



Raising OE-Free Monarchs

by Rose Franklin

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This presentation should answer these questions:

What is OE?

How do Monarchs become infected with OE?

Why should we strive to prevent our hand-reared Monarchs from being infected?

How do we protect our Monarchs from OE?

OE is the abbreviation for Ophryocystis elektroscirrha (pronounced O-free-us-sis-tus electra-sceer-rah).

While many refer to OE as a disease, it is not a bacterial, fungal, or viral disease. OE is a microscopic protozoan (a one-celled organism) parasite.

OE is neither a plant nor animal, but it is a living thing, a living thing that has many of the same characteristics as animals.



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Q
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Note: Queens and Soldiers closely resemble one another, and it is difficult to differentiate between the two.

OE can only reproduce inside the body of a Monarch (*Danaus plexippus*), Queen (*Danaus gilippus*), or Soldier (*Danaus eresimus*) caterpillar. Monarchs, Queens, and Soldiers are closely related and each of them uses milkweed as its larval host. OE is present in nature and probably coevolved with the Monarch, Queen, and/or Soldier.

While Monarch butterflies might be seen in any of the 48 contiguous U.S. states during the summer, Queens are usually only seen in the southern third of the U.S., and Soldiers are restricted to Florida and Texas. Many Northerners, in fact, have never seen a Queen or Soldier. Since the Monarch is the most well-known among the three, I will, from this point on, refer to only the Monarch. Remember though, that if you live where Queens and Soldiers reside, they too can be infected with OE.



Leech



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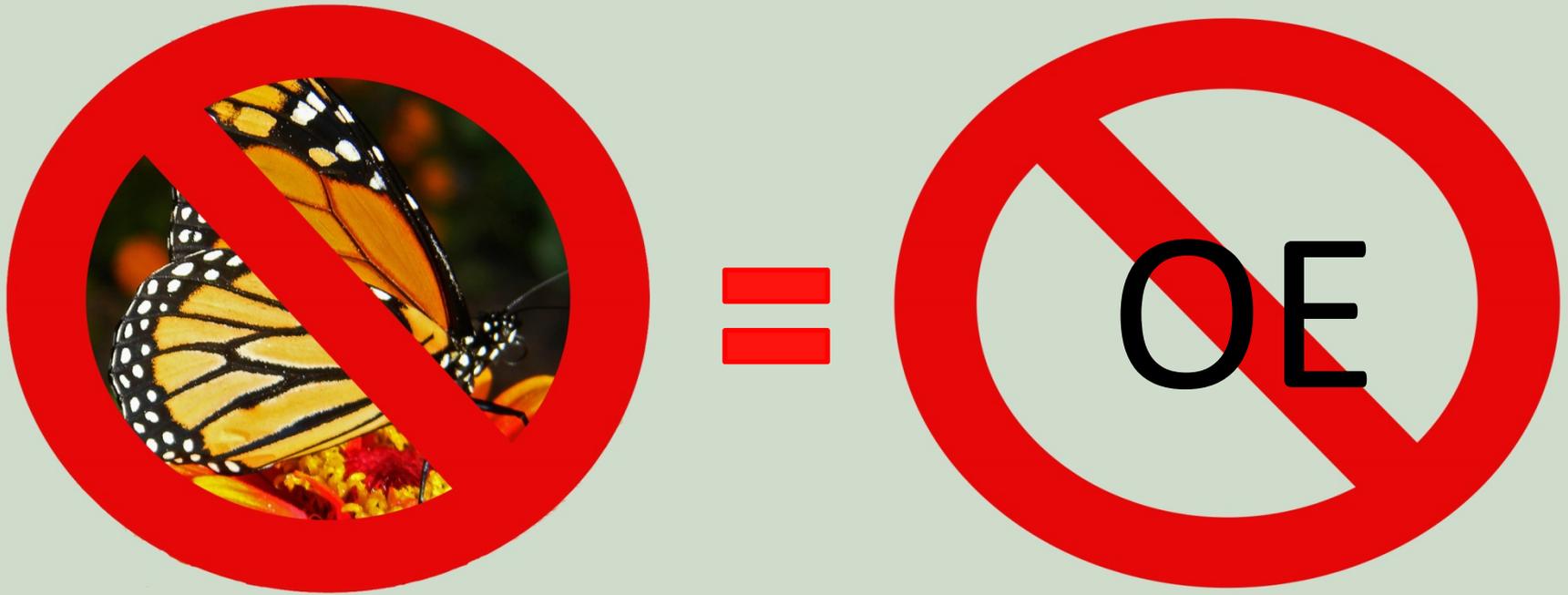
Flea



