UNIVERSITY OF COPENHAGEN

Control of *Podosphaera pannosa* in roses with use of induced resistance

Mads Wadum Larsen CLR689

Supervisors

Hans Jørgen Lyngs Jørgensen

Frida Helgadottír (HortiAdvice)

Department of Plant and Environmental Sciences
14th June 2019

Contents

Introduction 3

Research questions 4

Biopesticides 4

Induced resistance 6

Podosphaera pannosa 8

Induced resistance against *Podosphaera pannosa* 10

Discussion 11

Conclusion 12

References 13

Picture on font page: Symptoms of powdery mildew on rose flower buds, Clemson University – USDA Cooperative Extension Slide Series, www.forestryimages.org

Introduction

Pests and diseases are one of the determining factors for yield potential in both outdoor fields and greenhouses across the world. Control of pathogens and pests rely mainly on the use of conventional chemical pesticides. Pesticides have a wide range of uses, both in agriculture, on non-agricultural areas and in public health protection programs, and are therefore widely used (Nicolopoulou-Stamati *et al.*, 2016). Over the years there has been an increase in the knowledge of numerous possible negative side effects caused by pesticides, both in the environment and health effects such as neurological, carcinogenic, respiratory, reproductive and endocrine effects (Nicolopoulou-Stamati *et al.*, 2016).

An increasing dependency on chemical pesticides in agriculture in combination with the general awareness about possible adverse health effects has led to directives by the European Union (2009/128/EC) that strives to implement alternatives to chemical pesticides (European Union, 2009). These alternatives include cultural practices along with promoting the use of biopesticides to control plant diseases.

Potted roses, of the genus *Rosa* in the family Rosaceae, is one of the world's most widely sold ornamental plants. The annual Danish production from Rosa Danica alone, the leading producer of potted plants in the world, is around 14.5 mio. potted roses (Rosa Danica, 2019). Roses are subject to a number of phytopathogens, but powdery mildew, caused by *Podosphaera pannosa,* is one of the most damaging (Leus *et al.*, 2006). It causes visible powdery growth on the surfaces of leaves and flowers, leaf distortion and premature defoliation, dramatically reducing commercial value of potted roses (Kaufmann *et al.*, 2012).

With the recent ban on more pesticides and the shift in consumer demands for a more sustainable and organic pesticide-free grown crop, there has been an increase in the incentive to look for alternatives to conventional pesticides (Miljø- og Fødevareministeriet, 2017). A promising candidate is biopesticides, biochemicals and microbials, which the EU also strives to be implemented in conventional production and also organic production (Miljø- og Fødevareministeriet, 2017).

A particular mode of action, induced resistance, which can be induced by products such as biopesticides has previously been shown to be a promising mode of action in pest control with most reducing disease by between 20 and 85% (Walters *et al.*, 2013). It is defined as the process of active resistance elicited by biotic or abiotic factors (Kloepper *et al.*, 1992). Since the 1990s, several plant growth-promoting rhizobacteria have become commercially available and they have been found to elicit induced resistance in plants (Kloepper *et al.*, 2004). The use of cell wall fragments, plant extracts and synthetic chemicals has also been shown to induce local and systemic resistance in field trials (Walters and Fountaine, 2009).

This review will focus on the use of induced resistance in rose production to control the pathogen *P. pannosa* with emphasis on induced resistance as the main mode of action.

