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Sudden Death Syndrome in Soybeans: Catastrophe Next Door

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Diagnostics, in many respects, is similar to detective work. Detectives aim to capture all the clues and evidence that are possible to collect from a crime scene. In this case, everything matters. Moreover, assuming any scenario based on first impressions can be misleading for further investigation, since the true crime can be purposefully camouflaged. Samples of soybean leaves with minor mottling and chlorotic lesions were sent to our virology lab for further diagnosis of the disease. A suspected viral infection turned out to be a much more serious condition. Providing only samples of symptomatic leaves resulted in a delayed diagnosis, which in turn led to further development and spread of the disease. This case exemplifies the fact that the localization of symptoms can be different from the point of entry for infection and the place of further localization of the causal agent. Therefore, for diagnostic purposes, it is important to examine the condition of the entire plant and not just the parts that are currently exhibiting symptoms.

INTRODUCTION

It was a usual day at a plant virology lab. We were on the way to the thermostat room to prepare media for the next experiment with virus cloning in a bacterial vector. A new research assistant from the lab next door ran into us. He asked if we had a spare bacterial loop to borrow since he desperately needed one for his next experiment, and his lab previously did not work with plant pathogenic bacteria. We sure did have a bacterial loop. It was surprising how much stuff had been accumulated in the lab over the years that did not seem to be directly related to viruses.

The case we would like to discuss in this study came from a lab next door. This lab did research on soybeans, and their space in the greenhouse was next to ours. Several rows of soybeans that they had planted in a greenhouse started to show symptoms of disease. At first, the symptoms were minor, with some yellowing areas on leaves. They collected some symptomatic leaves and showed them to us wondering if it could be a viral infection.

CAST OF CHARACTERS

Researcher at a Soybean Research Laboratory

Researcher at a Plant Virology Laboratory

CASE OBJECTIVES

The major goals of this case study are

1. To expose students, researchers, and plant growers to the main problems related to diagnostics.
2. To demonstrate that restricted views and false assumptions made during the early stages of the diagnostic process can be misleading and harmful, since they can significantly delay the diagnosis and postpone subsequent disease management interventions.
3. To encourage people who are involved in the process of disease diagnostics to think outside the box and take into account a picture that is as complete as possible when they try to identify the source of a problem.

THE CASE

Part A

Our virology laboratory received soybean leaf samples with mottling and chlorotic and necrotic lesions for investigation and diagnosis of disease. Preliminary examination of the soybean leaves showed the absence of mycelia growth, spores, or bacterial colonies, which are indicative of fungal or bacterial infections. The leaves did not show traces of insect invasion and/or damage. The symptoms observed on the soybean leaves suggested the possibility of a viral infection, probably Soybean yellow mottle mosaic (**Fig. 1**).

We made an extraction from a sample of the symptomatic leaves, purified it using differential centrifugation, and prepared grids for transmission electron microscopy (TEM) screening (see Ageratum Case Study [Lenskaia et al. , 2022] for more detail about viral extraction and TEM). Although sample preprocessing for TEM screening is a long and laborious task, this screening allows researchers to obtain an image of a virus if it is present in the plant tissues. However, in this case, TEM screening showed no evidence of plant viruses. Therefore, we continued to look for clues to identify a possible source of the disease.



Figure 1. Example of symptoms caused by Soybean yellow mottle mosaic virus. Courtesy L. L. Domier—© APS. Reproduced, by permission, from Hartman, G. L., et al., eds. 2015. *Compendium of Soybean Diseases and Pests*, 5th ed. American Phytopathological Society, St. Paul, MN.



Figure 2. Example of severe symptoms of sudden death syndrome. Courtesy T. K. Herman—© APS. Reproduced, by permission, from Hartman, G. L., et al., eds. 2015. *Compendium of Soybean Diseases and Pests*, 5th ed. American Phytopathological Society, St. Paul, MN.

