Practical exercises with microscopy and molecular methods for identification of fungal pathogens

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Background to teaching laboratory classes

Students typically following agricultural science programs at the University of Copenhagen is expected to link theory and practice in various disciplines. This also includes within the plant pathology world. The course content considered for my project was the course "Plant Pathology: Crop Diseases and Food Security" (NPLB21000U) which aims to provide both practical and theoretical knowledge about plant diseases and how to manage them around the world. Lectures are in English, 7 ECTS during block 4 and at bachelor level with course capacity of 40 students.

Link to course homepage: Plant Pathology: Crop Diseases and Food Security - 2021/2022 (ku.dk)

Course content: The course intend to teach the students why it is important to study, understand and control plant diseases by which threaten food security and food safety as it reduces crop production and final yield. Methods for teaching and learning strategies included in the course are via lectures, problem based learning via case studies, journal clubs, excursions, laboratory demonstrations via molecular lab and microscopy exercises.

I was teaching on this course for the first time, and the course was newly initiated by course coordinator and teacher, Professor David B. Collinge, in which he gave me the opportunity to impact the teaching and course content for 2 teaching days. The curriculum are based on newly published book called "Plant Pathology and Plant Diseases" which is written by David B. Collinge, Associate Professor Hans Jørgen Lyngs

Jørgensen and other experts within plant pathology from Northern Universities (Tronsmo et al., 2020). I have a background w molecular plant pathology and been taught by both teachers and the Co-authors of the book during my undergraduate studies and as PhD researcher. Thus, it was a privilege to help out with this course for my own teaching development. During my own time as undergraduate student, I felt the lack of acquiring practical experience on applying more molecular methods such as PCR analysis for the identification of plant diseases and integrate the molecular data with visual observations, assessments, isolation and microscopy of the causal agents (the pathogens). In fact, this is typically how both the biotechnological and agricultural industry and university use as verification tool in order to determine which microbe harmed the plant tissue (Hariharan & Prasannath, 2020). However, this was a missing part during my own undergraduate studies.

Nowadays, PCR analysis is an important molecular tool especially after COVID-19 detections and is even in the public eye known as analytical method of choice for more accurate verification. Thus, students are expected to know how to use such methods for identification of fungal and bacterial pathogens once completed the undergraduate studies especially by the industry. Unfortunately, to my knowledge it is still not addressed as practical exercises at plant pathology courses provided by KU science instead it is emphasized important from lectures presented by the teachers. It is only during BSc, POCS and MSc thesis projects the students may have the opportunity to get hands-on experience to the methods. The molecular tools are used in several other courses but for other purposes, thus students can learn the methods via these courses and only in this way apply the knowledge to plant pathology courses. Consequently, to me it leads to a gap integrating the more core methods used for diagnostic of plant disease, which is unsatisfied, as the molecular methods are not today considered challenging or demanding to perform as it used to be.

Justification for choice of topic in the project

As of today, various lab chemicals are significantly improved, easier to use, rapidly perform as required along with instruments (e.g. PCR machines, electrophoresis, imagine systems and sequencing platforms) are typically now available and easily accessible in most working environments at KU Science. As the observation by Shulman and Tamir (1973) in which experienced the laboratory classes is one of the most distinctive feature of science instructions and is the core of science learning process (Shulman & Tamir, 1973). Based on these criteria it was a necessity and feasible to apply it to this course for the students to learn more from the practical exercises and strengthening the gap between the intended learning outcomes (ILOs) and teaching and learning activities (TLAs). An important criteria for good ILOs are pre- and post-discussions as Woolnough and Allsop (1985) argue it often causes failure of practical science courses when pre- and post laboratory discussions are left out (Woolnough & Allsop, 1985). Thus, it is important to making sense of laboratory experience on the day and shortly after (to recap). It also gives great opportunity to social interactions during laboratory classes, which I will take advantage off with small "easy" questions to the work addressed as way to make the working environment as friendly as possible. In addition, it has known that as an effective way to reduce cognitive load and to increase meaningful learning laboratory classes via effective pre-laboratory preparation (O'Brien & Cameron, 2008) by which pre-lab videos as a way to demonstrate and enhance student understanding and learning before actual lab exercises (Onyeaka et al., 2022).

