

Insect Pollination in Guayule, *Parthenium Argentatum* Gray¹

ELDON J. GARDNER² (1947)

CROSS pollination is known to occur in nature in most strains of guayule, *Parthenium argentatum* Gray, which have been studied (2, 3, 4, 5, 6, 9, 11, 12).³ Natural agents of pollination are of interest and have a practical significance in the guayule breeding program. Insects and wind are the two most likely transporting agents. The efficiency of the wind as a pollen carrier at Salinas, Calif., and the effectiveness of wind-borne pollen in fertilization were discussed in a previous article (4). Observations concerning insect pollination are reported in this paper.

Numerous insects inhabit the guayule fields during the flowering season some of which become intimately associated with the pollen-producing parts of the plant. The adhesiveness of the pollen and the spiny character of the outer surface of the grains suggest an adaptation to insect transportation. The role of insects as agents of pollination in guayule has been a subject for speculation, but little experimental work has been done on the problem. There is reason to suspect that insects may be an important natural agent in cross pollination. Noncontrolled crosses among different strains of guayule, which occur readily in the field, and interspecific crosses which Rollins (12) has shown also to occur in nature may be dependent to a large extent on insect pollination.

A more practical reason for pursuing the problem of insect pollination at this time lies in the possibility of utilizing insects for making experimental crosses. Hand pollination is a tedious, time-consuming task. With the development of self-sterile strains of guayule possessing characters desirable in the breeding program, the prospect of working out a technique whereby insects can be transferred from one cage to another and thus affect pollination stimulates much interest. Kalashnikov (5) attempted such a procedure by introducing ants into paper bag isolators. He reported that this method does not lead to as high a percentage of seed set as hand pollination with a brush.

MATERIALS AND METHODS

Large numbers of insects were collected in the guayule fields and subjected to microscopic inspection for pollen. Guayule pollen was distinguished morphologically from other kinds. A staining technique (10) making use of cotton blue lactophenol, as given by Lee (7), was employed to determine whether the insect-borne pollen was normal or consisted of aborted and unfilled grains. When it became obvious that insects associated with the plants were carrying pollen which appear-

¹Initiated as a part of the Special Guayule Research Project, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Dept. of Agriculture, Salinas, Calif. Received for publication November 11, 1946.

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³Figures in parenthesis refer to "Literature Cited", p. 232.

ed to be good, the next step was to study the importance of insects as natural agents of pollination and to determine whether or not it would be feasible to use insects for experimental cross-pollination. Both field and greenhouse experiments were devised to study these problems.

FIELD STUDY

Field experiments were conducted during the summer of 1945 to determine the effectiveness of insects as pollen carriers. Cheesecloth cages placed around the plants were used to hold the insects and isolate the plants from wind-borne pollen. The experiments were well designed and carefully conducted, but the checks showed a high percentage germination. This indicated that the cheesecloth bags were not effective isolators for guayule pollen and the experimental results were thus confounded. The data were discarded and the experiments were repeated during the next season. The problem of effective isolation for plants being selfed and crossed has since been investigated and the results are reported in another article (4). Kraft paper and percale bags were used in the experiments reported here.

Self-sterile 36+ chromosome plants from Powers' (8) seed collection (accession 4255) were used for the experiments. All showed the yellow color indicating that they were homozygous for the recessive factor, xanthous. Each plant had been selfed and none produced more than 1% germination of seed, thus demonstrating the high degree of self-sterility inherent in the selection. The plants were randomized in four replications with provision for seven different treatments. Six plants in each replicate were covered with percale bags supported in the center with metal stakes and tied at the top and bottom (Fig. 1) 1 week before the introduction of the insects. The mature flowers were picked and only those which were at or approaching the receptive stage were present on the plant when the insects were introduced. One plant in each replicate was left uncovered as a check and allowed to be pollinated in the natural way. One covered plant in each replicate was reserved to check the effectiveness of the isolation and no insects were introduced.