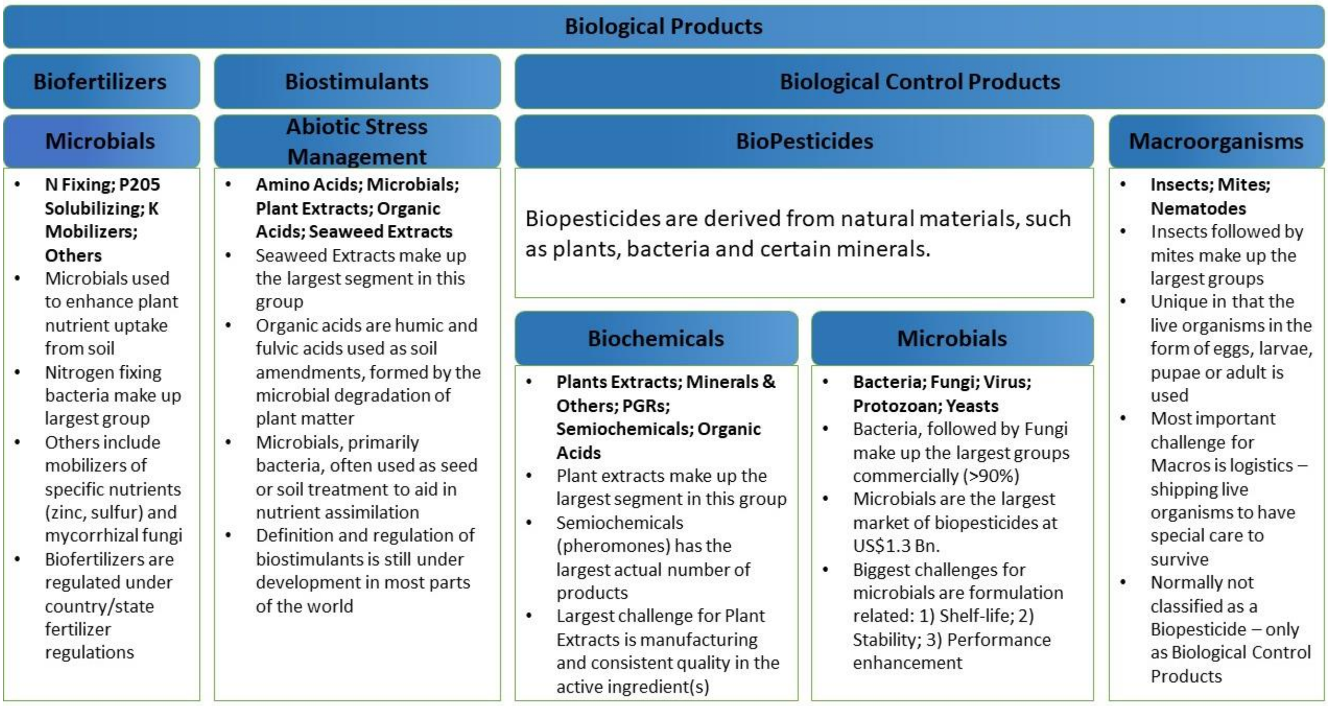
Research questions

1. What is induced resistance and how can it be used to control phytopathogens in plants?
2. Have experiments been made to elicit induced resistance in roses and how can it reduce symptoms of *Podosphaera pannosa?*

Biopesticides

Many phytopathogens are controlled using conventional chemical pesticides. Because of potential negative side effects of pesticides (Nicolopoulou-Stamati *et al.*, 2016), directives by the EU to lower the use of pesticides (European Union, 2009) along with the recent ban in Denmark on pesticides currently used (Miljø- og Fødevareministeriet, 2017) alternatives of conventional pesticides are sought after which include biopesticides.

A clear definition of biopesticides is lacking which makes the industry seem a bit blurred. The American Environmental Protection Agency (US EPA) has divided biopesticides into three major classes. **1)** Biochemical pesticides (including growth regulators, pheromones, oils, soaps and minerals), **2)** Microbial pesticides and **3)** Plant-Incorporated-Protectants (PIPs) (US EPA, 2016). Even though they have a definition for biopesticides it is still difficult to determine whether a substance meets the criteria as biopesticide and a special committee was therefore established to make such decisions. The definition from US EPA include GMOs which are not allowed in the EU making it not suitable for use in the EU. A definition was proposed by Dunham Trimmer LLC summarized in figure 1. The group of biopesticides include biochemicals, (including plant extracts, minerals, plant-growth regulators (PGRs), semiochemicals and organic acids) and microbials (including bacteria, fungi, viruses, protozoans and yeast).



Biological Products

Biofertilizers

Microbials

- **N Fixing; P205 Solubilizing; K Mobilizers; Others**
- Microbials used to enhance plant nutrient uptake from soil
- Nitrogen fixing bacteria make up largest group
- Others include mobilizers of specific nutrients (zinc, sulfur) and mycorrhizal fungi
- Biofertilizers are regulated under country/state fertilizer regulations

Biostimulants

Abiotic Stress Management

- **Amino Acids; Microbials; Plant Extracts; Organic Acids; Seaweed Extracts**
- Seaweed Extracts make up the largest segment in this group
- Organic acids are humic and fulvic acids used as soil amendments, formed by the microbial degradation of plant matter
- Microbials, primarily bacteria, often used as seed or soil treatment to aid in nutrient assimilation
- Definition and regulation of biostimulants is still under development in most parts of the world

Biological Control Products

BioPesticides

Biopesticides are derived from natural materials, such as plants, bacteria and certain minerals.