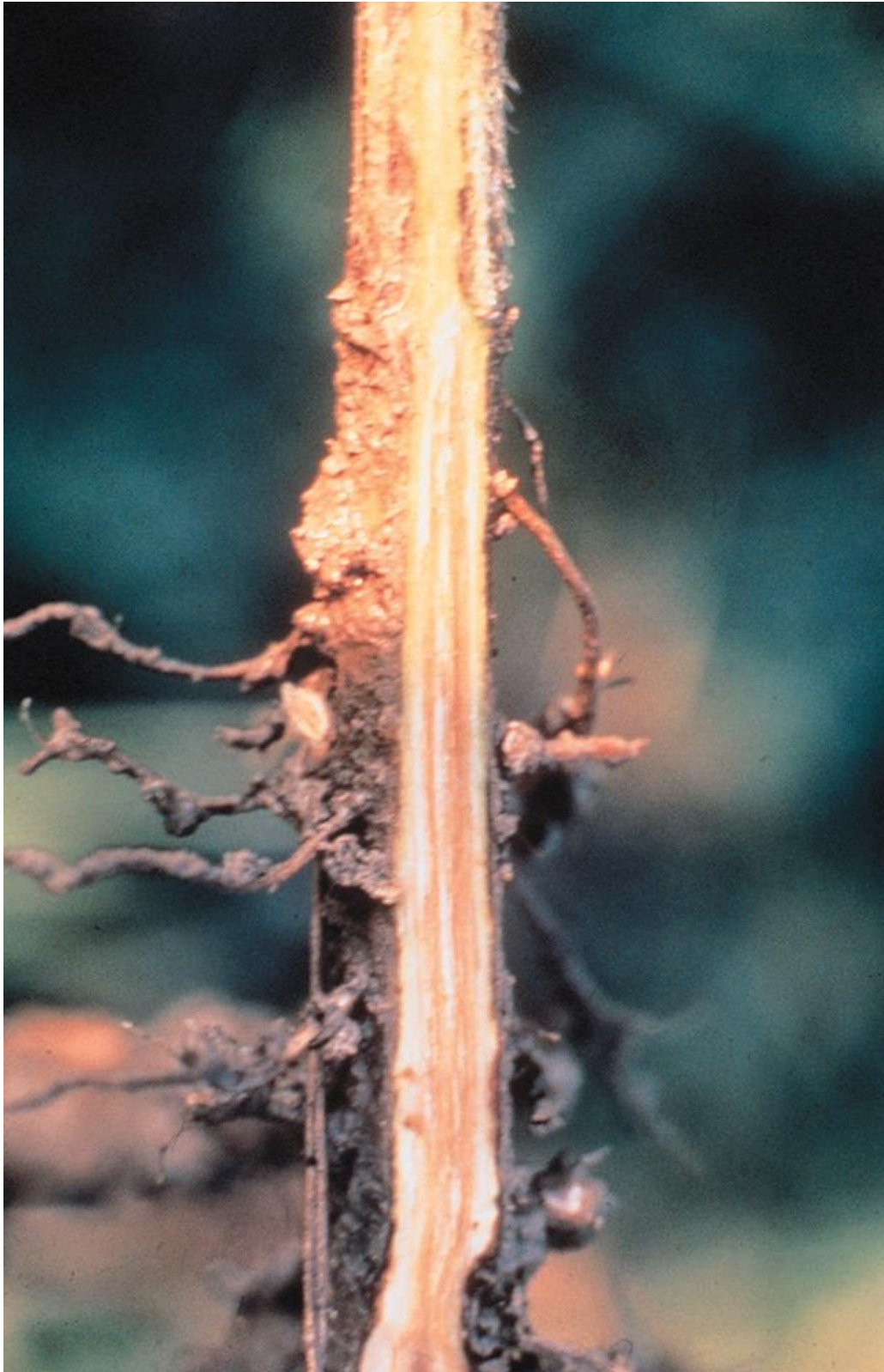


Figure 3. Example of stem and root symptoms of sudden death syndrome. Courtesy J. C. Rupe—© APS. Reproduced, by permission, from Hartman, G. L., et al., eds. 2015. *Compendium of Soybean Diseases and Pests*, 5th ed. American Phytopathological Society, St. Paul, MN.

