Molecular identification of predatory mites

An army of different predatory mites are at the front line of biological pest control. They all seem to share an identical appearance, but they differ mutually in biological characteristics and functionality. Dutch research institute PPO Lisse has created a DNA fingerprint of the various species of predatory mites currently deployed in greenhouse horticulture, or being investigated for that purpose.

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In the 1990s the numbers of natural enemies available for use in greenhouses threatened to dry up. Fortunately, however, in recent years the numbers have risen, particularly in the category of 'leaf-borne predatory mites'. This is a large group of tiny, inconspicuous predators, who nevertheless have a crucial role in controlling populations of plant eaters in both natural and commercially grown vegetation.

Their appearance is incredibly boring. They are not blessed with bright colours, graceful tufts or characteristically shaped bodies. In fact, they have no use for such adornment: they are completely blind, and recognise each other and their prey through the senses of smell and feeling. In the field, most species are indistinguishable from each other. In the artificial situation of a greenhouse, where few natural predators are present, we simply assume that any specimens observed are the descendants of the mites released a few months earlier. Unfortunately, this is not always the case.

At the time when just a few species were used, this was not such a problem. If you found an orangey red predatory mite in a colony of spider mites, it was most probably *Phytoseiulus persimilis*. Today, however, the situation is more complicated. A growing number of species are available on the market. In addition, the number of suppliers to the market is also growing, and not all these companies share the same level of expertise. And last but not least: as growers become more successful in the long-term avoidance of broad-spectrum insecticides, the chance of spontaneously occurring natural enemies increases.

Specialists

Identifying a certain species in the field by using a magnifying glass is impossible. These animals are even too minute for the binoculars currently used by advisors that magnify up to 40x. Mites and small insects should first be macerated and cleared until they are completely transparent, after which their fine structure is examined under an optical microscope. Species identification is work for specialists. As more species become available, even these specialists encounter problems. The current arsenal contains a few 'tricky pairs': the indigenous *Amblyseius andersoni* compared with *A. swirskii* from the Middle East, and the North-American *A. fallacis* compared with the cosmopolitan *A. californicus*. These have many morphological features in common, but function differently. Crossing females of one species and males of the other would provide certainty, but this method is far too convoluted for daily use.

Molecular markers

A year ago, PPO Lisse started identifying predatory mites based on their DNA. The researchers used pure cultures from Wageningen UR Greenhouse Horticulture that are regularly checked microscopically, as well as a number of cultures from the commercial circuit. For each predatory mite sample, it was tested whether a certain piece of DNA could be multiplied using special primers. Tests were first done on a general primer set for this family of predatory mites (the Phytoseiidae), followed by tests on primer sets specific for a certain species of mite. These sets were already on hand for certain 'old friends' such as *P. persimilis*, but for most species PPO Lisse defined the characteristics itself.

Khanh Pham in the PCR laboratory at PPO Lisse: "Using a simple PCR test we can identify between the different types of predatory mites released in the greenhouse quickly and with 100% certainty."

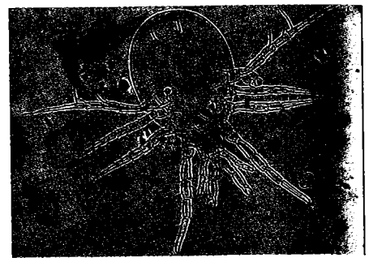
As expected, all the samples reacted to the general Phytoseiidae set. The good news is that all the samples studied reacted exclusively to one species-specific primer set, namely their own. The DNA test fully matches the traditional morphological identification. This also applies to the presumed mutual relationship between species. One example is *A. fallacis*, which gave a weak reaction to the primer of *A. californicus*. This pair is also difficult to differentiate between microscopically. Currently, work is in progress on better primers for these two species, and on primers for the new species that will be included in the study.

From each continent

If required, these molecular techniques can be used to investigate the lower levels of sub-species and local populations. This is mostly interesting for species that occur worldwide. The origin of the predatory mites bred commercially can be found in each of the continents of the world (with the exception of Antarctica) and is sometimes not even known. For example, *A. cucumeris*, the most widely used natural enemy in greenhouses today, has been mass-reared for more than 20 years. As far as we know, the commercial production lines of this predator originate from either a Dutch greenhouse population or a field population in New Zealand. In the meantime, this strain is raised in dozens of places from Japan to British Columbia, with a constant likelihood of them interbreeding with local populations. Are all these 'products' one and the same? What we are talking about here is not only correctly labelling the mite, but also possible differences in their functioning.

Molecular techniques are becoming faster and more efficient. For one test about ten mites are generally used, but testing is also possible with one specimen, maybe even with just one egg. The results are available within 2 days. What is also significant is that any lab technician can learn this technique, whereas using traditional morphological keys requires high levels of skill and experience. However, the optical microscope is not set to leave the stage just yet. The microscope will continue to be used to identify mixed populations, and to assess the vitality of a population (fertility, infection with pathogens). Microscopy will also remain important in investigating new species and as a calibration method to enable further development of this highly promising DNA technique.

A predatory mite in action, magnified. Except for the person who took the photograph, no one knows which predatory mite this is.



Identifying the species of predatory mite is done based on minute differences only visible under a microscope.

Practical applicability

DNA identification is not just a handy tool for entomologists. Companies active in biological pest control are also showing interest, both in current and future options.

Directly applicable is the verification of the trueness to species. Serious producers of biological predators regularly examine their populations under a microscope. They would like to confirm the identity of their mother lines occasionally using a molecular technique.

Predatory mites are usually bred in a 3-dimensional system on a natural substrate preying on storage mites. This breeding method is by far the cheapest, but susceptible to all types of contamination. Storage pests, unintentional predators and pathogens may occur. The larger the scale of mass-rearing, the greater the risk. And, of course, the risk is high if several species are raised by the same people in one and the same location. Producers of natural enemies must continually monitor this. Molecular techniques can probably indicate contamination in mass rearing at an earlier stage, provided the expected contaminant is known and identified. The sensitivity of such a method is potentially much higher than with traditional sampling.

Quantitative application

Most of the interest, however, focuses on the quantitative application of molecular techniques, with the basic question being: how many predatory mites of a certain species are contained in a certain volume or certain type of packaging? This is a very time consuming (and thus costly) part of the daily routine in commercial mass rearing and a constant source of confusion and dispute in the distribution channels. Testing based on DNA would by far be the preferred method in this case. A considerable amount of homework remains to be done, however. The most suitable molecular method still has to be selected. It should be calibrated thoroughly, because conflicting interests are at stake here (suppliers versus customers). The major producers will want to have these techniques in house and under their own supervision. Smaller competitors, as well as distributors, advisors and growers' associations will probably prefer to use the services of independent laboratories.

Summary

On crops grown in greenhouses, one may encounter different species of predatory mites, either released deliberately or occurring spontaneously. Identification keys for the microscopic determination of these mites are available. These keys can only be used by highly experienced specialists. To support their work, PPO Lisse has constructed molecular markers for the major predatory mite species.