Biochemicals

- **Plants Extracts; Minerals & Others; PGRs; Semiochemicals; Organic Acids**
- Plant extracts make up the largest segment in this group
- Semiochemicals (pheromones) has the largest actual number of products
- Largest challenge for Plant Extracts is manufacturing and consistent quality in the active ingredient(s)

Microbials

- **Bacteria; Fungi; Virus; Protozoan; Yeasts**
- Bacteria, followed by Fungi make up the largest groups commercially (>90%)
- Microbials are the largest market of biopesticides at US$1.3 Bn.
- Biggest challenges for microbials are formulation related: 1) Shelf-life; 2) Stability; 3) Performance enhancement

Macroorganisms

- **Insects; Mites; Nematodes**
- Insects followed by mites make up the largest groups
- Unique in that the live organisms in the form of eggs, larvae, pupae or adult is used
- Most important challenge for Macros is logistics – shipping live organisms to have special care to survive
- Normally not classified as a Biopesticide – only as Biological Control Products

Fig. 1 Biological market overview of different biological product types as proposed by Dunham Trimmer LLC (2017).

There are many advantages to using biopesticides in agriculture and horticulture, some of the more traditionally believed advantages include faster deterioration time, inherently less toxic and more targeted to specific pests (Peabody *et al.*, 2009). The target range can be quite narrow as the mode of action tends to be more specific towards the target organism. The specificity in turn gives a lower impact on non-target species which can be beneficial. However, the target range depends on the mode of action/mechanism of the active ingredient, so this is not always the case. These advantages also bring with them disadvantages such as a shorter shelf life following the faster deterioration time and being slower acting compared to conventional pesticides. As the targeted pests can be more specific it may be necessary to include more than one product to broaden the range. These traditional advantages as mentioned are alleged but we must critically evaluate them. Not all mechanisms of biopesticides are narrow target e.g. induced resistance is characterized as being broad spectrum. Furthermore, we cannot be sure that biopesticides are in fact inherently less toxic to the environment although they are ‘natural’ products. For example, a component of several citrus essential oils, d-limone, is naturally derived but regulated as a conventional insecticide due to its toxicity. This differs from product to product and cannot be said generally for all biopesticides. It must be thoroughly tested and supported by toxicological data before we can be certain.

Biopesticides possess various modes of action including production of volatile metabolites, parasitism, competition and antibiosis caused by microbial antagonism, plant growth regulators affecting various parts of the plant which overall enhances the plant (Alabouvette *et al.*, 2006; Peabody *et al.*, 2009). Modes of action of microbials pesticides including *Bacillus subtilis* and *Pseudomonas fluorescens* are reported to be competition and induced resistance respectively (Vidhyasekaran *et al.*, 2001; Shafi *et al.*, 2017) but the actual mechanisms are poorly understood, and further research is needed to determine modes of action.

Induced resistance

Induced resistance can be caused by many different biotic and biotic factors and can be elicited by factors including biochemical and microbial biopesticides as previously mentioned (Walters and Fountaine, 2009). A definition for induced resistance was proposed by Kloepper *et al.* (1992) as the process of active resistance by the host plant, by physical or chemical barriers, activated by biotic or abiotic agents. Plant recognition of these elicitors lead to activation of a signalling pathway leading to production of plant defences. These defences include mechanisms like the hypersensitive response (HR), production of reactive oxygen species (ROS), lignification and reinforcement of plant cell walls as well as the expression of a broad range of pathogenesis related (PR) proteins (Walters and Fountaine, 2009; Jamalizadeh *et al.*, 2011).

Quantification of defence mechanisms is key in determining if induced resistance is present. There are many defence mechanisms in plants including mechanisms previously mentioned e.g. HR, ROS, lignification, PR proteins. Application of *Peseudomonas fluorescens* Pf1 in rice leaves showed an increase in defence mechanisms such as lignification and enzyme activity of peroxidase, ammonialyase and CoA ligase when challenged by *Xanthomonas oryzae* pv. *oryzae* (Vidhyasekaran *et al.*, 2001). These mechanisms where then seen to drop when no resistance was observed in susceptible interactions. Many enzymes and defence mechanisms can be measured but it is important to consider if the pathogen is affected by the mechanisms to ensure induced resistance is the reason of reduced disease severity.

It has been established as early as the 1930s that plants can develop enhanced resistance following infection by a pathogen (Chester, 1933). Experiments conducted on cucumber, watermelon and muskmelon showed enhanced resistance against diseases caused by fungi, bacteria and virus following prior inoculation by different microbial pathogens e.g. *Colletotrichum lagenarium* (Kuć, 1982). Through induced systemic resistance, the plant can systemically enhance defensive capacity from subsequent pathogen attacks in sites not locally infected by pathogens. Several different types of elicitors have been used against many different types of phytopathogens, e.g. chemical elicitors and non-biological inducers such as acibenzolar-S-methyl (ASM), β-Aminobutyric acid (BABA) and silicon (Walters *et al.*, 2013). Biological inducers such as plant growth-promoting rhizobacteria (PGPR), notably different strains of *Bacillus subtilis* have also been used as well as fungi and algal extracts (Walters *et al.*, 2013).

