Use of supplemental lighting towards efficient management of powdery mildew in greenhouse roses

Bruk av tilleggslys for bedre kontroll av meldugg ved produksjon av roser i veksthus

Philosophiae Doctor (PhD) Thesis

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Abstract

Powdery mildew caused by the *Podosphaera pannosa* is one of the most widespread and destructive diseases in roses and has been an increasing problem in Norwegian rose production due to lack of disease resistant cultivars. In practice, roses are frequently treated with fungicides to control powdery mildew, and the most common treatment today is with evaporated sulphur. Some fungicides used against powdery mildew, including sulphur may be toxic to biological control agents.

Supplementary lighting in Norwegian rose production has been practised since the late1980s. Previous experiments clearly indicated that continuous lighting strongly reduced powdery mildew in roses, but keeping quality was significantly reduced. The main goal of this study was to investigate the effect of different components of light on rose powdery mildew, and use this knowledge to manipulate lighting regimes for improved management of the disease.

This thesis consists of three papers. Experiments with day length, light quality and light intensity were conducted with pot roses (*Rosa* interspecific hybrid 'Mistral') in growth chambers with constant temperature and air humidity. Humidity boxes with two rooms were developed and used to test for germination and formation of conidia. Inoculated leaflets were placed on water agar in the upper room of the boxes. The lower room contained saturated salt solution (KCl), which maintained constant high air humidity (96 ±2% RH) in both rooms. For testing of conidia formation and release from whole plants, wind tunnels were developed. Microscope films attached to clock cylinders collected the conidia released from the diseased plants.

The first two - investigations was on how day length, light quality and light intensity influenced germination, production of conidia and disease development. The third study was a molecular investigation to find a phytochrome like photoreceptors in *P. pannosa*.

A significant reduction in conidia formation and release were noticed when exposed to 20 - 24 h day length compare to 18 h day length with white light. When trapping conidia from whole plants placed in wind tunnels, exposure to continuous lighting gave only 22 % of the conidia trapped at 18 h day length. There was no significant difference in number of trapped conidia between 20, 22 or 24 h lighting.

Germination of conidia was slightly, but significantly reduced when exposed to continuous full spectrum white light or 18 h day length of blue light. While far-red light

induced conidia formation, red light suppressed conidia formation and release. Red light supplied in the 6 h period following 18 h white light strongly suppressed formation and release of conidia relative to a diurnal cycle of 18 h white light and 6 h darkness. If plants were exposed to a day length of 18 h provided with a full spectrum of white light, a 1 h night break with red light was sufficient to strongly suppress formation and release of conidia compared to a 1 h night break with far-red light or no night break. However, red light night break followed by far-red light night break almost nullified the suppressive effect of red light. Under full spectrum white light, formation and release of conidia increased with increasing light intensity.

PCR amplification with degenerate primers were attempted to find a phytochrome like gene(s) in *P. pannosa*. It was not successful. Further work is needed to confirm whether *P. pannosa* has photo sensory receptor. At the moment, designing of more specific primers that are suitable to detect the photo sensory receptors in powdery mildew fungi by PCR is difficult. However sequencing of its genome, proteomic analysis or transcriptome sequence analysis would be other alternatives.

Key words: Conidia, day length, light intensity, light quality, *Podosphaera pannosa*, phytochrome, *Rosa* × *hybrida*.

Sammendrag

Rosemeldugg er forårsaket av soppen *Podosphaera pannosa* var. *rosae*. Dette er en av de alvorligste sykdommene på roser i Norge og er et økende problem på grunn av mangel på gode sorter som er resistente. I praktisk rosedyrking blir plantene behandlet med soppmidler, og mest vanlig er svovelfordamping. Noen av soppmidlene brukt mot rosemeldugg, inklusiv svovelfordampning, er skadelige for nyttedyr som brukes for biologisk kontroll av skadedyr. Videre har det utviklet seg resistens mot flere av de syntetiske soppmidlene.

Tilleggsbelysning er brukt i norsk roseproduksjon siden slutten av 1980-årene. Tidligere forsøk har vist at kontinuerlig belysning reduserer angrep av meldugg i roser, men holdbarheten ble ofte dårlig. Hovedhensikten med dette forskningsarbeidet var å undersøke hvordan belysningstid, -styrke og lyskvalitet virket på rosemeldugg for å kunne bruke denne kunnskapen i bekjempelsen av rosemeldugg.

Denne avhandlingen består av tre individuelle arbeider. Alle forsøkene med daglengde, belysningsstyrke og lyskvalitet foregikk med potterosesorten Mistral i vekstrom under konstant temperatur og luftfuktighet. Plantematerialet bestod enten av hele planter eller enkeltblad som ble holdt i live på kunstig medium. Det ble utviklet bokser med to rom som kunne brukes til å teste spiring og konidiedannelse på enkeltblad som ble lagt på vannagar i det øverste rommet. I den nederste delen var det en saltløsning som ble brukt til å holde en konstant høg luftfuktighet. Luften beveget seg fritt gjennom huller langs kanten av veggen mellom de to kamrene. For testing av konidiedannelse og spredning av konidier fra intakte planter ble det utviklet vindtunneler som samlet opp sporene som ble frigitt fra plantene på en mikroskoptape.

I de to første arbeidene ble det undersøkt hvordan daglenden, lyskvaliteten og lysstyrken virker på spireevnen til soppens konidier, konidiedannelse, konidiespredning og sykdomsutvikling. Målet for det tredje arbeidet var å identifisere en eventuell fotoreseptor hos soppen ved hjelp av molekylære teknikker.

Kontinuerlig belysning reduserte spireevnen og konidiedannelsen til soppen og ga kun 22 % av tallet på konidier som ble dannet ved 18 t belysning i vindtunnel. Det var ingen forskjell i mengde konidier fra planter som var plassert ved 20, 22 eller 24 t belysning. Ved å eksponere planter inokulert med meldugg for sykluser med 18 t vanlig vekstlys (50 µmol m^{-2} s^{-1}) + 6 t med rødt lys (5 µmol m^{-2} s^{-1}) ble mengde konidier redusert til 13 % sammenlignet med 18 t vekstlys + 6 t mørke. Mørkerødt lys gav ingen slik reduksjon, mens blått lys ga noe,

men ikke signifikant reduksjon. En time rødt lys i den 6 t lange mørkefasen var nok til å redusere konidiedannelsen kraftig, mens 1 t mørkerødt lys etter 1 t rødt lys nesten fullstendig opphevet effekten av rødt lys. Det var en økning i dannelsen av konidier ved økende lysstyrker fra 5 til 150 μmol m^{-2} s^{-1}. Fra de to første arbeidene i avhandlingen kan det konkluderes at ved å øke daglengden fra 18 t (tidligere mye brukt i roseproduksjonen) til 20-24 t, reduseres danning og spredning av konidier og sykdomsutvikling hos rosemeldugg. Små mengder med rødt lys gitt i daglengdeforlengelsen (utover 18 t) er tilstrekkelig for å oppnå denne effekten.

PCR med generelle primere med etterfølgende sekvensering av genproduktene ble prøvd for om mulig å finne fytokromliknende gener i *P. pannosa.* Det lyktes ikke. For øyeblikket synes utvikling av mer spesifikke primere nødvendig for å identifisere organer i *P. pannoa* som er følsomme for lys. Ved sekvensering av hele genomet, proteomikk analyser og transkriptom sekvence analyser til *P. pannosa* er trolig mulighetene større for å lykkes.

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List of papers

This thesis is based on the following papers, which are referred to by their Roman numerals

I. Continuous lighting reduces conidial production and germinability in the rose powdery mildew pathosystem.
Suthaparan, A., Stensvand, A., Torre, S., Herrero, M.L., Pettersen, R.I., Gadoury, D.M. and Gislerød, H.R. 2010.
Plant Disease 94: 339-344

II. Specific light emitting diodes can suppress sporulation of *Podosphaera pannosa* on greenhouse roses.
Suthaparan, A., Torre, S., Stensvand, A., Herrero, M.L., Pettersen, R.I., Gadoury, D.M. and Gislerød, H.R.
Plant Disease- Accepted with revision

III. A molecular genetic approach to identify possible phytochrome-like photoreceptors in *Podosphaera pannosa*.
Suthaparan, A., Klemsdal, S.S., Torre, S., Stensvand, A. and Gislerød, H.R.

1. INTRODUCTION

Roses are among the most important ornamental plants cultivated outdoors and indoors worldwide. It is a perennial flower shrub or vine belonging to the genus *Rosa*, within the family Rosaceae (8). The genus *Rosa* comprises more than one hundred botanical (wild) species, of which mainly ten species contributed to the development of cultivated roses; *R. canina*, *R. chinensis*, *R. foetida*, *R. gallica*, *R. gigantea*, *R. moschata*, *R. multiflora*, *R. phoenicea*, *R. rugosa*, and *R. Wichuraina* (33, 53). Most of the roses grown today are not true species but are derivatives of interspecific hybridization (22, 53), leading to a wide diversity among cultivated roses. The world production under protected cultivation is annually about 8 500 hectares, including 15-18 billion stems for cut roses (16) and 60-80 million pot roses (94).

Flower production of rose plants depends on different components of yield, such as the number of lateral buds released from inhibition (sprouting of axillary buds), the rate of flower bud abortion, the formation of renewal shoots and the growth rate of the flower stems (119). All of these variables are dependent on environmental factors, such as light, CO_2 level, temperature, and RH. Influence of light on plants can be explained in terms of day length, light intensity, light quality, and light integral (lighting period × light intensity). Photosynthesis together with photoperiodism, phototropism and photomorphogenesis are the four representative processes related to interaction between light and plants (107). Plants consist of specialized photo receptors to capture the light energy to mediate these important biological processes. Photoreceptors are proteins or protein complexes that absorb light. By absorbing photons the molecules become excited. This excitation energy can then be re-emitted as light (luminescence), thermally dissipated, or transferred to other molecules (9). However, most importantly the energy in the absorbed photons can drive chemical transformations, such as electron transfer, phosphorilation or conformational changes (9, 44).

1.1. Photoreceptors in plants

Photo receptors in plants can be classified into two main groups. Mass pigments: These pigments are in high concentrations in plant tissues (e.g chlorophyll, anthocyanin and flavanoids). The amount of energy absorbed by these pigments is relatively high. Mass pigments or mass photoreceptors either absorb photons which drive metabolic processes (i.e. they harvest energy, e.g. chlorophylls) or absorb potentially damaging photons (i.e. they

screen light away from sensitive tissues, e.g. anthocyanins or flavanoids), and by dissipating this energy safely, they afford protection to other cell components (9). Sensor pigments**:** These pigments appear in relatively low concentration in plant tissue, and only absorb a small fraction of the incident light (9). They sense (i.e. gather information about) the light environment. Plants use a number of different photo sensory receptors to perceive different bands of the electromagnetic spectrum that adjust the developmental program and behaviour of plants to the prevailing environmental conditions (photo morphogenesis and photoperiodism) (106).

1.1.1. Plant photo sensory receptors

These photo sensory receptors are red/far-red sensing phytochromes, blue/UV-A sensing cryptochromes and phototropins, and UV-B photoreceptors (88, 106).

1.1.1.1. Phytochrome

Phytochrome was the first photo sensory receptor family identified nearly 50 years ago in plants (106). They are well defined photoreceptors known in higher plants and mediate plant growth and development in response to red and far-red light.

Phytochromes consist a large apoprotein part (120-130 kDa, size depending on the specific phytochrome) and a chromophore part - covalently attached pigment molecule of linear tetrapyrrole (bilin). The chromophore (bilin pigment) sense and absorb specific light spectrum of red and far-red that causes slight structural changes in the chromophore part (cis-trans photoisomerization) and conformational changes in the protein part. Phytochrome exists in two photo interconvertible, stable forms, mainly red absorbing Pr (phytochrome red) form (λ_{max} = 660 nm) and the mainly far-red absorbing Pfr (phytochrome far-red) form (λ_{max} = 730 nm) (100, 107).

