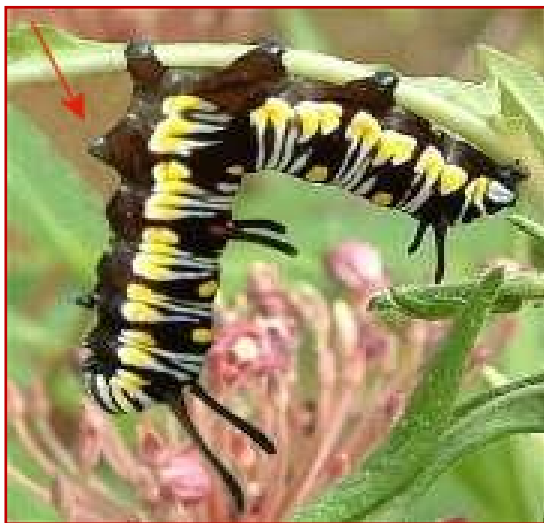


PROFESSIONAL BUTTERFLY FARMING – PART I
Workshop Article 3: The Livestock and Production Processes
Raising Healthy, Parasitoid and Disease-Free Butterflies
Workshop Article 3.6.4

Disease and Parasitoid Prevention
By Nigel Venters



An African Monarch larvae (*Danaus chrysippus*) showing the first signs of disease. Always worry when you see this drooping action in your larvae, and especially when you start to see the first set of pro-legs, (claspers) nearest the head lose their grip, as is shown here (see red arrow).

The stages that follow are the other claspers will lose their grip, and the larvae are seen hanging on by their rear end claspers only.

By this time disease will be obvious, and many times, the end result is a black sack of stinking fluid!

Introduction

One of the most depressing aspects when first starting to breed butterflies is to find that one day, when everything looked so good and hopeful on the previous day, is to find that all your breeding stock is dead or dying from disease, and/or parasitoid infection. These problems can cause you a complete “wipe out” and the only way forward is to start all over again! I have to add a word of warning here - many breeders have a great first year, and then it all goes downhill, and successive seasons can be even more disastrous!

This article provides prevention and control measures that will hopefully prevent you from experiencing such loss, as I did, so many years ago, when I first started breeding my butterflies! It's so much better if you can learn through my experience and not have to go through it and learn the hard way. Please enjoy, and read on, as this article will save you endless misery in the longer term!

O.e. (Ophryocystis elektroscirrha)

It is important when reading the information that follows, that this article begins with the eradication of O.e. from your breeding stock. This is the simple name we butterfly breeders give to the awful scientific name, “Ophryocystis elektroscirrha”. So now you know why we just call it “O.e.”! Why is this important information to start with?

IMPORTANT:

O.e. is a problem for you only if you are breeding Monarch butterflies! O.e. is an infection which can wipe you out in no time! However, O.e. ONLY infects Monarchs, and Danaids, (the Monarch’s butterfly family). **ALL** other butterflies in other butterfly families are totally unaffected (*or should I say, “never infected”*) by O.e.! When breeding any butterfly which is not in this family, relax! Except for the issues presented later in this article, you will never have to worry about the “dreaded” O.e.!

It is worth repeating that O.e. is a **protozoan infection** that has evolved with Monarchs, and evolved to ONLY infect Monarchs (and other species in the Danaid butterfly family). It is a “species-specific” infection that has evolved over millions of years in conjunction with the Monarch. Being a protozoan infection, although it may well closely fit to the fungus family, it is a separate group. Common traits that O.e. shares with fungus are, like a fungus, it has a “reproductive stage” (for want of a better description) and new “spores” are formed just under the pupal shell. Wild Monarchs mostly cope with this infection, as it is found in low levels in many of them. The Monarchs are unaffected by low levels of this parasite. Throughout their adult lives, wild Monarchs spread O.e. spores as they lay their eggs, and on the surrounding leaves of their host plant as they land. Don’t forget! Monarchs will nectar at flowers, and spread O.e. here too. Basically, they spread hard-coated spores that are amazingly durable, and can survive for some years in this “spore” stage.