To get a better understanding, induced resistance is commonly divided into categories and can, in broad terms, be split up into two main types: systemic acquired resistance (**SAR**) and induced systemic resistance (**ISR**). However, our knowledge of induced resistance is limited, and categorizations of induced resistance can lead to confusion and hasty conclusions. Other types of induced resistance include cross-protection, localized acquired resistance and localized induced resistance (Van Loon, 2000).

SAR is when the plant develops a broad-spectrum systemic resistance followed by a microbial pathogen or treatment with plant activators e.g. chemicals such as Oryzemate® with the active ingredient probenazole (Oostendorp *et al.*, 2001). SAR is characterized in the plant as an increase in levels of salicylic acid (SA) as well as expression of genes corresponding to synthesis of pathogenesis related (PR) proteins (Walters and Fountaine, 2009).

ISR is a phenomenon that happens when the roots of the plant are colonized by certain PGPR. ISR has similarities to SAR in that it also elicits a broad defence in the host plant against different pathogens (Pieterse and Van Loon, 2007). Characteristics include accumulation of phytoalexins and alterations in the cell wall composition (Walters and Fountaine, 2009). The signalling pathway was shown to be through jasmonic acid (JA) and ethylene along with dependency on the regulatory gene *NPR1* when elicited by several strains of *Bacillus* spp. (Kloepper *et al.*, 2004; Pieterse and Van Loon, 2007). However, other cases have shown that ISR is independent of JA and *NPR1* and instead dependent on SA again when elicited by other strains of *Bacillus* spp. (Kloepper *et al.*, 2004). Hormone signalling seems to be diverse depending on possibly both pathogen, host and elicitor.

Methods of hormone detection in plants have become more sensitive and straightforward in the last decade leading to elucidation of more signalling pathways to try and better understand mechanisms of plant defence. There have been many reports of SA, JA and ethylene playing important roles in deployment of induced resistance. However, as described by different strains of pathogens, the pathways of plant hormones in SAR and ISR are not always clear, and it can be difficult to characterize differences by signalling pathways. It is therefore important to be careful and critical when blindly trusting signalling pathways previously established.

Deployment of plant defences are costly for the plant and has led to theories of negative effects on plant fitness including yield loss when plants have been elicited with induced resistance (Heil and Baldwin, 2002; Walters and Heil, 2007). Stunted growth and seed loss have been seen on plants with induced resistance grown in enemy-free conditions. A grain yield loss of 7% was reported in the 1980s following inoculation of *Blumeria graminis* f. sp. *hordei* in barley due to an increase in respiration during successful resistance (Smedegaard-Petersen and Stølen, 1980). The plant allocates important resources when actively defending against pathogens but can be wasted if no pathogens are present. This topic is still understudied, and reduction of disease is still seen in the field when induced resistance is applied therefore it can be discussed whether yield losses are present when plants are exposed to pathogens.

Priming is a form of induced resistance where no changes in gene expression or levels of resistance traits are detectable when the plant is exposed to a priming agent prior to a pathogen. The defences are activated once the plant is challenged by a pathogen thus eliminating waste of plant resources. Priming agents might be a chemical elicitor, a challenging pathogen or volatile compounds released by neighbouring plants (Walters and Fountaine, 2009). Seeds of tomato treated with JA showed enhance defence gene expression only during pathogen attack and exhibited long-lasting priming responses for up to 8 weeks after priming and did not result in reduction of growth (Paul *et al.*, 2011). Priming therefore might be a promising candidate for induced resistance in plants as the plant does not waste important resources before actively being targeted by a pathogen.

Podosphaera pannosa

Powdery mildew of roses is caused by the obligate biotrophic ascomycete *Podosphaera pannosa* (formerly *Sphaerotheca pannosa*), belonging to the family Erysiphaceae, the same family as other significant powdery mildews such as the ascomycete *Blumeria graminis* of cereals. It is one of the most important rose pathogens found both under open-air and in greenhouses. Symptoms include reduced flower production, visible powdery growth on the surfaces of leaves and flowers, leaf distortion and premature defoliation (Kaufmann *et al.*, 2012). To properly combat a pathogen, it is important to know the disease cycle to identify where control of the pathogen will be most feasible.