Part B

Samples of soybean leaves collected from the same greenhouse a few weeks later demonstrated more severe symptoms, including large areas of interveinal chlorosis and necrosis. These symptoms definitely resembled sudden death syndrome (**Fig. 2**) caused by a soilborne fungus. Sudden death syndrome in soybean is a devastating disease that can lead to significant losses in yield (see the Background Information section for more detail). Prevention is the most effective control measure for this disease. To distinguish between viral and fungal infections in early stages would require having a more comprehensive sample collection (not only leaf samples from the affected plants but also stems and roots) and no prior misleading assumptions about the source of the disease. Also, careful examination of the environment of the affected plant and disease dynamics could provide very important information. Conducting a holistic assessment of the diseased plant and its environment prevents diagnostic errors and critical delays. The delayed diagnosis resulted in further development and spread of the disease, which in turn collapsed and significantly delayed the reinstalment of several other ongoing research experiments.

QUESTIONS FOR DISCUSSION

1. What false assumptions were made that led to the delay in diagnosis?
2. What are the key steps in constructing as complete a picture of disease as possible?
3. What recommendations would allow plant pathologists to anticipate and prevent similar problems related to disease diagnostics in the future?

CLASSROOM MANAGEMENT

Case Summary

This case exemplifies the fact that providing only symptomatic parts of the plant for disease diagnostic can be misleading and not suitable for making a timely and correct diagnosis. Symptoms (visible effects of disease) and signs (physical evidence of the pathogen) related to a given plant disease can be found in different parts of the plant affected by the disease. For example,

chlorotic and necrotic lesions on leaves (symptoms) are consequences of the released toxins produced by a fungus that colonizes plant roots (signs). Therefore, for diagnostic purposes, it is important to examine the condition of the entire plant and not just the parts that are currently exhibiting symptoms. Observation of conditions for neighboring plants and weeds can also help in understanding the source of the problem. Getting as complete a picture as possible can provide useful clues for identifying sources of disease, methods of transmission, and potential vectors.

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 disease diagnostics for undergraduate and graduate students in plant pathology, horticulture, and other areas. This case study aims to explore a process of plant disease diagnostics and to communicate examples of the diagnosed cases that extend beyond the virology scope of the diagnostic laboratory.

Background information review about the disease diagnostic process and sudden death syndrome in soybeans can be assigned as a reading before the class starts, 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 3–4 people by randomly assigning one of the topics to each group. After the in-class 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 preliminary diagnostic steps that were taken. The goal of this step is to set the scene for a further discussion between students to clarify the case. At the end of Part A, students should come up with a plan for the next steps in the plant disease diagnostic process. The instructor guides the discussion of the next steps to elucidate possible sources of the disease other than viral infection and asks about necessary steps to check this possibility.

At the beginning of Part B, the **instructor summarizes the evidence collected** so far from the preliminary diagnostic steps and the viral detection results and formulates a diagnostic challenge. The instructor can **stimulate a discussion of 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 instructor can write a list of suggested questions for discussion on a whiteboard or distribute it as a handout. Students can be divided into groups of 3–4 people. Each group should pick a question from the list and formulate 2–3 key points to address it. Alternatively, this conversation can be role played by dividing students into two groups representing a diagnostician and a researcher that have encountered symptoms of a disease in soybeans. The answers suggested by groups can be discussed in class and summarized by the instructor in the context of the case. **The class session can be concluded with a short quiz.** Students also can share their feedback and comments about the lessons learned during the case study and formulate recommendations to avoid possible problems related to disease diagnostics in the future.

[Instructor Slides](#) [Detection Slides](#)

Possible Adaptation

This case study can be adapted to meet specific education needs and utilize available resources. The discussion can be enriched by demonstrating the viral detection techniques in a laboratory setting (see Slides) and encouraging students to conduct various stages of the analysis themselves, including viral extraction, viral purification, and viral identification (see Ageratum Case study). 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 possible interdisciplinary nature of disease diagnostics and its complexity. Often a diagnostic case cannot be fully addressed within one given field of interest or primary expertise. Stimulating students and researchers to think outside the box is the primary outcome of this educational experience. Availability of laboratory equipment for viral detection is beneficial but not required to achieve the learning goals. This case study can be useful to include in a class discussion about major sources of problems related to the process of disease diagnostics. The discussion can

promote critical thinking and stimulate careful exploration of any assumption in the process of disease diagnostics. The case can be modified to make it relevant to the audience's background and interest in terms of a host and pathogens involved. One of the important outcomes from studying this case is the exploration of how a partial view and false preliminary assumptions can interfere with the process of proper diagnostics of disease and delay the entire process.

