Welcome to My layman's talk for the defense of my thesis titled Light Stress and Plants Insights from Chlorophyll fluorescence. I will do the Laman's Talk today in Dutch, and the rest of the defense will follow in English. Hello, all-a-allel. Welcome by my leaky-preighture for my thesis, get titled, Light Stress and Plants, Insights from Clorophyll fluorescence. I'm going today at Leukeprighter for you in the Netherlands do, and the rededering of my proofstrift and stelling is, well then in the English, three-quarters.

Therena is there even time for the commission to overleg, and then, hopefully, the outreaking.

Therenaz, are you all welcome by Under the Linde for the feast.

I have four years long to look at how plants with light on-goan. Plants have light needed to grow, but in the during the day, and in the various seasons, can't the light conditions not all forerangering. In the bos, for example, see you delet with very light, but also many plants are in the shadow. Tudence my thesis, I've done to the effect of too much light on plants. Too-vehirt is namely for plants, so as people also can burn through the sun. This was the underwerp of my research. For many people, is an oprisser of photosynthesis for their place. With the energy of light can the plant of photosynthes outforing. Photosynthesis can use the energy of sunlight and coldsop-dioxide to produce. So, the energy of the sunlight upgeslaken in the sugars that can use to grower. This is so a very important process for the foodsel production. I'll let here a gawassing, namely maize, but actually I the greatest idea of my research done on on model plants that that are much to growes and where we're very over be quite in the blood-groom corals or chloroplasts that in the bladers in the bladers of plantes. This are a sort photosynthese fabricies. In these chloroplasts is it a very very difficult membrane called the telecovene membrane. This membrane can you see as the looping band whereop which ultimately the production of chemical energy place find. The machines on the looping band of the telecovene membrane, are the photosystems, photosystem 1 and photosystem 2. These are two eyewit complexes or biological machines that are needed for photosynthesis. Verwarrant enough, let we actually photosystem 2 always see. As I earlier said, can too light photosynthesis' shatter to bring. Photosystem 2 is more than too much more than photosystem 1. And my research went that's for all over photosystem 2. We know a shard on photosystem 2, by too much photo inhibitivity. Through photoinibitia works photosynthesis more good, and that's them can gwashed and more foodsel produceer, and that's it important to be studer. I had over this order of this other questions. First, I've got to look at the shatter of photo-inibitia. In the Hector two, I've looked, how we these shatter actually mete, and in the hostok three, I've got to how this shatter of my I've got to look to look to the short-term security mechanisms that plant to use to be able to So, at my undertitel, Insights from chlorophyal fluorescence all bleak, I have I chlorophyll fluorescension to do with the great deal of my metings to do. As light on a blad falls, can energy three-cantle. It can be used for photosynthesis, it can back to get used as fluorescension, and it can rexed to be light of a larger energy. As warmth and fluorescence would light very light is, because if there too light is, can there namely shard on a plant stures then more light energy as warm as a body, these three ways of outstote are related.

And that's you can you the one use to look how it with the other two go. As there more energy for photosynthesis, is there, for more energy over for fluorescension. A manner where I have had to keken to shade is with a streak camera setup. There come this sort of very in-givocaled plaidies out.

We met here the fluorescence of a blood. The color that you see gives the intensity of the fluorescence on, with red, very, very intenseity, and dark-blow, a little intensity.

On the horizontal as, I'm looking to different color of fluorescencey, and we They're going very sai, from fell-rode to dark-rode, because of plants, now, red fluorescensia outstral. The differences by these different color of fluorescensi, tell me about how different components of photosynthesis are going

to be. On the vertical as, can you the time whereop I have met. In this case, 5,000 picosecond. That is 5,000 x. 10 to the minus 12th second. So that is a fraction of a second, we know it also ultra-fast measurements. Light, let in this time but over 1/2 meter off. Here I've got to keaken in a gosontblad. But I can it's the same to do for bladering that I have besched by too much light. That see we here. The greatest deal of the differences have I catered with this whitterecthock. We see in this whittancey, more fluorescensity as there shardt place.