It is generally accepted that the Pfr form is responsible for biological action, and the degree of action is quantitatively related to the concentration of Pfr (56, 100). The sensitivity of phytochrome for biological response varies. In some cases very low levels of Pfr (less than 1 % of the total phytochrome) elicit a maximum response, while others require almost all of the phytochrome to be in Pfr form to elicit biological response. Phytochrome mediated responses have been classified into three modes of action, depending on their light exposure requirement for biological response. Those are high irradiance response (HIR), low fluence response (LFR), and very low fluence response (VLFR) (56).

Molecular genetics has revealed the existence of several genes for phytochrome protein in a given plant. A plant species can have more than one phytochrome. The model species *Arabidopsis thaliana* has five phytochrome genes termed *PHYA*, *PHYB*, *PHYC*, *PHYD* and *PHYE*. Similarly, five phytochrome genes of *PHYA, PHYB1, PHYB2, PHYE, PHYF* have been identified in tomato, and three phytochrome genes (*PHYA*, *PHYB*, and *PHYC*) have been reported in monocot rice (75).

Phytochromes are essential for all major developmental transitions such as germination, de-etiolation, and the commitment to flowering. They also fine-tune vegetative development of the plants by influencing photropism and photomorphogenesis. The different phytochromes have been shown to play different, but overlapping roles in plants. Both phyA and phyB affect seed germination. Germination of many small seeds with a low food reserve is controlled by phyA under very low fluence (VLF). The low fluence (LF) germination response is red/far-red reversible; controlled by phyB. It has been shown that phyA is necessary for the perception of incandescent day length extension (107). PhyB plays a major role in the perception of prolonged red light in processes like inhibition of hypocotyl elongation, cotyledon, leaf expansion and the synthesis of anthocyanin. Furthermore, it is apparent that phyB is involved in the red (R) to far red (FR) ratio signal perception that leads to shade avoidance responses (107). In general, it is also known that plant morphology is regulated by phyD and phyE in combination with phyB (106).

1.2. Supplementary lighting in greenhouse production

The low availability of day light in northern latitudes and year-round demand for horticultural products leads to the use of supplemental lighting in greenhouse production. Supplementary lighting has been used to grow plants for nearly 150 years. It took three general paths of development; i) the era of artificial lighting started with incandescent lighting which was refined by Edison's invention of the incandescent filament lamp in 1879, ii) open arc lighting that typically used carbon rods and became popular for street lighting in the late 1800s, iii) enclosed gaseous discharge lamps developed initially with mercury vapour in the late 1800s (114).

The first attempt to study the effect of electric light on plant growth was done in the 1860s, and Siemens was the first scientist who did extensive studies with carbon arc lamps for growing plants (104). Extensive experiments with fluorescent and high pressure mercury lamps were carried out in the 1950s in Norway, and it was found that fluorescent lamps were

more effective for plant growth than high pressure mercury vapour lamps (68). However, the use of fluorescent lamps in commercial greenhouses is impractical. High pressure sodium lamps have much higher luminous efficiency per unit of electrical energy, high radiant emission and long life expectancy combined with low price. These factors have made the high pressure sodium lamps as the preferred light source for year-round production in greenhouses.

Currently, light emitting diodes (LEDs) are gradually becoming a popular light source. LEDs generate light through an electroluminescent principle (32) and are fundamentally different from other lamps used for plant growth. LEDs have several advantages compared to light sources that are in use now. It is possible to specify the desirable spectrum, they have a long life time and high energy efficiency, and they contain no toxic gasses (19, 81). The radiated heat of LEDs is very low compared to HPS; makes it suitable light source for interlighting. LEDs were invented in 1920s, but the practical visible version (red) was developed in 1962. The first practical blue LED was developed in 1995, and the first white LED was developed in 1996 by phosphor coating applied to blue LED (5). In 1999, a major advancement was achieved in this technology through the development of high power LED (5).

Testing of LEDs for plant growth was concomitant with the development of the first crude LED arrays in the late 1980s and early 1990s (12, 21). At the same time, use of LEDs were investigated for germination of seeds and rooting of cuttings in the Netherlands (91) and for tissue culture systems in Japan (76). The crop research group at the Kennedy space centre investigated the effect of LED based light system on the yield and physiological responses of several crop plants, including wheat, radish, spinach and lettuce (50, 116).

1.2.1. Effect of supplemental light on rose growth, yield and quality

The importance of supplementary light in rose production has been thoroughly studied. Productivity has increased with increasing light intensity and day length. It has been found that if increasing the day length from 18 to 24 h, this increased the number of flowers by 34 % and reduced the number of days to flowering by 12 % in pot roses (96). Extension of the day length by use of low intensity light treatments of 30 μmol m^{-2} s^{-1} photosynthetic active radiation (PAR) increased the number of flowers, but did not increase fresh weight production (79, 121). Increasing flower yield may be due to the effect of supplementary lighting on assimilate translocation within the plant through promotion of sink activity. In roses, the duration of day length was important in determining daily carbon gain. When roses were

exposed to a constant daily radiant energy dose of 17.6 mole m^{-2} day^{-1} provided either as 12 h irradiation at 410 µmol m^{-2} s^{-1} PAR or 24 h of irradiation at 204 µmol m^{-2} s^{-1} PAR, the plants exposed to 24 h of continuous irradiation at the lower photon flux density (PFD) retained 80 % more carbon (61).

Light is probably the most important factor that controls bud break and blind shoot formation. Increasing light intensity increases the number of buds that break and reduces the proportion of blind shoots (117). Supplementary light will be most effective during the first two weeks of shoot growth to reduce the flower bud abortion (87). The effect of light intensity on the flower yield of rose plants was also demonstrated by comparing flower production at different locations within the greenhouse. Outside rows of a rose bed produced more flowers than inside rows (74), and a reduction in light intensity increased the number of blind shoots, but photoperiod had no effect on flower bud atrophy (117).

Supplementary PFD had a consistent effect throughout the year on yield and quality of two cut rose cultivars. The response to increasing supplementary light in the range from 0 to 174 µmol m^{-2} s^{-1} PFD was generally linear (20). It has been reported that, yield of cut roses increased with light intensity, and the yield increased (average for different varieties) by 18, 41 and 53 % at 190, 250 and 370 µmol m^{-2} s^{-1} PFD respectively, compared to 130 µmol m^{-2} s^{-1}. In addition, increased light intensity increased the quality of roses by longer stem formation and enhancement of keeping quality (48). Experiments conducted in Israel showed that reduction of 10, 35, 60 and 70 % in light transmitted into the greenhouse by shading of plants during winter, reduced the number of flowers per rose plant by 43, 65, 75, and 90 %, respectively (120). The decrease in yield and plant deterioration in relation to light intensity was accompanied by severe damage to the root system, and average weights of rose plant roots were reduced greatly with shading (27).

Decrease in light intensity was followed by a decrease in the activity of gibberellin-like substances in roses, and this reduction was much more pronounced in the lower shoots, which are more prone to flower abortion (118). On the other hand, the content of endogenous cytokinin in the shaded shoots was higher than in shoots exposed to light (111), and it was speculated that shading may cause inactivation of endogenous cytokinin. Supplementary lighting by high pressure sodium or fluorescent lamps promoted bud sprouting (23). The formation of renewal shoots was also promoted by supplementary lighting (65). Enhancement of growth and flowering by supplementary lighting obtained in miniature rose cultivars became a common practise (26). In most experiments, supplementary lighting reduced the

time period required for completion of flower development of roses, and this was correlated with the total amount of irradiance and varied among different cultivars (23).

In addition to their position along the shoot and decapitation of shoots above the buds, the sprouting of buds is also correlated with light intensity and the red to far-red ratio of the light spectrum. A high red to far-red ratio promotes sprouting of buds, while a low ratio inhibits it (80).

Colour development in rose petals depends on light intensity, spectral composition of the light as well as by other environmental factors, including temperature. Various pigments were affected selectively by the decrease in light intensity. A reduction of light intensity from 425 to 212 μmol m^{-2} s^{-1} PAR (photosynthetically active radiation) was followed by a proportional reduction in the content of cyanidin without affecting pelargonidin, the two major anthocyanins of the red rose cv. Baccara (15). The change in the ratio between these two pigments resulted in blueing of the petals (15). Similarly, a decrease in light intensity also reduced the pigmentation of yellow roses containing carotenoids as the major pigment, but the pink rose cv. Carol was not affected (69). It has been reported that increased intensity of visible light along with UV irradiation enhanced anthocyanin formation in the floribunda rose cv. Ehigasa (73).

1.3. Powdery mildew disease development and its effect on growth and yield of roses

The powdery mildew fungi are biotrophic parasites only invading living host epidermal cells. Two to six hours after deposition on the rose leaf surface, conidia germinate with one or two thick-walled germ tubes (71) (Fig. 1b). A nipple shaped unlobed appressorium forms at the end of the germ tube (Fig. 1c), and the appressoria are usually characterized by a large, centrally located vacuole (54). Then, the fungus attempts to penetrate the leaf cuticle and cell wall of the underlying epidermal cell. A very fine penetration hyphae (penetration peg), originating from the appressorium (Fig. 1d), enters the epidermal cell in two stages; enzymatic degradation of the cuticle and cell wall in addition to mechanical penetration (43). In the compatible interaction, the penetration peg forms an elongated structure from which a feeding organ, a globose multilobed haustorium forms (Fig. 1e) (54). The invasion of haustoria into intact host cells creates an area of intimate contact between the fungus and its host. The invading haustorium is encased within a modified host plant cell membrane, called extrahaustorial membrane, with a gel-like extrahaustorial matrix in between, all together called a haustorial complex (Fig. 1e-g) (67). Prior to host cell

penetration, fungi use storage compounds from the conidia as energy sources for growth (18). When the fungus starts to take up nutrients from the host through haustoria, it continues its growth epiphytically by elongating secondary hyphae, from which it penetrates further into epidermal cells (52). The growth of the hyphae on the host surface and formation of conidiophores develop the characteristic symptoms of whitish velvety pustules that may cover leaves, stems, flower sepals or petals of infected plants.

(a) Conidium - plant contact

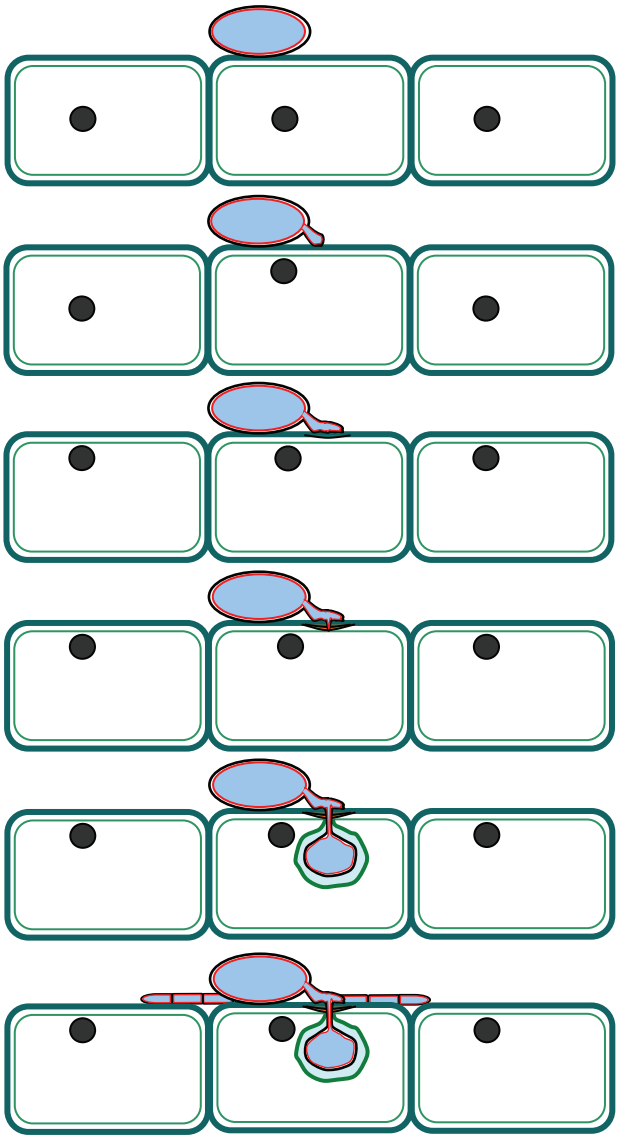
(b) Conidium germination

(c) Appressorium formation

(d) Formation of penetration peg

(e) Development of haustorial complex

(f) Hyphal (mycelial) growth



(g) Formation of conidial chains and release of conidia

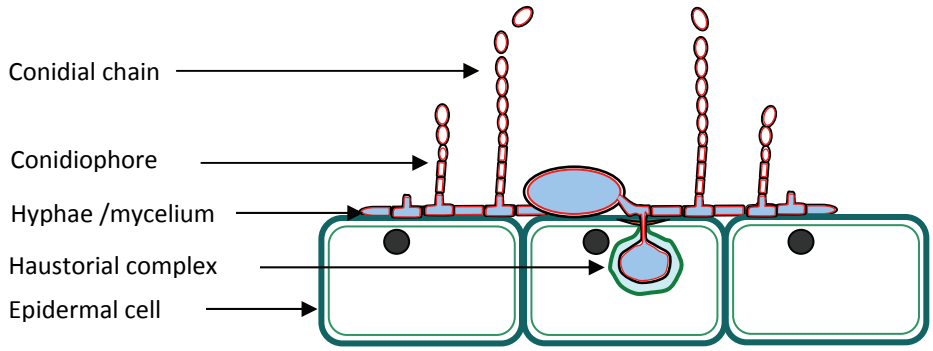


Fig. 1. Schematic illustration for the stepwise progress of a powdery mildew fungus on the host epidermal cell.