The disease cycle of *P. pannosa* is pictured in figure 2. Under favourable conditions, if the temperature and humidity is right, ascospores or conidia geminate on the surface of the plant tissue. The germ tube forms a thin hypha which can develop haustoria in the host epidermal cells to obtain nutrients from the host plant. The growth of hyphae continuous on the leaf surface which can further develop into conidiophores. Each conidiophore might hold 5 to 10 egg-shaped conidia, strung together in chains, which can further spread infection as secondary inoculum and spread to other nearby roses. The production of conidia ceases later in the season when the weather is colder and cleistothecia may be formed instead, mainly on canes. Clestothecia are usually covered in appendages of hyphae and produce ascospores throughout the end of the season which are ready for dissemination in the spring as primary inoculum. The fungus can survive through the winter as cleistothecium and mycelium in the buds but only survives as mycelium and conidia in greenhouse production (Agrios, 2005).

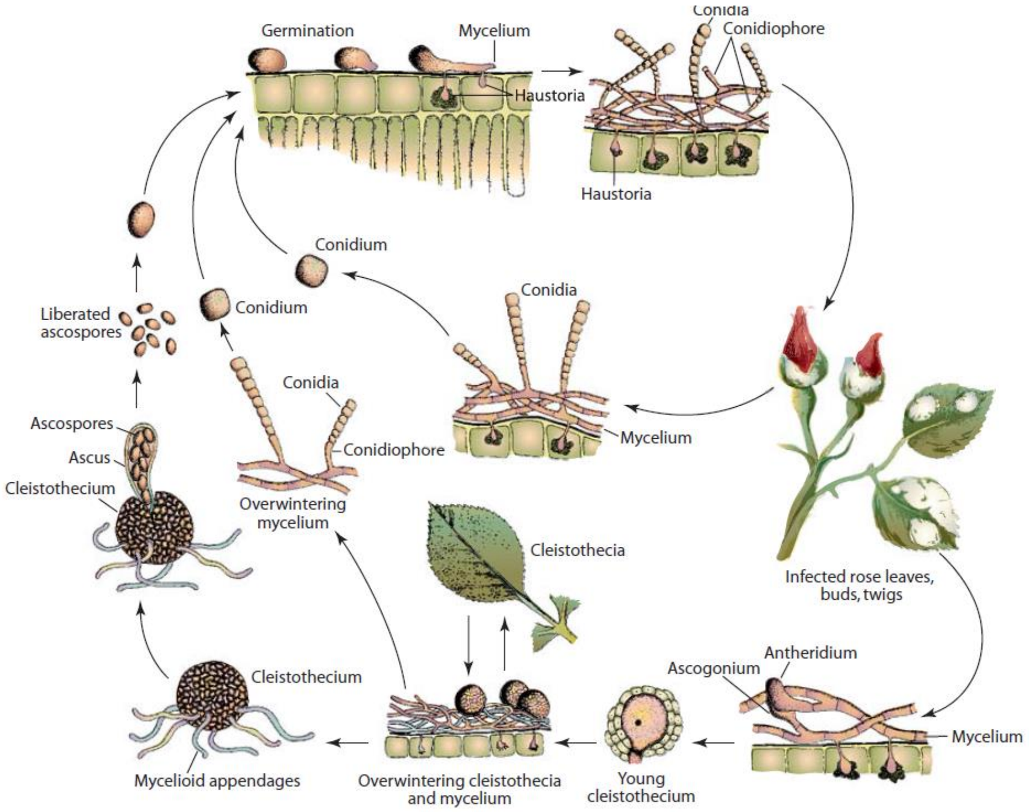


Fig. 2 Disease cycle of powdery mildew on roses caused by *Podosphaera pannosa* (Agrios, 2005).

Induced resistance against *Podosphaera pannosa*

Not many experiments aimed at eliciting induced resistance in roses against *P. pannosa* have been made. Of the few experiments made, induced resistance have been elicited with silicon and possibly UV-B lightning, both which will be discussed along with some microbial biopesticides commercially available.

Silicon (Si) is not generally regarded as an essential element for plants, but it is still known to be beneficial for the plant in terms of growth and production (Wang *et al.*, 2017). Si has been shown to contribute to plants overcoming many biotic and abiotic stresses and improve the mechanical and physiological properties of the plant (Wang *et al.*, 2017). This include deposition of Si compounds in cell walls, phenolic compounds and enhanced production of PR proteins. Experiments were made on potted miniature roses in greenhouse treated with a nutrient solution containing Si and a control group without Si (Shetty *et al.*, 2012). Symptoms of disease was delayed by 1-2 days and disease severity was decreased by 48.9% in the largest reduction. Plants treated with Si displayed an increase in papilla formation, fluorescent epidermal cells, deposition of callose and H_2O_2. Increase in these mechanisms indicate an induced resistance in the host plant. Si is easy to apply to plants in greenhouses but did not substitute the use of conventional pesticides for disease control. With the strong potential for use in integrated pest management it could be used as a supplement reducing the need for pesticides.