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Tick

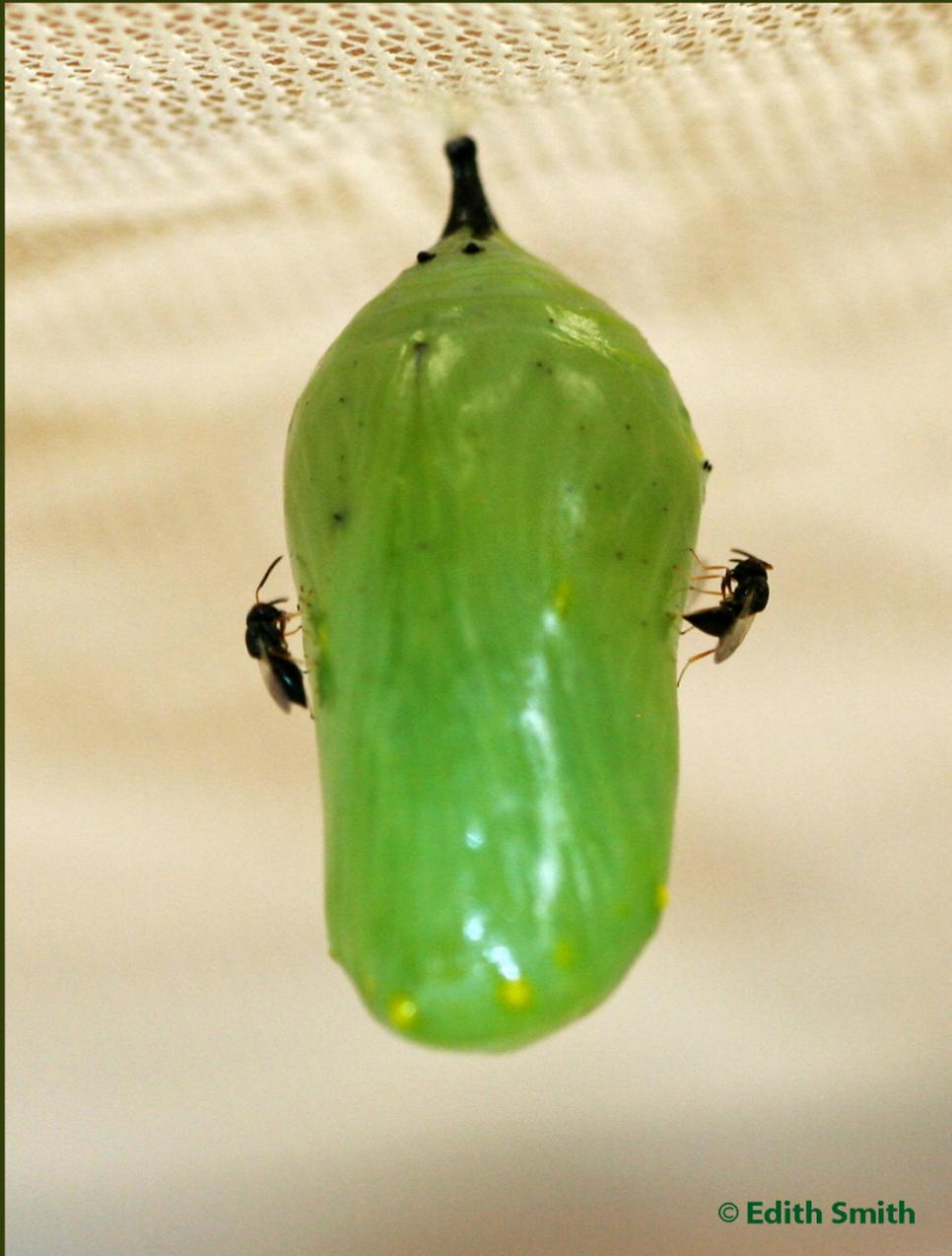
OE is technically a *parasite* and not a *parasitoid*. Parasites are organisms that live in or on a host organism, generally weakening the host as the parasite grows and/or reproduces. The parasite benefits from its host, while the host is usually harmed in some way by the parasite. Well known parasites include tapeworms, leeches, fleas, and ticks. Parasites do not intend to kill their hosts.



Actually, to survive and multiply, OE (and other parasites too) must keep its host alive. If the host dies, so too will the parasite.

Parasites are usually dependent upon their hosts during a very specific period of their life cycle. In the case of OE, it is dependent upon the Monarch for that period of time in which it reproduces.

Parasitoids, on the other hand, generally live their entire lives inside or attached to their host, and eventually the host is killed. The host's death is a direct result of being drained of nutrition by the parasitoid(s).



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Chalcid Wasps are parasitoids. They are tiny, about the size of fruit flies. They lay their eggs on soft, freshly formed Monarch pupae (chrysalis). When the wasp larvae hatch, they consume the developing Monarch inside the butterfly pupa. The wasp larvae will pupate inside the Monarch chrysalis and then emerge from the Monarch pupa as adults. The developing Monarch will be sacrificed while Chalcid Wasps increase in number.



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Brachonid Wasp



Brachonid pupae on a tomato hornworm

The Brachonid Wasp, which is only 1/32" to 1/4" in length, is also a parasitoid. The *Cotesia congregates* is a species of Brachonid Wasp which often lays its eggs on tomato hornworm caterpillars. The adult female wasp will stun the caterpillar with a sting and then wound the skin of the caterpillar to make a cavity for the eggs to be laid in. The wasp usually lays over 50 eggs inside the cavity. When the eggs hatch, the wasp larvae (tiny, cream-colored grub-like creatures) feed on the insides of the caterpillar. When the wasp larvae are fully grown, they exit the caterpillar and pupate, forming cocoons on the skin of the caterpillar. By the time the adult wasps emerge from their cocoons, the tomato hornworm caterpillar is near death. It will die within a few days.

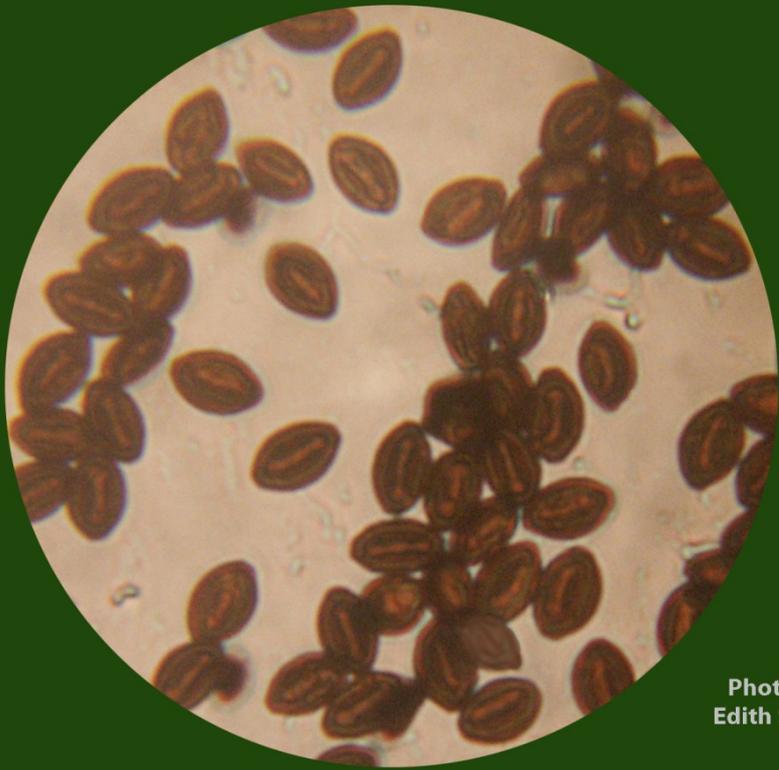


Photo by
Edith Smith

OE spores can be seen under a microscope at 40X to 100X magnification.

Outside a Monarch caterpillar, OE survives as spores or, more precisely, tiny, microscopic OE parasites survive inside the spores. Inside each OE spore are eight OE parasites. OE spores are resistant to heat and cold, and also to water and drought.

