The Plant Health Instructor
Volume: 22
Year: 2022
Article Type: Case Studies

Ageratum: The Dose Makes the Poison

[1]Tatiana Lenskaia, Ph.D., [2]Benham Lockhart, Ph.D, and [3]Sergei Lenskii, M.D., Ph.D

[1]Bioinformatics and Computational Biology, University of Minnesota
[2]Plant Pathology, University of Minnesota
[3]Minnesota Center for Philosophy of Science, University of Minnesota

Date Accepted: 16 Jul 2022
Date Published: 26 Aug 2022
DOI: 10.1094/PHI-C-2022-0826-01

Keywords: Case study, diagnostics, fertilizer overuse, leaf scorch, plant disease, plant pathology, wilting

---

This case study emphasizes the interdisciplinary nature of disease diagnostics and its complexity, which usually cannot be fully addressed within a given field of interest or primary expertise. In medicine, an overdose is considered a serious concern. Thus, individual sensitivity to therapeutics is carefully examined before the treatment is applied. Unfortunately, insufficient attention is usually paid to possible variability of individual plant characteristics when making decisions about disease management and agricultural practices. In our opinion, the question of dose is very important for both medicine and plant science.

INTRODUCTION

According to Paracelsus (1538), a Renaissance philosopher who was one of the founders of modern pharmacology and the father of toxicology, "*Sola dosis*

facit venenum" ("the dose makes the poison"). In our opinion, the question of dose is very important for both medicine and plant science.

Often, a plant disease diagnostic case is sent to our virology lab as a last resort of plant pathology diagnostic efforts, when all the other possible sources of disease have been eliminated. Usually, the Plant Disease Clinic diagnosticians at the University of Minnesota do the preliminary examination of samples for possible causal agents using light microscopy, microbe plating, and other techniques. There are not usually many potential causal agents left to blame except for viruses, when fungal and bacterial infections are rejected and other factors, such as abiotic agents, changes in agricultural practice, and environmental conditions, are eliminated.

CAST OF CHARACTERS

- An Ageratum plant grower in Minnesota
- Researchers in a plant virology lab at the University of Minnesota

CASE OBJECTIVES

The major goals of this case study are as follows:

1. To emphasize the intrinsic complexity of disease diagnostics and its interdisciplinary nature. Thanks to this interdisciplinary component, over time professionals involved in disease diagnostics substantially broaden the scope of their diagnostic experience, networks, and expertise.
2. To familiarize researchers, students, and plant growers with the usual workflow of a plant virology laboratory.
3. To demonstrate a possible scenario, using a real-life example, when the diagnosed case extends beyond the virology scope of a diagnostic laboratory.

THE CASE

Part A

The grower sent in plants exhibiting symptoms of a possible disease directly to our lab. No preliminary examination had been done to eliminate sources of disease, including abiotic agents and common plant pathogens. We started

our diagnostics from scratch. The sample was Ageratum plants (*Ageratum houstonianum*) sent to our lab with noticeable symptoms that included wilting and leaf scorch. Preliminary examination of the symptoms by eye (based on morphology) and light microscopy did not show any typical signs of fungal or bacterial disease, such as mycelia, spores, or bacterial colonies. Therefore, fungal and bacterial infection seemed very unlikely. Also, we did not find any evidence of insect damage to the plant.

We decided to contact the grower to learn more about the disease settings.

We suggest that a reader pause here to formulate a list of 3 to 5 questions that they could ask the grower. Imagine what questions a plant pathologist could/would ask to reduce the circle of suspects or potential causal agents. Some of the suggested areas that need questions to clarify are listed below.

Possible areas for questions:

1. Occurrence of the disease
2. Disease spatial and temporal characteristics/dynamics
3. Agricultural practice
4. Fungicide application practice
5. Impact of environmental conditions

Part B

After a conversation with the grower, possible fungicide damage and environmental factors were eliminated. The grower had many years of experience growing Ageratum in controlled indoor settings with minor occasional applications of fungicides. He did not report any recent changes in agricultural practice. Everything seemed to be business as usual. However, the symptoms appeared on several plants of a new breed the grower had recently planted. Although the observed symptoms did not provide any clue that would lead us to recognize a disease caused by known viruses, this combination of factors made a possible viral infection a plausible explanation for the disease outbreak. But, what virus was in charge?

