, seed-set as also relative fruit or seed yields and quality under three treatments. Yield differences between (SP) and (BP) indicates the extent of self-sterility, self-incompatibility, relative preponderance of auto or allo-gamy and the relative increase in crop-yield due to bee-pollination. Yield difference between (BP) and (OP) indicates whether the density of pollinating bees is adequste for securing maximum pollination so as to reach the potential increase in yield through providing bee-pollination. If yield under (BP) are higher than (OP) need for additional bee hives per acre is suggested. Such additional hives may be provided until yields under (BP) and (OP) approach equality without any significant difference as in table 2. This therefore, gives additional information on optimum stocking capacity in terms of bee colonies per acre of a crop.

Compared to conventional routine methods of confining crops with or without bees in wire-mesh enclosures, this procedure has the following advantages:

1. It provides a simple method of conducting experiments on assessment of increased crop-yields due to their cross pollination by bees.

2. Being inexpensive and easy to operate, it permits sufficient replications. We usually take about 100 replications for each of the three treatments (SP), (BP) and (OP).

3. A team of a few co-workers can easily tag over hundred flowers under each of the three treatments on the same day, thus minimising any vitiating effects of variation in climate or phenological factors during successive days and making the comparison between the three treatments more valid.

4. It does not expose the entire plant to effects of artificial micro-climate under wire-mesh enclosure, except for individual solitary flowers under (S.P.) for a limited period of one to three days between blooming and withering of its corolla following fertilisation.

5. It secures normal foraging behaviour of pollinating bees without causing any annoyance due to their enclosure under prolonged confinement in limited space.

6. It provides a fairly indicative measure of increased yield due to bee-pollination.

7. Simultaneously, it provides a very useful additional information about an optimum density of bees per acre of a given crop and easy procedure to adjust the number of average sized hives per acre for the crop until the yields under (BP) and (OP) are nearly equated. Over-stocking or over-pollination can thus be avoided.

This procedure, however, has to be modified more or less, in crops with compound inflorescences with very high densities of minute innumerable florets such as clovers, alfalfa, lupins, grapes, brassica, sunflower, onion, etc.

By employing the above experimental design. The Central Bee Research Institute at Poona has collected data on increased yields due to bee pollination of some Indian crops. This has been condensed in Table 1. A programme of collecting similar data on other Indian crops is continuing, for which help from statisticians is sought to improve this design further and make it more accurate and informative.

In the context of certain peculiarities about behavioural biology of bees and floral biology of crops they pollinate, we have formulated this procedure so as to comply with the elementary principles of design of experiments such as randomisation, replication and controls. It is our usual practice to locate bee-hives so as to ensure their even distribution over the experimental field. We select at random about 100 samples under each of the three treatments (SP), (BP) and (OP) in succession preferably on the same day. This virtually amounts to taking about 100 replications which provides enough reliance over the end-results and an un-biased comparison of the means of the three treatments (SP), (BP) and (OP). Professor P.V. Sukhatme considers

TABLE 1

Data on the effect of ee-pollination on yield of some Indian crops

Crop/Year	*Yield under*			
	Self Polli-nated (SP)	*Bee-Polli-nated (BP)*	*Open Polli-nated (OP)*	*Per cent increase in yield by bee-pollination over self-pollination*
Oilseeds :				
Brassica compestris (Mustard) Yield (g) per sq.m. 1973-74	21.5	49.8	—	131.63
Carthamus tinctorius (Safflower) Yield (g) per sq.m. 1973-74	9.72	59.44	—	511.52
Guizotia abyssinica (Niger) Yield (g)/75 flowers 1958-59	0.390	4.763	4.240	1121.3
Seed/head (1958)	14.8	40.45	—	173.3
Helianthus annuus (Sunflower) Yield per head (g)				
var. EC 68414 1973-74	11.96	53.15	49.29	675,42
var. Ramson Record	2.66	76.29	—	2768.04
var. Sunrise Selection	6.63	66.09	—	896.83
Linum usitatissimum (Linseed) Yield (g) per sq. m. 1973-74	1.54	5.12	—	232.46
Vegetable Crops :				
Allium cepa (Onion)				
Mean Wt. (g) per 1958-59	1.694	4.715	4.588	176.3
inflorescence 1959-60	1.56	3.91	3.09	150.6
Daucus carrota (Carrot) Mean wt. (g) per inflorescence 1973-74	1.03	6.19	—	500.97
Raphanus Sativus (Radish) Yield (g) per sq. m. 1973-74	3.45	27.80	—	705.79
Solanum melongena (Brinjal)				
Per cent fruitset 1958-59	66.7	90.0	80.0	34.9
1959-60	30.0	50.0	40.0	66.7
Mean Wt. (g) per fruit 1958-59	66.9	83.7	76.1	25.1
1959-60	1.56	3.91	3.09	150.6
Fruit Crops :				
Citrus paradisi (Grape fruit)				
Per cent frutt-set 1958-59	68.2	92,4	—	35.5
1959-60	21.1	32.0	—	51.7
Citrus reticulata (Santara)				
Per cent fruit set 1968	9	90	47	900.0
1969	14	80	40	471.4
Citurs sinensis.(Musambi)				
Per cent fruit set 1958-59	58	79	—	36.2
1959-60	8	35	—	337.5
1960-61	4	34	17	750.0
Fragaria sp. (Strawberry)				
Per cent fruit set 1958-59	64.5	89.1	85.7	38.1
Mean Wt. (g) per fruit 1958-59	3.31	5.57	4.31	68.3
Psidium guajava (Guava)				
Per cent fruit set 1958-59	25	60	—	140
1960-61	40	68	60	70
Mean Wt. (g) per fruit 1959-60	33.8	106.1	82.0	213.9
Plantation Crop :				
Coffea arabica (Coffee)				
Per cent fruit set 1965-66	47	86	80	83.0