It is important to remember that O.e. relies on the Monarch to exist and spread its spores, and it is in O.e.’s own interest to NOT kill its host under normal conditions. After all, O.e. also wants to survive, just like any other living creature!

As I mentioned, O.e. has a “spore production stage” as does a fungus. Remember, the life of a fungus is usually underground or lurking inside a plant or other creature. An example is the familiar mushrooms that we all love to eat. The stage we eat them, is only a small part of its lifecycle beneath the earth - the fruiting stage.

The flowering stage, or the stage that O.e. reproduces, is inside the pupa of the Monarch butterfly. When we consider this point, it makes a lot of sense! The spores are produced at exactly the time the adult butterfly forms, so that when the butterfly emerges, it can spread the spores far and wide!

Now in my view, there are simple solutions for the Monarch butterfly breeder to prevent O.e. from ever entering the breeding operation or livestock. These solutions are not difficult. However, we must remember that we breed Monarchs in far higher numbers and concentrations than are found naturally in the wild. A little bit of care to start with will make all the difference to your breeding without O.e. infection. I describe the problems with available solutions below, and offer advice on how to eradicate them.

Avoiding O.e. In Your Stock

As I mentioned earlier in this article, O.e. usually lives quite well in conjunction with its host, the Monarch in the wild. It does not want kill it, but instead, simply wants to survive and spread. The O.e. reproduction stage is at exactly the same time of the pupal stage of the Monarch.

So beware when you buy in Monarch pupae. You could be bringing in more problems that you think! Find a reliable supplier, and ask what O.e. eradication scheme they have in place. No matter who you buy from, you must keep the pupae in an isolation area well away from where you are breeding, to allow these pupae to emerge.

Have a good look at the pupae. Use a magnifying glass, and look for pupae that have irregular (although they may be only tiny) black spots. This is a sure way of seeing O.e. spores “fruiting” just under the pupal skin! Reject these pupae.



Here are some extreme cases of O.e. in Monarch pupae!

It's been so long since I had O.e. infection in my breeding stock, that I had to breed some particularly infected butterflies in an isolation unit to produce pupae to show infections in photos.

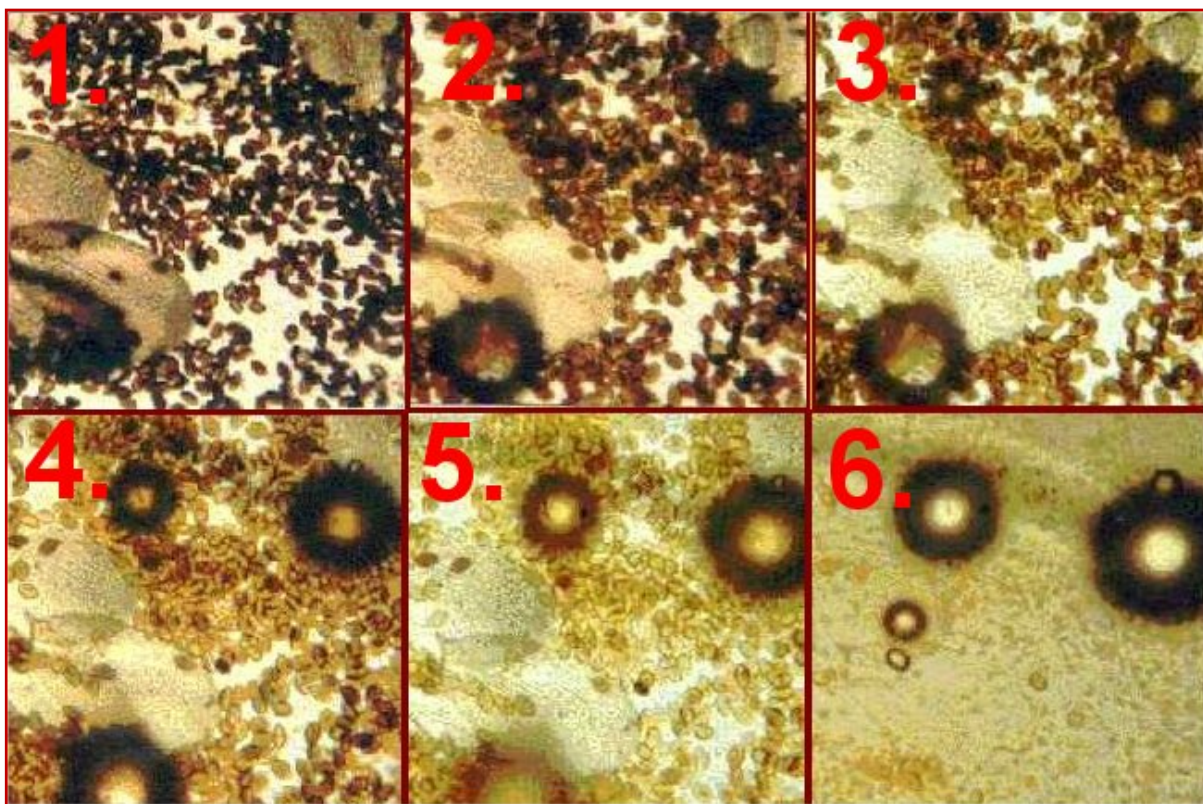
OK, these are totally extreme cases! The usual small black irregular spots have become huge patches. In fact, these pupae are dead, having been totally overwhelmed by O.e. The beautiful green colour has gone, because I sun dried them to show contrast. Left alone, they would go black all over.