Conidiophores of most powdery mildews typically arise from superficial hyphae. The basal cell, usually called the foot cell, and the cell above the basal cell are responsible for the conidiogenesis (28). The process of conidia production has three stages; formation (cell division), maturation (growth and formation of elliptical cells), and release (29, 45, 92). Powdery mildew fungi form conidiophores with single conidium (non-catenate, e.g. *Erysiphe polygoni*) or conidiophores with chains of conidia (catenate, e.g. *Podosphaera pannosa*). Some of them may form pseudo-chains, e.g. *Oidium neolycopersici*.

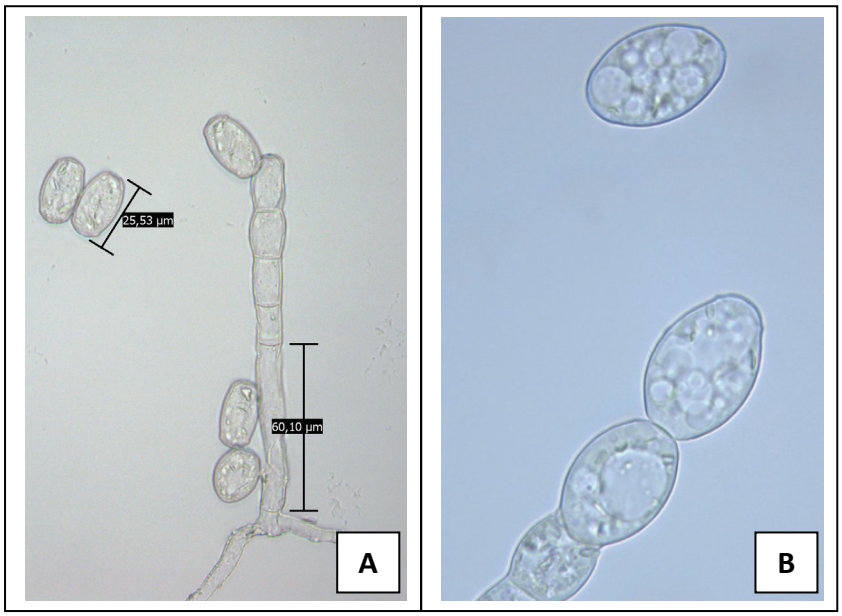


Fig. 2. Light microscopic view (400 X) of conidial chain of *Podosphaera pannosa* of rose powdery mildew (A) and *Podosphaera xanthii* of cucumber powdery mildew (B). Photos: A. Suthaparan,

Powdery mildew caused by *P. pannosa* (Wallr.: Fr.) de Bary is problematic worldwide in greenhouse and garden roses. The fungus absorbs nutrients from the host, reduces the host vigour and esthetic value, and may greatly reduce photosynthesis. Infection of young tissue also causes uneven growth of the affected and surrounding cells. The growth of severely infected shoots is inhibited. Infected flower buds often do not open, and if they open, the flowers become infected and may not develop properly. In severe case, the disease can cause distortion and death of the infected leaves and shoots (71).

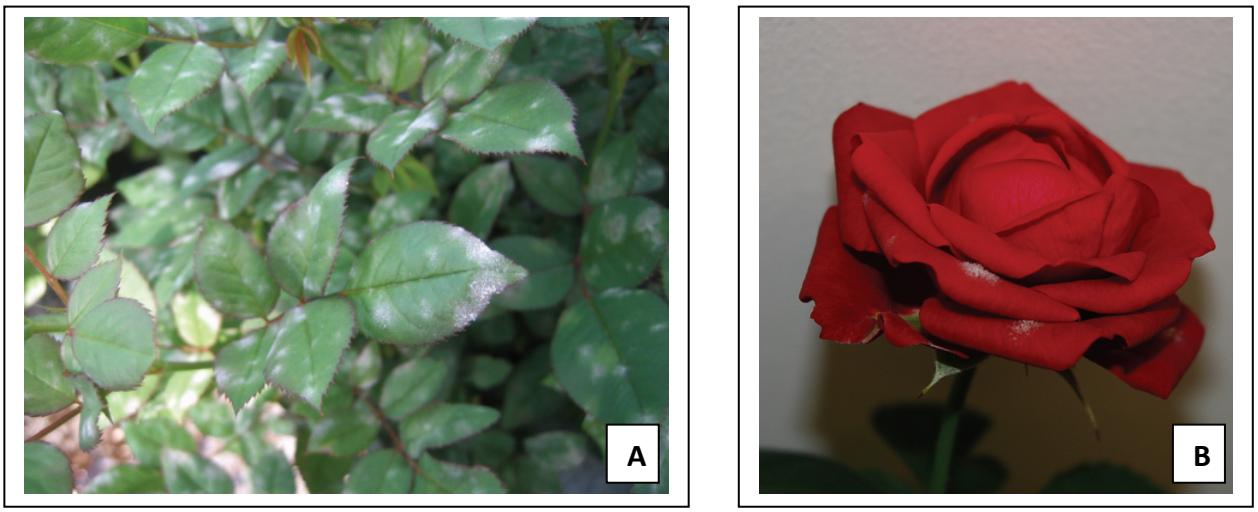


Fig. 3. Powdery mildew (*Podosphaera pannosa*) in rose leaves (A) and flower petals (B). Photos: A. Suthaparan.

When conidia land on a rose plant surface, they may start to germinate in 2 to 4 h, reaching maximum number of germination in about 25 h. The minimum, optimum and maximum temperatures for germination are about 5, 22 and above 30 °C, respectively (72). Optimal RH for conidia germination is nearly 100 % (71). However, conidial germination and colony growth have been reported to occur at 50 % RH, and increasing the RH had no significant effect on germination of conidia placed on rose leaves (101). Furthermore, germination of powdery mildews is reduced by free water (105).

Dissemination of conidia, as inferred from spore trapping, was associated with rising temperature, increasing solar radiation and decreasing RH (1). Once conidia released from the conidiophores, viability reduced with time. Two hours after release, germination of rose powdery mildew conidia reached 95 to 100 % at a specific temperature (32 °C) and RH (≤70). It was dropped to 8 to 20 % in 5 h after release. Although conidia of *P. pannosa* remain viable longer at RH of 80 to 90 %, essentially all conidia are dead after 48 hours at 21 °C and after 24 hours at above 32 °C (3). Rose leaves inoculated with powdery mildew and kept at 0 °C under moist conditions showed that conidia did not lose viability after 10 days (97). The time from infection to formation of new conidia in *P. pannosa* can be as short as 72 to 96 h (3), and on detached leaves the latent period range from 4 days at 20 °C to 28 days at 3 °C (97).

1.4. Light and fungal development

It has been reported that conidial germination of *P. aphanis* (syn. *Sphaerotheca macularis*) on detached strawberry leaves were not affected by light (95), while in another work, the highest germination occurred in complete darkness if compared to 12 h day length (2). In barley powdery mildew, infections developing in the dark for the first 12 h after inoculation formed more haustoria compared to when exposed to light for the first 12 h (40).

Diurnal periodicity in conidial production was recorded in several powdery mildews, including rose powdery mildew (28, 70, 92). The role of light in different stages of the conidia production has varied among powdery mildew species. In barley powdery mildew, formation of conidia was independent of light and occurred continuously (92). In *Erysiphe polygoni*, conidial formation and release was light dependent, while the maturation was not (92). However, in tobacco powdery mildew caused by *Golovinomyces cichoracearum* (syn. *E. cichoracearum*), conidia formation was independent of light, while maturation and release was light dependent (29).

In tobacco powdery mildew exposed to 12 h day length with light sources of either near ultra violet or fluorescent light, conidia developed faster in light than in the dark, and most conidia were released in the light periods (30). In rose powdery mildew, conidia formation was independent of light; however, maximum formation took place during the dark period, and maturation and release occurred only during day time (45). It has been reported that release of conidia in rose powdery mildew primarily depends on RH, but not on light (1). Further, it was reported that, no conidia were released in light or darkness in still air (0.04 ms^{-1}) at 100% RH, but when the humidity was lowered rapidly to below 50%, conidia were released independent of light. It was speculated that high RH may have caused the abstracted spores to adhere rather tightly to the conidiophores, and that the almost still air was not sufficient to release the conidia. It has been reported that changes in RH levels from 100 to 25 % had no effect on spore release of *Blumeria graminis* in wheat at a wind speed of 4.5 m s^{-1} (55).

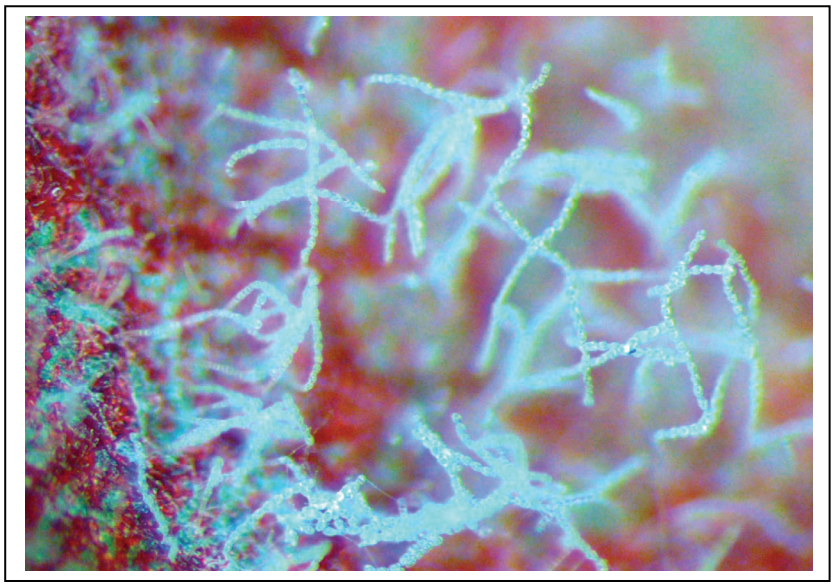


Fig. 4. Stereo microscopic view of *Podosphaera pannosa*. Long chains of conidia formed in detached rose leaflets placed in a Petri dish containing water agar. Photo: A. Suthaparan

1.4.1. Light quality

In *Aspergillus nidulans*, formation of conidia and ascocarps were significantly reduced by either red or blue light, while the highest conidial formation was recorded in full spectrum white light (98).

Powdery mildew caused by *P. xanthii* (syn. *Sphaerotheca fuliginea*) in cucumber plants grown under different spectral compositions with 12 h day length showed that the number of powdery mildew colonies per leaf was highest under metal halide lamps (broad spectrum of 300 - 800 nm), intermediate under red + far-red (spectral composition of 83 % red and 17 % far-red) or red + blue (98 % red with 1 % blue and 1 % far-red), and lowest under red light (99 % red and 1 % far-red) (103). Furthermore, the same authors reported that colonies grown under red light contained very sparse mycelium while the other treatments gave a more robust mycelium (99). Recently it has been showed that the number of powdery mildew colonies in cucumber leaves caused by *P. xanthii* was significantly less under red light (peak 628.6 nm) compared to purple, blue, green, yellow and broad spectrum white light (112).

