BIOLOGICAL ASSOCIATION OF INSECTS: PARASITE AND HOST POPULATIONS

JAMES K. HOLLOWAY

UNIVERSITY OF CALIFORNIA, BERKELEY

PROBLEMS IN ENTOMOLOGY pertaining to studies of ecology, life history, population trends, and control are numerous and varied. In the Orthoptera, a group of insects which includes grasshoppers, roaches, and a few others familiar to us, it is estimated that more than 12,000 species have been named. In the order Coleoptera, which includes the beetles, there are an estimated 100,000 species described. Insects are distributed from the equator to the arctic. Some are adapted to live in fresh water, others in salt water. They can thrive on many different kinds of food media, some of which are wood, leather, stored grains, dung, carrion, and growing plant and animal tissue. Many insects are detrimental to our interests, whereas others are beneficial.

Economic entomologists are concerned with the control of the insects that are detrimental to our well being. The methods employed may be grossly classified in the following groups: chemical, mechanical, cultural, and biological. As its title suggests, the Division of Biological Control of the University of California is engaged in research on biological control methods. We endeavor to utilize insect parasites, predators, and diseases to reduce and maintain populations of insect pests below a level at which they become a problem of economic importance. Many of the insect pests of agriculture in this country have been brought in from foreign countries. Under such conditions the natural enemies of the pest are seldom introduced at the same time. It then becomes necessary to search for the natural enemies so that they can be imported. Before importation actually begins, however, a detailed study is made of the world-wide distribution of the host and its parasites and predators. From this information a program is arranged, and an explorer is sent to collect, classify, and ship to this country entomophagous insects. The parasites and predators are reared here under quarantine conditions. After the necessary preliminary observations are completed, the entomophagous insects are liberated in the hope that they will become established and bring about an effective control. Methods must then be devised to study the pests and their natural enemies in order to evaluate the results obtained.

The first problem for discussion is one in which an attempt was made to study the influence of fungus diseases on populations of purple scale (*Lepidosaphes Beckii* Newm.) in Florida. Many species of scale insects in Florida show evidences of entomogenous fungi. Frequent showers during the rainy season aid in disseminating the fungus spores, and the warm, humid weather in this season is ideal for the germination and growth of fungi. They develop during all seasons of the year but the greatest activity occurs in the summer, which is the rainy season in Florida. The most frequently encountered fungus associated with purple scale is the red-headed scale fungus, *Sphaerostilbe aurantiicola* (B. & Br.) Petch, and next in importance is the pink scale fungus, *Nectaria diploae* B. & C.

Purple scale comes under the general classification of armored scale. During most of its life, it is covered with an opaque waxy shield which, in the mature female, greatly resembles an oyster shell in outline and contour. Both sexes occur; the adult male is winged, whereas the female is wingless and remains for most of its life beneath the scale covering. The first-stage scale is motile; the newly hatched larvae are frequently referred to as crawlers. They hatch from eggs which the female has deposited beneath the scale cover, each female averaging sixty eggs. The crawlers issue and wander about over leaves and twigs until a suitable location is found. They then insert their slender beaks into the plant tissue, commence feeding, attach to the host plant, and secrete the waxy scale covering. Sometimes the crawlers become attached within two hours, and again a day or two may elapse before attachment is completed. After settling, the female must remain attached throughout its entire life; if disturbed, it is unable to re-insert its mouth parts, and death results. The male must remain attached until the transition takes place, when the immature-stage scale changes to a winged adult. In Florida, all stages from egg to adult are found throughout the year; however, the crawler is the predominant stage in March, July, and September. There are approximately three generations a year.

It is frequently necessary to use fungicides to control citrus diseases such as melanose and scab. Abnormal scale populations resulted following the use of Bordeaux mixture, a fungicide prepared from copper sulfate and lime. The entomogenous fungi were killed or inhibited by this treatment. It was commonly believed that this permitted an unchecked increase in the scale insects. The U. S. Department of Agriculture, Bureau of Entomology and Plant Quarantine organized a project to study the influence of fungicidal sprays on entomogenous fungi and on purple scale. A survey of previously published investigations and verbal reports indicated a diversity of opinion with respect to the cause of scale increases following the use of fungicidal sprays. Some believed that the elimination of fungi was the major factor; others, that the inert granular residue contained in the spray was the most important factor; and still others, that the increase was the result of the combination of the two factors. This diversity of opinion was used as a basis for choosing the combination of spray materials. Two fungicidal sprays containing copper were used: one had 12 pounds of inert residue per 100 gallons of liquid spray, whereas the other contained but 1 pound. A third spray was used having 12 pounds of residue, with physical properties similar to the copper spray but without fungicidal action.