In another experiment made, potted roses in greenhouses were treated with UV-B irradiance from 0.1 to 1.2 W m^{-2} at exposing times ranging from 2 min to 2 h (Suthaparan *et al.*, 2012). Disease severity of powdery mildew were significantly reduced by more than 90% compared to controls. Although there was an increase in flavonoids and phenolic compounds, which can act as antifungal compounds, disease severity increased once treatment stopped which indicate that induced resistance was not the case. Furthermore, plants treated prior to inoculation did not show a reduction in disease severity which also indicate that there was no induced resistance.

Experiments have been made on rose with the use of benzothiadiazole (BTH), an artificial inductor, and JA to induce resistance against *P. pannosa* reaching inductive effects of up to 66% for BTH and 54.5% for JA (Yan *et al.*, 2018). Biotrophic pathogens are traditionally believed to be regulated by defence activated by SA (Glazebrook, 2005) and active defence regulated by JA is therefore remarkable. Levels of phenolics, flavonoids and lignin were increased significantly as well as an increase in defensive enzymes and secondary metabolites in leaves. Results indicate that there was induced resistance and it could be used as a supplement to control *P. pannosa*.

The field of induced resistance against *P. pannosa* is limited and only a small fraction of elicitors of induced resistance have been tested. Other biological control products like *Gliocladium catenlatum* (Prestop® WP) and *Bacillus subtilis* (Serenade MAX™) have been used against powdery mildew of roses which provided significant control of disease comparable to conventional chemical pesticides (Elmhirst *et al.*, 2011). It is possible that microbial biopesticides like these elicit induced resistance in the plant, but our knowledge is limited, and further testing must be made specifically on roses against *P. pannosa*.

Discussion

Induced resistance as a mode of action has received increasing attention to supplement pesticide use. It has been possible to reduce disease severity of many different pathogens including *P. pannosa* as with the case with the use of Si, BTH and JA. Besides targeting of one pathogen it also acts as a broad-spectrum mode of action to combat many different pathogens.

A combination of elicitors of induced resistance and conventional pesticides could be a promising strategy while meeting the IPM goals set by EU to lower the use of pesticides. As induced resistance only offers partial resistance farmers will most likely not be enthusiastic about using a product that does not completely remove the pathogen. Although induced resistance does not offer full disease control, management of diseases could become much easier with use of induced resistance. Some elicitors like Si is simple for the farmer to adopt but many other elicitors will have to be tested for the use against *P. pannosa* with focus on induced resistance.

The current practices for pest control have relied heavily on the use of chemical pesticides. Some of which were first introduced in the 1980s is still in use today while others lost efficacy in only a couple of years. Agrochemical companies are always on the lookout for new pesticides with new modes of action. According to the European Crop Protection, on average a new crop protection product costs $286 million (roughly 1.900 mio. DKK), with 11 years of development (ECP, 2016). These costs have increased 55% since the turn of the century. Much can be attributed to regulations of increased requirements of toxicology data to ensure environmental safety. The process of developing new chemical pesticides is becoming more and more difficult. The field of biopesticides is most likely to be the green chemistry alternative to some of the pesticides currently used. The market of biopesticides has been expanding since its introduction by an estimated 10% per year, mostly driven by conventional farmers (Peabody *et al.*, 2009).

The Danish pesticide strategy for 2017-2021 seeks to implement principles of IPM which include biological and non-chemical methods to reduce disease severity in crops (Miljø- og Fødevareministeriet, 2017). The high cost of making new biopesticide products have likely halted the development in alternatives to conventional pesticides. Some would argue that the data portfolio required for biopesticide registration should be less than chemical pesticides as there is a belief that biopesticides are inherently less toxic because of their natural origin. This is not the case as seen with natural derivatives such as the concentrated extract of d-limone being regulated as a conventional pesticide because of its toxic mode of action (Peabody *et al.*, 2009). Toxicology data still must be necessary to determine if biopesticides are safe for use but a modified form of data portfolio for registration that promotes the use of biopesticides might be beneficial.

The term biostimulants has come to attention in the recent years as products that overall enhance the plant. A definition for biostimulants proposed by du Jardin (2015): *"A plant biostimulant is any substance or microorganism applied to plants with the aim to enhance nutrition efficiency, abiotic stress tolerance and/or crop quality traits, regardless of its nutrients content"*. The substances might be single compounds or group of natural compounds e.g. plant extracts. These biostimulants are not regulated in the same way as biopesticides and require different registration of products. Biostimulants might provide the plant with some of the same mechanisms of induced resistance and broad-spectrum basal resistance, e.g. increased resilience of plant pathogens by papilla formation, lignification of cell walls, increased activity of PR proteins to name a few. The elicitors of induced resistance must be applied before the onset of disease to effectually lower disease severity and the use of biostimulants before onset of disease could be useful in controlling pathogens like *P. pannosa*.