In this case, Photocyssin 2, a slender fluorescence year, and there comes minor fluorescensy free. That is, because there more energy would be offered as warmth. I have this for many different plants, I have this sort of metings for many different plants done, to look to look at how shatter on how fosystemt two. How fosystem 2, have I also bekekeke with a very special form of microscopy. Here we're going a light flit and then we how long the blood blueriselser. This is the levensstur e. This form of microscopy also flim or fluorescensi levensensi microscopy. For every pixel of a microscopy plaatje, I gave information over the levensstub. And because the schade the fluoricensi levinsstur coter makes, can I here see how the shard is in different of a chloroplast. I know the telecovide membrane, the membrane in chloroplasts what the looping is for the photosynthesis fabric. This membrane is very very indivocal, and normal is FOTysystem 2 in this upgestapled pannelcoek of the membrane. But if FOTysystem 2 has stelt would be going to the other different of the membrane, where it more more room has more for restel. I have the a that-vercropie-plata of an individual blood-grong-correl. Helaus, we're by this technique more on the black-groomcourt in place of these more schematic that I've already have to see. This betekent that we the felled, here also encircled with greenerontes. That are the up-gasteled pannecook-gedealtes of the membrane, and the minor-velled are the rest of the membrane. I can you look at what the fluorescensy do in both the end. This is a gloreplast, and we can see that the hook very is from this graphic. If we this forgelike with the chloroplast where I've well have done through too few light, then we can we see that the helling more stiler is more. This means that there more fulricentia place found. And that gives on that that the shadow for more offgifting of light energy as warmth. I have also to look at the shatter in the different of the gloroplasts and these are actually the same. That gives on that that beschated SOOTus 2 is not uphoct in one of the gloeplast but that affraq and reflation efficient stay under the community conditions. The off-feworthy of light energy as warmter, it's not only if there's not only when the plant can it also in short of the short of a sort of and then he's not express light energy off as warmth as a sort of a vitality fantiel. This know me also not photochemising. I have this undersoct in a moss, and normally it there's like here boven out, very green, but I've the last last two years also indruk-beck many shimels to create and that I would I didn't you not hold. This moss has more other actors that are important for this not-photochemis damping. These are LSCS-S-S-S-S and Xxantina. And I've out out of Italy all different mossa get created that one or more of these components not had, and I have rekekeke to how these actors add to different light intensities. Here we see we a graphique of the howveveh the plant to do on the plant to do the different light intensities going from green, Lager-Lignt light-intintintintit to dark-blown high-light intensity. We see that on a high-light intensity there more bescherming place-fint, or NFD, on the vertical as, while on the horizontal as the plate, the time seen.

What we can see is that the form is on large-light and on high-light-intensitite. This is because of different processes add-draging on these bescherming that different light-intensitites, and I have wiscunded to use-cundersed to isoler.

On high-light intensities, see we that the security long-to-neemes. This process is for a very very much but it dured even to start. On the low-light have you a peak in the security, so that you all enough security in a minute of the light-intensitite. And, and there now, the bescherming there off, because Lager-Ligthensitite actually so very much security is. Now I can look at the actors are very important for these processes. We see actually that LSCSR and Xxtantine

for all in some working very important for this process that it's also that they're very important for the process that sorts for short-beshrim.

But as you maybe can remember, was there also another component, genaimed PSPS, and it is maybe not even doubt what he then does. Now, this works actually for the same sort of processes. He's also for a longsame component that at the end upendly that at the end uphearing bids and for a a small peak, only it gives he just gives it more better so the form is the form is the same, but the total howveveil is more. This research gave on that all components, PSBS, LSSR and CXantina and are other light intensities. Now we more we know about their individual role we can we can we maybe plan to make that better better and this was the last slide from my leke prait. I thank for listener. I hope that you there something have upgestoken and now will, and maybe the defense better going to understand. Over, I think, two minutes comes the Paddell again with the commission and then will the defense of three-quarters follow. Please be seated. I hereby open this ceremony, convened by the Academic Board of Wagening University, in which Cleo Roberto Paola Baches is offered the opportunity of defending a thesis with propositions entitled, Life Stress in Plans, Insides from Chlorophyll fluorescence. The defense will take place before an examining committee appointed by the academic board as a prerequisite for conferring the degree of doctor. Good afternoon. I want to welcome you all to this graduation. My name is Jogsvon de Gucht. I'm a professor of physical chemistry and soft matter, and also a member of the academic board. And in that capacity, I represent the Rector Magnificus today. I now call on the first examiner, Professor Avers. And Professor Aves is a professor of crop physiology here at Wagening, University and research. The floor is yours. Thank you very much. Dear candidate, I've read your thesis with a lot of interest. Some of the it is close to the things that I work on myself, some of it is, or actually, well, quite a lot of it is a little bit further away, so it made a very interesting read for me, and I learned a lot. Also, my compliments for the beautiful cover. It's absolutely stunning. I would love to discuss a couple of points with you regarding this thesis, and I would like to start at the start, so at the general introduction.

On page 12, you mentioned in this section, I will describe some of the photoprotective mechanisms plants take to protect themselves against highlight with a special focus on MPQ, non-photochemical quenching.

And then you highlight that you go deeper into the mechanisms, but I'm not reading anywhere explicitly the reasoning you chose to focus on MPQ among the different mechanisms available to focus on. So could you say a little more, yeah, a little bit about your reasoning behind focusing especially on this?