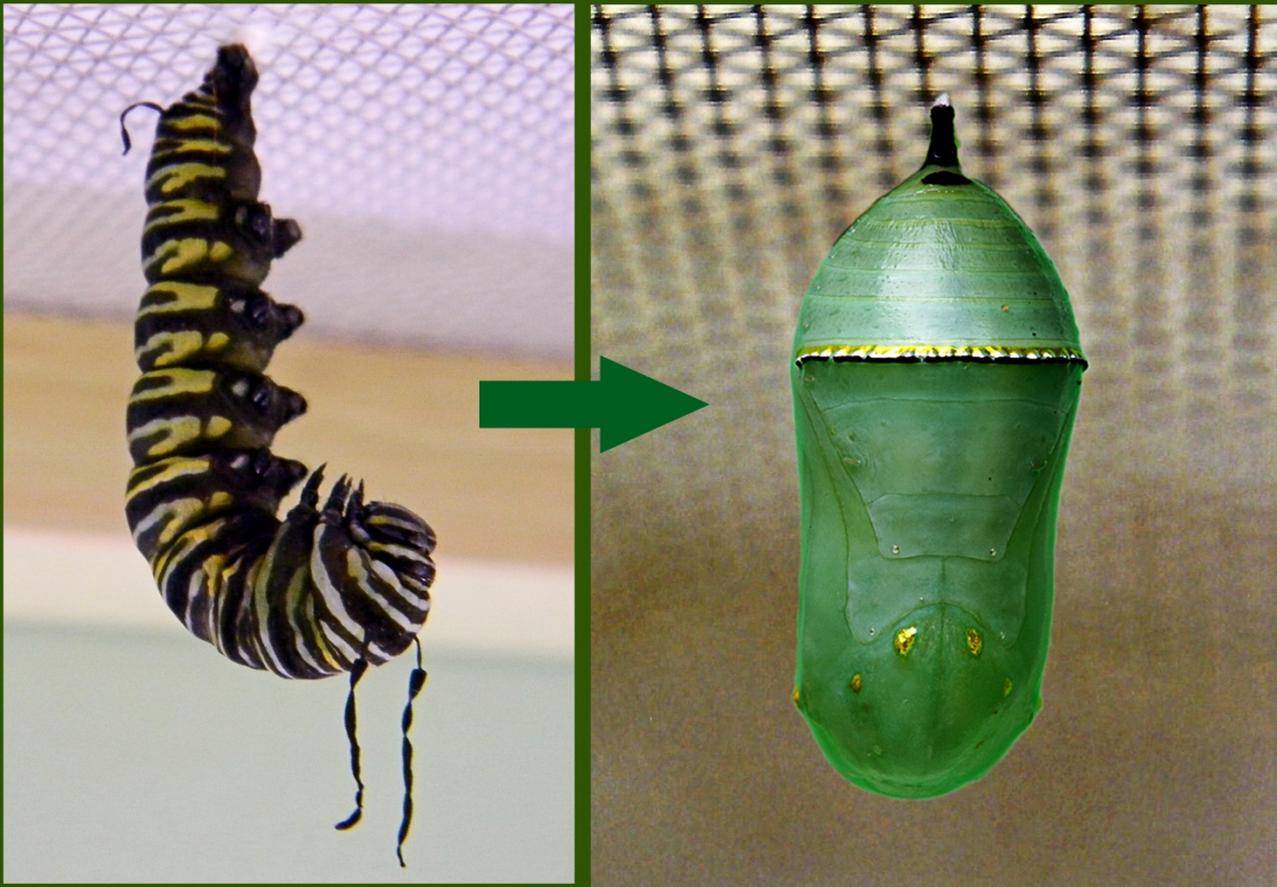
In the photo to the left are dozens of OE spores. Each of the brown, football shaped objects is a spore.

Think of an OE spore as a dormant seed.

Just as a seed splits open and begins to grow when it is put into soil and given the necessary amount of water and light for germination to occur, the OE spore, once inside a Monarch caterpillar's intestinal tract, is in an environment where conditions are perfect for the parasite to reproduce.



After being consumed by a Monarch caterpillar, the dormant OE spore passes through the caterpillar and into its midgut, where digestive fluids dissolve the spore. Eight microscopic, one-celled OE parasites emerge from the spore. Once the parasites are released, they pass through the intestinal wall and take up residence in the hypodermal cells which lay just beneath the caterpillar's skin. There in the hypodermis, and also in the hemolymph (the blood-like substance of insects), the parasites multiply asexually by cell division.



Later, after the caterpillar pupates, the parasites pair up to reproduce sexually, and at that point in time, OE multiplies at an astronomical rate. A few dozen parasites can easily produce thousands upon thousands of offspring in just seven to ten days. It is also at this time that the parasites are causing the most damage to the developing Monarch.



OE Spore



8 OE parasites
inside

The last generation of parasites produced inside the Monarch pupa is borne inside spores, eight parasites per spore. Since OE parasites cannot survive and multiply outside the Monarch caterpillar, the parasites must be inside protective casings by the time the adult Monarch emerges from its pupa.



Two to three days before the adult Monarch emerges from its chrysalis (pupa), spores begin to appear among the scales of the butterfly's abdomen. If the butterfly is heavily infected, you might see dark-colored splotches through the pupa shell. These splotches will appear a day or two before the chrysalis naturally "blackens" prior to the butterfly's emergence. These splotchy spots might be OE spores.

Note: Dark splotches, if they are symmetrically placed on the pupae, could be normal maturing. It's when the patches are not symmetrical from side to side (mirror image), that they might be an indication of OE.