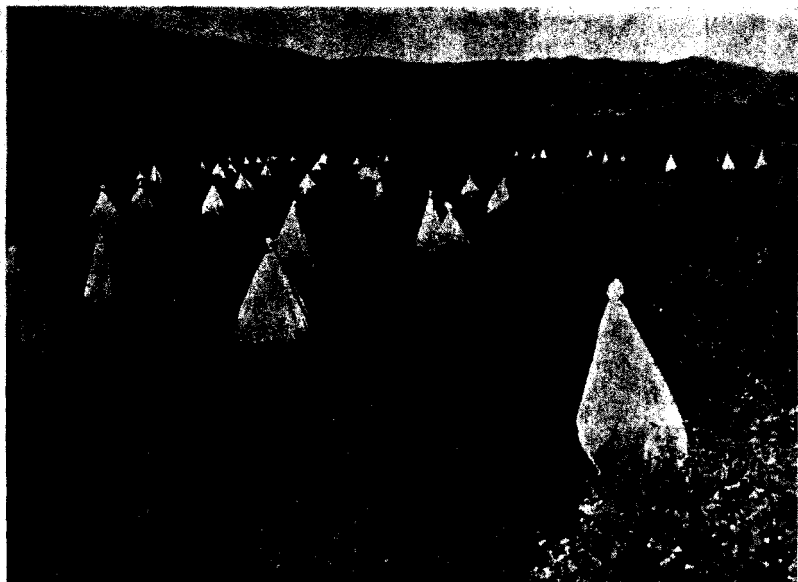


FIG. 1.—Guayule field showing cloth cages placed around yellow plants for insect pollination experiments.

The insects which were most abundant in the guayule fields were used in the experiments. Ladybird beetles, mostly *Hippodamia convergens* Guerin, were numerous at the time the experiments were begun. Counts made on 20 plants chosen at random showed an average of 26 ladybird beetles per plant. Not only were these beetles present in abundance, but they were intimately associated with the flowers. During the warm part of the day they were observed to fly actively from one plant to another. Small black flies, *Hylemya* spp. Robineau-Desvoidy, were also abundant. The season was early for lygus bugs, *Lygus hesperus* Knight, but they were present in appreciable numbers. Romney, *et al.* (13) reported that lygus represented 64% of the total insect population on guayule in the Salinas and Bakersfield areas in the 1943 season. Western 12-spotted cucumber beetles, *Diabrotica sorro* Lec., were also present in appreciable numbers. These four insects seemed to be dominant and universal guayule insects which could be expected to carry pollen from one plant to another if any insects do.

Insects for the first series of field experiments were collected in nets from the guayule fields. Microscopic inspection showed that all carried guayule pollen on their bodies. The insects were removed from the nets in sterile pipettes and introduced into bags surrounding plants through temporary openings near the bottom. Every precaution was taken to avoid mechanical transfer of pollen. Each insect species collected in the fields was introduced into the cages surrounding one plant in each replicate in the following numbers: 100 ladybird beetles, 100 flies, 50 lygus bugs, and 50 cucumber beetles. One hundred ladybird beetles collected elsewhere and observed not to carry guayule pollen were introduced into the cages surrounding each of four other plants as checks. The insects were allowed to remain with the plants 7 days. Pollen had been shown in a previous study (4) to be viable under field conditions for that long a period. The immature flowers and buds were picked at that time. The bags were allowed to remain on the plants until the flowers which were in a receptive stage while the insects were present had dried up. When the seed was mature it was picked, treated by the method described by Benedict and Robinson (1), and 500 seeds from each plant were sown in soil in the greenhouse.

The other part of the field study was designed to follow more accurately the transfer of pollen by insects. Ladybird beetles were collected from another location some distance from any guayule. Microscopic examination showed these insects to be free from guayule pollen. One hundred were placed in the cages surrounding each of four self-sterile plants as a check and allowed to remain for the same length of time as those carrying pollen. About 500 beetles were placed in a cage surrounding a large, yellow plant, homozygous for the color factor (xanthous). This plant was producing an abundance of pollen. The insects were allowed to remain 48 hours and 100 were transferred to each of four other yellow plants, also homozygous for the color factor. Sterile pipettes were used in making the transfers and the insects were allowed to crawl into the cages from a temporary opening near the bottom. Every precaution was taken to avoid mechanical transfer of pollen. It was expected that the proportion of seeds which germinated would indicate the effectiveness of pollen transfer by the insects. All progeny should be yellow if the isolation was complete.

Another 500 beetles were placed in a cage with a large green plant presumed to be homozygous for the dominant green color factor. The insects were allowed to remain for the same length of time and transferred in the same manner described above to four other yellow plants. All progeny from these crosses, except the few resulting from natural selfing of the seed parent, should be green. The seeds (500 seeds from each plant) were picked, treated, and germinated as described above.

GREENHOUSE STUDY

Cages built on a wooden frame 10 inches square and 14 inches high, each constructed with one side of glass and three sides of fine silk (Fig. 2) were used in the first series of greenhouse experiments. The silk was removed from one side of each cage and the cages were joined together in pairs with the open sides facing each other. The plants were selected from a self-sterile, 36+ chromosome strain (Powers' 4255 (8)) on the basis of uniformity in size and numbers of flower heads. They were about 6 months old at the time the experiments were begun. The plants were placed inside the cages before the flowers matured and were not removed until the seed was picked.