1.4.2. Light intensity

Increased light intensity from 1000 to 5000 lux reduced the conidial germination of *Oidium neolycopersici*, the cause of tomato powdery mildew (60). On the other hand, conidial

formation and disease severity increased with increasing light intensity (60). However, disease severity of strawberry powdery mildew was reduced significantly at light intensities of 7000 lux compared to either 1200 or 3800 lux (2).

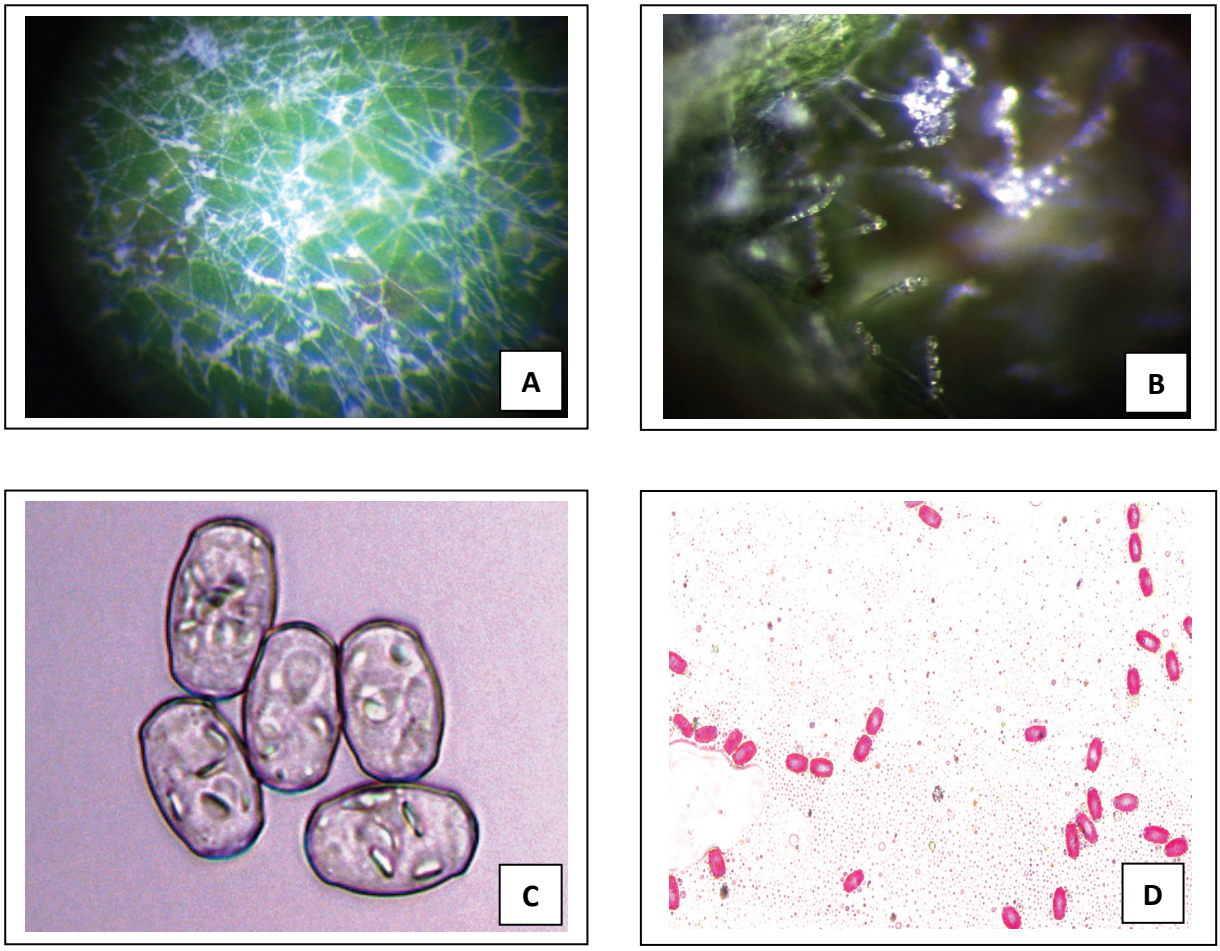


Fig. 5. Rose powdery mildew (*Podosphaera pannosa*); Stereo microscopic views of a colony on a rose leaflet (A), conidiophores with conidia in chains on a rose leaflet (B), light microscopic views of conidia containing fibrosin bodies (C), and conidia stained with lacto fuchsin after being trapped on a microscopic tape in a wind tunnel (D). Photos: A. Suthaparan.

1.5. Fungal photo sensory receptors

Recent advances have revealed that phytochrome related genes are present in almost all forms of life on earth, except Archaea (78). The existence of red light sensing systems beyond the photosynthesizing organisms have been confirmed (17, 98). This allowed to extend the phytochrome super family that is now divided into families, including plant

phytochromes (Phys), cyanonobacterial phytochromes (Cphs), bacteriophytochrome photoreceptors (BphPs), and fungal phytochromes (Fphs) (64).

All phytochromes consist of both a photo sensory domain at the N terminus and a regulatory domain at the C terminus. Each domain consists of several sub domains. Fungal phytochromes share several characteristic domains with plant and bacterial phytochromes. Instead of phytochromobilin or phytocyanobilin that are present in plants and cyanobacterial chromophore, fungal and bacterial chromophore has biliverdin as pigment (100).

The fungus *Neurospora crassa* has two phytochrome gene sequences, called *PHY 1* and *PHY 2*, however, the biological functions of these photoreceptors are not yet clear (46). *A. nidulans* produced the least conidia in darkness and most in white light. Less conidia was produced in either blue (450 nm) or red (680 nm) light compared to white light, but in a combination of red and blue light, formation of conidia was similar as in white light (98).

Phytochrome FphA and a fungal specific protein (VeA) have been described as important components of light responses in *A. nidulans*, and red light inhibition of sexual development was acquired in the *fphA* mutant strains (17). The two genes of LreA and LreB activate the sexual cycle in *A. nidulans*, and these genes were repressed by the action of FphA (98).

Light sensitivity of *A. nidulans* depends on gene VeA which encodes a 573 amino acid polypeptide. A mutant for gene VeA favoured asexual sporulation, independent of light control (66). Deletion of a single phytochrome gene of *A. nidulans* (FphA) resulted in the loss of red light inhibition of sexual reproduction, the first function assigned to a specific fungal phytochrome (17).

1.6. Plant resistance against powdery mildews

Powdery mildews attempt to penetrate the leaf cuticle and cell wall with a penetration peg by means of enzymatic and mechanical pressure (43). If the fungus overcomes the preformed physical barriers, then plants activate its defence mechanisms. This resistance can be controlled by a few major genes or several minor genes or in combination of both.

Resistance controlled by single or a few major genes are usually called vertical or race specific. They act against pathogen development through the formation of penetration barriers (papillae formation) and prevent nutrient uptake by death of infected cells and a few adjacent cells via hypersensitive reactions (31, 57, 58). This will completely stop the pathogen

development, but this resistance can be overcome by the pathogen after a certain time period through the development of virulent strains.

Resistance controlled by several minor genes are often called horizontal or partial resistance. It acts against pathogen development through the formation of penetration barriers (papillae formation), abnormal haustorium formation and reduced haustorial efficacy in the nutrient uptake. This finally reduces the growth and reproduction potential of pathogens, and may be expressed as smaller lesions and fewer number of conidia produced (37, 38, 57, 110).

Plants or plant parts normally susceptible to a disease may become resistant in response to an extrinsic stimulus, but without any alteration of the genome. This is called acquired or induced resistance. The inducing agent can be of biotic or abiotic nature and the resistance can be localized or systemic (113).

Long-term resistance, or systemic acquired resistance (SAR), involves communication of the damaged tissue with the rest of the plant using plant hormones such as jasmonic acid, ethylene, abscisic acid or salicylic acid. Because of biotrophic nature of powdery mildew, SAR is regulated mainly by salicylic acid path way (49). The reception of the signal leads to systemic changes within the plant, which induce genes that protect from further pathogen intrusion (7).

1.6.1. Effect of light on plant disease resistance

Resistance induced by ultraviolet (UV) light in host plants against pathogens has been well documented for many plant pathogen interactions (14, 39, 90). The resistance of a Sekiguchi lesion (Sl) rice mutant to *Magnaporthe grisea* infection was enhanced by the accumulation of indole alkaloid tryptamine under long wavelength and among the visible light (400-700 nm), red light was the most effective (10).

Red lights (600-700 nm) suppress lesion development caused by *Alternaria tenuissima* on detached broad bean leaves (99). Yellow (maximum at 590 nm) and red (maximum at 650 nm) light significantly inhibited the formation of infection hyphae (penetration pegs) from appressoria of *Botrytis cinerea* on both detached and attached broad bean leaflets. Pretreatments of leaflets with either yellow or red light for 24 h before inoculation also inhibited the formation of infection hyphae from appressoria, and it was concluded that yellow or red light induced resistance in broad bean against *B. cinerea*. Irradiation of broad bean leaflets with red light activated production of antifungal substances in leaf tissues that seemed to induce resistance in the plants (59).

In soybean cotyledons, light amplified the elicitor induced production of phenylpropanoid derived phytoalexin precursors (51). Both the development of hypersensitive cell death during an incompatible interaction and salicylic acid induced pathogenesis related protein (PR)-1 accumulation have been shown to be light and phytochrome dependent in Arabidopsis (47). In oat powdery mildew, day length during plant growth prior to inoculation was an important factor determining the level of resistance, and plants grown in 16 h day length showed higher level of adult plant resistance compared to plants grown in 8 h (62). Red light induced resistance of cucumber plant against powdery mildew caused by *P. xanthii* has been proved recently. Exposure to red light up-regulated PR-1, WRKY6 and WRKY30 (encoding two transcription factors involved in systemic aquired resistance pathway), whereas exposure to purple, blue, green and yellow light down-regulated. Further it has been reported that, exposure to red light resulted in higher levels of H_2O_2 and salicylic acid (SA), and stronger expression of defence genes such as PR-1 than exposure to white or other monochromatic lights (112).

1.6.2. Powdery mildew and disease resistance in roses

Rose genotypes have a variable level of constitutive or inducible resistance to powdery mildew, and several studies of this pathogen have shown that both horizontal and vertical resistance exists in roses (38). One or two major genes as well as a few minor ones likely control resistance to powdery mildew in roses (71). It has been reported that papillae formation, cell reactions (total cell collapse or cell walls were strengthened and haustoria were surrounded with cell material without cell collapse), induction of fungitoxic phenolic compounds (phytoalexins), inefficient haustorium formation (abnormal haustorium) are the resistance mechanisms present in four rose genotypes (37).

2. OBJECTIVES OF THE PRESENT INVESTIGATIONS

In Norway, greenhouse crops constituted 60 % of the first hand value in commercial horticultural production in 2006. The ornamental plant production constituted 43 % of the sales value of the total greenhouse production that year (6). Roses are an important cut flower and pot plants produced in Norway. However, the industry, which traditionally has benefited from relatively restrictive import regulations, is now faced with more liberal imports of horticultural products as a consequence of new international trade agreements. The Norwegian roses have thus to be of high quality in order to compete with imported products. In addition, the environmental aspect of plant production is very much in focus. Much of the research efforts are therefore focused on quality and less use of chemicals for pest and disease control.

Powdery mildew is the most destructive disease in greenhouse roses (71) and has been an increasing problem in Norwegian rose production. The pathogen is an obligate parasite and in greenhouses it survives exclusively as mycelium and conidia in living host tissue (71).

Conidia carried by wind to young green tissues, germinate and infect these tissues, if conditions are favourable (71). It has been reported that cool nights combined with warm days are favourable for mildew development (113).

