danger in the use of powdered arsenate of lead, we would by all means seek to learn the truth and to inform the public accordingly. At the present time we know of no reason why anyone should hesitate to use powdered arsenate of lead in preference to Paris green or any other arsenical poison now commonly obtainable on the market.

The department of entomology of the Alabama Experiment Station is now making an investigation covering the use of various forms of arsenate of lead with a view to finding exactly what form is most effective, economical and generally satisfactory, for use with various insect pests, and we believe that this work will in the future be of real service, not only to the planters who may use these poisons but to the manufacturers as well.

THE WILT DISEASE OF GIPSY MOTH CATERPILLARS¹

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INTRODUCTION

In August, 1912, we published a paper in *Science* entitled: "Studies on the Wilt Disease or Flacherie of the Gipsy Moth." More extensive observations and experiments conducted during the past year have led the authors to modify some of their original views concerning this disease. The work last year was carried on under rather unfavorable circumstances making elaborate experiments impossible. This difficulty was overcome through the courtesy of the Bureau of Entomology who supplied us with all of the necessary help and material.

Our observations in 1912 on the general behavior of the disease as influenced by external conditions, conclusions concerning the mode of infection and studies on the general pathology were on the whole correct, as far as they went, but a large series of experiments performed during the past summer have shown that we were too hasty in connecting etiologically a micrococcus with the disease.

GYROCOCCUS FLACCIDIFEX AND THE BLOOD TEST

We described this micro- or diplococcus last year (1912) and believed it to be etiologically connected with the wilt disease. Since then it has proved to be simply a casual intestinal parasite in nature with which much of our laboratory material became infected. This micrococcus was isolated in pure culture and caterpillars inoculated or fed with it died of wilt after a comparatively short time. Our

¹This will be followed by a bulletin of the Bureau of Entomology in which this disease will be treated more fully.

only explanation for this phenomenon at present is, that the caterpillars we thought healthy and used for the inoculation experiments were really infected with the virus and by giving them great quantities of this bacterium, we simply lowered their resistance to the disease and hastened death, which, in all probability, would have occurred sooner or later anyway. Another matter not to be overlooked is the apparent great resistance of many caterpillars towards the virus, provided other invaders or unfavorable conditions are excluded. The caterpillars used in the control experiments in 1912 did not die although doubtless some of them were infected, and the only feasible explanation for this seems to be that in one case, *i. e.*, in the inoculation experiments, the introduction of great quantities of a specific bacterium lowered the resistance of the animals towards the virus and death followed while in the other case, *i. e.*, in the controls no such bacterium was introduced.

Observations made on the blood of a great many individuals followed by a study of their tissues in section have clearly demonstrated that the polyhedral test for diagnosing the health of caterpillars is by no means absolutely reliable. The blood corpuscles of a caterpillar may not reveal polyhedral bodies, but this does not necessarily mean that the caterpillar is healthy, for sections through such an individual are very likely to show polyhedral bodies in the nuclei of the tissue cells. On the other hand polyhedral bodies may be present in the blood corpuscles and not in the tissues. These observations showed the futility of the blood test in diagnosing. It is a pity that it cannot be used for we have been compelled to substitute a rather more indefinite for a presumably more definite method.

WHY *GYROCOCCUS* WAS ELIMINATED

The reasons why we abandoned *Gyrococcus flaccidifex* are the following: If smears are made from caterpillars dead but a short time no bacteria can be found as a rule. Cultures made from such caterpillars on caterpillar and the ordinary nutrient media remained sterile, except in a very few cases when the bacterium in question again appeared. Of course, after a caterpillar has been dead for a certain length of time bacterial invaders entered the body, but cultures made from dying or freshly dead caterpillars rarely manifested growth. At the beginning when the disease first became epidemic in nature we made smears and cultures out in the field by taking with us all the necessary sterilization appliances and in so doing on quiet windless days we found the chances for contamination were very slight as compared with those in our laboratory where thousands of caterpillars had previously died.

As stated in our first paper dead caterpillars cannot be sectioned owing to the fact that they disintegrate completely after death. If serial sections are made of diseased caterpillars obtained in the field, polyhedral bodies will be found in abundance, but no bacteria in the tissues and in most cases the intestinal lumen will be perfectly free from microorganisms in general. This is a very striking fact when compared with the abundance of intestinal flora in the higher animals. Last year we stated that we had observed small wriggling things in the tissues which after being stained were revealed as Gyrococci. Small wriggling or rather dancing things are certainly found in the tissues of diseased caterpillars, and it is possible that we mistook these for Gyrococci. Pigment granules are also easily confused with bacteria as anyone knows who has studied the pigment granules in the basement membrane lining the hypodermis, and in the ganglia. The microorganism described as *Gyrococcus flaccidifex* when found proves to be an intestinal invader pure and simple. We will return to the small wriggling particles later.