A highly esteemed opponent. Thank you but thank you for your kind words about my thesis and for your question. I must admit that the main reason that I focus on MPQ in the general introduction is that I focus on MPQ in my later work. Also, I think MPQ is really interesting from a chlorophyll fluorescence standpoint in that it's easy to measure with chlorophyll fluorescence, which are the techniques that I applied in my thesis. and other things that plants do to increase their highlight resistance, might also not focus on the timescales that I used in my thesis, such as, I don't know, thickening leaves, salt depositions on the leaves, to scatter light, increased VAS pools. Okay, yeah, indeed, my question was not so much, why do you focus on MPQ in your introduction, but your thesis as a whole? But I think you answered that question as well. Yeah, so could you elaborate a little bit on MPQ as a mechanism in plants? I mean, clearly you describe its role to help plants deal with highlight. Does that mean that a mechanism such as MPQ is present in most plant species, or does it have to do with whether you as a species have evolved in highlight situations or in situations where highlight can occur? as I know, most plants, and for example, so vascular plants and also mosses, have a version of MPQ, but there are also species that have different versions of MPQ, I think like antioxidant properties to protect against damage, that are not necessarily haven't encountered high-intensity lights in their environment. I think some desert algae also don't necessarily use MPQ, but more antioxidant properties to protect themselves.

Right. So now a very question. I mean, if you think about plants that grow on a forest floor, would you find similar levels of MPQ activity, if you expose them to highlight as other species that do experience highlight a lot? There is. So I did in chapter four and five, I focused on a moss, fiscometer and patent, that does grow on forest floors, but also next to roads and open pools. And that one has quite a high level of MPQ, especially compared to, for example, Arbidopso-Staliana, which also grows in open fields. So I think it depends. I think both can happen. Okay, thank you. then I would like to move on to, well, I linked it in my notes to Chapter 4, but it's actually, yeah, also related to some of the other chapters. And this is actually about the fact that you've been using both Arabidopsis and this moss as your model plant species. So you choose different species. In Chapter 2 and 3, you used Arabidopsis. That's, of course, plant scientists, pet species. In chapters 4 and 5, you used a moss species. and your reasoning, if you're doing that on page 1004, is that, and I want to read that out properly, that the moss is a descendant of an evolutionary intermediate between green algae and vascular plants, you're saying, and it contains multiple members of the light harvesting complex superfamily of proteins, right? You just mentioned that as well. It has the LHCSR, so the stress-related. protein and the PSBSS, photosystem 2 subunit S proteins, in the effect on MPQ. So they interact, and then you find an effect on MPQ. So, and you also mention on the top of this page, that vascular plants lack LH CSR, right? So could you, and you discuss this a little bit in your general discussion about moving to vascular plants, but could you elaborate a little bit on the sense that it makes to move to a moss that has both proteins present to study the interactive effect on MPQ while one of those elements is not present in vascular plants, the kind of plants that you ultimately want to work with with moss being a model species. So could you elaborate on the reasoning behind that choice? Yes. I think at first I must say that I am a fundamental scientist and I think studying things for the benefit of studying things is very important and I think just PSBS, LCSR and an active center of a cycle inside a single organism is just very interesting. Super cool. Super cool. I must admit that when I started the research, I did hope that there would be more benefit of introducing LCSR into vascular plants. and when I started the work and started writing my general discussion, I actually figured out that work has been done to introduce LSCASR infestular plants and it hasn't been super successful. And I think it's mostly due to the lack of CXentin, so the amount of CXentin matters because we could see that in MPQ2 mutants with constitutive expression of CXentin, there was not a lot of benefit of introducing LSCSR, but maybe some. I had hoped that by doing this research, I could potentially figure out how we could introduce LCSR and Vascular Plans in a way that actually benefits them, but I don't think I've reached that goal. Okay, so continuing that a little bit, what would you propose then to do in order to reach that goal? So what are the unknowns still that we need to clarify if we want to get there eventually? The exact quenching mechanism of LCSR and the pH dependency of LSCSR in FISCO isn't super clear to me yet. The LSCSR, Fiscomitometheus, has less protonable residues compared to LSCSR from Glaminomones, and that might make it less pH sensitive. I'm also not sure whether if we could understand, LSCSR better, it would be a good substitute to introduce intervascular plants. It could also be that we could understand it better, and then it still wouldn't make sense to introduce it. I'm also not completely sure that higher MPQ would actually yield more crops, result in higher crop yield. I think most of the focus on improving crop yield via changing MPQ is more changing the, increasing the speed at which it decreases after. there are periods of highlights. Okay, right. Yeah, I'm not at all criticizing fundamental science. It's super important. I was just wondering, yeah, what scope you see for moving to beyond the most species that you've been working on. So, okay, thanks so much. Yeah, actually, well, you constantly give passes. Foursetches, how do you say this English? Yeah. For my next question. So that's great. I wanted to move to your general discussion, Chapter 6, page 174. mentioned something there, which is interesting, and I think it's a thing of debate.

Somewhere halfway, I'll just read it out. Photosynthesis is a prime target to improve crop yields. Well, you just mentioned it.

Solar energy conversion efficiency of photosynthesis is typically below 1% in crops, right, which is lower than a theoretical maximum of approximately 5%.

So you're not going deeper into it here. So maybe you could elaborate a bit on why on earth is the theoretical maximum only 5% after millions of years of evolution?

How does that happen? Yeah. I, when I started by PhD thesis, I was also a bit confused.

I think part of the reason is that there could be, once I once I heard a very interesting talk on the function of Robisco, where they actually said that while you would, it evolved in an environment that it's not necessarily reflects the environmental conditions of today, but then you get stuck in one of these like evolutionary local minima and you can't really get out of there. So you can like optimize it to a certain degree. But maybe you should actually start like completely over if you want to have like the most optimal photosynthesis that you could have today, which we aren't able to do.