Now have a close look at your emerged adults. Check the adults, even though they emerge perfectly. Here are some warning signs to look for.

Even if they emerged successfully from the pupae, have a look at the wing shape. Extreme O.e. infection can often be seen quite easily by looking at the forewing edges. The wings may be symmetrical, but often the exterior edge can be scythe shaped. Also look for thinly scaled wing tips. These can look almost transparent in extreme cases. Once you have rejected butterflies with these obvious signs of problems, further testing is required to ensure these butterflies are safe to use in your breeding operation.

We'll take this further. Once you are happy that obvious signs of O.e. infection are not present, it is now necessary to check the wings and body with a small bit of cellophane or clear scotch tape. Carefully take clear tape samples from the wings and the body. I also use an artist's brush to gently brush the hind-wing folds onto the tape. The hind-wing folds are where the hind wings meet the body. In my experience, this is a sure way of seeing O.e. spores in the least infected Monarchs if they are present. Now have a look at these with a microscope. You don't have to spend a fortune here, and even a junior microscope is just fine with a magnification of around 150X. In fact, too high of a magnification can hinder you, and make the spores harder to see! What you are looking for is black oval discs amongst the wing scales on the taped sampling. You can't really miss seeing them at this magnification.

Even after going to all this trouble to detect any signs of O.e., I don't despair even if I can't find any completely O.e.-free Monarchs. I simply choose the least infected adults, pair them, obtain eggs in isolation from my main breeding stock, and then sterilize the eggs. In fact, I actually sterilize eggs of any new stock I am going to introduce to my breeding stock, but I am a bit paranoid about O.e. prevention! Egg sterilization process is provided later in this article and I am very grateful to Melanie McCarthy and Jacob Groth (seasoned Monarch breeders) for sharing their methods and processes. Dr. Sonia Altizer has also generously given her permission to share her article on O.e.



The six microscope photos above show the step-by-step process of removing O.e. by sterilizing the eggs. Starting with photo 1 - This is a heavy infection, and the small disc-like spores can easily be seen. By photo 6 the spores have all been destroyed.

Here are a few further precautions I take when thinking about new Monarch stock. I actually don't ever buy in stock now. I don't need to, as I can always catch it in my garden! But, again being totally obsessed with keeping my stock clean, I do take precautions with it! But again, it's only a couple of times a year that I need to do this. Compare that with the misery of a meltdown in the breeding operation! Well worth the time and effort to check.