There was no possibility of direct contact between the members of each pair of

plants in the cages. Since guayule pollen is heavy it was presumed that it would not move any great distance in the air of the cages in the absence of wind. Micro slides smeared with glycerine were placed in the cages and allowed to remain several days before the experiments were begun. Microscopic inspection showed that no pollen penetrated the cages under greenhouse conditions. The experiment was conducted in a protected section of the greenhouse and every effort was made to avoid drafts during the critical period. The insects were introduced with a glass pipette through a temporary opening in the side of one of the cages in each pair. They could move freely from one compartment to another, and it was presumed that they would be responsible for all pollen transported back and forth from one member of the pair of plants to the other.



FIG. 2.—Plants in small cages prepared for insect pollination experiments in the greenhouse.

The insects used in the experiment were collected from fields and secured from laboratories widely separated from guayule. Ladybird beetles and cucumber beetles were collected from a chard field west of Salinas, Calif., thrips, *Thrips tabaci* Lin., were collected in an onion field adjoining the chard field and the two species of fruit flies, *Drosophila melanogaster* Meigen and *D. hydei* Sturtevant, were obtained through the courtesy of Dr. Everett Dempster, Department of Genetics, University of California.

The pairs of cages containing plants were identified alphabetically from A to F. The A pair was reserved as a check and no insects were introduced. Twenty-five ladybird beetles were introduced into the B cages, 50 fruit flies, *D. melanogaster*, into the C cages, 50 fruit flies, *D. hydei*, into the D cages, 25 cucumber beetles into the E cages, and at least 50 thrips were introduced into the F cages. The insects were allowed to remain in the cages 1 week. At the end of that period all plants were dusted with 5% DDT without opening the cages. When the seed was mature, it was picked, treated (as above), and germinated between blotters in the germinator.

Since selfing cages considerably larger than those described above were being used in greenhouse studies on guayule an experiment was designed to determine whether or not insects could be used for transferring pollen from one plant to

another in these cages in place of the usual method of brushing. The cages were 24 inches square and 36 inches high. They were constructed on wooden frames and covered with cheesecloth (Fig. 3). Appropriate tests had shown them to be suitable for isolating plants from foreign pollen in the greenhouse. Ladybird beetles free from guayule pollen were used in the experiments because they had been shown to carry guayule pollen from one plant to another in the field and in other greenhouse experiments and they caused no injury to the plants. Plants which had been crossed before and were known to be compatible were used for the experimental crosses. Six pairs were placed in cages, as shown in Fig. 3. Two pairs were brushed

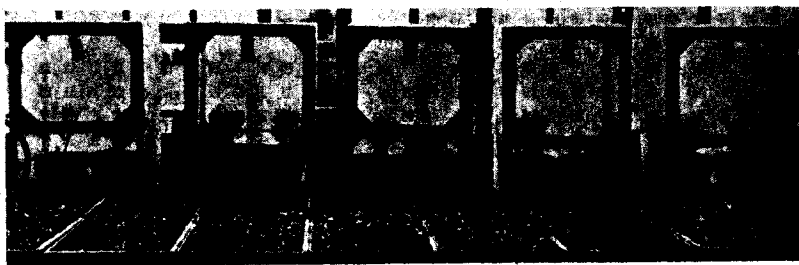


FIG. 3.—Large cages containing pairs of plants used in insect pollination experiments.

daily, the pollen from one member of each pair being transferred to the other and *vice versa* with a camel hair brush. At least 50 ladybird beetles were maintained in each of two other cages. Fresh beetles were introduced at three different times during the 3 weeks period. The other two pairs of caged plants were reserved as checks. At the end of the 3-weeks period all plants were dusted with 5% DDT, and all immature flowers and buds were removed. The plants were kept in the cages until the seed was mature. The seed was picked, treated, and germinated by the method described above for other greenhouse experiments.

EXPERIMENTAL RESULTS

FIELD STUDY

All insects collected in the guayule fields during the flowering season and subjected to microscopic examination were observed to be carrying some guayule pollen on their bodies. Pollen grains washed off the bodies of captured insects accepted the cytoplasmic stain (cotton blue lactophenol) uniformly and appeared to represent normal pollen. The proportion of aborted grains was not greater than that reported for samples taken directly from the experimental plots in a previous study (10).