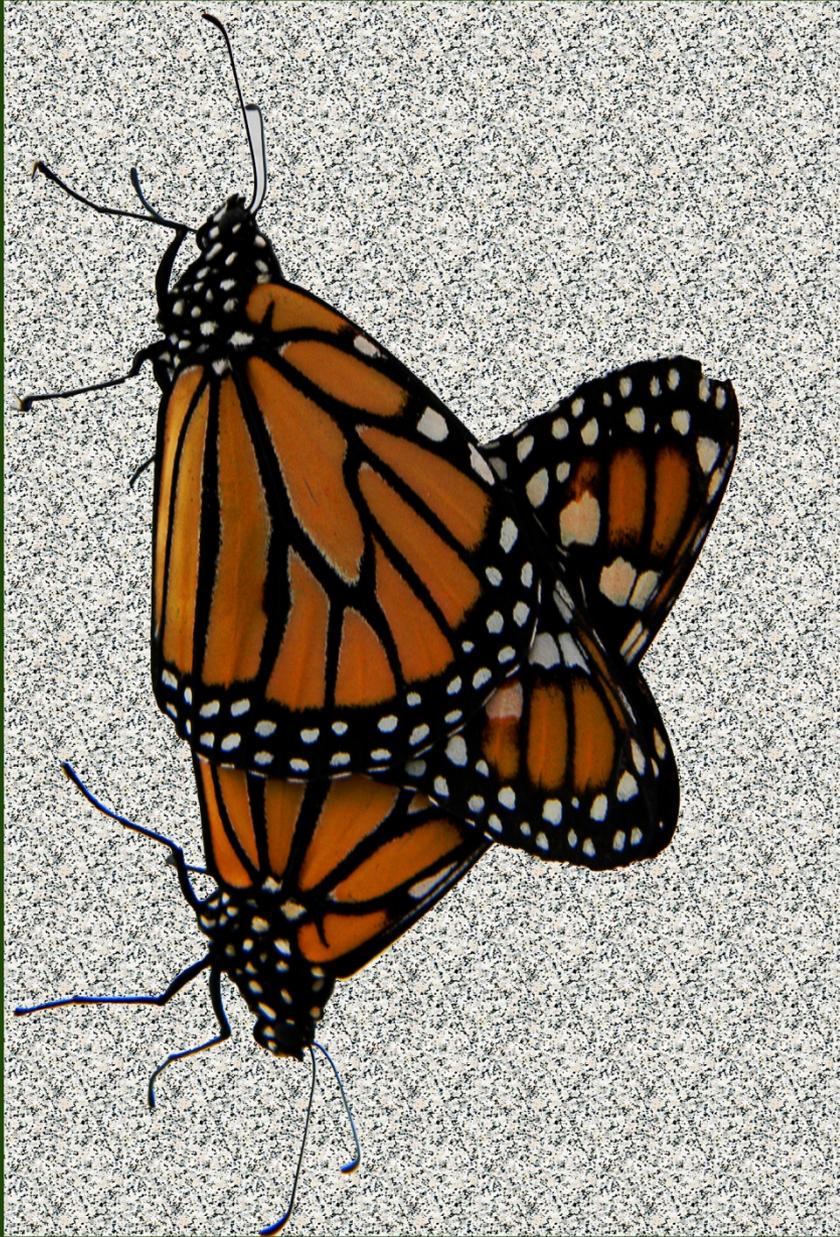


Once a Monarch butterfly is infected with OE, it will be a carrier for OE until its death, now having OE spores scattered among the scales that cover its body. The heaviest concentration of OE spores is generally on the butterfly's abdomen, but sometimes they will also be on its wings and/or other body parts.

Remember though, that you cannot see OE spores with your naked eye. Only under a microscope, can OE be detected.



But the OE spores aren't clinging tightly to the Monarch's body. Instead, some of the OE spores will drop off the Monarch onto milkweed on which it is nectaring, resting, or laying eggs.



OE spores will be passed from infected Monarchs to uninfected ones during mating.

© Rose Franklin



OE spores will be deposited on eggs as eggs are being laid by infected females.

Only by consuming OE spores can a caterpillar become infected with OE parasites.

The severity of OE (the number of OE spores present on the adult Monarch when it emerges from its chrysalis) is determined by how old the caterpillar is when it ingests the OE spores, and how many spores are consumed.



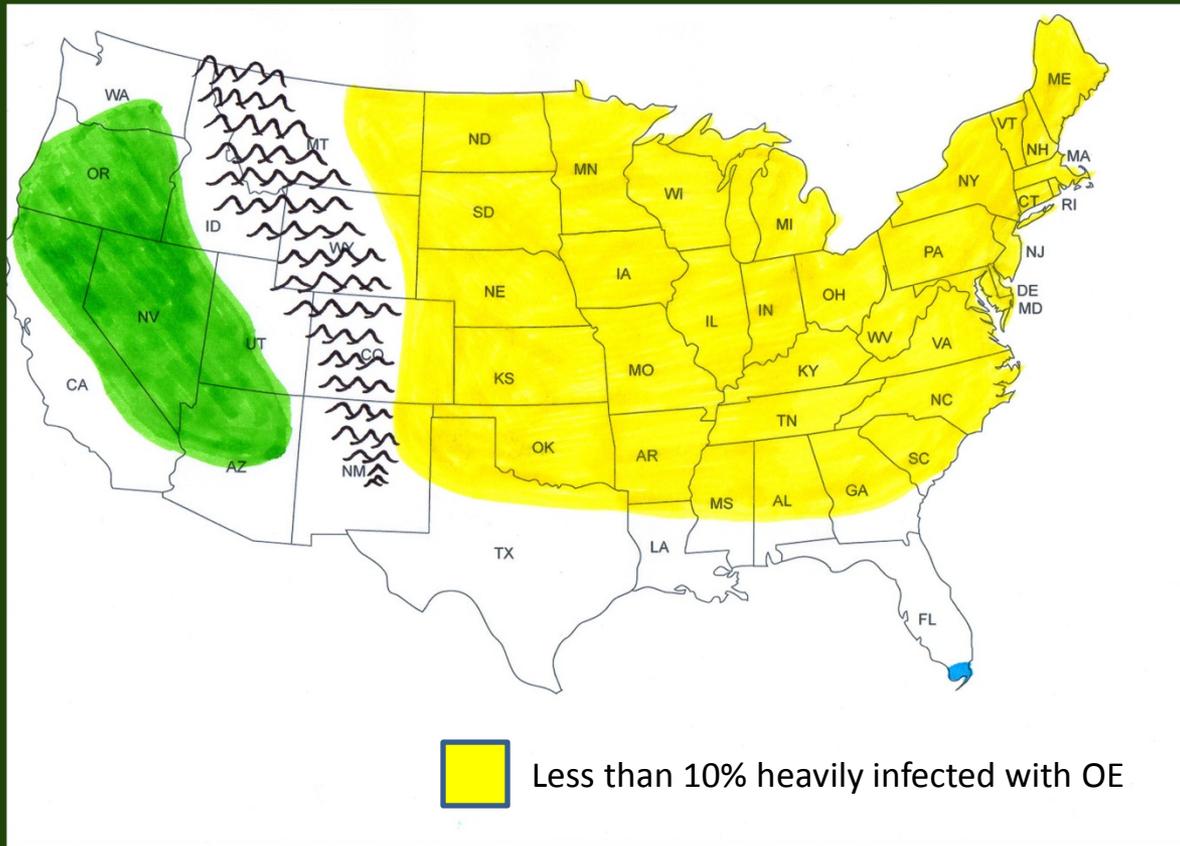
If OE spores are on the shell of a Monarch egg, they will likely be consumed soon after the tiny caterpillar hatches. This is because a Monarch caterpillar usually consumes its eggshell before it takes its first bite of milkweed. If a newly hatched caterpillar consumes dozens of spores while eating its eggshell, each of those spores will replicate themselves many, many times inside the caterpillar. By the time this caterpillar becomes an adult Monarch, it will likely be heavily infected with OE.