METHODS USED FOR OBTAINING HEALTHY MATERIAL

After we realized that it was futile to depend upon the blood test for obtaining healthy material and that it was impossible to be certain of any material in the vicinity of the laboratory, field men were asked to report any places in the infested territory where the disease was not evident. As soon as such places were reported, we visited and carefully examined them ourselves. If any dead caterpillars were found and later on microscopical examination showed polyhedral bodies, living material from such a locality was not used. Caterpillars were only taken from places where the disease could not be found at the time of collection. The objection will at once be raised and rightly, that the disease may have been there in a latent form and that no one can tell by an external examination whether a caterpillar is healthy or not. We have studied the disease in the field as well as in the laboratory and have found it in all of the infested territory from Maine to Rhode Island. It will at once become evident what a difficult matter it is to obtain healthy material for experiments. Apparently the healthiest looking colonies harbor a number of diseased individuals, for sooner or later, if the place be carefully watched, the disease will appear. Then after all, the critic will say, you obtained your experimental material from a diseased locality. This is quite true, but the disease if present had not taken the form of an epidemic in such localities when the collections were made and the chances of obtaining some healthy individuals were very great. In places where the infestation is light and the caterpillars widely separated, the disease never reaches

an epidemic form throughout the entire summer and naturally many caterpillars escape since infection can probably only take place through the mouth by means of the food. Dead caterpillars disintegrate and their fluids soil the leaves, and other caterpillars on eating these become infected. Some experiments, to be mentioned later, seemed to show that this is the only way infection can occur. It at once becomes apparent why the disease plays such havoc in a heavily infested locality.

After being collected from a certain place some of the caterpillars were isolated in separate autoclaved boxes and placed in rooms which had never been used for this purpose. Others were kept together in what were known as stock boxes. Since it has, we believe, been accepted that unfavorable conditions assist the development of the disease, provided infection has occurred, the individuals in the different stock boxes were treated in a variety of ways. Some were starved, others were subjected to the heat of the sun, still others were fed with leaves soaked in water for forty-eight hours. After several days in case no disease appeared in either the isolated boxes or in those thus treated, it was thought safe to use them for the experiments. Several times during such health tests one or more caterpillars would contract the disease and then the entire collection was thrown away, the boxes were again autoclaved, and another lot brought in to be submitted to a similar physical examination. Such a method of obtaining healthy individuals proves to be much more satisfactory than the blood test as our controls show and if large enough numbers are used is perfectly safe. It was only owing to the large series of experiments which we performed as will be seen from our next publication that we were able to come to any conclusions at all concerning the virus of the wilt disease.

EXCITING AGENTS THOUGHT TO BE RESPONSIBLE FOR THE DISEASE

A disease very similar to the wilt disease of the gipsy moth is known to attack the silkworm and the nun moth in Germany. In this country we have another similar disease in our two species of tent caterpillars, *Malacosoma disstria* and *M. americana*.

In Europe there is a tendency to group all of the caterpillar diseases which are characterized by the formation of polyhedral bodies under the name of "polyederkrankheit" or polyhedral diseases. This is an excellent way to distinguish them from those diseases not characterized by the formation of such bodies for, though we do not believe that the polyhedral bodies are the exciting cause of the disease, we are convinced, nevertheless, that they are absolutely specific for a certain type of malady.

European investigators have advanced various theories concerning the exciting cause of the polyhedral diseases. At one time bacteria were thought to be responsible, especially by Hofmann and Tubeuf. The polyhedral bodies were regarded as being formed by the tissue cells as a reaction against the bacterial toxins. In 1911 Escherich and Miyajima expressed the opinion that we must look upon the polyhedral bodies as the carriers of the virus. Bolle at one time believed a certain Microsporidian (*Microsporidium bombycis*) to be responsible for the disease. According to Prowazek's latest paper in 1912, on the "Gelbsucht" of the silkworm, Knoch delivered a lecture in Stuttgart on the 14th of March, 1912, in which he said that little refractive granules appear in the blood corpuscles. These multiply and infect the nuclei of tissue cells where their amoeboid membrane hardens and they change into polyhedral bodies. He further said that the minute granules which resemble the Chlamydozoa of Prowazek are the vegetative; the polyhedral bodies the resting stages of the causative organism.

Prowazek, in the paper above mentioned, infected some fifteen or sixteen caterpillars with emulsified diseased material which he passed with difficulty through the Berkefeld and through his Agar-Ultra filter. The filtrate contained no polyhedral bodies and no bacteria, as control cultures showed. The caterpillars died typically and so he concluded that in some cases healthy caterpillars could be infected with material free from polyhedra. These experiments are suggestive, but cannot be taken seriously because no controls were used and because the blood test was the only precaution taken in diagnosing the health of caterpillars.