But just what we have to work with is stuck in a local minima. Interesting observation. Thanks. I think my time is up. I give the word back to the deputy director. Thank you, Professor Avers. And then we move on to Professor Janz. Professor Janz is a professor of plant physiology at the Heinrich Heine University in Dusseldorf, Germany.

The floor is yours. The candidate, I also really enjoyed reading your thesis for several reasons.

The most important reason for me it covers a lot of topics that I have been studying and in contact with during my career, which actually, and this is a second point, ends in two weeks, and this is my final defense, I will act as opponent, so I'm really happy that I have the chance to be here. I want to start with a very general question with the title of your thesis is light stress in plants what is a molecular basis of light stress why is light stressful okay hi sorry highly esteemed opponent thank you for your kind words about my thesis and for doing this two weeks before your retirement i'm really grateful thank you for your question as well um light stress if there's more photons being absorbed then can be used for photosynthesis. They encounter closed reaction systems. And then I think there are several methods, several pathways, to eventually lead to cinglet oxygen, and cinglet oxygen is a very potent reactive oxygen species, which can lead to damage. Okay, yes, completely correct. And when you think, so this is a situation that is quite normal for most photosynthetic, organisms and obviously they survive quite well and each of them has found some specific strategies to overcome these problems and at different stance the the problems are quite different depending whether there is fluctuating light whether there's constant high light low light and so on what can you summarize or tell tell me what general strategies plans of photosynthetic organisms have to avoid or to overcome light stress? Yeah. So we have MPQ, which I cover a lot of in my thesis, to protect against light stress. There's also the antioxidant function of santa fields, which can help minimize light stress. There's also stuff like functions like moving away from the light, tilting the leaves, getting salt depositions on leaves, minimizing the Bs 2 antenna size to limit the amount of light absorbed. I think there's probably like a hundreds more that I'm currently forgetting. Yes, but you mentioned the most important one. The most important strategy in principle I would suggest is really that what you mentioned last, is a reduction of absorption. So it's the same when we go into the shadow when it's too hot or too sunny. Plants cannot move, at least not vascular plants. And in principle, this is the most efficient strategy to overcome the problem. What is the chloroplast movement? Do you know how this works and at what time scale? Yeah, so chloroplast movement is so you have a leaf and inside the leaf is chloroplast and then instead of if you have incumbent light like this instead of chloroplast like this they move to the walls to protect themselves against incumbent light the time scale I would say is like half an hour or like 30 minutes between 5 to 30 minutes and it depends a bit on the color of the

incident light whether it's activated or not. I think there was some debate about which colors activated in fiscometrium patents. Both blue and red light activates chloroplast movement. Actually, it's the same time scale when one of these MPQ components is QZ is occurring. Yes. And there's also a debate whether the fluorescence changes are really due to quenching or to the movement of chloroplast, for example. And this you can, as you mentioned correctly, discriminate by different light qualities that you apply. Okay, so reduction, reduction of light absorption is a perfect strategy. It does not cost anything, and it's very efficient. The dissipation of energy, you also mentioned the MPQ, so when you have too much energy, you dissipated. And at the end, of course, is when all these mechanisms did not work, you form some reactive oxygen species. And do you know which raw species are formed in the chloroplast? Singlet oxygen. Yeah. Peroxide. Yeah, it's 50% good. Yeah. No, sorry. So there are two principles that can, in which way radioactive oxygen species can be formed. One is energy transfer, and this is what you meant. mentioned, the cinglet oxygen formation, and this is more or less specific for plants, but also in our case animals, men, we also have a problem with formation of reactive oxygen species, and this is due to direct electron transfer.

Do you know where electrons can be transferred to oxygen in the chloroplast?