We applied transmission electron microscopy (TEM) for virus detection. However, in the described case, the results of TEM screening showed no evidence of plant viruses. We were back where we had started—questioning what could cause the symptoms now that all major suspects, including fungi, bacteria, and viruses, were rejected. We also excluded the possibility of fungicide damage, the impact of new agricultural practice, and change of environmental conditions during the growing season. Although the symptoms somewhat resembled damage from excessive application of fertilizers, the overuse of fertilizers was denied by the farmer.

Next, we took a close look at the condition and disease progression of the samples. We received two types of samples associated with this case: one type was plants transferred in a pot and a second type was seedlings with roots immersed in gel placed in bottles with lids. The plants in a pot quickly died within a few days. The seedlings in a bottle survived longer. For the seedlings, some of the leaves showed marginal yellowing. The seedling roots left outside the gel looked healthy, and the roots in the gel looked much less so.

We suggest that the reader pause here and write down what they think the problem could be. Then, continue with the study.

The causal agent of the described plant conditions was probably not a pathogen. The possible cause was likely to be a higher sensitivity of a new breed to the use of fertilizers. In this case, previous experience in growing Ageratum and usual practice in applying fertilizers did not prevent the grower from doing more harm than good to the plants. We reported our findings to the grower. Our conclusion was that selecting for the desired consumer characteristics of a new breed had a side effect that included higher sensitivity to fertilizers.

In conclusion, we would like to remind readers about Paracelsus' seminal idea cited in the introduction and emphasize that the use of fertilizers can cause damage to plants. In medicine, an overdose is considered a serious concern. Thus, individual sensitivity to therapeutics is carefully examined before the treatment is applied. Unfortunately, insufficient attention is usually paid to possible variability of individual plant characteristics when making decisions

about disease management and agricultural practices. In our opinion, the fundamental principle of medicine, "Do no harm," is as relevant for plant health as it is for human health.

CLASSROOM MANAGEMENT

Case Summary

This case study emphasizes the interdisciplinary nature of disease diagnostics and its complexity, which usually cannot be fully addressed within a given field of interest or primary expertise. To stimulate students and researchers to think outside the box is the primary outcome of this educational experience.

Suggestions on How to Use This Case

This case study can be used to stimulate a class discussion (20–45 min) or fill in a laboratory session (60–120 min) on plant viruses for undergraduate and graduate students in plant pathology, horticulture, and other areas. This case study aims to explore the routines of a plant virology laboratory and also to communicate examples of diagnosed cases that extend beyond the virology scope of the diagnostic laboratory.

Background Review

Background information about the following three topics—major characteristics of plant viruses, viral detection, and TEM—can be assigned as a reading before the class starts and followed up with a short quiz at the beginning of the class. Alternatively, the course instructor may allocate time at the beginning of the class session to review the materials in groups of 3 or 4 people by randomly assigning one of the three topics to each group. After the review, the groups should formulate at least two key points about the assigned topic and share them with the class.

After the background information review is completed, the instructor introduces Part A of the case study to the class by briefly describing the case settings and the preliminary diagnostic steps that were taken. The goal of this step is to set the scene for a further conversation between a diagnostician and a farmer to clarify the case. The instructor can write the list of suggested areas

for questions on a whiteboard or distribute it as a handout. Students can be divided into groups of 3 or 4 people. Each group should pick an area from the list of the suggested areas for questions and formulate 2–3 follow-up questions for a farmer to clarify the case and decide on the next step in diagnostics of this plant disease. Alternatively, this conversation can be role-played by dividing students into two parties representing a farmer and a diagnostician. The suggested questions are discussed in class and addressed by the instructor in the context of the case. At the end of Part A, students should come up with a plan for the next steps in the plant diagnostic process. The instructor guides the discussion of the next steps to emphasize the possibility of viral infection and asks about the necessary steps to check this.

In Part B, the instructor summarizes the evidence collected so far from the preliminary diagnostic steps taken, the conversation with a farmer, and the viral detection results and formulates a diagnostic challenge. The instructor can stimulate a discussion of other possible causes of the observed symptoms and insights about its diagnostics by students before the class dives into the solution of the diagnostic challenge outlined in Part B. The class session can be concluded by a short quiz. Alternatively, students can share their feedback and comments about the lessons learned during the case study in groups and share the key points with the class.