TABLE 2

Bee-Pollination of Sunflower (Variety EC : 68414), Kharif—1973
Locations : Poona, Rahuri and Digraj (Condensed data for all locations)

Treatment		*No. of wellfilled seeds*	*No. of empty seeds*	*Total No. of seeds*	*Weight of wellfilled seeds (g)*	*Weight of empty seeds (g)*	*Total weight of seeds*			*Ratio of well-filled empty seeds weight*	*Per cent increase in filled-seed weight over self*
							Mean per head (g)	*Range (g)*	*S.D.*		
Self-Pollinated (in bags)	Average Yied per head	83·37	702.71	786.08	5.94	6.02	11.96	0.5 to 50.6	11.81	49.7 : 50.3	—
Open-Pollinated	Average Yield per head	847.75	403.68	1251.43	48.28	4.87	53.15	6.5 to 103.0	24.37	90.8 :9.2	712.7
Bee-Pollinated	Average Yield per head	739.85	277.72	1017.57	46.06	3.23	49.29	13.3 to 98.0	23.35	93.5 : 6.5	675.4

Remarks : 1. All figures denote mean yields per head of sunflower.

2. Yield difference between OP and BP not significant; therefore, field density of bee-pollinators adequate, with the number of bee-colonies kept on the plots.

this as a case of *unrestricted randomisation* and has suggested a suitable method for statistical analysis of such data, either by simple *t* test, or by employing analysis of variance corresponding to the procedure of unrestricted randomisation, after ensuring that the variances of each of the three treatments (SP), (BP) and (OP) are not heterogenous.

By employing the same design with cetain modifications this group at the Central Bee Research Institute has recently estimated the increased yield in sunflower due to bee-pollination. In view of the contemporary importance of sunflower as a new crop under trial in various parts of the country, this data on sunflower pollination has been summarised in Table 2. It will be seen from the table that since the sunflower variety EC 68414 used in the experiments is highly self-sterile, it correspondingly responds to bee-pollination by giving an increased yield of about 675%. This will explain why the yields of sunflower in India are relatively low compared to its reported yields in Russia and other eastern European countries, where farmers invariably keep about 1 to 2 bee colonies per acre during the flowering time of sunflower as an established routine.

Bee laboratories from several western countries have reported voluminous data on increased yields due to bee-pollination in various temperate crops. Published reports run into several thousands. The ranges of increased yields extracted from these reports are as follows:

Sunflower: 21 to 3,400 per cent; Onion: 353 to 9,878 per cent; Alfalfa: 23 to 19,733 per cent; Berseem and other Clovers: 23 to 33, 150 per cent; Vetches: 39 to 20 000 per cent; Apple varieties: 180 to 6,950 per cent; Pears : 240 to 6, 014 per cent; Plums: 6 to 2,739 per cent; Cherries: 56 to 1,000 percent; Grapes : 756 to 6 700 per cent.

The ranges of enhanced yields due to bee-pollination in each of these cases in fact reflect corresponding ranges of varietal differences in self-sterility or self-incompatibility in these crops. In highly self-sterile varieties there can be near-zero seed-set under self-pollination which explains the unbelievable reponse of over 33,000 per cent increase in yield due to the cross pollination by bee. These are the results of the carefully planned experiments conducted by bee laboratories in various countries.

If a crop variety is completely self-sterile giving actually a *zero* seed-set when selfed under (SP), any seed-set resulting from its bee-pollination under (BP) may have to be considered as a near infinite response. Therefore, these apparently fantastic figures for percent increases in yields in (BP) compared to (SP) have to be properly understood in their relevant context as above. These figures for percent increases in yields have to be taken as relative measures for varietal differences in their self-sterilities and imply corresponding need for their bee pollination to attain their optimum yield-potentials.

Honey and beeswax are to be looked upon merely as minor by-products of the bee keeping industry. The essential utility of bees in fact consists in securing higher crop yields. For instance, the valuation of the annual production of honey and beeswax in U.S.A. has been estimated to be $ 40 million (Levin 1970). As against this the valuation of increased crop-yields due to bee-pollination has been estimated at $ 5,000 million. This will serve to emphasize the importance of bee keeping as an essential component of the mixed farming pattern,

The total number of bee colonies in U.S.A. is estimated to be 4.3 million. As against these figures, India at present has about 0.5 million bee colonies managed according to modern agricultural techniques. The total area under oilseed, pulse, vegetable, fruit orchard, other crops responsive to bee pollination in India comes to about 50 million hectares. At a minimum rate of about 3 colonies per hectare, we must have atleast 150 million bee colonies in India to pollinate such crops and exploit their maximum yieding potential.

Bee keeping is an essentially migratory occupation in all countries and has to be so in India. We have to migrate bee colonies between agricultural farms and adjacent forest areas so as provide bee forage and sustain bee colonies all the year round. For this purpose our reafforestation programmes have to be rationalised so as to include appropriate arboreal species which, besides being economic, also provide adequate bee-forage.

In case of our highly self-sterile crops we are getting only a fraction of their potential yields. Even these fractional yields are obtained due to natural populations of bees and other pollinators in the localities. However, the wide-spread use of insecticides and particularly indescrete aerial sprayings are leading to wholesale destructions of bees and other beneficial insects. Therefore our entire approach to pest-control needs to be rationalised before it is too late.

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