The donor side of Bs 2? The principle, yes, but this is, but at what side, we are going the, Electrons on the electron transport chain. Where are they going to? Pfeo phythin. Further? Phyophyting, plestocyanin, plestocyanin. And then? Plastocycinine, plestopinol, cytogram B6-F. And at the end? P is 1? P is 1. The P is 1 give the electrons to pharodoxin or to NADP and so on. And so on. And when this is, then it's the point. Because the highest possibility to transfer electron to induce the formation of reactive oxygen species is indeed at the acceptor side of photosystem 1. And this is, you may know or not, the so-called Mela reaction. And in fact, this has also been thought that this is not only a stress signal, but also photoprotective mechanism to remove the electrons in this way. But you must take care that you get rid of the reactive oxygen species. Okay. Finally, next topic is the xanophils. What are the functions of the xanophils in photoprotection? What different properties do they have? They're antioxidants, so they remove ross, and zhexanthin induces quenching, so is important for MPQ. And when you say they act as antioxidant, what do you think what happens they react with reactive oxygen species yes all right yeah exactly and this is something that has been studied indeed in vitro experiments but it's nobody knows directly when it takes energy on electron from from reactive oxygen species what happened with a with a product then okay um you might also know that the xiaxanine that you have have also investigated, is present in different lineages and different organisms. And obviously, the function is quite different in the different species. Particularly, the green alde climatomonas. Do you know what is specific with the xanthofal function in chlamidomonas? No, sorry, I wouldn't know. So it has an active xanthal cycle, but in fact, there's one very important. interesting thing, the de-epoxidase is not in the lumen side, but in the stroma side, but nevertheless responds to the membrane energization. And indeed, when you knock out the BDE and have no xerxentin formed in chlamidomonos, then the impact on the MPQ is much less than in land plants, for example, or in other species. Thank you very much, Professor Jans. and then we move on to Dr. Navrochki. Dr. Navrochki is a scientist at the CNRS and the Sorbonne University in Paris in France. The floor is yours. Director, thank you. Esteemed, respected candidate. I also wanted to congratulate you on this work, which I read with a lot of interest. It's also quite close to the research I am doing, but more on the plan side. So it was fascinating. That said, I wanted to challenge with a few mechanistical questions, maybe more speculative. And I will start with the xanthin that we just heard about. So do you actually think that it can replace the pigments in the late CSR scaffold? And if so, how would you test this? Would you come up with some experimental ideas to see if it really goes inside, at which time scales and so on? Highly assumed opponent. Thank you for your kind words about my things. and the question. I think the interaction between LSCASR and CX-X-EFentin is very interesting because I could observe in Chapter 4 an interaction, really the ability to produce X-X-Exanth for Fiscomitium patents is really

important even on timeskills where not a lot of CX-Xentin has been formed. So even within one minute and at low light intensities, I could see very clear differences between plants that are able to synthesize CXentine versus plants that are unable to synthesize CXen.

So I think the function isn't necessarily linked to the changing of the binding of CXentine to the pigments of LHCSR.

But probably I would say that CXentine could bind to LHCSR. And then your question must further what it would, how I would test this. Maybe some in vitro experiments. Yeah. There are like pull-down, there are pull-down essays where you could see whether you pull down the pigments together with the LSCSR. I think I would start with those. Sounds good. On page 15, you mentioned partitioning of different types of photo damage. I'm thinking here about the acceptor and donor site. photo damage in PS2. There's not a lot of mentions of this further down the line. And so I was wondering if you could also think of how, whether it would be possible to account for the two types and the partitioning of the two types of damage in natural conditions. Through the scope, for instance, of in which way PS2 is damaged? Could you think of, you know, of a way of actually quantifying this in natural and not laboratory conditions where I suppose you only look at one type of damage so by natural conditions you mean I cannot change the color of the incident lights to yeah in plants in plants in the field in microalgae in the in the ocean I suppose that the partitioning of the damage will be specific to an environment to the environment but I could if I would do experiments in the lab to check for this, I would change the color of the incident light because UV lights, the manganese clusters way more sensitive to UV light instead of red lights.

But as you discussed in your talk this morning, it gets really difficult to account for the absorption of a leaf for the different types of lights to then check for how many charge separations photo system to actually does. So I would definitely need to take that into account. We would start in the lab. I think that makes sense. All right. So in chapter 2, page 35, but this is not so important, you were looking at chlorine a mutant. And so you mentioned that it's difficult to separate the signals from PS2 and PS1 in this train. But from what I understood, you conclude that PS2 has a shorter life. time in the closed state in chlorine mutant compared to the wild type. And I'm not really understanding why this would be the case. If PS2 is closed, why are you not at the lifetime of excited state of just chlorophyll 2 nanoseconds?

Why is it quenched? So for the decay-associated spectra for piece 2 and piece 1, I indeed wasn't able to completely separate them out as I've done for the other mutants that I studied in chapter two. But if there is a quencher formed in the PS2 reaction center, as I suggest in chapter two that has occurred, then there would be quenching in the... So this is after some photoinhibition time, but you also conclude that at time zero, right, before we even look into photo inhibition, that the lifetime of closed PS2 is shorter in in chlorine, assuming you are able to separate it perfectly from PS1. So in other words, is it just a question of mixing of the signals between the two and do you think that the closed PS2 has a long lifetime in this train? Or is it that you think that there is a quencher in chlorine even before we start looking at photo inhibition? So the shorter lifetime that I show in the figure 2.6 on page 37 is because of the mixing of both PS1 and PS2 and the decay associated spectra before and after photo inhibitory treatment on page 51 the fluorescent lifetime is slightly shorter slightly shorter but it doesn't differ by that much okay so just the mixing that explains it all right so you also I also mention a bit later on, I think in the experiment with the long-term experiment exposure to lycomycin, and that there are partly connected antenna that account for the high F0. And so I would like you to explain us, how do you actually physically envisage partly connected antenna versus very rare occurrence of photochemical quenching, which I think can be also an explanation. In a sense that if your antenna size becomes so huge that the losses in energy transfer will take place, as I think is the case in your mutant, why do you need to talk about this partly connected antenna that I think is difficult from the endpoint of how many antennas are there in

the membrane and which concentration and so on. So I just wanted to get your feeling about what do you mean by partly connected antenna. I discussed partly connected antenna because if there would be no connected antenna, the FV over FM would be zero, while for the long-term linkomycin mutant, the FV over FM is not zero.