Possible Adaptation

This case study can be adapted to accommodate specific education needs and available resources. The discussion can be enriched by demonstrating viral detection techniques in the laboratory setting and encouraging students to conduct various stages of the analysis themselves, including viral extraction, viral purification, and viral identification. This additional learning experience can be provided in collaboration with a plant virology laboratory if time, resources, and interest allow it. This case study emphasizes the interdisciplinary nature of disease diagnostics and its complexity. To stimulate students and researchers to think outside the box is the primary outcome of this educational experience. Availability of TEM equipment is beneficial but not required to achieve the learning goals. We provided a link to TEM training and basic information about this technique in the Background Information section.

Precase Quiz

1. What methods are commonly used to detect plant viruses?
    a. Light microscopy
    b. Plating
    c. Electron microscopy
    d. All of the above
2. Arrange methods in decreasing order of the amount of preparatory work required for viral diagnostics (from most work to least work):
    a. Dip-stick test
    b. ELISA
    c. TEM screening
3. Transmission electron microscopy is a necessary step in diagnostics of any viral disease in plants:
    a. True
    b. False

Postcase Quiz

1. Disease diagnostics, in essence, is close to:
    a. Technology
    b. Science
    c. Art
2. In-depth knowledge in a particular area of expertise like plant virology is enough to recognize the cause of a plant disease:
    a. True
    b. False
3. What factors are NOT relevant to plant disease diagnostics for identifying a potential cause of the disease?
    a. Agricultural practice
    b. Environmental factors
    c. Abiotic agents
    d. Pathogens
    e. Vectors
    f. None of the above

Answers to the Quizzes

Precase Quiz:

1. (c)
2. (c) – (b) – (a)
3. (b)

Postcase Quiz:

1. (b)
2. (b)
3. (f)

BACKGROUND INFORMATION

Plant Viruses

Viruses are intracellular parasites that are difficult to propagate under artificial conditions. They cannot be detected using light microscopy or agar plating. For some viruses, there are premade test kits available on the market, such as dip-stick tests, that make their diagnostics much easier and faster. Premade test kits require minimal preparatory work and can often be done on the spot under almost any conditions, even in the field. The number of these tests will only grow in the future. Particular viruses can also be diagnosed in the lab by serological tests such as enzyme-linked immunosorbent assay (ELISA) using the existing antibodies against these viruses (Dijkstra and de Jager, 2012). Genome-based techniques such as PCR tests and sequencing are also used in viral diagnostics. However, if the virus in question is less well-studied, then more sophisticated techniques and equipment are required to bring it out of the shadows (e.g., TEM screening).

However, the story does not end here. One of the key issues with regard to viruses is their transmission. Viruses do not have the means to penetrate plant cell walls, and they lack motility. Identifying ways/modes of transmission is an essential component in disease diagnostics and disease management, especially for infections caused by viruses.

Virus Detection

We applied TEM screening for viral detection (Figure 1). In most cases, the usual process starts with freezing the plant tissue sample with liquid nitrogen and grinding it in a buffer to release the viral particle from plant cells (Figure 1, Step I). Plant virologists usually extract viruses directly from infected plants rather than propagate them under artificial conditions, as researchers often do for human and animal viruses. The extracted mixture of plant remnants, viral particles, and other components released from broken cells is purified using differential centrifugation (low speed–high speed) to separate viral particles from other components (Figure 1, Steps II and III). Little droplets of this preparation are placed on a meshed grid (a tiny metal plate coated with a thin film [Figure 1, Step IV]). These prepared grids are placed in the TEM vacuum chamber for screening (Figure 1, Step V). TEM allows the microscopist to achieve a very high level of magnification (15,000–25,000´). The TEM equipment is usually connected to a computer that allows the microscopist to view magnified images on a screen and store pictures taken by the built-in camera (Figure 2). These pictures can provide evidence of the presence of viruses. The captured images of viruses (Figure 3) also can provide clues for preliminary viral identification using a library of images taken previously for various viruses.