I mentioned that I isolate all new stock from my other breeders. Even before this, I take steps to prevent cross infection. Each time I catch new stock, I carry a hand mister filled with 96% alcohol and an alcohol-based hand-sterilizing bottle with me. Each time I catch a Monarch, I put it in an envelope or triangle and then put it into a sterile plastic box. Then, I mist my butterfly net with pure alcohol. (Pure alcohol at 96% by volume may be difficult for you to get, as folks drink this stuff. However, I suggest that Vodka at 40% is also effective!) You don't need too much of it. Alcohol is instant death to pathogens, and it dries off in seconds. I then use a hand gel on my hands before I catch the next Monarch, and so on. These butterflies are then taken into isolation, and I go and have a shower. I test each butterfly, and sterilize between testing.

Having gone to all this trouble to isolate and produce your O.e.-free stock, there is one further precaution you must consider. You all know you are going to need a lot of milkweed plants to feed your larvae, and care must be given to where you grow these plants. The problem is, of course, that unprotected milkweed will be visited by wild Monarchs. They will lay eggs on, and infect your plants - and the infection process will continue on!

Many breeders use covered enclosures to prevent this, but that may not always be an easy option for everyone. One cheap way of doing this is to use a simple fruit frame designed to keep wild birds off soft fruit. You can use a netting with a fairly open mesh, around $\frac{3}{4}$ inch (2 cms.). Finally, you can take a different approach and grow some of the vines in the milkweed family that adult Monarchs never visit or use for laying eggs! The larvae love these milkweed vines. They eat and thrive on them! *Cynanchum* species are found in the U.S., but they are occasionally visited by wild Monarchs. So, a better option is to use *Morrenia* vines. Seeds are sometimes available by seed merchants on the internet.



Or, grow some *Araujia sericifera* (photos above) - also known as Cruel Plant, sometimes known as the Moth Plant. It is sometimes listed as *Araujia hortorum*. These vines are extremely vigorous, and **they produce a huge amount of large leaves for your larvae to feed on in safety.**

All these precautions may seem as though I am a sad case to you, but I haven't had a single case of O.e. or any other pathogens in my stock for, oh, too many years for me to remember! It helps me sleep well at night!

Avoiding Other Diseases That Can Infect Any Butterfly Species You Are Breeding

The following section will provide you with an understanding of the diseases, such as virus, bacteria and fungus, and the problems they can cause butterfly breeders. We'll also cover how best to avoid and eliminate these problems in your breeding set-up. Disease has to come from somewhere! Often when breeders have a wipe-out, they are left wondering, "Where did this come from?"

Some breeders will take the trouble to send off diseased stock for analysis to determine the exact pathogen that killed it. My take on this is that it is all a bit pointless, as prevention and eradication steps are the same, regardless of the actual, specific disease. When you think of it, the larvae are still all dead regardless of the actual pathogen that killed them! If anyone is interested, just enter "Insect diseases" on an internet search and read on from there. However, in this article I generalize the problems to prevent overcomplicating the issues. As a general rule of thumb, if your larvae is hanging on by its rear claspers only, and is a black, wet stinking bag of skin, the cause is likely to be bacterial. Or, if the larvae look pretty much the same, are slightly dryer and don't stink so much, the problem is likely to be a virus. Of course, there are many other symptoms. A fungal infection is evident when larvae stop eating, then contract in size and lose color, or just collapse and dry out. Of course, once a larvae is weakened it will be more susceptible to a combined attack from all three of these causes.

Self-Induced Disease



Above is an example of a virulent virus. Here we see that even the internal organs have been evacuated, when passing diarrhea.

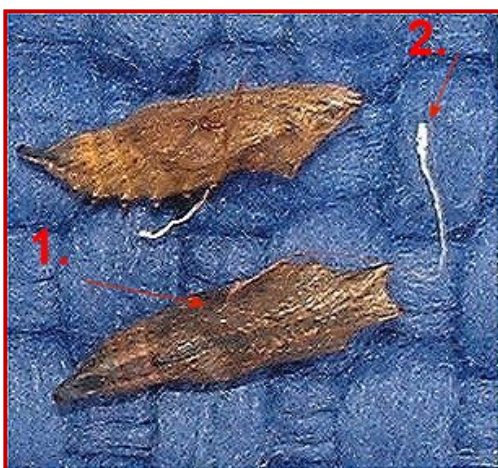
In the wild, disease is relatively uncommon. (We are not discussing O.e. here, which is a separate issue described in the previous section). In my 56 years of breeding and searching for butterflies, eggs and larvae in just about all the climate zones and countries around the world, I have only seen a few cases of disease in the wild. These were all gregarious species, and it is a sad sight to see a host plant with so many larvae hanging dead on it, looking like a strange fruit!