At present, powdery mildew in greenhouse roses is mainly controlled by application of sulphur vapour or synthetic fungicides, and about 40 % of all fungicides used in rose production are for control of powdery mildew (71). It is not environmentally friendly, may reduce the quality of roses and may be toxic to biological control agents used against insect and mite pests (4). In addition, resistance of *P. pannosa* against the most widely used synthetic fungicide groups in the management of rose powdery mildew has been reported(34). There is an increasing interest in the reduction of the use of chemicals in horticulture worldwide; it is becoming increasingly desirable to find an alternative to control powdery mildew.

Many rose cultivars show a moderately high level of resistance to powdery mildew, but most popular cultivars are highly susceptible to the disease. The development of modern cultivars with improved resistance has been a major breeding challenge for a long time, but successes are scarce. Introduction of the corresponding resistance genes into modern cultivar is very time consuming, these crossing often restricted by incompatibility or different ploidy level of the parents. Further, crossings may completely disrupt the given valuable phenotype of the cultivar. Several fungi have been reported to parasitize or antagonize powdery mildew

in several crops including roses (41). Although this control approach appears promising, so far it has not been developed sufficiently to be used for practical control of powdery mildews.

Manipulation of environmental conditions may be an alternative for powdery mildew management in greenhouses. Constant high RH (>90%) greatly reduced powdery mildew in strawberry (2), tomato (60), and roses (85). However, in practice it is very difficult to maintain high RH most of the year (except in parts of the winter period), and in roses, a constant high RH significantly reduced the keeping quality (108, 109). In addition, high RH may induce the development of other fungal pathogens such as *Botrytis cinerea.*

Temperature regulation has also been proposed as a management means to control powdery mildew. It has been reported that powdery mildew may be strongly reduced if infected plants are exposed to high temperature (≥ 30 °C) for a short time (6 h) (42, 93). However, rose growth is optimal at around 25 °C, and high temperature may greatly reduce yield and quality of roses (16).

Supplementary lighting of 150-200 μmol m^{-2} s^{-1} PFD for 18 to 20 h per day is generally practised in Norwegian greenhouse rose production (77). Studies conducted in Norway revealed that continuous lighting reduced powdery mildew development in cut roses compared to 16 to 18 h lighting (85, 86). These findings indicated the possibility to manage the disease by day length manipulation with supplementary lighting. However, the post harvest keeping quality of roses significantly reduced under continuous lighting (84).

The main goal of the present study was to further investigate the effect of day length and light quality in order to use this knowledge to more accurately manipulate the light environment to suppress powdery mildew in roses. Subsequently this may increase the efficacy of biological control measures against insect pests, because living organisms used for biological control may be sensible for fungicides used against powdery mildew.

Paper 1

Continuous lighting reduces conidial production and germinability in the rose powdery mildew pathosystem.

Paper 2

Specific light emitting diodes can suppress sporulation of *Podosphaera pannosa* on greenhouse roses.

Paper 3

A molecular genetic approach to identify possible phytochrome-like photoreceptors in *Podosphaera pannosa.*

3. MATERIALS AND METHODS

The experiments were conducted with detached leaves or whole plants of pot rose cv. Mistral. Plants were propagated and grown under 18 h day length at 200 µ mol m^{-2} s^{-1}, 20 °C and 80 % RH. Experiments with detached leaves took place in double room humidity boxes (Fig. 6). In the lower room, there was a salt solution providing constant high air humidity (96 ±2 % RH). Air flow between the two rooms was secured by holes along the edges of the wall separating the rooms. In the upper room, rose leaflets were placed on top of water agar, with the abaxial side facing the light source. Germination and formation of conidia was investigated on detached leaves. Wind tunnels were constructed to study the development of powdery mildew on whole plants (Fig. 7). Powdery mildew was recorded as number of trapped conidia (included the process of both formation and release of conidia). Two mildewed plants (inoculated 12 d before start of the experiments) were placed in each tunnel for 4-7 d. There was a constant air flow (0.06 ms^{-1}) passing over the plants, and conidia were trapped on a microscope tape attached to a 24 h rotating clock cylinder.

Mercury lamps with PFD of 50 µmol m^{-2} s^{-1} were used for all day length experiments, both for detached leaves and whole plants. Conidia on leaflets were exposed to 0, 12, 18 or 24 h day length, and examined for germination 24 h after inoculation.

When studying the effect of day length on conidia formation, inoculated leaflets were first kept at 18 h day length for either 2 or 4 days, followed by exposure to 0, 12, 18 or 24 h day length. Nine days after inoculation, the leaflets were examined for number of conidia by shaking the leaflets in water and counting them in haemocytometer. To better understand the effect of day length on formation of conidia between 18 to 24 h, inoculated leaflets were also treated with 18, 20, 22 or 24 h day lengths.

Whole plants were exposed to day length treatments in wind tunnels in one series of experiments with 0, 12, 18, or 24 h and in another with 18, 20, 22, or 24 h. The plants were inoculated 12 d before the day length treatments started and kept at 18 h day lengths. To examine the possible effect of adaptation of plant and pathogen to day length, control

experiments were conducted with inoculated plants kept in 12 h day length for 12 d before treatment, and exposed to 0, 12, 18 or 24 h. Number of trapped conidia was recorded.

Inoculated plants with three marked leaves on each plant were exposed to 0, 12, 18 or 24 h day length. Eight days after treatment, marked leaves were detached and assessed for disease severity.

Following inoculation on detached leaflets, conidia were exposed to 18 h day length with either blue, red, far-red or full spectrum white light followed by 6 h darkness, and then assessed for germination. The PFD was kept at 3.6 µmol m^{-2} s^{-1} for blue, red, far-red, and 5 µmol m^{-2} s^{-1} for full spectrum white light. To investigate the effect of different light qualities on formation of conidia, inoculated leaflets were kept at 18 h day length (white light, 50 µmol m^{-2} s^{-1}) for the first 4 d and then exposed to 18 h light cycles of either blue, red, far-red or white light at PFD as mentioned for germination. Samples were assessed for numbers of conidia formed as mentioned in the day length experiments.

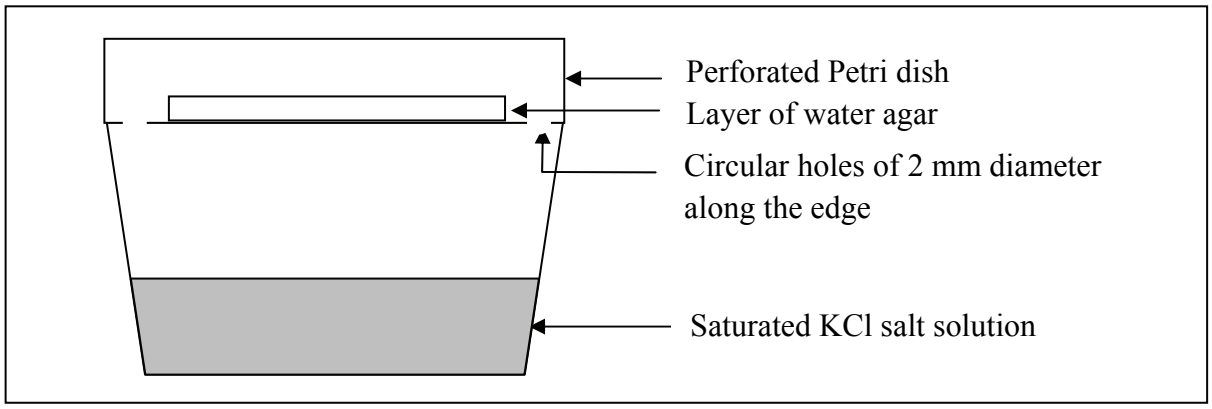


Fig. 6. Schematic illustration of a double room humidity box (further described in paper 1). The upper part is a perforated Petri dish with water agar placed in the centre. The lower part is a 500 ml plastic container with 100 ml of saturated KCl salt solution providing a stable RH of 96 ± 2 % in both parts of the box at 20°C.

Plants grown at 18 h day length for 12 d in white light following inoculation were moved to the wind tunnels and treated with diurnal cycles of either i) 18 h blue, red, far-red, or white (5 µmol m^{-2} s^{-1}) + 6 h darkness, or ii) 18 h white light (50 µmol m^{-2} s^{-1}) + 6 h of blue, red, far-red (5 µmol m^{-2} s^{-1}) or darkness. Plants were also treated with short 1 h night breaks within the 6 h dark period with either red or far-red light, also including one treatment with 1 h far-red following 1 h red light (paper 2, Fig. 2).

Whole plants were also exposed to light intensities of either 5, 50, 100, or 150 µmol m^{-2} s^{-1}, to develop a better understanding of the effect of light intensity on conidia production and release.

Genomic DNA of *P. pannosa* was extracted and investigated for possible presence of phytochrome-like genes. Polymerase Chain Reaction (PCR) was performed to amplify the genes of interest. Degenerate primers that were used in PCR, designed based on most conserved regions of known fungal phytochrome (like) protein sequence data published in National Centre for Biotechnology Information (NCBI). Amplified genes were cloned and the DNA sequences of the cloned PCR products were analysed, and searched for similarities to the translated nucleotide sequence with an existing sequences available at the NCBI, using tblastx software. The Pfam database was also searched for homologies.

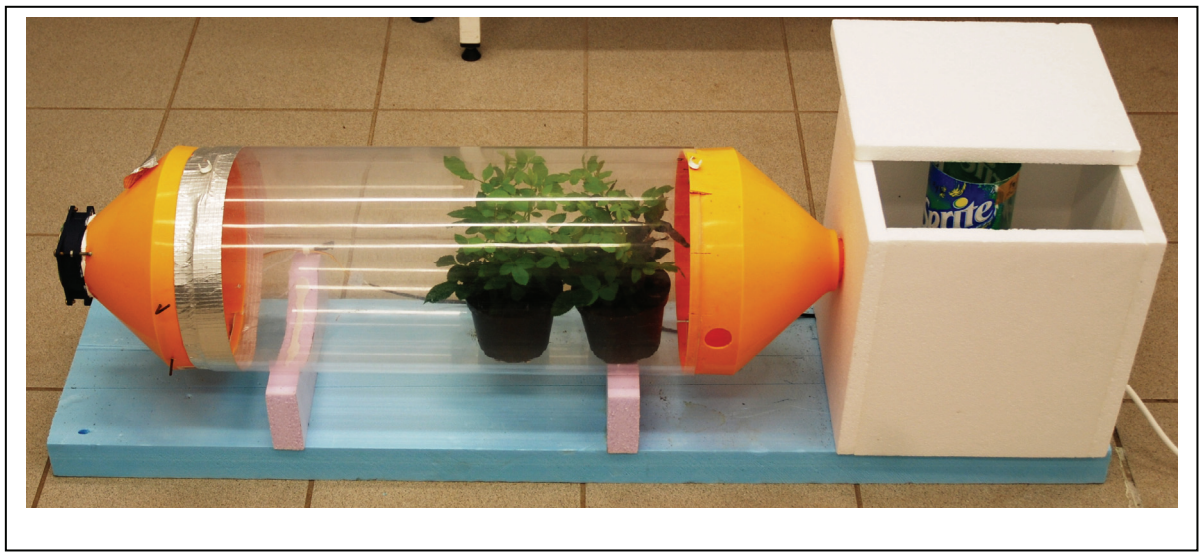


Fig. 7. Wind tunnel with mildewed roses (further described in paper 1). Ambient air entering the tunnel on the left side, and exiting through a small orifice on the right side. A microscope tape on a rotating clock cylinder trapped conidia of *P. pannosa*.

4. MAIN RESULTS AND DISCUSSION

Under constant temperature and RH, there was a significant reduction in powdery mildew with increased day lengths beyond 18 h (paper 1).

Low intensity red light strongly reduced conidia formation and release in *P. pannosa* (paper 2). A diurnal periodicity of 18 h full spectrum white (50 µmol m^{-2} s^{-1}) light followed

by either 6 h low intensity red light or a night break (1 h) with low intensity red light, effectively suppressed formation and release of conidia. At 18 h diurnal periodicity, there was a significant increase in formation and release of powdery mildew conidia with increasing light intensity between 5 and 150 μmol m^{-2} s^{-1} (paper 2).