Objectives of the project

Thus, the objective of the project reported here were to

- Implement molecular lab exercises and integrate the method with common classical methods
- Provide proper introduction and theoretical background of plant diseases relevant to lab exercises

- To increase the relevance and understanding why the practical exercises are beneficial to the students in the real-world via post-discussions

Methods

Context and activities

The course content intend to provide a basal understanding of how microorganisms interact with plants and other microbes, but also on how the environment influences diseases. It also introduces the methods used to study these interactions as well as management options (how to prevent) and their effect on food security via lectures, exercises in lab and excursions. In general, several occasions (at least 7 times) laboratory classes are included in the curriculum which consist of inoculation experiments (add spores) with plant pathogens followed by observations of diseases symptoms as diagnostics tools. In addition, once during these practical exercises an integration of the molecular tool using PCR analysis as verification are performed.

A case study was decided to be used as example which was based on previous work I had developed as teaching material for courses used as Post doctoral researcher in Sweden at SLU, Alnarp by using two important fungal pathogens of two different genus. Both of these pathogens (Colletotrichum acutatum and Botrytis cinerea) typically infects strawberry plants in field. However, both are also known as post-harvest pathogens of strawberry fruits as they continue to develop and grow on fruits once harvested, stored and are typically observed in supermarkets or by the consumers once bought and stored at home as unpleasant molds. With proper training, it is possible at certain developmental stages (reproductive stage) to differentiate between the two pathogens as symptoms are morphological different. Classical microscopy can be applied when the fungal pathogens emerge with their reproductive stages (producing spores) on strawberry fruits where size and shape of spores clearly differentiate. In addition, molecular tools via PCR analysis can also verify the origin of the fungal pathogen either by

gene sequencing the amplified PCR products or simply using fungal-species or genus specific primers which can amply specific pathogens of interest that are further visualized on gel electrophoresis. Both the classical (microscopy) and molecular methods (PCR analysis) are permed by students on the course in order to differentiate the pathogens. The example considered for the course forms basis for how the research community typically characterize fungal pathogens for accurate identification. It was the first year of the course (block 4, 2022), unfortunately only few BSC students participated in the course as originally ten signed up for the course but in the end only seven showed up during the whole course period.

Discussions and descriptions on how the project evolved

The case study with setting up the experiment, observations, microscopy and molecular work, me and another teacher, Assistant Prof. Chatchai Kosawang, had arranged for the first time and in coordination with course coordinator David Collinge (also my departmental supervisor) and teacher Hans J.L. Jørgensen. First, we prepared preliminary power slides and presented the ideas, then later as a presentation step by step on how the lab exercises was going to take place and with time schedules for how we would organize the course days with our experimental settings for the exercises. In agreement with them, a decision was made that it would require one full day, which included introduction to exercises via power point presentations, followed by the lab exercises (molecular method and microscopy) and a second day with post-discussions concerning the learning outcome. These were further modified in accordance with available lab facilities as it require both molecular and regular microscopy labs. We decided to split the students on lab exercise day (1st day) into two teams. At first, we got to know each other in plenum by names, education and what science interested us followed by arranging the two teams. Both teams are giving the same introductions about the two teaching days whereas one first team get the microscopy and the other the molecular exercise. Hereafter, they shifted which were after about two lecture hours. In addition, an email was sent out via Absalon to all students one week in advance, concerning two video links with focus to

the molecular part on the theory behind PCR analysis and gel electrophoresis with good illustrations. Pre-lab videos were carefully chosen by the four of us as studies has proven it as an effective way to demonstrate and enhance student understanding and learning before laboratory exercises (Onyeaka et al., 2022).