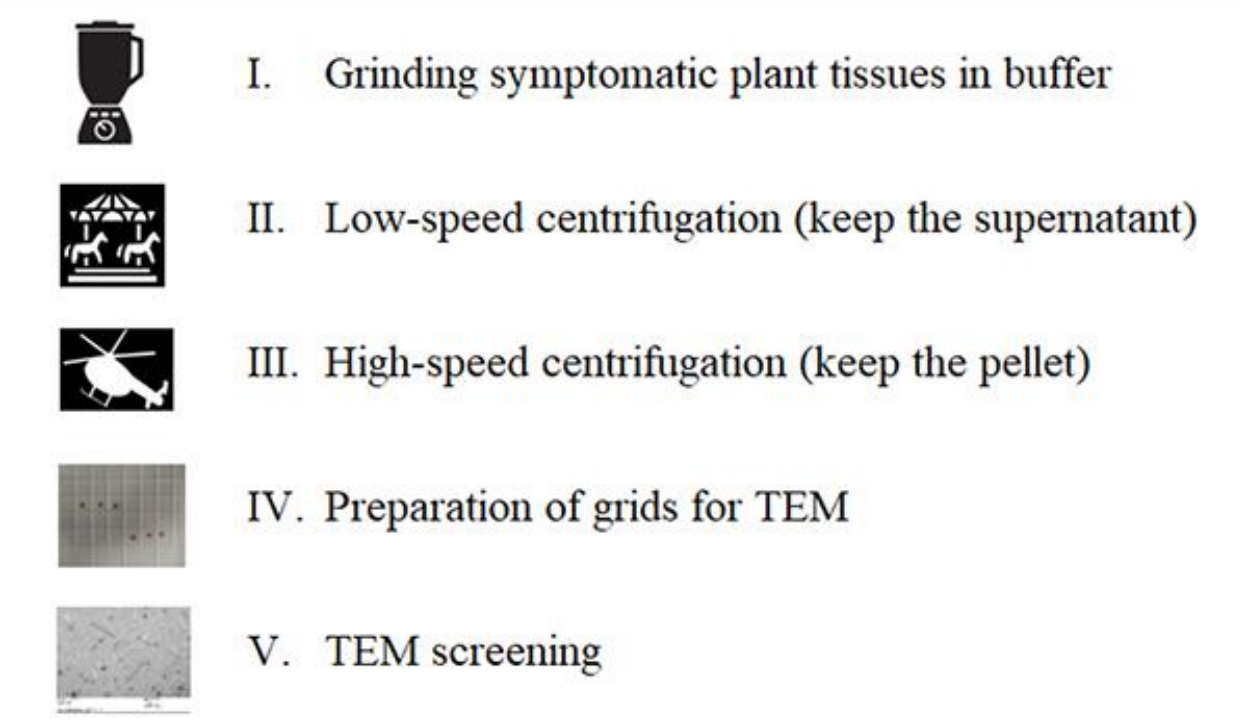

Figure 1. Steps in the process of sample preparation for viral detection by transmission electron microscopy (TEM). (Step I: black blender silhouette by Pixabay under Pixabay License; Step II: carousel icon by Delapouite under CC BY 3.0; Step III: Helico silhouette by Pypaertv under CC BY 3.0)