A short 1 h red light night break followed by a 1 h far-red light night break almost nullified the suppressive effect of red light (paper 2). This opens the question if *P. pannosa* has a photo sensory receptor that responds to various light qualities and day lengths. In spite of indications that one or more phytochrome-like genes may exist in rose powdery mildew, we did not manage to find it (paper 3).

Light is an important environmental factor that regulates many aspects of growth and developmental processes in living organisms, including plants and fungi. Roses are able to utilize continuous lighting, and it was previously found in Norway that increasing day length from 18 to 24 h reduced the number of days to flowering (juvenile period) and increased the number of flowers (82, 84). Furthermore, day length seems to be a more important component of the light factor than its intensity under similar light integral. Roses exposed to continuous lighting at a lower photon flux density (204 μmol m^{-2} s^{-1}) retained 80 % more carbon than roses exposed to 12 h day length at a higher photon flux density (410 μmol m^{-2} s^{-1}) (61). In addition to improved rose yield (82, 84), the present study showed that increasing the day length beyond 18 h significantly reduced powdery mildew. This confirmed the earlier observation in Norway that rose powdery mildew is suppressed in continuous light (86). In peas, continuous lighting reduced hyphae elongation of *E. pisi* compared to 12 h day length (89). Haustoria formation of *B. graminis* f. sp. *hordei* was stimulated in short days (6 h) if compared to long days (18 h) (25), and formation of haustoria was higher under 16 h day length than continuous light (24). Even though continuous lighting has advantages in terms of rose yield and powdery mildew management, growing of roses under continuous lighting at a high light intensity has not been recommended due to significant reduction in post harvest keeping quality (84). The poor keeping quality in roses grown under such conditions is due to stomata malfunctioning (fail to close) (86). However, diurnal variations in relative humidity under continuous light can improve the post harvest keeping quality of roses (96). On the other hand, large fluctuations in relative humidity may increase the conidial release in rose powdery mildew (1), and may thus increase powdery mildew disease severity. In our study, there were no significant differences between 20, 22 and 24 h in formation and release of conidia. Our findings clearly suggest that, instead of using continuous light, growing roses

under day lengths of 20-22 h may give similar effects regarding management of powdery mildew. This may also be helpful in improving the post harvest keeping quality of roses (83, 102).

In addition to day length, the significance of the other components of light such as quality and intensity on rose growth and yield has been well documented. Yield of roses depends on axillary bud break, and a high red to far-red ratio induce axillary bud sprouting (80). Light quality also plays a significant role in powdery mildew disease development. We found that day length extension of 18 h full spectrum white light followed by low intensity red light significantly reduced the conidia formation and release of rose powdery mildew and the effect was valid even under a short night break of 1 h. It has been reported previously that cucumbers grown under broad spectrum light had the most powdery mildew and that the least was found under red light (103). Formation of conidia was also greatly inhibited under red light (103). Similar results of significant reduction of powdery mildew in cucumber grown under red light has been reported recently (112). However, those two studies were conducted under high light intensities of 300-350 μmol m^{-2} s^{-1}. In our work we used a very low intensity (5 μmol m^{-2} s^{-1}) of red light. As mentioned previously, light intensity is an important factor in rose growth and yield (20, 48). Increasing light intensity increase the number of buds that break and reduce the formation of blind shoots (117). In addition, increasing light intensity increased the quality of roses by formation of longer stems and enhancement of keeping quality (48). Furthermore, reducing the light intensity for a long time reduces the colour development of rose petals (15, 69, 73). On the other hand, our study showed that increasing light intensity of full spectrum white light increased the conidial formation and release when exposure to a 18 h diurnal periodicity. Similar results with increased severity of powdery mildew in tomato caused by *O. neolycopersici* (60) and conidial release in rose powdery mildew (1) has been reported with increasing light intensity.

Based on the present work, day lengths of 20-22 h white light followed by low intensity red light for a short period (paper 2) may provide successful management tools to better handle powdery mildew in greenhouse roses, without extensive use of fungicides. Furthermore, the combination of high intensity long day (18-20 h) white light followed by low intensity short day red light (1-6 h) may induce the stomata functioning and improve the postharvest keeping quality of roses compared with high intensity continuous lighting. Plant growth and yield are dependent on production of carbohydrates during photosynthesis and the subsequent allocation of the available assimilates. Day length extension by use of low

intensity light of 30 μmol m^{-2} s^{-1} PAR increased the number of flowers in roses without increase in fresh weight, and it was speculated that low intensity light is sufficient for translocation of photo assimilates synthesised under high intensity growth light (79, 121).

Our work showed the influence of day length, light quality and light intensity on rose powdery mildew. However it is not clear if these factors affect the fungus directly or indirectly by influencing the host plant. Powdery mildews are caused by obligate biotrophic fungi. If there was a direct effect of light on the fungus, it should have one or more photoreceptors. The presence of photoreceptors, including phytochrome related genes, has been discovered in almost all forms of life on earth (78), and existence of red light sensing systems beyond the photosynthesizing organisms has been confirmed (17, 98), as discussed earlier in this part of the thesis. Our results with suppression of conidial formation and release after short night breaks of low intensity red light, and an almost nullification of this effect by exposure to a short period far red light, gave indications of the presence of phytochrome-like photoreceptors in *P. pannosa*. Fungal phytochrome genes have been found in *A. nidulans* and *N. crassa*, both belongs to phylum ascomycota as *P. pannosa.* The loss of red light inhibition of sexual reproduction resulted in deletion of a single phytochrome gene (FphA) of *A. nidulans*, and this was the first function assigned to fungal phytochrome (17). However, we did not succeed in finding any phytochrome-like genes in *P. pannosa*.

It is known that plant defences against pathogens are generally affected by environmental conditions. Our findings of the light effect on rose powdery mildew could at least partly be due to induced resistance in host plant by light as shown for other crops (59, 99, 112). Photoreceptors present in the rose plant may be involved in regulation of induced resistance. In Arabidopsis hyper sensitive cell death and the accumulation of the pathogenesis related protein (PR)-1 induced by salicylic acid, both have been shown to be light and specifically phytochrome dependent (47). Oat plants grown under 16 h day length prior to inoculation with powdery mildew showed higher level of adult plant resistance than plants grown under 8 h (62). Different quality light may induce different levels of resistance (59, 99, 112), and it has been proven that red light induced resistance of cucumber plants against powdery mildew is due to higher levels of H_2O_2 and salicylic acid (SA), and stronger expression of defense genes such as PR-1 than exposure to other monochromatic lights or full spectrum white light (112).

5. GENERAL CONCLUSIONS AND FURTHER PERSPECTIVES

- Increasing day length from 18 h to 20-24 h reduced powdery mildew in roses grown at constant temperature and RH. To avoid the keeping quality problem related to continuous lighting, day lengths of 20-22 h light may be a good alternative.
- Low intensity red light strongly reduces the formation and release of conidia in rose powdery mildew. Brief periods (1 h) of red light may be sufficient to improve the efficacy of day length manipulation to reduce powdery mildew.
- Conidia production increased with increasing light intensity. Further research is needed to provide better knowledge regarding the possible role of light intensity in management of rose powdery mildew.
- Further research is necessary to confirm whether *P. pannosa* has a phytochrome-like system.

The severity of an epidemic is mainly determined by the number of conidia that successfully infect the host tissue, the rate of colony expansion and the length of the latent period. Environmental factors such as temperature, RH and light play important roles in their growth and development. The obligate biotrophic nature of powdery mildew often make it difficult to distinguish between effects on the plant and the fungus.

The effect of light intensity on disease severity of the strawberry powdery mildew pathosystem was opposite to tomato, where the powdery mildew disease severity increased with increasing light intensity in tomato (2). Furthermore, the germinability and virulance of the conidia produced under different light treatments may vary. Studies in *E. pisi* showed that conidia produced in light had higher germination percentage than the conidia produced in dark (11).

Further research is necessary to find if *P. pannosa* has phytochrome-like photoreceptors. Very little is known about phytochromes in fungi, and there are no previous reports from powdery mildews, making it difficult to design specific primers to detect the possible phytochrome gene(s). Another possible strategy to characterize the phytochrome function in powdery mildew fungi are through assessment of mRNA produced in the fungus under different light quality treatments. This mRNA could be used to construct subtractive expression libraries, and the sequences of differentially expressed clones could be compared with phytochrome sequences available in NCBI. Proteomic analysis using mass spectrometry may be yet another alternative.

On the other hand the response of fungi to low intensity red and far-red light could be due to the different levels of resistance induced in the host plants by light. Thus identifying components of induced resistance and their levels may also be an option to better understand the role of light in rose powdery mildew.

Powdery mildew is problematic in several greenhouse vegetable and ornamental crops. Epidemiology of powdery mildews is complex, and knowledge from one pathosystem may not be directly applicable in another. All crops may not benefit from near to continuous lighting with high intensity light, e.g. tomatoes and cucumbers. It has been reported that under high light intensity, 14 h day length was optimal for growth and yield in greenhouse tomatoes (35). Longer days did not further increase the tomato yield, and day lengths of 20 h or more for more than 6 to 8 weeks caused leaf chlorosis and reduced growth and yield (35, 36).

Spectral quality manipulation and variable temperature regimes may be used to reduce the severity of leaf chlorosis caused by long day lengths (36), but there are no reports regarding the effect of day extension by very low intensity lighting. This leaves the possibility to use present knowledge of day extension of high intensity white light followed by low intensity red light as a management tool to control powdery mildew in crops like cucumbers and tomatoes. Day length and light quality manipulation with modern lighting technology might also be useful in several greenhouse grown and selected field grown crops where powdery mildews are an important disease.

The spectral quality of light plays an important role in the healthy growth of the crops. The main advantages of using LEDs as a light source is the possibility to modify the spectral balance by selecting and combining the different peak wavelength emissions that are most efficient. It has previously been reported that use of UV – B radiation reduced powdery mildew infections in several crops (63, 115). Filtration of the far-red part of natural light may turn out to be efficient in improving management of powdery mildew in greenhouse crops. Based on the present findings and new light technology, it may be possible to combine optimal day lengths for plant growth with manipulation of light quality for better powdery mildew management in crops other than roses, and thus reduce the need for fungicide applications in several crops. Our work will be continued in a new project with investigations of day length and light quality in powdery mildew on tomatoes and cucumbers.

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Paper I

Continuous Lighting Reduces Conidial Production and Germinability in the Rose Powdery Mildew Pathosystem

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ABSTRACT

Suthaparan, A., Stensvand, A., Torre, S., Herrero, M. L., Pettersen, R. I., Gadoury, D. M., and Gislerød, H. R. 2010. Continuous lighting reduces conidial production and germinability in the rose powdery mildew pathosystem. Plant Dis. 94:339-344.

The effect of day length on production and germinability of conidia and severity of disease caused by *Podosphaera pannosa*, the causal agent of rose powdery mildew, was studied. Whole potted plants or detached leaves of *Rosa* interspecific hybrid 'Mistral' were inoculated with *P. pannosa* and exposed to 0, 12, 18, 20, 22, or 24 h of artificial light per day in growth chambers equipped with mercury lamps. Increasing duration of illumination from 18 to 20 to 24 h per day reduced production of conidia by 22 to 62%. Exposure to 24 h of illumination per day also strongly reduced disease severity compared with 18 h. Our results suggest that increasing day lengths from 18 h per day to 20 to 24 h may suppress the disease significantly and, thereby, reduce the need for fungicide applications against powdery mildew.

Powdery mildew (*Podosphaera pannosa* (Wallr.) de Bary) is a widespread disease of roses that is particularly troublesome in greenhouse production of hybrid roses (*Rosa* × *hybrida*). Although the pathogen rarely kills the plant, infection reduces host plant vigor, productivity, and esthetic (and therefore economic) value. In greenhouses, the pathogen is disseminated as conidia produced primarily on diseased leaves. The ascigerous stage of the pathogen has no known role in the epidemiology of the disease in greenhouse rose production.

In general, relatively cool atmospheric conditions with moderate temperature and reduced light (shade) are favorable to powdery mildews (28,29). In *P. pannosa*, colony growth and conidial germination occur at 50% relative humidity (RH) (22) and optimum RH for conidia germination is nearly 100% (10). Furthermore, germination is reduced by free water (24) and temperatures above 30°C (21). Dissemination of conidia, as inferred from spore trapping, was associated with rising temperature, increasing solar radiation, and decreasing RH (1).

