Fungicide impairs silk production, according to study

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The silkworm is the larva or caterpillar of the domesticated silkmoth Bombyx mori. The best-known silk is obtained from cocoons spun by larvae of the mulberry silkworm reared in captivity (sericulture). Credit: Daniel Nicodemo (FCAT-UNESP)

One of the problems caused by the intensive use of pesticides is their effect on organisms other than those they are designed to combat—the most notorious example of which is the global mortality of honeybees.

However, there are other economically beneficial insects besides honeybees that are also implicated. "The silkworm is another managed insect whose performance may have been impaired by pesticide poisoning," said Daniel Nicodemo, a professor at São Paulo State University's School of Agrarian & Technological Sciences (FCAT-UNESP) in Dracena, Brazil.

In a study supported by the São Paulo Research Foundation, Nicodemo partnered with Professor Fábio Ermínio Mingatto and students at FCAT-USP to investigate the effects of pyraclostrobin, a strobilurin fungicide widely used as a crop spray, on the mitochondrial bioenergetics of silkworms (Bombyx mori) and their production of cocoons.

The researchers found that application of the fungicide to mulberry plants—silkworms' single source of food—increased caterpillar mortality up to threefold and significantly reduced the size of the cocoons spun by surviving caterpillars, causing silk production losses. The study was the subject of an article published in the *Journal of Economic Entomology*.

Brazilian silk farmers have reported a fall in the production of silkworm cocoons—even in the absence of factors such as disease, malnutrition and inappropriate management, which impact negatively on the development cycle of the mulberry silkworm, in Nicodemo's assessment.

Brazilian silk farmers are smallholders who grow mulberry plants to provide food for the silkworms. Most Brazilian silk farms are found in northern part of Paraná State, but there are also sericulturists in São Paulo State and Mato Grosso do Sul. They are small isolated concerns

surrounded by vast sugarcane, soybean or corn plantations where pesticides are heavily used.

"This is one of the possible causes of the silkworm crop losses," Nicodemo said. When the big plantations are sprayed with insecticides, herbicides or fungicides, the wind often blows these agrochemicals into neighboring farms, where mulberry plants may be growing.

Pyraclostrobin: from promising asset to sericulture villain

Nicodemo said that initial goal of the FAPESP-funded study was to determine whether treating mulberry leaves with pyraclostrobin would improve cocoon quality. "Besides being used as a fungicide, pyraclostrobin also delays leaf senescence and strengthens oxidative stress tolerance in many crops."

Mulberry leaves with high-protein content have a direct influence on the quality of larvae-produced silk. However, the art of growing a highly nutritive mulberry plant follows very particular standards. "The quantity of leaves produced by each plant is inversely proportional to their nutritional quality," Nicodemo said, explaining that the nutritional value of the leaves peaks approximately 60 days after pruning.

On the other hand, the average Brazilian silk farmer harvest the leaves about 90 days after the last pruning, when the mulberry plants show a larger amount of leaves with lesser nutritional value. That is because a typical silk farm is about three hectares in size, with mulberry planted throughout. Leaves are harvested monthly in each hectare by turns, so that plants pruned in January, for example, have until the end of March to produce new leaves, which, therefore, will be harvested only in April.

According to the FAPESP-supported researcher, the use of pyraclostrobin is suggested to maintain leaf quality to the end of the 90-day period. This would enable a bigger harvest of high-quality mulberry leaves. "Pyraclostobin action would then contribute to mulberry leaf quality enhancement and enable silk farmers to maximize production of high-quality leaves at pruning time." As for the outcome of the test, Nicodemo answered fast: "The results were the opposite of what we expected."

Respiratory chain inhibitor

A commercial fungicide with pyraclostrobin as the active ingredient was chosen for the field experiment. The methodology used in the experiment consisted of treating a mulberry plantation with pyraclostrobin in three doses (100, 200 and 300 grams of active ingredient per hectare) applied as 1,000 liters of solution 60 and 75 days after each pruning. A control area was sprayed with water only. Leaf production, chlorophyll content, macronutrients and micronutrients were analyzed after each pruning.

Mulberry branches from plants treated with the fungicide were fed to the caterpillars 15 and 30 days after application. Head and intestine mitochondrial bioenergetics were evaluated in vitro and in vivo, along with leaf consumption and caterpillar mortality.

Because mortality was very high when the caterpillars were fed leaves from plants treated with pyraclostrobin 15 days previously, the tests were continued using only leaves from plants treated 30 days before. In this case, the researchers found that at doses of 50 micromolar (μM) in vitro and 200 grams per hectare in vivo, pyraclostrobin inhibited oxygen consumption, dissipated membrane potential and inhibited ATP synthesis in mitochondria. ATP (adenosine triphosphate) is the nucleotide responsible for energy storage in cells.

"Pyraclostrobin acted as a respiratory chain inhibitor, affecting mitochondrial bioenergetics," Nicodemo said. "Thus, the main effect expected for fungi also occurs in silkworms."

Mortality rates were statistically similar for the groups fed 60 days after pruning on leaves from untreated plants and plants treated with pyraclostrobin. However, mortality was 30.7 percent higher for the group fed leaves from plants treated with the highest dose of pyraclostrobin than for the control group, evidencing a dose-dependent effect.

At the end of the feeding period, the larvae were managed so as to be allowed to spin cocoons. One hundred cocoons were weighed per experimental group, and the silkworm pupae were then removed. At the end of the process, the cocoon shells were weighed separately.

The results showed that the presence of the fungicide in leaves did not significantly affect their consumption by the silkworms but negatively affected cocoon weight when the dose of pyraclostrobin applied was 100 grams per hectare or more. The weights of the cocoons (-10 percent) and cocoon shells (-7 percent) were lower for caterpillars fed leaves treated with pyraclostrobin than for caterpillars fed untreated leaves. The dose of fungicide applied made no difference to cocoon quality.

According to Nicodemo, the toxicity of any substance is a key variable to determine its direct impact on the survival of individuals in a given species after exposure to a specific dose, concentration or both.

"Nonlethality doesn't mean the substance is harmless. Organisms may be harmed in various ways even if they survive," Nicodemo said. "In our study, while silkworm mortality was relatively low when they were fed mulberry leaves that had been treated with pyraclostrobin 30 days previously, damage to head and intestine mitochondrial bioenergetics

negatively influenced energy production by mitochondria as well as cocoon production."

More information: Daniel Nicodemo et al, Pyraclostrobin Impairs Energetic Mitochondrial Metabolism and Productive Performance of Silkworm (Lepidoptera: Bombycidae) Caterpillars, *Journal of Economic Entomology* (2018). DOI: 10.1093/jee/toy060

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