The treatments were classified into two levels of residue and two levels of copper. The low-residue level included unsprayed check plots. Each plot contained a single tree, and each treatment was tested on 5 plots. The plots were sampled by collecting 14 leaves from each. The purple scale are found on leaves and twigs alike, but since the populations on twigs are accumulated over a longer period of time they would not give so good a record of current conditions as would the populations on leaves. Flush tip growth would have an infestation of newly attached scale, whereas the oldest innermost leaves would have mostly mature and dead scale. Leaves taken adjacent to flush growth would show a goodly number of mature and young scale; leaves in this location had their origin in the spring at the time scale activity was beginning and the sprays were applied. A sample taken in this location could be expected to give a good index of conditions during the period of the experiments. With the exception of selecting a leaf-age zone, the sample was collected at random from all sides of the tree. The records of scales on leaves were kept for each leaf. It

TABLE 1

Total Infestation of the Purple Scale after the Use of Sprays with Two Levels of Inert Residue and Two Levels of Copper (1938)

Levels of residue	Number of living and dead scales		
	Copper absent	Copper present	Total
Low	1,420	1,848	3,268
High	7,686	5,793	13,479
Total	9,106	7,641	

was then possible to make an estimate of the portion of error variance associated with leaf and tree variation. An interesting observation was made while analyzing samples of purple scale. In samples of 14, 25, and 50 leaves the distribution was asymmetrical, in which the mean exceeded the mode in all instances. In samples of 14 leaves from 5 trees in which the total number of scales was 5,793, the average number of leaves in 14 which exceeded the mean was 5.00 ± 0.44. In another sample in which the total population was 731 scales, the number of leaves which exceeded the mean was 4.40 ± 0.51 per 14 leaves. Difference in population was not greatly influenced by the number of leaves in a sample on which the population exceeded the mean, but was dependent on the population found on the leaves.

Counts of scales on the leaves were made with the aid of a binocular microscope. In order that any variability due to individual judgment during the microscopical examination might be reduced, the leaves of each sample were distributed equally between two observers. The number of living and dead scales were recorded as total infestations. The dead scales were classified as dead associated with fungi and as total mortality (scale dead from all causes).

A summary of the total numbers of living and dead scales is given in table 1. The only significant difference in total infestation was between the two levels of inert residue. Therefore the indications are that the inert residue was the influencing factor associated with the high total infestation.

The records on mortality associated with fungi are entered in table 2, and those on total mortality in table 3. Percentages of dead scale were used to compare the rate of mortality in both tables.

It is evident that the fungicidal, or copper, sprays virtually eliminated the infection of the scale by entomogenous fungi. The absence of fungi within each level of residue did not cause any measurable influence on total infestation, as is shown in table 1. A comparison of the records of the two treatments, with

TABLE 2

MORTALITY ASSOCIATED WITH FUNGI AFTER THE USE OF SPRAYS WITH TWO LEVELS OF INERT RESIDUE AND TWO LEVELS OF COPPER (1938)

Levels of residue	Mortality associated with fungi (*In percentages*)		
	Copper absent	Copper present	Average
Low	32	0.6	16.3
High	14	0.0	7.0
Average	23	0.3	

TABLE 3

TOTAL MORTALITY OF THE PURPLE SCALE AFTER THE USE OF SPRAYS WITH TWO LEVELS OF INERT RESIDUE AND TWO LEVELS OF COPPER (1938)

Levels of residue	Total mortality (*In percentages*)		
	Copper absent	Copper present	Average
Low	83	75	79
High	58	57	58
Average	71	66	

and without copper, within the low level of residue shows that mortality associated with fungi was 31.4 per cent higher in the treatment without copper (table 2), whereas the total mortality was only 8 per cent higher (table 3). Within the high level of residue a similar comparison shows a 14 per cent higher mortality associated with fungi. The total mortality within each level of residue was not significantly influenced by differences in the numbers of scales infected by fungi. Therefore the differences between the two levels of residue were dependent on factors other than the entomogenous fungi. It would seem, however, that the fungus population was to a certain degree dependent on scale mortality. In the two treatments that did not include copper, the higher percentage of scale infected by fungi was associated with the higher rate of mortality.

The conclusions drawn from the experiment were that the use of copper fungicidal sprays decreased the infection of purple scale by entomogenous fungi. The scarcity or abundance of fungi does not influence the rate of mortality, nor is any abnormal scale increase associated with the fungicidal properties of the sprays.