THE EXCITING CAUSE OF THE WILT DISEASE A FILTERABLE VIRUS

Thirty filterable viruses are now known to be responsible for certain infectious diseases in man and the lower mammals, but to our knowledge only one has been described in insects, namely that of Sacbrood, a bee disease discovered by White in 1913.

In our infection experiments last year, we obtained negative results with the material passed through the Berkefeld filter. This was due to the fact that the material was so concentrated that a slimy film was deposited on the outside of the candle and withheld the virus. A concentrated emulsion of diseased material is so full of polyhedral bodies, cellular débris, hairs and pigment granules that it very soon plugs up the pores of the filters. This year caterpillars, which died typically of the disease, were crushed with just enough sterile water to facilitate the crushing. This material was then strained through cheese cloth and filtered by means of suction through filter paper. The filtrate was diluted in one case with fifty, in another with twenty-

five times its volume of sterile water. This was then passed through a Berkefeld "Grade N" filter and used for the infection experiments. The filtrate was sterile in so far as bacteria were concerned as control cultures on nutrient agar, gelatine and caterpillar soup showed. Microscopical examination showed no polyhedral bodies. Nothing could be observed even in a dark field except some very minute dancing granules also noticed by Prowazek in his experiments with silkworms (p. 272). These little granules may be identical with those observed in diseased tissue mentioned in the first part of this paper. This matter, however, is still so indefinite that speculation seems to be useless, but, since they were never found in the tissues of healthy caterpillars, their presence is worth recording.

A large number of caterpillars were fed with the Berkefeld filtrate smeared on red oak leaves. In order to establish uniformity in the experiments, all caterpillars of the same series were kept in one room where they could be subjected to the same degree of heat and humidity. Each caterpillar was kept in a sterilized pasteboard tray by itself, and fed with fresh red oak leaves after the infected leaves had been partially eaten. The oak leaves all came from a vicinity which was not infested with caterpillars. Thus the possibility of bringing the infection from the outside into the laboratory was excluded. Another matter considered was the age of caterpillars used in these experiments. If caterpillars are near the pupal stage, they cease feeding and are, therefore, difficult to infect, so it is important to get them young enough so that they will be sure to feed.

In one series of experiments fifty caterpillars were fed with the Berkefeld filtrate, in another forty, in a third twenty. The same number was fed with material before it was passed through such a filter and a large set of controls fed with Berkefeld filtrate which had been sterilized by autoclaving accompanied each series.

Out of the entire lot of caterpillars (110) fed with the unsterilized Berkefeld filtrate, twenty-eight died with typical wilt symptoms. Microscopical examination showed polyhedral bodies to be present in abundance and stained smears and cultures in nutrient broth showed that bacteria had taken no part. Quite a few of the caterpillars died of another cause which we will discuss more in detail at another time. Suffice it to say that the extreme heat during July of the past summer had much to do with the death of a great many of the caterpillars. Their death was due to disturbances in the normal physiological functions and not due to infectious disease. Caterpillars dying from this cause were not very flaccid, their skin was tough; they were not disintegrated, but more or less dried out. Polyhedral bodies were not present, but usually a great many crystals with radiating lines could

be found, a sign of some metabolic disturbance. Quite a few larvæ were killed by the imported tachinid parasite, *Compsilura concinnata*, and the rest transformed into moths. The only way we can account for these moths is on the supposition that they arose from immune caterpillars or from such as had escaped infection in some way. A greater number of caterpillars (48 out of 85) died in the experiments with unfiltered virus. This seems to show that the organism is filterable but with difficulty. The filter became clogged up after a short time and, therefore, only a certain amount of the virus could pass through it and, in order to overcome this difficulty in part, the old filters were replaced by new ones every few minutes. Among the entire number of controls, 162 caterpillars, only three died of wilt. This is equivalent to about 1.8 per cent, a very small percentage and one which can almost be overlooked as an experimental error in such a large set of controls when compared with the numbers that died in the experiments. In the controls as well as in the stock trays a great number of caterpillars died of the physiological cause already mentioned. Especially significant are those which died of this cause in the stock trays for these caterpillars were not at all infected, so that it is impossible to attribute the death-rate in the controls to some toxic effect of the sterilized virus. Laboratory conditions are poor at the best and our records show that very high temperatures were probably responsible for this "physiological death" as we have called it. In the laboratory, especially during high temperatures, the food plants put into trays dry out rapidly, no matter how often they are renewed. Dew and rain cannot wet them and very likely the lack of water, in other words thirst, was responsible for the mortality. We did not care to dampen the leaves artificially very much for fear that we might produce a condition still more unnatural and one that might favor the development of moulds in the trays.