Conclusion

The use of induced resistance against *P. pannosa* in roses has proven effective in controlling disease severity by up to 48.9% with the use of Si by activating host defence responses as seen in previous experiments. Root application of Si in production of roses would be simple compared to spraying of pesticides and could prove useful for farmers as supplement to conventional pesticides. Experiments with application of exogenous BTH and JA also inhibited *P. pannosa* by induced resistance in the host plant by increased activity of defence enzymes and accumulation of secondary metabolites in the leaves. Goals set by the EU and Denmark to reduce the use of pesticides, promote the use of eco-friendly solutions and to meet consumer demands, could be met if induced resistance is used in combination with pesticides. The current cost of developing and thoroughly testing new pesticides, including biopesticides, have likely halted the development of new biopesticide products. Re-evaluation of the current data portfolio requirements for biopesticides could promote the development of new biopesticide products but our judgement of toxicity should not be clouded by the belief of a natural origin. There are countless of potential induced resistance elicitors, many of which have not been tested yet. Our knowledge of induced resistance is still limited, and more research must be made on other potential elicitors and their mode of action.

References

Agrios, G. N. (2005) *Plant Pathology*. Fifth. Academic Press.

Alabouvette, C., Olivain, C. and Steinberg, C. (2006) ‘Biological control of plant diseases: The European situation’, *European Journal of Plant Pathology*, 114(3), pp. 329–341.

Chester, K. S. (1933) ‘The Problem of Acquired Physiological Immunity in Plants’, *The Quarterly Review of Biology*. The University of Chicago Press, 8(2), pp. 129–154.

Dunham Trimmer LLC (2017) *Biological Control Global Market Overview*. Available at: http://wrir4.ucdavis.edu/events/2017_SLR_Meeting/Presentations/GeneralPresentations/1 Trimmer - Global Biocontrol Market 2017.pdf (Accessed: 8 June 2019).

ECP (2016) *Cost of Crop Protection Innovation Increases to $286 Million per Product, European Crop Protection*. Available at: https://www.ecpa.eu/news/cost-crop-protection-innovation-increases-286-million-product (Accessed: 20 May 2019).

Elmhirst, J. F., Haselhan, C. and Punja, Z. K. (2011) ‘Evaluation of biological control agents for control of botrytis blight of geranium and powdery mildew of rose’, *Canadian Journal of Plant Pathology*, 33(4), pp. 499–505.

European Union (2009) ‘Directive 2009/128/EC of the European parliament and of the council’, *Official Journal of the European Union*. Available at: https://eur-lex.europa.eu/legal-content/EN/ALL/?uri=celex%3A32009L0128 (Accessed: 20 May 2019).

Glazebrook, J. (2005) ‘Contrasting Mechanisms of Defense Against Biotrophic and Necrotrophic Pathogens’, *Annual Review of Phytopathology*, 43(1), pp. 205–227.

Heil, M. and Baldwin, I. T. (2002) ‘Fitness costs of induced resistance: emerging experimental support for a slippery concept’, *Trends in Plant Science*, 7(2), pp. 61–67.

Jamalizadeh, M. *et al.* (2011) ‘A review of mechanisms of action of biological control organisms against post-harvest fruit spoilage’, *EPPO Bulletin*. Wiley/Blackwell (10.1111), pp. 65–71.

du Jardin, P. (2015) ‘Plant biostimulants: Definition, concept, main categories and regulation’, *Scientia Horticulturae*. Elsevier B.V., 196, pp. 3–14.

Kaufmann, H. *et al.* (2012) ‘Isolation, Molecular Characterization, and Mapping of Four Rose MLO Orthologs’, *Frontiers in Plant Science*, 3(November), pp. 1–13.

Kloepper, J. W., Ryu, C.-M. and Zhang, S. (2004) ‘Induced Systemic Resistance and Promotion of Plant Growth by Bacillus spp.’, *Phytopathology*, 94(11), pp. 1259–1266.

Kloepper, J. W., Tuzun, S. and Kuć, J. A. (1992) ‘Proposed definitions related to induced disease resistance’, *Biocontrol Science and Technology*. Taylor & Francis, 2(4), pp. 349–351.