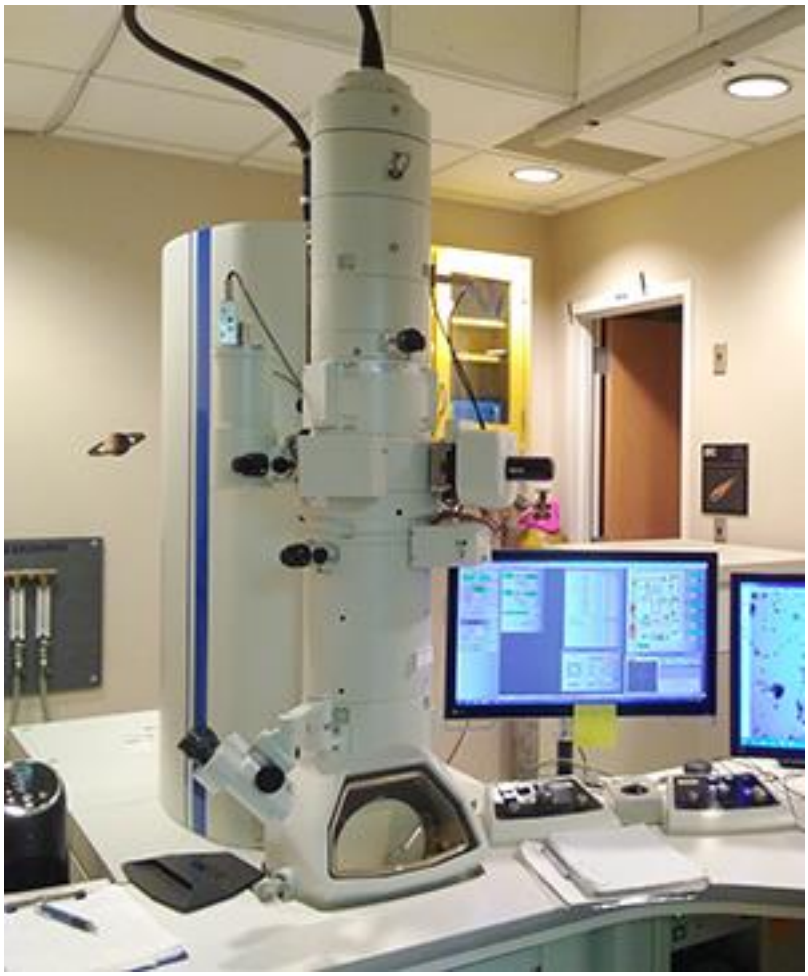

Figure 2. Example of a transmission electron microscope. (Photo by Tatiana Lenskaia)

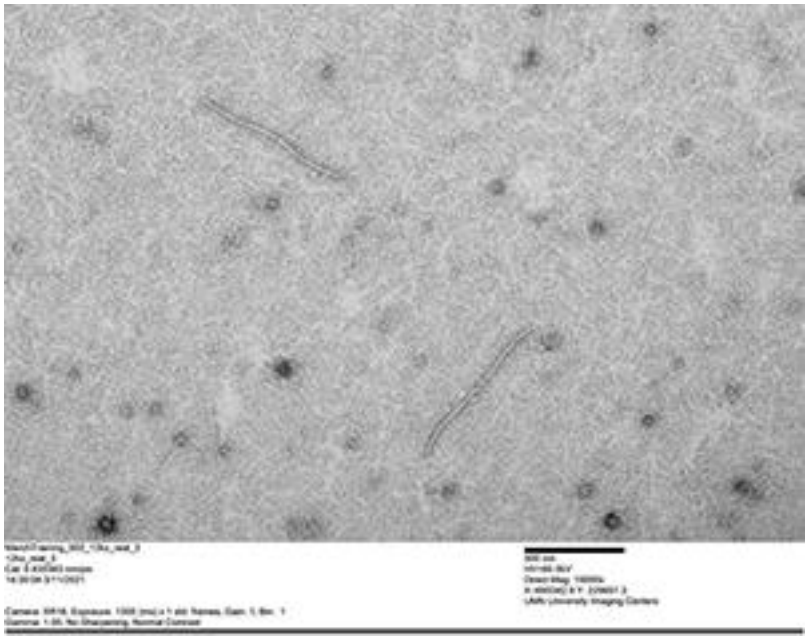

Figure 3. Example of an image of viral particles taken during transmission electron microscope (TEM) screening. (Microscopist: Tatiana Lenskaia)

Transmission Electron Microscopy

This screening requires preprocessing and sample preparation in the lab. Also, specific knowledge and skills are required to use the TEM equipment. Preprocessing can be long and laborious, with multiple rounds of viral extraction and purification if the previous protocol fails or mistakes in the process of sample preparation are made.

Although TEM screening allows researchers to visualize viruses that might be involved in the disease, it does not provide a definitive answer on many important questions regarding disease diagnostics, such as what virus is it and is this virus a causal agent of the disease? To elucidate the connection between the virus and disease, it is necessary to fulfill Koch's postulates (Agrios, 2005) and confirm that this virus can cause similar symptoms in a healthy plant upon infection. This is a critical step for studying new diseases and their causal agents.

An overview of TEM can be found on the Myscope Microscopy Training website (Microscopy Australia): Myoscope Microscopy Training: Transmission Electron Microscopy.

References

Agrios, G. N. 2005. Plant Pathology, 5th ed. Pages 26–27. Elsevier Academic Press, San Diego, CA.

Dijkstra, J., and de Jager, C. 2012. Practical Plant Virology: Protocols and Exercises. Springer Lab Manuals. Page 348. Springer Science & Business Media, Berlin, Germany.

Paracelsus. 1538. *Dritte Defensio*.