The influence of light on biological activity of fungi has been extensively studied in *Aspergillus nidulans* (3) and *Neurospora crassa* (2,27). However, little is known about the effect of light on *P. pannosa*. Importance of supplemental lighting in rose cultivation is well established, and there is an increase in productivity with increasing light intensity and day length (4,11,14,16,25). The use of supplementary lighting is essential for year-round rose production in Norway due to low natural irradiance during the winter (12). Our preliminary studies indicated that severity of powdery mildew was reduced on roses grown under continuous lighting compared with day lengths of 16 to 18 h (15,17). Our objectives in the present study were to (i) investigate the effect of day length on germination, formation, and productivity of conidia by *P. pannosa*; and (ii) quantify the effect of supplemental lighting on severity of rose powdery mildew. Preliminary accounts of this work have been published (17,20,26).

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MATERIALS AND METHODS

Production of host plant material. Mildew-free potted miniature rose plants (*Rosa* × *hybrida* 'Mistral') were grown in limed and fertilized peat (Floralux; Nittedal torv industri A/S, Norway) and perlite (25% by volume) in a greenhouse compartment to produce plant material for the experiments. Mercury lamps (Powerstar HQI-BT 400W/D day light; OSRAM GmbH, Augsburg, Germany) at photon flux densities (PFDs) (400 to 700 nm) of 200 μmol m^{-2} s^{-2}1 recorded at the top of the plants were used as a supplementary light source (18 h per day). The lights were automatically turned off when natural light exceeded 200 μmol m^{-2} s^{-1}. Temperature was set at 20°C and RH at 80%. Plants were exposed to evaporated sulfur for 4 h per day during the night and watered with complete nutrient solution when needed. The plants were pruned to induce young, active shoot growth to ensure sufficient supply of uniformly aged, mildew-free leaflets.

Production of inoculum. Rose leaves bearing powdery mildew colonies were obtained from a commercial greenhouse located in southeastern Norway. Detached young and newly unfolded rose leaflets from the abovementioned greenhouse compartment were surface sterilized by soaking them in sterile distilled water for 15 min followed by 4-min soaking in 0.5% sodium hypochlorite solution with Tween 20 (Sigma-Aldrich Chemie GmbH, Steinheim, Germany) at 30 μl/liter followed by three rinses in sterile distilled water, each lasting 3 to 4 min. This process also removed any sulfur residue. The leaflets were placed in petri dishes containing water agar (15 g $liter^{-1}$) and were inoculated by touching them gently with mildewed leaves from the commercial greenhouse. The petri dishes with the leaflets were sealed by Parafilm and kept in an incubator for 7 days with 16-h diurnal light periods provided by cool white florescent lamps (Philips, The Netherlands) at PFD (400 to 700 nm) of 30 μmol m^{-2} s^{-1} at the top of the petri dish lids and 20 ± 1°C.

These mildewed leaflets from the incubator were then used to inoculate another set of clean leaves by transferring single conidial chains. An eyelash attached to a fine glass needle was used to trap the single chain from diseased leaves, and a chain

of conidia was deposited directly on the clean leaves under a stereomicroscope. The petri dishes were sealed and incubated for 7 days in the incubator with conditions as detailed before. Following incubation, conidia were transferred two more times as described above to reduce the risk of contamination with other microorganisms.

Disease-free plants of Mistral were inoculated by gently touching them with mildewed, detached leaves kept in the incubator as described above. Inoculated plants were kept in an isolated growth chamber throughout the experimental period, with 18 h of diurnal lighting provided by mercury lamps of the same type as described above, at PFD of 200 μmol m^{-2} s^{-1}, 20°C and 80% RH. The inoculum was renewed by continuous replacement of healthy plants in the growth chamber.

In all experiments (detached leaves or whole plant), inoculum from 7-day-old cultures was used. Seven days before each experiment, cleaned, detached leaves were placed in petri dishes containing water agar and sprayed with a conidial suspension. To obtain the suspension, 20 young mildewed leaflets from diseased plants in the growth chamber were added to a 50-ml centrifuge tube containing 25 ml of distilled water and shaken by hand for approximately 1 min. Leaflets were removed from the centrifuge tube, and the conidial suspensions were sprayed onto cleaned, detached leaflets placed in petri dishes containing water agar with a hand-held sprayer (approximately 1 ml per 20 leaflets). Petri dishes were sealed by Parafilm and kept in the incubator for 7 days under conditions as described above. These 7-day-old cultures are hereafter called source inoculum.

Materials and equipment designed for experiments. Experiments were conducted both in vitro (detached leaves) and in vivo (whole plants) in growth chambers. Growth chamber lighting was with mercury lamps (same lamp type as described above) with PFD of 50 ± 5 μmol m^{-2} s^{-1} when lamps were on and temperature and RH were kept at 20 ± 2°C and 80 ± 5%, respectively. Investigations with germination and formation of conidia were conducted with detached, clean leaves placed in double-room humidity boxes. Petri dishes (90 mm) were perforated with 20 circular holes (each with a diameter of 2 mm) along the bottom edge of the dishes by using an electrical heater. The perforated petri dishes were glued on top of a 500-ml plastic container with the same diameter and made into double-room humidity boxes (hereafter called humidity boxes). The lower part (the plastic container) contained 100 ml of a saturated KCl salt solution (34 g in 100 ml of distilled water) and, in the upper part (the petri dish), a layer of water agar (50 mm in diameter) was placed. The agar did not cover the holes, allowing air to flow between the two parts, thus ensuring a constant high humidity (96 ± 2% RH) in the upper part. Detached surface-sterilized and cleaned rose leaflets were placed with the abaxial side up on the agar layer.

For production of conidia (combination of formation and release) and disease severity experiments, whole plants of the same age, healthy, disease free, and with uniform canopies were selected from the greenhouse compartment mentioned above. For conidia productivity experiments, four wind tunnels were constructed (Fig. 1). An electric wind blower (Enermax UC-8FAB, China), 80 by 80 by 25 mm, with manually adjustable speed control (1,000 to 3,000 rpm) fixed to the upwind end of the tunnel, drew ambient air into the tunnel. The intake air was drawn through a spongy synthetic air filter medium (FILTRAIR; Peregrine Industries Pvt. Ltd, Campbellfield, Victoria, Australia) of approximately 2 cm in thickness that ensured both laminar and cleaned air flow. The wind tunnel was composed of a light-transmitting Plexiglas tube (main tube: 500 mm in length, 250 mm in diameter, approximately 3 mm in thickness of the Plexiglas), with cone-shaped ends (each 26 cm in length). The downwind end had an overflow orifice (29 mm in diameter). A narrow metal nozzle orifice (27 by 2 mm) channeled the air flow toward a Melinex microscope tape (with a sticky substance made of 1 g of paraffin, 100 ml of Toluene, and 9 g of clear Vaseline added to its surface) fastened to a 24-h rotating clock cylinder (approximately 3 mm of distance between the orifice and the tape). The cylinders were the lower parts of 1.5-liter plastic bottles (17 cm in length) fixed to the clock by a rubber ring (5.2-cm inner diameter) glued onto the bottom of the bottle.

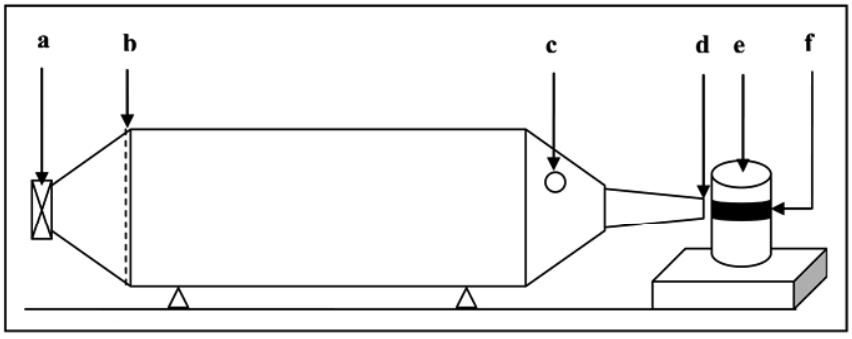

Fig. 1. Schematic illustration of a wind tunnel showing an electric wind blower with manually adjustable speed control (a); spongy filter (b); overflow orifice, 29 mm in diameter (c); nozzle orifice, 27 by 2 mm (d); clock cylinder (e); and microscope tape (f). Ambient air entered through the wind blower fixed inside the left plastic funnel of the wind tunnel (a); two diseased plants were placed in the main plexiglass tube (b); conidia exited the right plastic funnel through the overflow and nozzle orifices (c); and spores were trapped on the microscope tape attached to the rotating clock cylinder (plastic bottle, 24-h rotations) fixed to an electric timer (d).

Recording environmental conditions. Light intensity was recorded at the top of the lids of petri dishes or the double-room humidity boxes (described above) in detached-leaf experiments and at the level of plant height in the whole-plant experiments, with a Lambda LI-185B photometer (LI-COR Inc., Lincoln, NE) containing a quantum sensor. Light quality was recorded inside and outside of the petri dish or double-room humidity boxes and wind tunnel (described above) with a Spectra Wiz spectrometer Model EPP 2000 Fiberoptic (Stellarnet Inc., Tampa, FL). Temperature and RH were recorded inside the double-room humidity boxes, wind tunnels, growth chambers, and incubators with two-in-one sensors of SHT 75 (Sensirion AG., Staefa, Switzerland) with an accuracy of ±0.3°C and ±1.8% RH. Additionally, temperature and RH in growth chambers and in greenhouse compartments were recorded by a Priva greenhouse computer (Priva, De Lier, The Netherlands).

Germination of conidia. Three humidity boxes, each containing six detached leaflets, were placed in each of four growth chambers set to provide either 0-, 12-, 18-, or 24-h day lengths. Shortly before lights were turned on, the leaflets were inoculated by touching them gently with source inoculum leaflets, with one inoculum leaflet for each of the healthy leaflets. Two leaflets from each humidity box (six leaflets from each treatment) were removed 24 h after inoculation and kept at –2°C (to stop the fungal growth) until further examination. These leaflets were then placed individually on microscope glass slides; a few droplets of 50% lactofuchsin were added to stain mycelium and conidia, followed by examination under a light microscope with bright field at a magnification of ×200. Fifty conidia from each leaflet were assessed for germination. Conidia having a primary germ tube equal in length to at least half the width of the conidia were considered germinated. The experiments were repeated three times.

Production of conidia. Twelve humidity boxes, each containing seven leaflets of the same age, were inoculated by placing

one drop (15 µl) of a spore suspension on each leaflet. (The same method of inoculation was used in all experiments below with detached leaflets in humidity boxes). Immediately after inoculation, the humidity boxes were kept at 18 h of day length for the first 2 days to ensure uniform germination, and then they were transferred to 0, 12, 18, and 24 h (three boxes at each day length). All together, six leaflets with conidia from each treatment were removed 9 days after inoculation and placed in 50-ml centrifuge tubes, with 5 ml of distilled water added, followed by hand shaking for 2 min. The leaflets were removed from the centrifuge tube, and spores in drops of the resultant suspension were counted with a hemacytometer under a light microscope at ×200. Two spore counts were done from each resultant suspension. The experiment was repeated three times. An experiment was set up where samples were kept for 4 days at 18 h before transferring to the different day lengths. Here, samples were kept for 5 days in the four day length chambers before assessing production of conidia (9 days after inoculation). The experiment was repeated three times, and all other procedures were as described above. Inoculated leaflets were also exposed to an 18-h day length for 4 days, followed by 5 days at 18, 20, 22, and 24 h. All other procedures were as mentioned above, and the experiment was repeated three times.