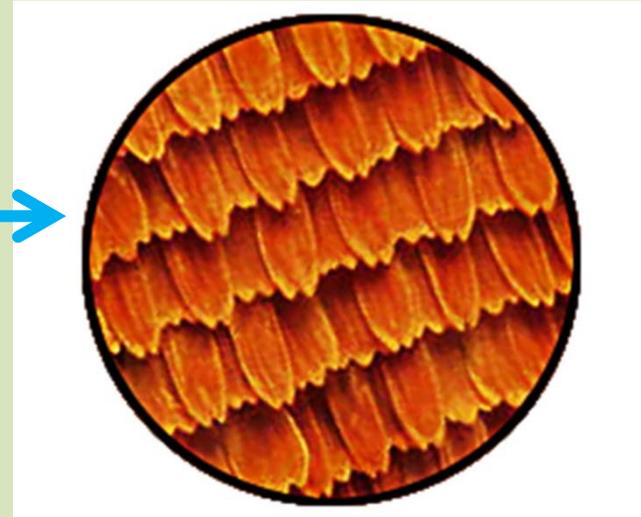
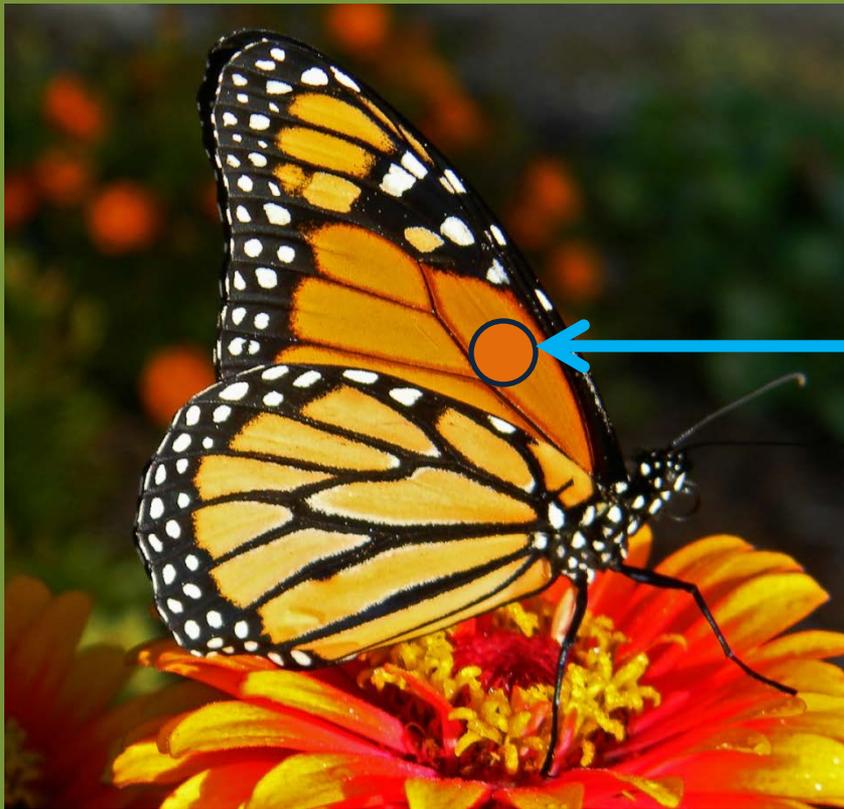
If spores aren't consumed until the caterpillar is almost fully grown, the OE parasites will have less time to multiply and thus, the adult might be only mildly infected.



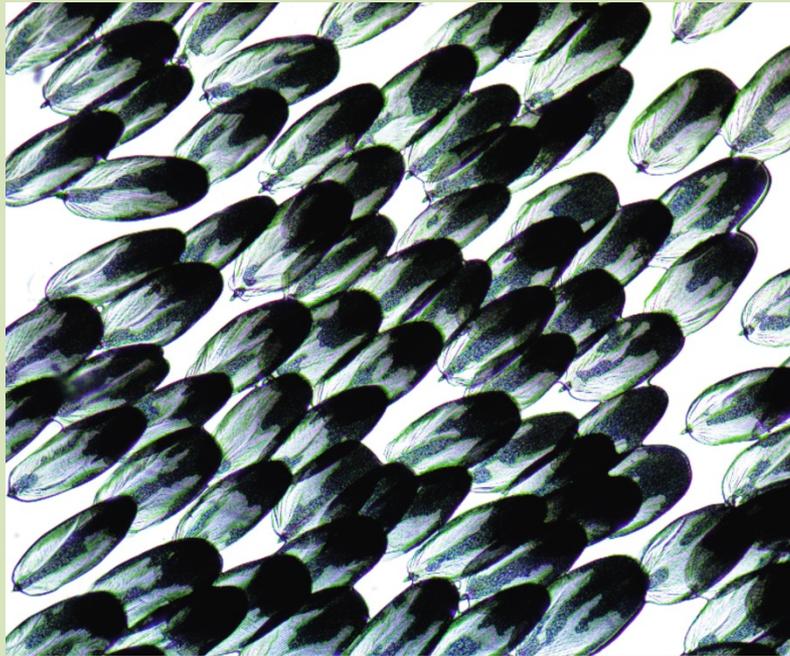
Some of the wild Monarchs have probably been infected with OE for thousands of years. There's no way to know this though, since OE wasn't discovered until the late 1960's.