I think it's like 0.3 or something from the top of my hat. So this is, I mean that sometimes they are able to reach a reaction center and actually perform charge separation. But you agree that if you had a perfectly connected system where you have huge amounts of antenna per PS2 that are perfectly connected, your F0 would still be very high because the distance would be just too large, right? Yes, I agree. For chapter 3, I still have one minute, I think, left. I was wondering if the reasoning about the damage and repair and the relocation of the broken PS2 would still hold in absence of link. In other words, what is your take on the effect of repair in translation on the observables and conclusions that you make in the chapter? Of course, it's easier to work with linkomycin for the care that you know, but how extrapolable they are too. So I used linkomycin to indeed block repair and with that I don't see any measure. difference between the fluorescence lifetime between the grana and the stroma lamella and rich regions using flim.

If I would do this, so even if I maximize damage, I wouldn't see a difference. So if I had less damage because I would have an active repair cycle, I also wouldn't be able to detect a difference with this technique, just because the technique is limited due to the, I think, mostly due to the Zed resolution that is actually larger than the average size of a Gana stack in the Arbidopsis grown on the right light. So it's always mixed.

Thank you for your answers. Thank you, Dr. Nauroski. And then we go to our last opponent of today, Dr. Pandit. Dr. Pandit is an assistant professor at the Leiden Institute of Chemistry. The floor is yours. Respected candidate. I also would like to start to congratulate you with your thesis and with the work. I mean, as you know, I mean, we're in the same consortium, and I followed it, and really interesting to read now all the results together. And also, I think you did very rough studies in four chapters, also publications. And I also have some questions, and I would like to go with you to chapter four. And actually, my first question is a general one. So I'm an experimentalist, but not on plants. So I know that in a palm fluorescence experiment, you take your setup and you put it, if you have a vascular plant, you take a leaf, and you put it next to it, and then you shine light and you measure your fluorescence. But now you have moss. So you have these tiny little plants, and I assume you illuminate them together and maybe collect fluorescence together or not, So my question is, are there any extra challenges to do fluorescence induction experiments on moss? Highly esteemed opponent, thank you for your kind words about my thesis and your question. You missed my layman speech, and then I had an image of a moss and the way I grow it, completely infected with fungus because I wanted to show them the realities of lab work with physical medium as well. So I have, I did my measurements on protonamal tissue in fiscometrium patents. That means that I had a petri dish filled with agar, covered with cellophane, and then I've grown like the, the fisco as a sort of carpet. I called it as a carpet. And then when I did my measurements, I just put the head of a mini palm relatively close to the, relatively close to the protonamal tissue. So you eliminate like a range of this tissue instead of a single leaf. The biological variation is relatively high in measurements done on fiscometrium patis, and I think one of the reasons is that you eliminate a little single leaf with like the whole carp. But the most challenging was that it's really sensitive to lights. So you really had to like minimize all of the parameters to make sure that you didn't actually induce damage just by your PAM protocol and I think probably still my PAM protocol was slightly actinic in nature. That was the most challenging. So do you also have extra challenges because it's more heterogeneous that you get some more scatter or shade or not with the way as you explained? I missed the pictures of... I didn't notice it with the way that I performed the measurements. Okay, so I learned something how to do it in Moss. So then you analyze your data and you can fit it with two components. So one is a slowly rising component and one is a