Kuć, J. (1982) ‘Induced Immunity to Plant Disease’, *BioScience*. [American Institute of Biological Sciences, Oxford University Press], 32(11), pp. 854–860.

Leus, L. *et al.* (2006) ‘Podosphaera pannosa (syn. Sphaerotheca pannosa) on Rosa and Prunus spp.: Characterization of Pathotypes by Differential Plant Reactions and ITS Sequences’, 28, pp. 23–28.

Van Loon, L. C. (2000) ‘Systemic Induced Resistance’, in *Mechanisms of Resistance to Plant Diseases*. Springer Netherlands, pp. 521–574.

Miljø- og Fødevareministeriet (2017) *Pesticidstrategi 2017 - 2021*. Available at: https://mst.dk/service/publikationer/publikationsarkiv/2017/okt/pesticidstrategi_2017-2021/ (Accessed: 11 June 2019).

Nicolopoulou-Stamati, P. *et al.* (2016) ‘Chemical Pesticides and Human Health: The Urgent Need for a New Concept in Agriculture.’, *Frontiers in public health*, 4(July), p. 148.

Oostendorp, M. *et al.* (2001) ‘Induced disease resistance in plants by chemicals’, *European Journal of Plant Pathology*, 107(1), pp. 19–28.

Paul, N. D. *et al.* (2011) ‘Treating seeds with activators of plant defence generates long-lasting priming of resistance to pests and pathogens’, *New Phytologist*, 193(3), pp. 770–778.

Peabody, K. O., Franjevic, S. and Jones, J. (2009) ‘Green Chemistry and Sustainable Agriculture’, *Advancing Green Chemistry*. Available at: http://advancinggreenchemistry.org/innovation/green-chemistry-and-sustainable-agriculture/.

Pieterse, C. M. J. and Van Loon, L. C. (2007) ‘Signalling Cascades Involved in Induced Resistance’, in *Induced Resistance for Plant Defence: A Sustainable Approach to Crop Protection*. Blackwell Publishing, pp. 65–88.

Rosa Danica (2019) *Fakta omkring Rosa Danica*. Available at: https://www.rosa-danica.dk/fakta/ (Accessed: 3 June 2019).

Shafi, J., Tian, H. and Ji, M. (2017) ‘Bacillus species as versatile weapons for plant pathogens: a review’, *Biotechnology and Biotechnological Equipment*. Taylor & Francis, 31(3), pp. 446–459.

Shetty, R. *et al.* (2012) ‘Silicon induced resistance against powdery mildew of roses caused by Podosphaera pannosa’, *Plant Pathology*, 61(1), pp. 120–131.

Smedegaard-Petersen, V. and Stølen, O. (1980) ‘Effect of Energy-Requiring Defense Reactions on Yield and Grain Quality in a Powdery Mildew-Resistant Barley Cultivar’, *Phytopathology*, 71(4), p. 396.

Suthaparan, A. *et al.* (2012) ‘Suppression of Powdery Mildew (Podosphaera pannosa) in Greenhouse Roses by Brief Exposure to Supplemental UV-B radiation’, *Plant Disease*, 96(11), pp. 1653–1660.

US EPA (2016) *What are Biopesticides?*, *United States Environmental Protection Agency*. Available at: https://www.epa.gov/ingredients-used-pesticide-products/what-are-biopesticides (Accessed: 20 May 2019).

Vidhyasekaran, P. *et al.* (2001) ‘Induction of systemic resistance by Pseudomonas fluorescens Pf1 against Xanthomonas oryzae pv. oryzae in rice leaves’, *Phytoparasitica*, 29(2), pp. 155–166.

Walters, D. and Heil, M. (2007) ‘Costs and trade-offs associated with induced resistance’, *Physiological and Molecular Plant Pathology*, 71(1–3), pp. 3–17.

Walters, D. R. and Fountaine, J. M. (2009) ‘Practical application of induced resistance to plant diseases: An appraisal of effectiveness under field conditions’, *Journal of Agricultural Science*, 147(5), pp. 523–535.

Walters, D. R., Ratsep, J. and Havis, N. D. (2013) ‘Controlling crop diseases using induced resistance: Challenges for the future’, *Journal of Experimental Botany*, 64(5), pp. 1263–1280.

Wang, M. *et al.* (2017) ‘Role of Silicon on Plant–Pathogen Interactions’, *Frontiers in Plant Science*, 8(May), pp. 1–14.

Yan, J. *et al.* (2018) ‘A study on JA- and BTH-induced resistance of Rosa rugosa “Plena” to powdery mildew (Sphaerotheca pannosa)’, *Journal of Forestry Research*. Springer Berlin Heidelberg, 29(3), pp. 823–831.