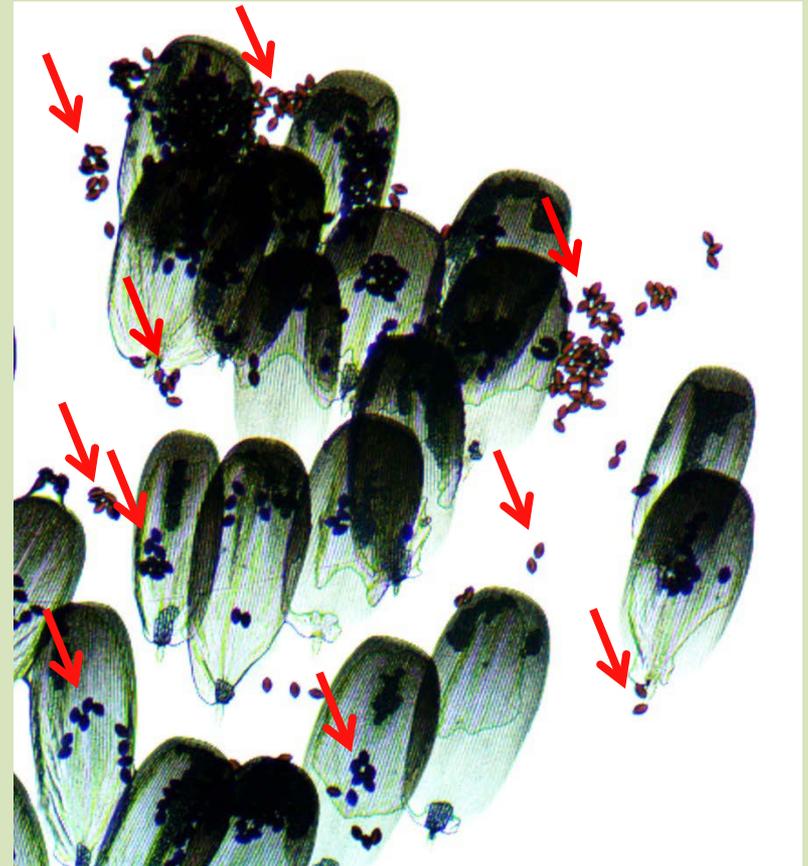
What is known is that the current rate of infection varies by region. The Monarchs that reside mostly to the east of the Rocky Mountains during the summer and then migrate to Mexico for the winter have the lowest infection rate, with less than 10% of them being heavily infected.



An adult Monarch's body and wings are covered with scales. On its wings alone are thousands of scales. Under a microscope at 40X to 100X magnification, Monarch scales somewhat resemble fish scales, and they overlap like shingles on a roof.



Since a Monarch's abdomen is mostly black and white, its abdomen scales are mostly black and white also.



OE spores, in comparison, look like tiny dark brown footballs lying on or around the Monarch scales. In this photo, red arrows point to some of the OE spores.



Infected with
OE???

Not infected?

Unless you look at a sampling of a Monarch's abdomen scales under a microscope, there is no way of knowing for sure whether or not the Monarch is infected with OE. The butterfly might look healthy and behave normally but still be infected, either lightly or heavily, with OE.



The white stripes on this caterpillar look cloudy. It might be infected with OE.

Sometimes, though rarely, signs and symptoms of OE are present in Monarch caterpillars. The white striping might not be pronounced but instead look grayish, cloudy, or mottled. You might think the caterpillar's skin looks dirty. Their appetite might be reduced, they may seem sluggish, or their antennae might be deformed. While these signs might signal the presence of a viral, bacterial, or fungal disease, any of them could be an early sign of OE infection.



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Look for the asymmetrical dark splotches that sometimes appear through the chrysalis a few days prior to the butterfly's emergence. These splotches are often tell-tale signs of OE.



Heavily infected Monarchs do often show signs of being infected just prior to, or immediately after, emerging from their chrysalises.

Sometimes the infected butterfly will have trouble emerging from its chrysalis; sometimes it will have deformed wings upon emergence; some might be too weak to cling to their chrysalis while their wings expand and dry. Sometimes the newly-emerged Monarch will be missing the pronounced white striping on its abdomen, or there may be green spots on the abdomen. But even if none of these signs or symptoms are present in the newly emerged Monarch, it may still be OE-infected.

Note: A Monarch's wings will always be wet and crimped upon emergence from the chrysalis. In a heavily OE-infected Monarch though, its wings might dry crimped and then the butterfly will be unable to fly.

When a caterpillar shows signs or symptoms of being infected with OE, isolate it immediately. Sanitize your hands, and then thoroughly clean the cage that the suspect caterpillar was in. Replace all the milkweed in that cage with fresh milkweed. Attach a note to the cage stating when and why the caterpillar was removed. Closely monitor the caterpillar that was removed and also the ones remaining in the cage. If, in the coming days, more caterpillars show sign of being ill, it might be time to euthanize all the caterpillars in that cage, dispose of the plant material inside, and sanitize the cage.

To euthanize caterpillars, put them in a plastic bag and tie the bag shut. Put the plastic bag in the freezer for 8 hours or so. Then dispose of the bag and its contents.

Do not squish the caterpillars as you could be releasing disease-causing pathogens into the environment in doing so.

Now we know what OE is, and we know that not until we sample the abdomen scales of Monarchs under a microscope, can we be assured that our Monarchs are OE-free.

But what's the problem with our Monarchs having OE since even in the wild, lots of Monarchs are infected with it?

In the wild, OE is kept in check. There are millions of OE-free Monarchs flying about, and millions of OE-free milkweed plants for the caterpillars to feed on. A few Monarchs will become infected, but many will not. This isn't the case with Monarchs that are reared in captivity. In captivity, where butterflies fly in small enclosures and caterpillars are reared in close quarters, if one butterfly is infected with OE, many others will likely become infected too. If one caterpillar has OE, many of the others will most certainly be OE-infected also. All the caterpillars have likely been feasting on milkweed that came from the same general area. And once OE is present in your rearing operation, it only increases in load from one generation to the next.