Most diseases suffered by breeders are actually self induced! Read on to discover why! The air is full of bacteria, fungal spores and virus, and all creatures are covered in them on their skins. This, for example includes us humans as well as butterfly larvae! In healthy conditions these never cause a problem for either us or the larvae. Some of these pathogens can be quite virulent. For example, there is quite a high percentage of humans that have meningitis in their noses!

However subject either humans or larvae to overcrowding, poor airflow, high humidity, wet condensation, poor food, etc., then all these previously “harmless” pathogens can multiply rapidly and overwhelm us with disease problems.

Consider this point. Unless the butterfly species has a gregarious larvae, most larvae we breed are very unnaturally crowded together. This in itself is not such a problem when you provide healthy conditions. As soon as the larvae become stressed by feeding cut, wilting host plants, poor airflow, heat, humidity, etc., then problems will soon follow. I can't stress enough the benefits of using growing host plants, in well-ventilated Pop-up cages for your breeding. Your disease problems will just not occur in the first place! Always beware of bought-in livestock, and if possible, isolate them from your main breeding area and stock until you are happy they are disease free.

Of course, this also means keeping everything you use and handle (all equipment) in this process, clean and sterilized after each use. Avoid cross contamination, and use a hand sterilizer gel between handling livestock in separate cages.



This photo shows a fungal disease on Nymphalid pupae.

The pupae are of course dead, but the fructing filament and head of the fungus is amazingly long.

1. (red arrow) shows the start of the filament, and 2. shows the fructing head with spores.

Effective Sterilization

Effective sterilization means everything you use in your set-up: cages, totes, sleeves, work benches and all equipment. Don't forget to regularly change your working overalls and wash them. Also, if at all possible, avoid using wood frames if you are making your own cages.

Consider using two separate areas to hold your milkweed plants, and alternate each season between them. If you use a fruit frame as described earlier in this article, it is not a difficult job to move it to another position at the end of the season. This is a sort of "lying fallow" effect!

A 5% bleach solution is a very effective way of sterilizing your set-up. Use it in a spray and spray liberally! You can of course use bleach on cleaning cloths, to wipe down benches, etc. I would look for a good quality brand of bleach. Some that claim to be effective on 99% of all germs are good. Be careful with bleach on metal items as it can cause corrosion. If you have any metal frames, then leave the spray on for 10 minutes and then rinse off with water. Pure or strong alcohol is a very effective sterilizer, but can be expensive.

One problem with both bleach and pure alcohol is that plants will not tolerate it! So it is difficult to use in a flight house without causing damage. You can overcome this problem by using a different product, described below.

I rarely use bleach as a sterilizer, as I prefer to use a sterilizing product that unfortunately does not seem to be available in the U.S. This is a baby bottle and equipment sterilizing solution called Milton, and I know of at least one U.S. breeder who imports it in tablet form. This product also comes in liquid form. My reasoning behind trialing this product (more than 40 years ago) was that if it's made for sterilizing baby bottles and baby equipment, it has to be very strong and very safe! It doesn't affect metal, either! It has proved a wonderful product for me! It is cheap and effective, and can also be sprayed on your plants without damaging them.

If you would like to learn more about Milton, visit,

http://www.milton-tm.com/sterilising_fluid.html

Oxine AH is a product that also can be used, and will not affect your plants. Both Oxine and Milton can be delivered using a "Fogger".

Here is a link to some information on Oxine that you may find useful.

<http://www.shagbarkbantams.com/oxine.htm>

Oxine is of course readily available in the U.S.