Approach and method for microscopy exercise

I was responsible for the microscopy lab exercises for each of the teams, whereas Chatchai were responsible for the molecular exercises, but I also were strongly involved in designing the molecular part. As only two days were available, we had to divide it up like this. If three days had been available it would had been better to divide it up into one day Microscopy and another for molecular method followed by the post discussion, however this was not possible. Thus, due to these circumstances we had carefully aligned our presentations (to make exercises coherent), which were used in the lab exercises. In my case, I first introduced common microscopy dissections, how to prepare microscopy slides, how to make morphological observations of infected fruits and most importantly how to distinguish the two fungal pathogens. It was very convenient with the low number of students for the two teams as I could help each student one by one and assured they made the observations of the specific fungal spores and other developmental structures. Each students were individually provided a light microscope to handle. I first prepared the microscopy slide from infected fruits, students observed, and hereafter the students performed the exercise themselves. Some had experience with microscopes and some had no experience, thus a bit more time were spent with them. When all students had the opportunity to differentiate the fungal pathogens, then I had power point slides presented in lab and we went through the different structures, added morphology terms (e.g. shapes, color, length etc.) to the structures. I had in weeks beforehand performed the experiments, identified the reproductive stages and taking photos via microscopes etc. and now we went through each of them again. Here, I could ask them questions concerning functions of the different structures in which I introduced in the pre-discussion and they from

several occasions been introduced to these terms by the other teachers. Chatchai used his teaching on the molecular methods via DNA extraction, PCR analysis and electrophoresis for visualizing molecularly, how to differentiate two pathogens and most importantly corresponded with the results found in the microscopy exercise.

Post discussions (after practical exercises)

Our power point presentations started out with ILOs on first slide. Both chatchai and I had our presentations aligned, mine in regards of the microscopy exercises and Chatchai for his molecular part. In my case (one lecture hour) I started by stating that by the end of the presentation (post discussion) there will be few questions related to what has been presented. This statement were made in order to assure the students would carefully listen to my presentation. I had specific questions presented by the end of the lecture and these were first addressed as group work, in two working groups (four and three students on each team) for about 10mins internal discussions and lastly discussed in plenum. Each of the question was carefully addressed by the students. Before starting the plenum, I said it would be nice if all students could provide answers to questions. Interestingly, they did accordingly and engage all by being actively involved. The questions was related to both the practical lab exercises but also with focus to the importance of plant pathology, diagnostic tools and why we use it for identifications (proper management and control measures).

Results

General feedback to the course content

I have extracted the written text prepared by the students which is based on the common course evaluation from Absalon. The following statements (see below) was giving in general to the course which included multiple positive responses. I have highlighted in bold those specifically related to the practical exercises performed by me and the other teachers.

1) What was good about the course? Why?

Student 1: “Generally, the course was extremely well organised. We were provided with a campus plan, showing where each of our lectures and practicals took place (with only a single exception/mistake), we were given well organised weekly plans with what to read for the coming days, and so forth. These were posted weeks before the course started, which was very nice, so that one could prepare ahead. The professors and teachers all seemed passionate about the subject, and very engaged in teaching it to us. The powerpoint slides are also excellent, both as a teaching tool, but also as a revision tool, because all the information is well summed up - not too much, not too little. The workload has been manageable, and relevant to the weeks teachings. **The practicals were interesting and engaging, even fun! I personally have not been able to do many practicals because of the corona-virus and subsequent closing down of the school, and the practicals in this course were neither too difficult or too easy, they seemed new to all of us, but all seemed able to participate and understand what we were supposed to do. And if questions arose, the teachers were quick to help.”**

Student 2: ”Det har ikke noget med planlægningen at gøre, men jeg synes, at det var rart, at vi ikke var så mange. **Derudover synes jeg generelt, at der har været en god sammenhæng mellem forelæsninger og øvelser.”**