A light infestation could quickly evolve into disaster. Instead of having beautiful Monarchs emerging from chrysalises, you might be watching the emergence of deformed, crippled creatures that have little resemblance to the majestic butterflies they should have been. Some of the butterflies will be unable to pry loose of their chrysalises while others will succeed in exiting, only to fall to the ground below because they are too weak to cling to their chrysalises long enough to dry and then fly. Some of the Monarchs might have green spots on their abdomens.

Anyone who has experienced the results of a heavy OE load in Monarchs reared in captivity will tell you that this is hard to handle. While it's not generally difficult to witness the death of a fly, mosquito, or spider, no one wants to see Monarchs in despair.

So how do we raise OE-free Monarchs?



We start by testing all of the adult Monarchs we are going to use for egg production. This means that we purchase a microscope and check our Monarchs for OE, or that we be willing and able to send abdomen scale samples to someone who has a microscope and is able to identify OE. It is essential that we start with clean stock and then do all we can to keep the operation clean.

It is also important that we utilize OE-free milkweed when feeding the caterpillars. To be OE-free, milkweed must either be kept where wild Monarchs cannot touch it, or it must be sanitized prior to feeding. A solution made of 10% bleach and 90% water will kill OE spores. To sanitize the milkweed, completely saturate the plants with the solution, let the bleach work for five minutes, and then rinse the milkweed several times in clean water. Allow the milkweed to dry before feeding it to the caterpillars. Wet milkweed might mold, and mold could lead to problems with bacterial or fungal disease in the rearing operation.

Because older caterpillars are more likely to have OE (and other diseases too) than the younger ones, it is important not to spread disease from the older ones to the youngest. This means that caterpillars be cared for (cages cleaned and new milkweed provided) in the sequence of smallest to largest caterpillars (youngest to oldest). In meeting the needs of the caterpillars, we should sanitize our hands, the utensils we use, and the work area (counter top, table, etc.) between each cage of caterpillars that we tend to. Unless we do this, we might be transmitting disease from one group of caterpillars to another. Then, when we are done cleaning cages for the day, we should wipe down the entire work area (counter, utensils, and floor) with a 10% bleach solution before exiting the area.

Note: To protect your hands from repeated exposure to bleach, you should wear latex, nitrile, or vinyl gloves while working with the caterpillars, and then just dip your gloved hands into the bleach solution after finishing one cage and before going on to the next.

While you are working to reduce or eliminate the occurrence of OE infection, you may as well do a few extra things to diminish the possibility of bacterial, viral, and fungal diseases, and also the chance that other parasites and/or parasitoids could attack your Monarchs.



Caterpillars should not be overcrowded when they are being hand-raised. In a rearing chamber about 12” square, there should be no more than 10 caterpillars. In a cage that is approximately 24” square, there should be no more than 25 caterpillars. Overcrowding facilitates the spread of disease among caterpillars.



Egg



**Caterpillar
(larva)**



**Chrysalis
(pupa)**



Adult



Different life stages (eggs, caterpillars, chrysalises, and adults) should be kept in separate areas, or preferably, in separate rooms. Separate buildings would be even better yet. Monarchs should not be emerging from chrysalises in the same room where caterpillars are feeding on milkweed and eggs are incubating and hatching. Adult Monarchs should not be flying in the same area where eggs, caterpillars, and pupae are being housed.

Before a new batch of caterpillars is put into a used caterpillar cage, the cage should be sanitized, inside and out.

To sanitize a used cage, the cage should be soaked in a solution of 10% bleach and 90% water. The bleach solution should be left on the cage for 10 minutes. Then, because the chlorine in bleach can be toxic to insects, the cage should be rinsed many times to remove the bleach residue.

In putting caterpillars into a cage that previously housed caterpillars but has not been sanitized, we would be immediately subjecting the new caterpillars to whatever disease-causing pathogens might be inside or on that cage.

Note: To protect your hands from repeated exposure to bleach, you should wear latex, nitrile, or vinyl gloves while working with a Clorox solution.

Optimally, we should raise Monarch caterpillars in a room where the temperature is 75 to 80 degrees F. and the humidity is 60% - 65%. We should not allow the temperature to rise or lower quickly as this causes stress for the caterpillars.

Caterpillars are also stressed when they run out of food, so we should ensure that they always have fresh milkweed to eat.

Just as stress makes a person more vulnerable to illness, so too does stress cause a caterpillar to be more susceptible to disease.

We should develop standard operating procedures to guide us in every step of rearing Monarchs, and then not deviate from those standard operating procedures. We should have a standard operating procedure (SOP) for cleaning caterpillar cages, one for sanitizing milkweed (or keeping wild Monarchs away from the milkweed we are growing for caterpillar consumption), and one for checking adults for OE. The SOP's should be printed and posted where each activity takes place. Then, whether you or someone else is doing the work, the work should be being done the same way.

OE infection can shut down a Monarch rearing operation rather quickly. Standard operating procedures designed to eliminate the occurrence of OE in the rearing operation can bring about the production of healthy, majestic butterflies month after month.

And in working to reduce or eliminate the occurrence of OE in our Monarch population, we will be maintaining cleanliness which will assuredly aid in reducing or eliminating the occurrence of other diseases too.

Raising Monarchs isn't easy, but it is extremely rewarding to those who are committed to raising healthy Monarchs.