Here is a small, portable fogger in action.

This is a great way to sterilize a breeding room or a flight house.

This fogger is not suitable for distributing bleach of course, but when used with Milton or Oxine AH, it works well.

You can of course also use a fogger to increase humidity in your flight house by just using water.

Managing a Melt-Down

If you do get a disease infection, although it is heartbreaking, you must take immediate action. I have not suffered from any disease in my butterfly breeding stock for more years than I can remember, but I am meticulous in hygiene, and following the principles of what I have written here in this article. If you do the same, disease will never be a problem for you! However, if you let your guard down and have diseased stock, there is no way back for the larvae. They will almost certainly all die if left to themselves. Those few larvae that do struggle through and make it to adults, will themselves be infectious and will pass it on to their offspring.

You will have to destroy the livestock that are all from the same cage (and hopefully your cross-infection prevention will have kept this to a single cage). Either incinerate them all or put the whole lot into a sealed plastic bag into the trash. Get rid of any host plants in the cage at the same time! Then, deep sterilize the cage and table as discussed earlier.

Frass

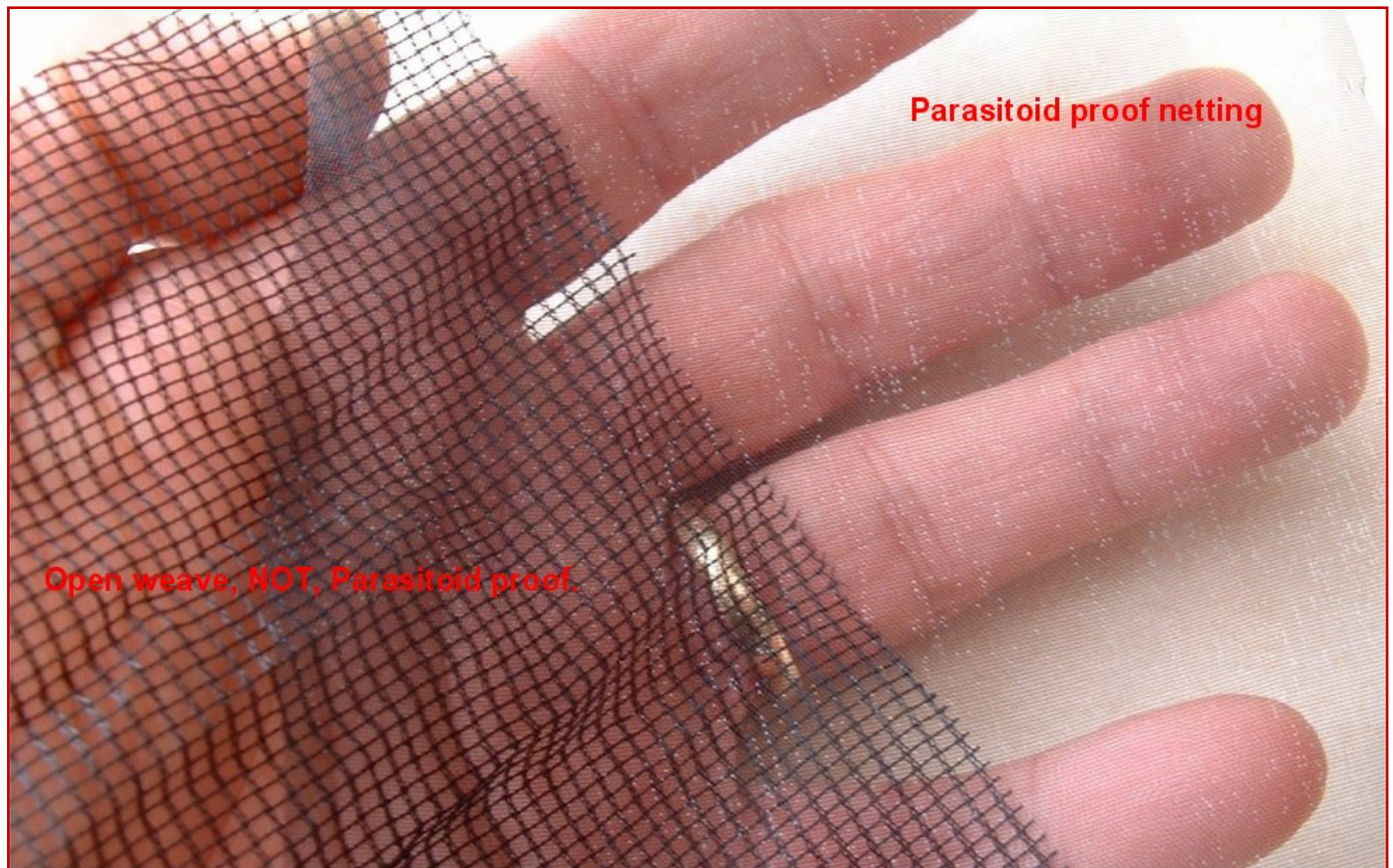
Frass, or larvae droppings, are actually relatively safe in healthy stock. And in the breeding set-up shown in Article 3.5, you can see the larvae do not make much contact with their frass. However, I have heard it suggested by some breeders that frass would make good compost! Never do this, and no matter how healthy your stock is, put it in the trash. It's not worth the risk! After all the trouble you take to avoid disease and subsequent cross infection, why take a chance with frass!