The polyhedral bodies have as yet revealed nothing of a parasitic nature. They may, of course, be a resting stage of a filterable vegetative form, but we are rather inclined to regard them as reaction bodies. They are possibly products of nuclear digestion produced by the virus invading the nuclei and digesting the chromatin.

We should like briefly to consider one more matter. It is claimed in this country, notably by Mr. William Reiff, that the disease is air borne and that caterpillars can be infected in the woods by hanging out small cheese-cloth bags containing the remains of individuals that have died of the disease. With this point in view we arranged a set of boxes near some windows through which the wind blew steadily for over ten days. The boxes were closed except on two sides, namely the side facing the window and the side facing the interior of the room.

These were simply covered with cheese cloth through which the wind could blow. Two boxes were placed in each of three windows, and twenty caterpillars, were placed in each with an abundance of red oak leaves. To the windward of one box a little bag of material was hung prepared according to Reiff's receipt. The cheese cloth lining the windward side of another box was smeared with the juices of disintegrated caterpillars. The leaves in two boxes were smeared with the same juices and the two remaining boxes were kept as controls.

Twenty-one caterpillars died of the disease in the two boxes containing leaves smeared with the virus and one died in each of the control boxes. Two died in the box to the windward of which the bag was hung and three in the box lined with the smeared cheese cloth. In the experiment with the bag one died the day after the experiment was begun, so in all probability it had already been infected before being placed in the box, for one day is entirely too short an incubation period. In this same box four died of tachinid parasitism; all the rest transformed. In the controls three died of tachinid parasitism and the rest transformed. In the boxes in which the leaves were smeared four died of tachinid parasitism and the rest transformed.

While these experiments are not very extensive they, nevertheless, indicate that the wind is an unimportant factor in the rapid spread of the disease and that infection in nature occurs when caterpillars feed on leaves soiled by the juices of dead individuals. During the height of the season we found leaves everywhere with brown spots on them and, on making smears of such spots with some sterile water, polyhedral bodies were found in great abundance.

A very striking phenomenon observed in all of our experiments was the large number of moths obtained from caterpillars infected several times, both with the Berkefeld filtrate and with the unfiltered virus. This seems to us to be very suggestive of immunity and agrees with observations in the field where, in a given locality, the disease raged for several weeks and yet moths were later seen in abundance. They may have been fortunate enough to escape infection, but this seems improbable in a heavily infested area and was entirely impossible in the laboratory where they were infected several times.

SUMMARY

Our experiments and observations may be summed up as follows:

1. The presence of polyhedral bodies in the blood corpuscles may be useful in diagnosing the health of nun moth caterpillars, but this test cannot be used for gipsy moth caterpillars with any degree of certainty.
2. The virus of the wilt disease is filterable with difficulty.
3. Such a filtrate is free from bacteria and polyhedral bodies.

4. Caterpillars dead from infection with the filtered virus are flaccid, completely disintegrated and full of polyhedral bodies.
5. Usually the complete absence of bacteria immediately after death is very striking as cultures and smears show.
6. Minute dancing granules were observed in the diseased tissue cells with very high powers.
7. These same granules were also noticed in the Berkefeld filtrate.
8. There is no evidence that the polyhedral bodies are stages of the filterable virus.
9. A large number of caterpillars used in the experiments died, due to disturbances in their normal physiological activities.
10. There is no evidence that the wind is an important factor in distributing the disease.
11. Infection naturally takes place through the mouth by means of the food.
12. Apparent immunity is a striking phenomenon.

CONCLUSION

In conclusion we wish to thank Professor William Morton Wheeler for the interest which he showed during the progress of our experiments. We also wish to thank Mr. A. F. Burgess, director of the Gypsy Moth Laboratory at Melrose Highlands, for the kindly coöperation which he has given us. Had he not provided efficient assistants to aid in feeding and looking after the trays, it would have been impossible to have accomplished much. We wish further to thank Miss Teresa Sheerin, one of our assistants, whose conscientious work and interest aided materially in our researches. Last, but not least, we would like to express our thanks to all the field men who assisted in this work.

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ARTHROCNO DAX CAROLINA N. SP.

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The species described below is allied to *A. apiphila* Felt from which it is easily separated by the lighter circumfili and the longer stems of the antennal segments in the male. It is easily separated from *A. occidentalis* Felt, a species recorded as preying upon red spider in California, by the shorter stems of the antennal segments in the male and by the rounded ventral plate. Mr. E. A. MacGregor, who reared this species in both 1912 and 1913 from red spider on cotton, is of the opinion that it is the most important natural agent in controlling this pest.

Male. Length .75 mm. Antennæ about as long as the body, sparsely haired, light brown; 14 segments, the fifth having the two parts of the stems with a length three-fourths and one and three-fourths times their diameters, respectively. The