Anatomical features and habits of the insects seemed to determine largely the amount of pollen carried. Insects which associate themselves intimately with the flowers and possess deep sutures on their ventral surfaces were observed to carry an abundance of pollen. Considerable variation in quantity was observed by microscopic inspection of insects collected in the guayule fields. Flies, *Hylemya* spp., were found to carry an average of about 50 pollen grains each. *Lygus* bugs, *L. hesperus*, carried an average of nearly 500 grains each. As a part of one field observation two ladybird beetles, *H. convergens*, were induced to walk through separate drops of cotton blue stain on a glass micro slide. One left 180 and the other 164 guayule pollen grains

on the stain. This pollen was washed from the legs and the ventral surfaces of the beetles. The same two insects were then dipped successively in several drops of stain. An appreciable amount of pollen was left in each drop. At the conclusion of several washings the beetles themselves were inspected and found still to have pollen on their bodies.

Table 1 gives the percentage germination of seed obtained from the self-sterile plants pollinated only by the designated insects in the first series of field experiments. Less than 1% germination was obtained from the plants preserved as checks. These results compare favorable with those obtained from previous selfing of the same plants with cloth and paper bags and demonstrate that the method of isolation employed for these tests was effective.

TABLE 1.—*Percentage germination of seed from plants pollinated by various insects collected from guayule fields, four replications.*

Insect or treatment	Percentage germination
No insects (checks)	0.425
Pollen-free ladybird beetles (100)	0.615
Ladybird beetles with pollen (100)	10.80
Lygus bugs (50)	3.40
Cucumber beetles (50)	3.10
Flies	0.72
Open-pollinated	27.10

A comparable low percentage germination was obtained when the ladybird beetles free from guayule pollen were introduced into the cages. This shows that the insects alone have no influence on pollination or germination of seed. A significant increase in seed germination was obtained from the plants with which ladybird beetles from the field were associated. The average germination was 10.8% compared with 0.425% for the checks. Smaller but significant increases were obtained through the agency of lygus bugs and cucumber beetles. It will be remembered that only 50 lygus bugs and cucumber beetles were introduced into each cage compared with 100 ladybird beetles. No significant difference was produced by the flies. An average of 27.1% of the open-pollinated seeds germinated. The data show that ladybird beetles, lygus bugs, and cucumber beetles are effective pollen carriers, but flies are not effective under the conditions of these experiments.

Data obtained from germination and classification of seedlings from controlled crosses in which insects were used as pollen carriers are summarized in Table 2. The percentage germination of seed is low but significantly higher than that for the checks (Table 1). The color classification shows that the pollen is actually carried, in the one case, from homozygous yellow pollen plants to homozygous yellow seed plants, thus producing yellow seedlings. Insects transferred from plants homozygous for green, carried pollen which was responsible for the high percentage of green seedlings. The few yellow seedlings likely are the selfs which might be expected even from such highly self-sterile yellow parents. These results show that pollen is carried by

insects from one plant to another. On the basis of these data, the amount of pollen carried by 100 ladybird beetles seems small. It must be remembered, however, that the insects were free from guayule pollen when collected and were allowed to remain only 48 hours with the pollen parent. About 500 insects were present on the same plant at the same time. Even though the plant was producing an abundance of pollen, the amount available to each insect may have been limited. Insects of the same species collected in the field obviously were better supplied. They carried enough pollen to provide for 10.8% seed germination compared with 2.12% and 1.85% for the controlled crosses. Other crosses involving these same plants made under field conditions by transferring pollen from one plant to another with a brush have been much more successful, yielding in the neighborhood of 35% germination. Plants allowed to be pollinated in the natural way produced seed showing an average germination of 27.1% (Table 1). Many factors are involved in controlled insect pollination in the field. Principal among these factors are methods of isolating plants with provision for the requirements of plants and insects; numbers, kinds, and behavior of insects; time factors concerned with the receptive stage of the flowers and viability of pollen; and source of pollen. Sterility factors known to exist in the plants cause added uncertainty in all types of crossing. Until better methods are developed, insect pollination does not seem practical for experimental crosses under field conditions.

TABLE 2.—Percentage germination and classification of seedlings from controlled crosses, four replications.