Parasitoids, And How To Eliminate Them From Your Production Process

First, I must say that it is vital to use parasite-proof netting in the confinement of all the life cycle stages, right up to the emergence of the adult butterfly. Actually, the correct scientific term used for these pests is parasitoids. Parasites do not usually kill their hosts, but parasitoids always do!

Adult butterflies are safe and are never infected by parasitoids, because their final life-cycle stage is too short for the parasitoids to take advantage of them.

Parasitoid Proof Netting



The difference between parasitoid proof netting, and an open weave netting. The parasitoid proof netting has a very fine weave, shown on the right in this picture.

Parasitoids

Many parasitoids are host specific, and do not infect other butterfly species. However, there are a number of opportunistic generalists that will! Wherever you live, there will be parasitoids present in the local environment. These parasitoids will have developed their own lifecycle, that even allow for the fact that in some times of the year butterfly caterpillars are absent.

Many breeders, regardless of where they live in the world, start off their breeding programs with no problems and assume it is all too easy. However, in time these pests will find their way into the breeding set-up, and unless the caterpillars and pupae are completely protected, they will wipe out your whole stock.

The problem is that if you do not protect your caterpillars from parasitoids, you will, in the end start to breed the parasitoids and not the butterflies! The parasitoids will have an endless amount of caterpillars to infect, and the whole system of attempting to breed butterflies fails.

Parasitoids can range from the tiniest wasp-like creatures that can infect a single butterfly egg, through to quite large wasps and flies. In fact, one group looks like a small housefly. One of the most dangerous to your breeding are the Chalcid wasps. These tiny wasps are often metallic in color, and once they have access to your breeding cages, the trouble starts.



This photo shows parasitoid larvae emerging from within a full grown caterpillar.

The next photo in this sequence shows the same caterpillar 30 minutes later, and the parasitoid grubs have all spun cocoons and pupated. In less than ten days, the adults emerge to re-infect more caterpillars.



Thirty minutes after emerging, the parasitoids have spun their cocoons and have pupated.

The next picture shows the adults. For this photograph, I obviously had to kill them to take the photograph. The American coin shows how small they are! But, they can be a lot smaller than the species shown here!



A general and a close-up picture of the adult parasitoid adults.

OK, you may think these are small, but they can be even smaller! See this next photograph of a parasitoid that has evolved to infect butterfly eggs!

The parasitoid is so small, that the photograph is not as clear as I would have liked!



A tiny parasitoid, which has evolved to infect butterfly eggs.
The egg (*Papilio thoas*) can be seen just in front of it!

I hope by now I have convinced you of the importance of parasitoid-proof netting!

If you would like to learn more about Parasitoids, see:

<http://en.wikipedia.org/wiki/Parasitoid>

Nigel Venters