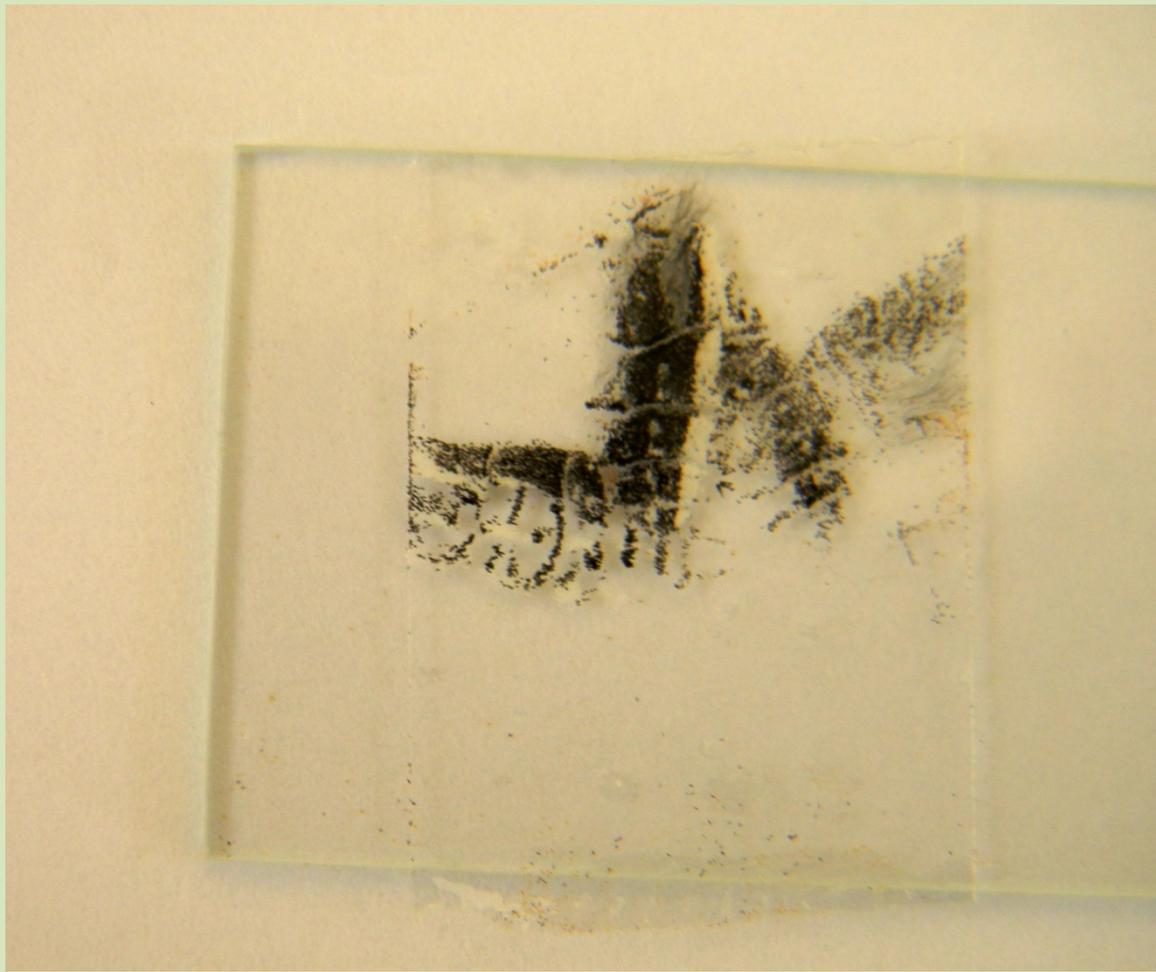
To check adult Monarchs for OE, you will need a microscope, some clear scotch tape, and some microscope slides. There are three steps to screening a Monarch for OE:

1. Use a piece of clear scotch tape to collect some scales from the Monarch's abdomen.
2. Place the scotch tape on a microscope slide.
3. Examine the slide under the microscope.



Hold the Monarch so
its abdomen is
exposed.

Place a piece of clear
scotch tape against
the abdomen to
remove some of the
scales.

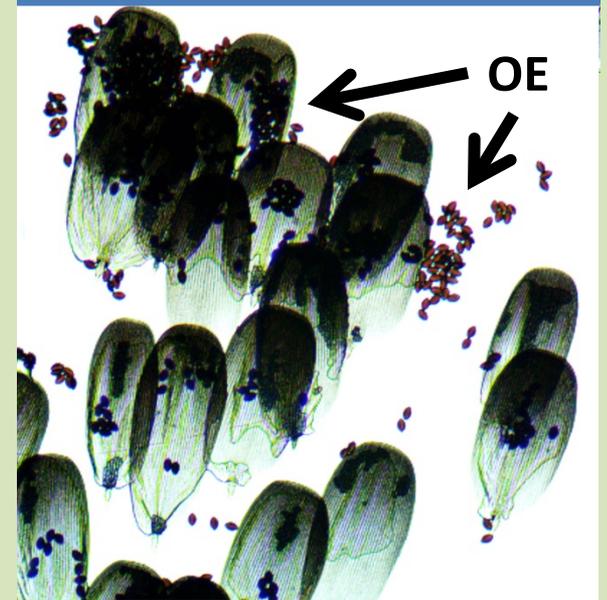
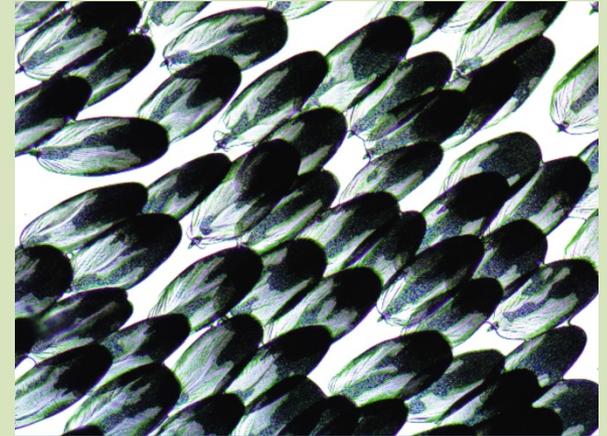


Put the tape on a microscope slide.

Rub across the tape with a paper towel to remove air bubbles that are between the tape and the slide.



Examine the slide under a microscope.



The small brown football-shaped objects in the bottom photo are OE spores.

If you decide to purchase a microscope, please get a student microscope, not a toy microscope. You will need only a 10X eyepiece and 10X optical lens. I suggest the microscope have bottom illumination.

You might be able to use a microscope in your local high school science department too, and there a teacher might be helpful in teaching you to use a microscope.

Please study the presentation until you can answer the following questions:

What is OE?

How do Monarchs become infected with OE?

Why should we strive to prevent our hand-reared Monarchs from being infected?

How do we protect our hand-reared Monarchs from OE?

Whether you are a citizen scientist, a school teacher raising Monarchs in the classroom, or a hobbyist, you should know what OE is and be willing to implement procedures that should help protect your Monarchs from the harmful effects of OE infection.

Please consider making changes to your rearing operation so the Monarch population might benefit. Even one or two small procedural changes could make a significant difference in the health of your butterflies.

Thank you for taking the time to read thru this presentation. That shows that you truly care about the Monarchs you are rearing.