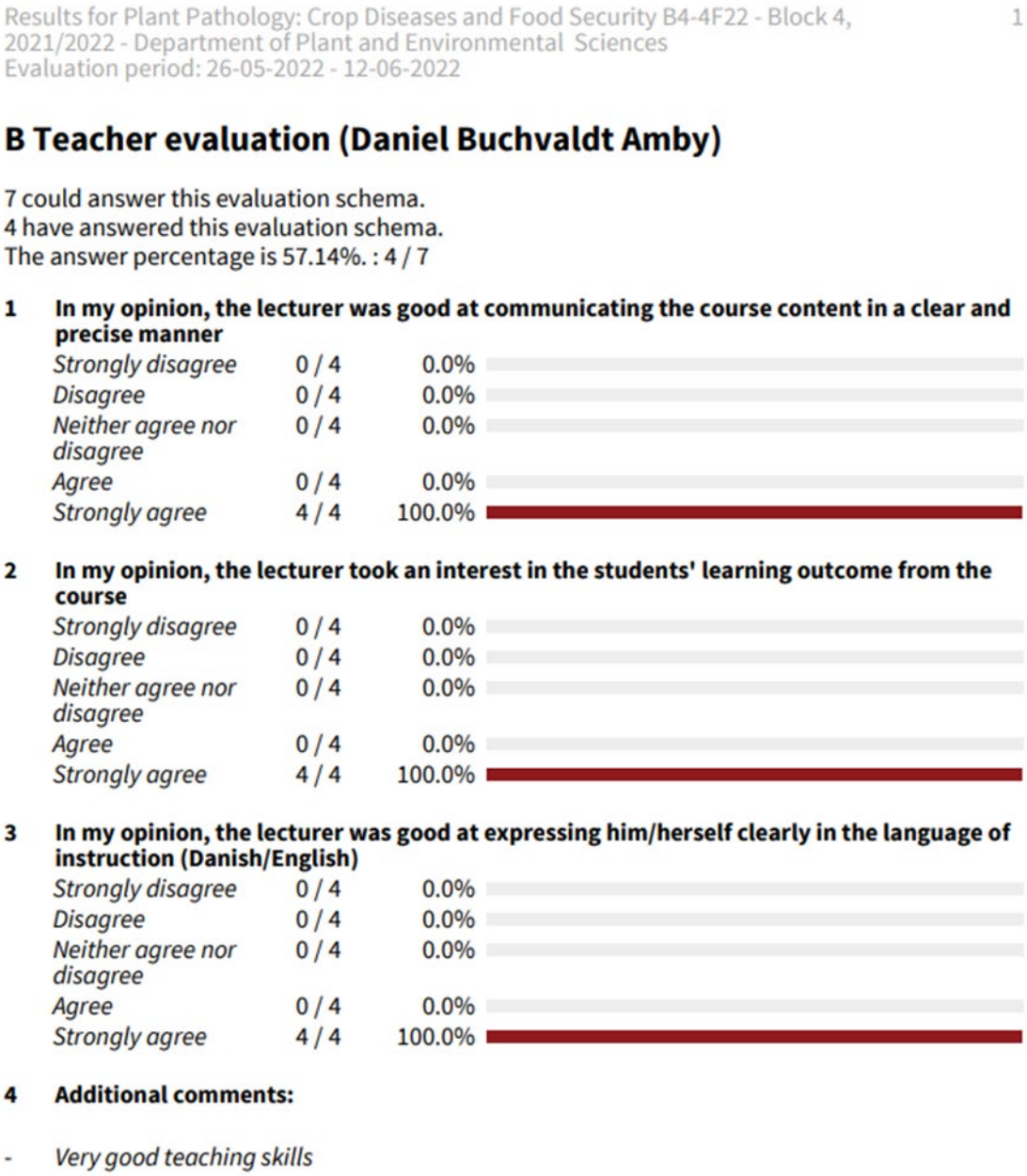
Student 3: **“The balance between lectures, exercises and lab is perfect”**

2) I would like to suggest the following improvements (NOTE: Comments regarding individual lecturers must be stated in the form for the specific lecturer.)

Student 1: *“In general I have no 'complaints' about the course whatsoever, I've really enjoyed it very much.* ***I only have a few comments - during the practicals, we sometimes did not get the results that the***

given professors were hoping we would get, and I would have liked a little bit more details about what they were hoping we would see from the practicals, such as pictures or other visuals, to understand what it is we were hoping to get from the practical. *Secondly, I would have like to - throughout the course - be a little bit more aware of what typical exam questions would look like, though it being a new course, I of course understand why this was hard to do. But for future reference, perhaps some of the colloquia we did throughout the course could be interspersed with some old exam questions, just for reference.*

Student 2: *"Det kunne være godt, hvis man kunne tage på nogle ture i starten eller midten af kurset, hvor man kunne prøve at kigge efter sygdom - måske bare en enkelt tur eller to. Jeg ved, at der ligger en tur til Pometet den 16. juni, men det er bare lidt sent ift. eksamen."*



Results for Plant Pathology: Crop Diseases and Food Security B4-4F22 - Block 4, 2021/2022 - Department of Plant and Environmental Sciences 1
Evaluation period: 26-05-2022 - 12-06-2022