The use of sprays containing inert granular residue materials, whether or not these sprays contain copper, causes abnormal purple scale increase and lowers the rate of mortality.

The next problem to be discussed is one in which the host scale insect is attacked by a small wasp-like parasite. The host is yellow scale (*Aonidella citrina* [Coq.]), which also belongs to the armored-scale group. The waxy cover of this scale is almost circular in outline and is slightly convex in the center. Both sexes occur; the female spends its entire life, after the crawler stage, attached to the leaves or fruit of citrus trees. The eggs of this species hatch within the female, and young are born. The crawlers may issue from beneath the scale covering at once, or they may remain in seclusion for several hours. Before the crawler settles down, wandering may continue for a day or more. The reproductive period averages two months. The time lapse between newly hatched larvae and mature adults will vary from 60 to 68 days in summer to twice that long in winter. In southern California, where the observations which follow were made, the scale had two generations a year.

The parasite is *Comperiella bifaciata* Howard, which was imported from Japan. It belongs to a general group called Hymenoptera, which includes other insect parasites as well as wasps and bees. There are three distinct changes in the general appearance, or morphology, of the parasite. The egg of the parasite is inserted by the adult female into the scale. The larva is a minute cream-colored grub which spends its entire life within the host scale. When the host is almost completely devoured, the larva becomes quiescent and changes to a pupa, which is the resting stage between larva and adult. The pupal stage is also passed within the body of the scale. In about a week the pupa changes to an adult, which emerges through a circular hole cut with its mouth parts in the top of the scale covering. The adults fly and are able to move around among the trees; this species, however, does not have a high rate of dispersal.

In the past, many entomologists have been intrigued with the potentialities of parasitic insects in view of their high egg productivity. Both Professor Harry S. Smith, of the University of California, and Dr. A. J. Nicholson, an entomologist in Australia, have suggested the searching ability of parasitic insects, rather than the egg production, as a gauge of their potentialities, for unless the parasite has the ability to find its host a large potential egg supply is of no importance.

An attempt was made to observe the ability of Comperiella in finding yellow scale under field conditions. Samples were taken when 10 per cent of the parasites had pupated; at that time most of the parasite population would be mature larvae. The mature parasite larvae rather than the eggs can be used to study the searching pattern of this parasite on infested leaves. This is an advantage, as it is less difficult to dissect and find mature larvae and pupae with the use of a low magnification microscope. However, to obtain ratios of parasitized to non-parasitized scale, or estimates of the mature scale which were available to the parasite at the time of oviposition, this method would be reliable only in the spring, since at that time of the year 90 per cent of the scale are reproducing, and for sixty days there is virtually no change in the mature scale population, thus permitting observations on at least two generations of parasite activity.

TABLE 4

PERCENTAGE OF LEAVES WITH ONE OR MORE MATURE SCALE WHICH WERE AVAILABLE TO THE PARASITES AND THE PERCENTAGE OF AVAILABLE LEAVES FOUND BY THE PARASITES

Number of scales per leaf	Percentage of available leaves	Percentage of available leaves found by parasites
1	14	50
2	13	67
3	8	67
4	6	81
5	3	80
6	3	94
7	2	89
8	1	83
9	1	80
10	3	92
11	2	100
12	1	100
13	1	100
14	1	100
15	1	100
16	1	100
17	1	100
18	1	100
19	1	100
20 to 28	2	100

A sample of 10 leaves was taken from each of 48 citrus trees. The trees were under observation during the period of activity of the adult parasite, and a record of infestation and parasitization was kept for each leaf, which permitted an examination of the performance of the parasite on the smallest unit of a sample. It was found that the parasites, in their apparent random search, visited uninfested as well as infested leaves on all sides of the trees. Therefore it was thought necessary to take into consideration the probability of occurrence of infested leaves of various densities, as compared to all the leaves of the sample. In table 4 a tabulation is given of infestation densities ranging from 1 to 28 scales per leaf. These leaves were infested with mature scales which were available to the adult parasites at the time they were actively searching for suitable hosts. The table shows the percentage of the total sample in each density group as well as the percentage of the infested leaves within each density group on which one or more scales were successfully attacked. It would seem that this parasite does a very thorough job of searching among the leaves, since whenever the infestation was 11 or more per leaf all of the leaves had scale which were parasitized, even though the probability of finding such leaves was a little less than 2 out of every 100 leaves searched. However, the parasite apparently had difficulty in finding the scales on the leaves, or in selecting a suitable scale after it was encountered, since the density groups which had the greatest probability of occurrence had the lowest percentage of leaves found by the parasites. An investigation of conditions similar to those mentioned above may prove of value in estimating the searching efficiency of parasites under field conditions.