transient one. And then about the transient component, so you refer to it that it might be the relaxation because of the counteraction that the minus MPQ, such as the activation of ATP synthase and these potassium efflux antiporters. So does it mean that it actually reflects a transient in the Lumen pH? Is that what you were suggesting? I wanted to be very careful with the way I worded things in this chapter because we see this dissipation. So this component also plays, it plays a smaller role at high light intensities, but it's still present there. And if it would be completely the difference across the pH difference across the thiodoids membrane, at high light, the pH of the lumen stays low. So that makes concluding that it's completely the pH gradient very difficult. So I think the pH gradient definitely plays a role, but I think there's another process there, that I just haven't pinpointed yet. But potentially also, I only separated out two components here due to the biological variation in Fiscal Mitrientatins. And it would be so... You might miss something. I might miss something, yeah. So then I want to challenge you a bit more. So what if you're quenching mechanisms that you don't know, but you try to unravel with your analysis, would contain sort of intermediate steps. Because then you would also have an intermediate fluorescence or quenched species that would sort of rise and fall. Yeah. But it would not be sort of two independent mechanisms, the slow and the fast component, but sort of one converting into the other. So would that look different in the way you do your analysis, or could something like that be a sort of alternative? explanation or part of an explanation? I think it could be part of an explanation. I think if it would vary at different light intensities, like the movement from one species to another species, it might be able to be disentangled. But I wouldn't know for sure. And I would definitely say that in FISCO, because we only see these two components, due to the biological variation, we wouldn't be able to completely separate these out. But then it's mostly due to the biological variation, which is relatively large. So the precision at which we can pinpoint the shape of these components is limited. Well, I guess if it would be sort of an intermediate that is formed and sort of converted in something else, then there should be a correlation with the amplitude of the two components. So would that help you, or do you see this correlation? If we could pinpoint the shape of the components and the amplitudes more precisely, that might help, yeah. And then you also say you draw conclusions that also at moderate light intensities. Already in the fast component there is an effect of Xxanth. so it reads like sea accentin might be either more rapidly formed or small amounts might be very important already than previous has been taught or measured in vascular plants. So do you think this is because it's moss and not a vascular plant or do you think we should revisit how it works in the vascular plants as well? I'm really trying to remember the work done in Widov's Saliana. I think this plays a big role in mosses just because of the interaction between LSSR and ZXen and this interaction between BSP and ZXentin, which are present in vascular plants, we don't see the interaction this much. So I think it's mostly due to it being a moss with the added component of LSCSR. So it's more sensitive to ZXentin? Yes. And you mean that even if you have only small amounts, forms formed, you were able to see an effect of those? That is currently my theory, but I would love it if people would do, if we could have done detailed pigment analysis on the production of, on the centrifugal cycle in fiscometrium patents to like say something about this, say something more about this. And then a very general question. It was actually very interesting or a bit intriguing to read how important LSCSR is for MPQ in Moss. It's like it's supposed to be an evolutionary, like an intermediate, going to land colonization. But actually, if you knock out PSPS, there seems to be a small effect. If you knock out LSCSR, the effect is huge in combination with X-X-Sentin. And so you start wondering, so how do fescular plants actually do this without LSCSR? There must be a huge leap because they only have PSBS, and I mean the protein is almost the same as it would be in patents. Yes, I am also very impressed with vascular plants. So one of the things that we see in fiscometrine patents that I haven't studied explicitly, but is that the amount of PSBS varies with the light conditions. So if you grow down under high light, they might increase the amount of PSPS. You can finish the sentence if you want. Increase the MPQ, that PSBS dependent MPQ. Thank you, Dr.

Pandit. And now I adjourn the meeting. The examining committee will withdraw for

consultation. Please be seated. I hereby reopened this meeting. The Academic Board of Wagening University, represented by the Deputy Director Magnificus and six committee members appointed by the academic board, having noted the content of a thesis entitled Light Stress In Plants, Insides from Chlorophyll fluorescence, with propositions, having heard the defense of that thesis, has decided to confer the degree of doctor on Cleo Roberto Paolo Baches, born in Amsterdam, the Netherlands on March 15, 1996, and to grant to this person all rights and privileges ensuing from that doctorate by law and custom. The Academic Board assumes that you accept your duty as a scientist to execute your future research ethically and with due diligence according to the Netherlands Code of Conduct for Research Integrity. I now invite the promoter, Professor von Amoronga, to present the new doctor with the degree. You have heard the decision of the Academic Board of Wageningen University confer on you, Cleo Roberta, Maula Bajus, the degree of doctor. It's now my honor to present you with the degree, signed by the Deputy Rector Magneticus, and the co-promoter and sealed with the great seal of Wagening University. I first invite you to sign the degree as well. With this signature you declare to act according to the Netherlands Code of Conduct for Research Integrity in the future. Deputy Rector Magnificus to offer my congratulations and to add a personal address. Dear Dr.

Bacchus. I will keep it very short. Of course, Emily, Dr.

Winches. It's your real supervisor. We just noticed that, in fact, she's the promoter now. And I'm the co-promoter. There's even more written on the screen. Even more reason for Aemly to address you. One, a few sentences. I was very impressed. We had a project together. We discussed many times and then evaluated every week or two weeks. And then after one week, we concluded that you had not achieved anything. At that moment, at least it's. seemed like that. So because you couldn't see you were measuring in different conditions. There were no differences at all. So it was very difficult for us to draw conclusions. But then from that moment on, yeah, things went on very well. So in three years, even within the time, schedule, you finished your complete thesis, four chapters even, and even managed to send everything to the committee, in fact, who I wouldn't like to thank, but the others will do that as well. So I give the word to Emily and I'm very impressed how you did everything. Dear Dr.

Wachas, dear Cleo, many congratulations with his great achievement and also to your friends, family colleagues, and especially Sophie Otto and your parents, of course.

Congratulations. So actually before you started your PhD with Herbert and me at Biophysics, you already did a master thesis with Johannes and he actually said to us, like, you should hire Cleo. And then with some work from Christo telling that photosynthesis is really cool, it all happened. And so, yeah, I think Herbert and me were happy when you applied. We were impressed by your CV. You did a better degree in Delft and then two master degrees in Wageningen. You attended an excellent high school. And all of that with great marks. And you were also the team captain of an IGM team that became. second place worldwide, so we were very impressed and we happily offered you the NWO PSBS nanoregulators position.