Insect and treatment	Germination, %	No. of yellow seedlings	No. of green seedlings
Pollen-free ladybird beetles.....	0.70	6.75	0.25
Ladybird beetles from yellow plant	2.12	20.50	0.70
Ladybird beetles from green plant	1.85	1.70	16.80

GREENHOUSE STUDY

Table 3 expresses the data obtained from the greenhouse experiments. The seed from the two plants making up each pair was lumped together. Since the plants used in these insect experiments were not subjected to routine spraying, considerable numbers of insects and red spiders common to the greenhouse accumulated on the plants. This probably accounts for the average germination of 5.5% from the A plants with only their natural population. The plants (B) associated with the ladybird beetles and one species of fruit flies, (*D. melanogaster*, (C) showed a percentage germination of 26.7 and 28.8, respectively. The D plants associated with the other species of fruit flies, *D. hydei*, germinated less readily. This was anticipated on the basis of observations made while the insects were in the cages. The *D. melanogaster* were observed to move from one blossom to another more freely and seemed to adapt themselves more favorably to the conditions in the greenhouse cages than did the *D. hydei*. A com-

paratively small amount of seed was obtained from the E plants associated with the cucumber beetles. These insects were observed to injure the plants severely and undoubtedly interfered with seed production enough to off-set any value they might have as pollen carriers. The lowest percentage germination was recorded for the F plants associated with the thrips. Those insects are abundant on guayule in the fields. The thrips were likely introduced in large enough numbers to devour much of the pollen produced by the two plants and thus limit cross pollination. Appropriate tests demonstrated the fact that no foreign pollen was penetrating the cages used in these experiments in the greenhouse.

TABLE 3.—Percentage germination of seeds following designated treatments of plants in small greenhouse cages.

Plant pair	Treatment	Total No. of seeds	Percentage germination
A	No insects introduced	880	5.5
B	25 ladybird beetles, <i>H. convergens</i>	785	26.7
C	50 fruit flies, <i>D. melanogaster</i>	735	28.8
D	50 fruit flies, <i>D. hydei</i>	775	6.7
E	25 cucumber beetles, <i>D. sorro</i>	230	7.0
F	25 thrips, <i>T. tabaci</i>	610	2.8

Results of the second series of greenhouse experiments for which the larger cages were used are summarized in Table 4. There is no significant difference between the percentage germination from the checks and the plants with which the insects were associated. The plants brushed produced a high percentage germination. These results were anticipated because the insects did not circulate back and forth from one plant to the other as in previous experiments but collected in groups in the corners of the cages. Evidently the conditions of the experiment were not as well suited to the activity of the insects as those involving the smaller cages. It is very likely that the efforts made to remove the usual greenhouse population from the plants and thus provide better control for the experiments were responsible for the results. Sprays containing such ingredients as DDT known to have a residual effect were avoided. The removal of the insect population removed the main food supply for the beetles and thus interfered with their activity. The results indicate that many factors are involved in problems concerning insect pollination and that the insects tested are not dependable agents of pollen transfer under controlled conditions.

TABLE 4.—Percentage germination of seeds following designated treatments of plants in large greenhouse cages, two replications.

Treatment	Total No. of seed	Germination, %
Selfed (check).....	1,784	0.61
50 beetles.....	1,406	0.64
Brushed.....	1,790	33.24

SUMMARY AND CONCLUSIONS

Large numbers of insects were collected in the guayule fields and examined microscopically and all were found to carry guayule pollen. Ladybird beetles and lygus bugs were observed to carry numerous pollen grains on their bodies. Flies were found to carry smaller but appreciable quantities. When stained with cytoplasmic stain and subjected to microscopic examination, insect-borne pollen appeared to be normal. Experiments demonstrated the fact that insects are capable of carrying pollen from one plant to another and the pollen thus transported is effective in fertilization.

One series of field experiments was conducted by placing cloth cages around self-sterile plants and introducing insects collected in the guayule fields. The percentage germination of seed showed ladybirds beetles, lygus bugs, and cucumber beetles to be carriers of pollen from one plant to another. Flies produced no significant effect under the conditions of these experiments. Controlled field crosses were made by transferring insects from one self-sterile plant to another genetically and phenotypically different self-sterile plant. The percentage germination of seed and classification of progeny demonstrated the fact that insects carry pollen which is effective in fertilization. Controlled pollination by insects in experimental field crosses does not seem practical.

Five different species of insects were introduced into small cages surrounding pairs of self-sterile plants in the greenhouse. Ladybird beetles and one species of fruit fly were found to be responsible for an increased germination of seed. The other three insects produced no significant effect. Results of further greenhouse experiments in which larger cages were used showed no significant effect from ladybird beetles. Many factors must be considered in insect pollination studies. It is doubtful that insects could be of practical importance as agents of controlled pollination in the greenhouse.

The results of both field and greenhouse experiments indicate that insects are effective carriers of guayule pollen from one plant to another in nature.

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