Another problem of interest in this connection is a study of how the infested leaves are distributed on the trees in various localities and how the parasites react under given conditions. An example follows: In a certain citrus district we had two orchards infested with yellow scale. In Orchard A the trees were large and close together, with the branches intermingling. In Orchard B the trees were small, with large open spaces between them. In A the scale were evenly distributed on all sides of the tree, whereas in B by far the heaviest infestation was on the northeast side of the tree. Evidently the scale were killed by the hot sun, or by some other condition in Orchard B, as during the summer months it was difficult to find a living scale on the south and west sides of the trees. It was found that the parasites searched on all sides of the tree regardless of the infestation pattern of the scales. After the first generation in the spring of the year, the parasites had difficulty in finding suitable hosts in Orchard B.

In one of the northern citrus-growing areas it was found that the infestation of yellow scale on the top surface of the leaf was significantly greater than the infestation on the bottom surface, whereas in the southern part of the state just the opposite condition existed. The parasite populations were greater on the lower surfaces of the leaves in both localities. No difference seemed to exist between the two sections with respect to the parasite populations on the upper surfaces of the leaves, even though many more scale were available on the upper surfaces of the leaves in the northern orchards.

The third insect pest which we shall discuss is black scale, *Saissetia oleae* (Bern.). This belongs to the group of soft-bodied scales, so called because they do not secrete a wax covering. Like yellow scale, which is almost completely devoured by the larva of its parasite, Comperiella, black scale also plays host to its parasite larva, but its destruction is hastened by the parasite adult, which feeds upon the scale as a predator. Black scale differs further from yellow scale, as well as from purple scale, discussed earlier, in that the scale is able to move about on the host plant until a short period before the mature female commences to produce eggs. The feeding, during the immature period, takes place on leaves, fruit, and twigs; the mature females, however, nearly always attach to twigs only. Both sexes are present, although the males are not necessary for species propagation. In the cooler and more humid coastal citrus districts there are two generations a year, whereas in the hotter interior districts there is only one.

Several parasites of the black scale have been introduced into California by the Division of Biological Control. The most promising at the present time is *Metaphycus helvolus* (Compere), introduced from Africa in 1937. According to Professor Smith, this is one of the most remarkable insect parasites ever used by the Division in experimental work. One of its outstanding characteristics is the longevity of the adult, making it possible for the parasite to span periods in which suitable hosts are not available. Another valuable trait has been reported by Flanders, who found that it is capable of producing as many as eight generations, to one of the host scale. As was previously mentioned, the parasite adult also serves in the role of a predator. According to Flanders, it is necessary for the parasite to feed on the scale in order that the female may produce the full complement of eggs. The female inserts her ovipositor into the body of the scale, and then feeds on the fluid which exudes from the wound.

In studies to determine the effectiveness of this parasite, consideration must be given to both the factors which destroy black scale. Smith and De Bach conducted a series of interesting experiments from which they obtained information on the two factors and on the ability of this parasite to reduce scale populations. They covered branches of citrus trees with large organdie sleeves. At the time the branches were covered, the then existing scale populations within the sleeves were eradicated by fumigation, and a parasite-free scale population was then started in each cage. Half of the cages were left open, making them accessible to parasites, whereas the other half were kept closed and protected from parasites. Since *Metaphycus helvolus* was the dominant parasite, the reduction of scale in the open sleeves could be attributed directly to the work of this species. A comparison of the closed and open sleeves showed a very marked reduction of scale in the open sleeves, resulting from scales being both fed on and parasitized. In another experiment, parasites were introduced into a closed sleeve cage containing immature black scales, and a record was kept on host feeding and parasitization. For the first two months about 60 per cent mortality resulted from feeding; this then decreased and parasitization commenced. During the last three months the remainder of the host population was parasitized and ultimately destroyed.

In our work with host and parasite populations, it has always been difficult to establish checks in which there are no parasites and also where the host populations can exist normally. The development of new insecticides may make it possible to obtain a differential in which the parasites could be eliminated without influencing the host population. We could then study fluctuations in insect populations and attempt to discover the dominant factor which brought about the changes. Once the factor was recognized, we could determine the conditions under which it proved to be the dominant influence, and attempt to answer the question of how the presence or absence of the factor, or some intermediate level of it, affects insect populations. In our problems of obtaining information on field populations, we are much in need of assistance and advice on sampling techniques and aid in developing new and workable methods of obtaining quantitative data, as well as suggestions in making logical interpretation of these data.

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