Then you started your PDD, you were supposed to start first of January in 2022, but then Cleo came so I first need to go on the winter sports. Can I start a bit later? Okay, half of January then you really started and I think a few weeks later you still needed to go on another one day. So, yeah, winter sports was a recurrent theme, but fortunately, you always returned healthy and in good shape. So that was a relief. Yeah, so for your first project, I asked you to photo damage photosystem two and look with fluorescent lifetime imaging, where are those damage complexes going, and what is their fluorescent lifetime, and then when they were repaired, how does this change? And I told it was a very nice idea, but it appeared to be. very complicated to actually figure this out. And, yeah, you first started with the analysis, then Leonard helped you with all kinds of different analysis methods, phaser, all stuff. But it's very difficult. It turned out to peel out these differences in the leaf, in the chloroplast, and

then in the granar versus the stromallemella. So, yeah, after you learned a lot of mathematics and coding, but the results were still hard to interpret. actually. And yeah, after a year, you had invested a lot of energy in measurements, in analysis, but it was still unclear. And that was, of course, hard for you. But then two things happened. First, you decided to see your PhD more as a job than like an identity. And second, you discovered forced optimism. And then it went actually very smooth. From that moment onwards, you had three years with lots of measurements, analysis, paper writing, and all three first author publications were spot on accepted during your contract and one co-author publication soon after.

So that was very good. And next to your publications of the project that are in the thesis, you also supervised three thesis students.

You did a lot of measurements for CV, C4 photosynthesis, which in you. And also, when there was an organizational task, you were always happy to take it up. So you did also several other things. And I especially remember you walking around with your notebook and your fill pen. And everything we discussed would go in that little notebook. And then we had the next meeting. Actually, you had done most of the list. You had really done it. Many people write things down, but they never happened. But Cleo was really doing these things.

And yeah, this became, was very useful. And yet, all the lifetime data or the pump traces from the fiscometrium at all kinds of mutants and all kind of light intensities so your organization skills they were very useful and not only with data but also when there was the major power failure in helix and afterwards you became somehow an organizer of these things that had to be arranged yeah and the last organizational thing when Herbert asked me a few weeks ago like do you know when the thesis of play will arrive I told him, I don't know, but I'm sure it's arranged all fine. Cleo is so organized. And then Herbert said, oh, yeah, I'm not worried either. He said, Cleo is the most organized PSD I encountered in my career. So, yeah, that's another title you bring today. Most organized PhD candidate. Yeah, then of course, I also would really like to thank the opponents for making it over to this room, to Wageninga, some from abroad even, so thank you very much for having the discussion, being here, evaluating the teasers, and yeah, thank you for all the work you did. It was very nice to work together with you. Yeah, I wish you, of course, all the best for the future, and I'm very curious to learn what other great things you would do. Thank you, Professor van Amarunga, Dr.

Wienchus, and dear Dr. Bacheras, or highly learned Dr. Baches, congratulations, also on behalf of Wagner University and the academic board and of myself, of course, and I also want to extend these congratulations to your partner Otto.

I understand that you still have to go, undergo this ceremony, so you've seen today now how to do it, and good luck.

Also to your parents, I presume somewhere here on the first row, your twin sister somewhere here, and your grandfather is here today, and many more people here.

So everybody, congratulations with the doctorate of Cleo.

Also to your paronyms, today there was no question about the proposition, so you didn't really have to do anything.

I would have loved to hear about forced optimism, too, but that will maybe come later.

So I very much enjoyed the discussion today. So you worked on how plants deal with very intense light and what protection mechanisms they have. And in our meeting that we had, you already told me how complicated that is. And we also heard that from your supervisor. Also from the discussion today with all the abbreviations, N-P-Q, L-H-C-S-R, Ross, PS-1, PS2, and so on. So it's definitely

a very complex subject. But it's a very important one. also, of course, fits very nicely with the motto of our university, which is to explore the potential of nature to improve the quality of life.

We've heard today how terribly inefficient plants are, and so maybe your work will in the end contribute to maybe somehow making things more efficient. Who knows? So now you have obtained the highest academic degree that we know in the Netherlands. This is something that you can be very proud of. And of course, with that doctorate, we also expect you to maintain high standards and quality of research in your future work, and maybe also to act as an ambassador of the university. You're an alumnus of the university, and you will remain that for the rest of your life. And I also hope that you will stay in touch with the university in some manner. So you now have moved on to a new job. You're a program coordinator of NWO, so in that capacity you will certainly, encounter scientists from Wageningen and again, and maybe also your former supervisors. Who knows?

I also want to express my thanks to the committee. It's a lot of work to prepare for this thesis, to read the thesis, to come here, to ask questions, but without that work, we would not be able to maintain the high standards of our research. So thank you very much. Of course, Dr.

Navoski and Professor Jan from coming from abroad. also Dr.

Pandit for coming from Leiden, but also our own Professor Avers coming from Wageneg, and very much appreciated, and thank you very much. And then also thanks to everybody in the audience for being present today, for supporting the candidate today, and I hope that now after this you have time to celebrate together with our new doctor. So finally, Dr.

Baches, again, congratulations, and I wish you all the best in the future, in your career, but also in your private life. And with that, I close this ceremony.