B Teacher evaluation (Daniel Buchvaldt Amby)

7 could answer this evaluation schema.
4 have answered this evaluation schema.
The answer percentage is 57.14%. : 4 / 7

1 In my opinion, the lecturer was good at communicating the course content in a clear and precise manner

Strongly disagree	0 / 4	0.0%
Disagree	0 / 4	0.0%
Neither agree nor disagree	0 / 4	0.0%
Agree	0 / 4	0.0%
Strongly agree	4 / 4	100.0%

2 In my opinion, the lecturer took an interest in the students' learning outcome from the course

Strongly disagree	0 / 4	0.0%
Disagree	0 / 4	0.0%
Neither agree nor disagree	0 / 4	0.0%
Agree	0 / 4	0.0%
Strongly agree	4 / 4	100.0%

3 In my opinion, the lecturer was good at expressing him/herself clearly in the language of instruction (Danish/English)

Strongly disagree	0 / 4	0.0%
Disagree	0 / 4	0.0%
Neither agree nor disagree	0 / 4	0.0%
Agree	0 / 4	0.0%
Strongly agree	4 / 4	100.0%

4 Additional comments:

- *Very good teaching skills*

Fig. 1. Personal teacher evaluation: Primarily represent survey question of practical exercises.

The above figure shows the questionnaire provided via Absalon and the results of my teaching. This is primarily based on the laboratory exercises, pre- and post-discussions as this is what I took part in for this course. All

the responses “strongly agree” (100%) to teacher evaluation of me but only 4 out of 7 had answered the questionnaire. Obviously, it would have been better with more students in general and have answers from all participated students. Thus, it would be worth for me in future to prepare such questionnaire myself and hand it out to students by end of the lectures. The questions should be addressing the lab exercises in order to improve the lectures even more, despite those who answered (shown above) gave very positive responses.

Discussion

Pre- and post discussions are extremely important as stated by Woolnough and Allsop (1985) and assure the students understand how to collect samples, why we should distinguish the pathogens (to find proper treatment), how it is done (i.e. visual observations of disease symptoms on fruits, microscopy and PCR analysis) and to put it into scientific and industrial context.

The learning outcome were documented via post discussion to assure the students had learned from the laboratory exercises in both the molecular and microscopy exercises. There are various ways to learn about biological science, the passive or the active processes, with both equally important. However, specifically laboratory classes provides rich learning experiences to students and bridging the gap between lab experiments and students’ conceptual understanding. Because of the low number of students attended plus we divided them into two teams in which gave me the opportunity to have even more closer interactions with the students. Thus, I could pay more attention to each of the students and assure everyone had understood the exercises such as having the time to look in the microscope with them. Thus, assured each had identified the specific structures of fungal pathogens, which are used for morphological characterization and to differentiate the fungal species used in the case study. In addition, the students had many more questions than usually and it felt more that each of the student dared to ask me or answer my questions I had. I was also told in the post discussion (day 2), that the pre-discussions based on the introduction to exercises and the videos links we provided before lab exercises was very appropriate and improved the

understanding. Especially several of the students are not biotech oriented or consider them self as plant pathologists, thus the video and introduction was important to them.

In general, I think based on the Absalon questionnaires that comments and scoring of the course clearly shows that students can gain a lot from practical exercises also as indicated in many other study evaluations published and how much they appreciate hands-on experiences. I would have wished more students would have signed up for the course and hope more will in future. Especially, because I have again the honor to be teaching on this course on the same part as previously (in block 4, 2022), but also in coming 2023 course to teach more theoretical and other practical exercises and lectures. Here, I would consider either in the end of the course or specifically after ending specific topic days to have more specific questionnaires addressing the practical exercises. For the written project report here it could definitely have improved the documentation and evaluated better the experiment of this current project as it was lacking herein. However, I am still very satisfied that now the teaching can in future continue to use this setup and prepare the students better for the plant pathology world as the industry (i.e. biotech businesses such as Novozymes, CHR Hansen etc.) has shown their interest to this world. Especially, since the industry have interest to prevent these plant diseases in agriculture using various bio-solutions where such methods from our practical exercises are specifically used.

References

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