Pre-case Quiz

1. Disease diagnostics can be mastered by using the following approach:

- (a) Mimicking detective work
- (b) Applying the scientific method
- (c) Following one's intuition
- (d) None of the above

2. Area(s) that heavily depend(s) on a diagnostic process include:

- (a) Medicine
- (b) Plant pathology
- (c) Car repair
- (d) All of the above
- (e) (a) and (b)

3. To be a good diagnostician, it is enough to be a good scientist.

- (a) True
- (b) False

Post-case Quiz

1. For plant diseases, the localization of symptoms coincides with the localization of causal agents:

- (a) True
- (b) False

2. Why is it important to be aware of possible sources of problems related to diagnostics?

- (a) It helps speed up the diagnostic process and reduces the risk of diagnostic errors.
- (b) It allows a person to become an outstanding diagnostician.

3. To speed up the diagnostic process, a diagnostician should focus on exploring only the following:

- (a) Symptomatic parts of the affected plant
- (b) Prior assumptions about the origin of the problem
- (c) Analogous cases in the past
- (d) Information about the case provided by a grower
- (e) None of the above

Answers to the Quizzes

Pre-case Quiz:

- 1. (b)
- 2. (d)
- 3. (b)

Post-case Quiz:

- 1. (b)
- 2. (a)
- 3. (e)

BACKGROUND INFORMATION

Disease Diagnostics

We would like to emphasize that a partial view and any false assumptions made before the evidence is collected can be misleading and deleterious to the disease diagnostic process. It can substantially delay disease diagnostics and application of disease control measures. Problems with diagnostics appear in many areas, including medicine, veterinary science, plant pathology, and other domains. But, what is diagnostics in its essence? The *Stanford Encyclopedia of Philosophy* mentions this term in different contexts, but it does not provide an answer regarding its definition. According to Stewart (2004), the process of diagnostics is much more extensively studied in medicine than in plant pathology. Medical professionals usually do several rounds of medical investigations, and they are very cautious when formulating the final diagnosis. In early stages, medical diagnosticians use the term preliminary diagnosis to emphasize the necessity to explore other possibilities.

Diagnostics, in many respects, is similar to detective work. Detectives aim to capture all the clues and evidence that are possible to collect from the crime scene. In this case, everything matters. Moreover, assuming any scenario based on first impressions can be misleading for further investigation, since the true crime can be purposefully camouflaged. Disease diagnostics has some elements of investigation, such as asking questions and narrowing the range of suspects. The ability to recognize analogies also plays an important role in disease diagnostics. However, cases often exhibit a set of characteristics that makes them unique. This **requires the use of scientific methods to explore them**. The challenge is to find explanations among different possibilities by formulating a hypothesis and developing an approach to test and validate this hypothesis. In our opinion, any diagnostic problem should be approached and addressed as a research problem in a new area.

Is a good diagnostician an outstanding scientist? Yes. However, the opposite is not true. Historically science has diverged into different areas that have followed different paths and distinct methodological heritages. Scientists are often experts in a particular domain. They are familiar with the state of the art in their domain, but advances in other domains are often obscure. This one-sided point of view can be misleading and limited in its ability to discover the

source of the problems related to diagnostics. In medicine, the primary point of contact for patients are physicians who are trained in general medicine. In general, they have rather limited in-depth knowledge in different specialties like endocrinology or dermatology, but their broad view allows them to see outside the box of a given medical specialty and take into account a broad range of possibilities regarding the origin of the disease.

Why is it important to be aware of the main problems related to diagnostics? These problems are relevant to the process of diagnostics regardless of the area in which the diagnostic procedure is applied. Ignoring these problems puts people at high risk of encountering diagnostic errors that are associated with time losses, **additional financial burden, and other costs and losses (including worsening the condition and death of a patient).**

Sudden Death Syndrome

The soilborne fungus that causes sudden death syndrome in soybean is *Fusarium virguliforme*, it colonizes the roots, and it is not present on leaves. A distinct feature of sudden death syndrome is vascular discoloration that can be observed inside the stem (**Fig. 3**). Examining the condition of the roots could also be helpful for diagnostics. If the entire plant had been sent to the lab initially, it would have made the diagnostic process much faster and more efficient.

For additional information about sudden death syndrome of soybean, please visit <https://extension.umn.edu/pest-management/sudden-death-syndrome-soybean>.

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