Mistral pot roses were also grown in the greenhouse compartment as described above. Ten plants were spray inoculated with a 25-ml suspension of conidia made from 1-week-old source inoculum. Immediately after inoculation, the plants were moved to a growth chamber with 18 h of day length provided by mercury lamps at 200 µmol m^{-2} s^{-2}, 20°C, and 80% RH for 12 days. Four growth chambers were maintained with conditions as described in previous experiments, with 0-, 12-, 18-, and 24-h day lengths. One wind tunnel was placed in each of the four growth chambers. Relative humidity of the intake air of the wind tunnels equaled that of the growth chambers, and was elevated by approximately 5% after passing through the canopy of the plant in the wind tunnel. Two diseased plants (inoculated 12 days before start of the experiments) were placed in each wind tunnel and, 1 h afterward, the air flow was switched on and maintained for 7 days (wind speed of approximately 0.06 m s^{-1} in the Plexiglas tubes and 2.9 m s^{-2} at the nozzle openings). A portable air-velocity meter (Model 1650; TSI Inc., St. Paul, MN) was used to measure the air flow speed through the plants and nozzle opening of the wind tunnel.

The clock cylinder revolved seven times during the 7-day test before the Melinex tapes were removed and prepared for microscope reading. Lactofuchsin was used to stain the conidia. There were 144 transect readings (2 mm or 1 h apart) at ×200 for each tape. Thus, the spores counted in these transects represented the pooled number of spores trapped at a particular hour of the day for the 7 days the trap was operated. The counts from these transects were further pooled within eight 3-h periods (i.e., 0 to 0300 h, 0300 to 0600 h, and 0600 to 0900 h) and the factors of day length, experimental repeat, and time period were included in the data analysis. The experiment was repeated three times and, before each repeat, the tunnels were cleaned with moist paper followed by 12 h of air flow to ensure that conidia were not inside the tunnel.

A similar experiment was set up with 18-, 20-, 22-, and 24-h day lengths (repeated twice). To clarify if there was a possible adaptation of the fungus and plant to the 18-h day length given prior exposure to the different day lengths that would influence the observed trends, inoculated plants were kept at the 12-h day length for 12 days followed by the various day-length exposures for 0, 12, 18, and 24 h (experiment repeated four times).

Influence of day length on severity of powdery mildew. On each of 12 mildew-free uniformly aged pot roses of Mistral, three newly unfolded leaves were marked. The sulfur evaporator was shut off 2 days before inoculation. The plants were then spray inoculated as before with 2.5 ml of conidial suspension per plant, and three plants (each considered as repeats/blocks) were placed at each of 0-, 12-, 18-, or 24-h day lengths. Eight days after inoculation, the three marked leaves from each plant (nine leaves from each treatment) were detached, and severity of the disease (percent area of leaf surface diseased) was assessed for each leaf, based on an assessment key for powdery mildew in roses (21).

Statistical analysis. Treatment effects were determined using Minitab version 14 for analysis of variance (GLM procedure) and mean comparison (Bonferroni test) at $P = 0.05$.

RESULTS

Germination of conidia, production of conidia on detached leaflets. The mean percentages of germinated conidia were 70.3, 77.2, 84.9, and 61.4 after exposure to 0-, 12-, 18-, and 24-h day lengths, respectively (Fig. 2), with a significant difference between 0 and 18 h, and 24 and 18 h ($P < 0.001$).

Significantly more conidia were produced on detached leaflets exposed to 18-h day length compared with all other day lengths or continuous darkness (Fig. 3). When pretreated for 2 days in 18 h of light, there were also significant differences in numbers of conidia produced between 12 and 18 h, and also between 24 and 18 h (Fig. 3A). The number of conidia produced under an 18-h day length was 1×10^4 conidia/ml and it was 1.6×10^3 and 5.2×10^3 for continuous darkness and continuous lighting, respectively**.** The 0-h day length treatments (continuous dark) always produced much fewer conidia than the others; however, it was not significantly different from the 24-h day length treatment when exposed to pretreatment of 18 h for 2 days. Four days at 18-h day lengths prior to exposure to the different day lengths showed the same tendency, with less spore production at 12 and 24 h than at 18 h, and there were no significant differences noticed between 12 and 18 h (Fig. 3B). *P* values were <0.001 for the *F* test in the two experiments. When comparing day lengths from 18 to 24 h, there was a significant and steep drop in sporulation between 18 and 20 h (Fig. 3C). There were no differences in spore formation between exposures to 20, 22, and 24 h ($P < 0.001$).

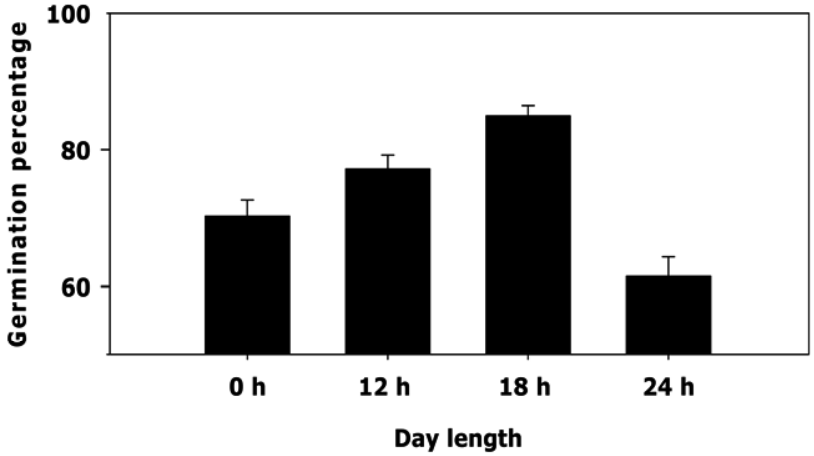

Fig. 2. Effect of day length on germination of *Podosphaera pannosa* conidia on detached leaflets of *Rosa* × *hybrida* cv. Mistral placed in double-room humidity boxes (photon flux density of light at 50 µmol m^{-2} s^{-2} with mercury lamps, 20°C, 96 ± 2% relative humidity). Assessments were made 24 h after inoculation. Each bar represents the mean of six randomly sampled leaflets in each of three replicated trials; bars indicate standard error of the mean.

Spore production and release from whole plants in wind tunnels. Exposure of mildewed plants to various day lengths in the wind tunnels strongly affected the total number of conidia trapped (Fig. 4). In the experiment, where the incubation (after inoculation) took place at 18 h of day length for 12 days, the number of conidia trapped under 18 h of day length during 1 week was 24,903. It was 101, 4,691, and 5,506 for continuous darkness, 12-h day length, and continuous lighting, respectively (Fig. 4A). When inoculated plants were incubated at a 12-h day length for 12 days, treatments of 0, 12, and 24 h significantly reduced the relative spore numbers to 0.8, 44.9, and 38.5%, respectively (Fig. 4B). In both experiments (with either 18 or 12 h of day length during incubation), the number of conidia trapped under the 18-h day-length treatment was significantly greater from other day-length treatments ($P < 0.001$ for the two experiments). Infected leaves exposed to complete darkness (0-h day length) in the wind tunnel also released significantly fewer spores compared with day lengths of 24 and 12 h.

Day lengths of 20, 22, and 24 h reduced the relative spore numbers to 28.3, 50.9, and 42.9%, respectively, relative to 18 h of day length (Fig. 4C). There was a significant reduction between 18 and 20 h ($P < 0.001$) but no significant differences between 20, 22, and 24 h.

Disease severity on whole plants. The highest disease severity was recorded on plants exposed to an 18-h day length (70%), followed by 12-h (48.3%), 24-h (22.6%), and 0-h (1.5%) day lengths (Fig. 5). All treatments were significantly different from each other ($P < 0.001$). Significant interaction between treatment and replicate (each plant within treatment considered as replicate) were noticed ($P = 0.01$).

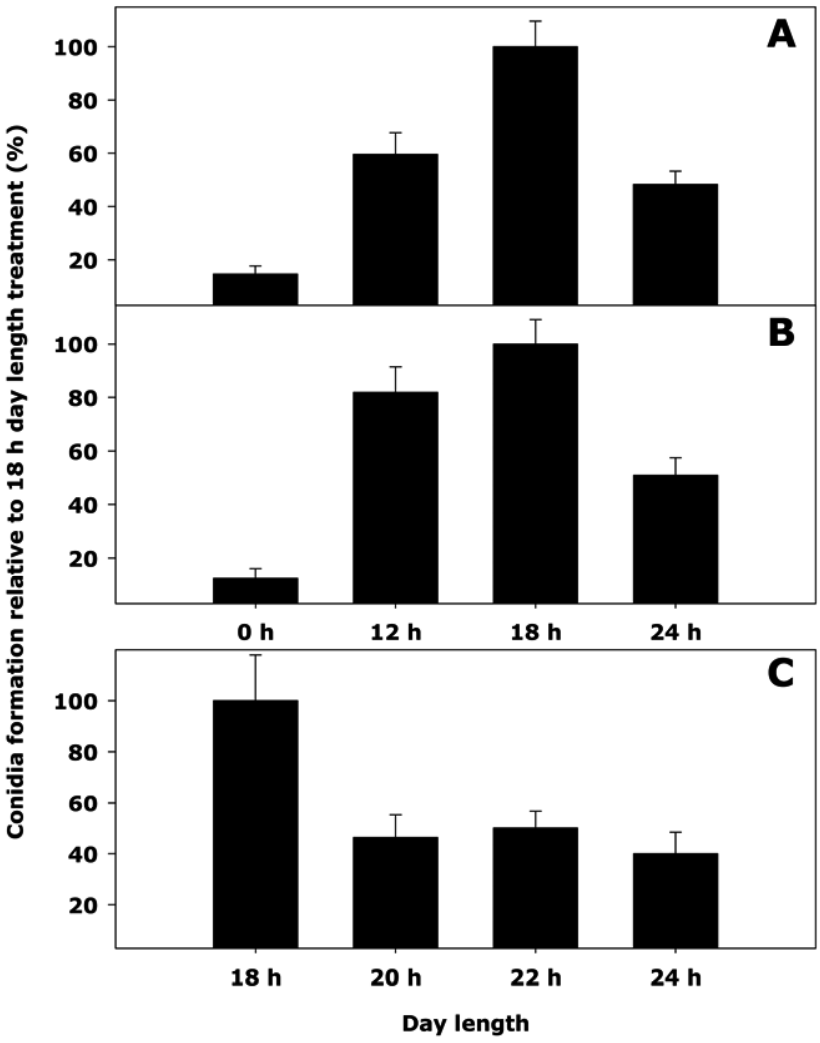

Fig. 3. Effect of day length on formation of *Podosphaera pannosa* conidia on detached leaflets of *Rosa* × *hybrida* cv. Mistral placed in double-room humidity boxes (photon flux density of light at 50 µmol m^{-2} s^{-2} with mercury lamps, 20°C, 96 ± 2% relative humidity). The boxes containing detached leaflets were kept at an 18-h day length for either **A,** 2 or **B** and **C,** 4 days after inoculation, and then transferred to the different day lengths. Samples were assessed 9 days after inoculation. Spore formation at the 18-h day length was set to 100%, and all other treatments are relative to that. Data presented are an average of six spore counts in hemocytometer (two counts per treatment per replicate). Experiments were repeated three times. Bars indicate standard error of the mean.

DISCUSSION

Our results clearly showed that day lengths of 20 to 24 h strongly suppressed powdery mildew development in roses and confirm what was earlier observed in greenhouse production of pot roses and cut roses (17,20). Furthermore, we have demonstrated that the treatment effects involved a reduction in the quantity of spores produced on mildew colonies exposed to illumination for 20 to 24 h per day, as well as reduced germination potential of conidia. Productivity of roses increases with day length and is maximized by continuous lighting (14,20). However, roses grown in continuous lighting with constant RH frequently exhibit stomatal malfunctioning (19), which reduces the keeping quality of roses due to severe water loss. Postharvest keeping quality of roses (vase life) was improved by reducing the day length to less than 24 h (H. R. Gislerød., *unpublished data*; 13). There was no additional suppression of powdery mildew when day length was increased from 20 to 24 h, indicating that a day length of 20 to 22 h would be sufficient to obtain most of the suppressive effect upon powdery mildew while avoiding the potentially deleterious effects on keeping quality.

When preparing plants for exposure to different day lengths in the wind tunnels, we kept them at either 18- or 12-h day lengths for 12 days after inoculation. If comparing the resulting spore counts after exposure to different day lengths following the 12 days of incubation time at either 18 or 12 h, the results were similar. This indicates that the high productivity of conidia and disease development at 18 h was not due to the environmental adaptation of plant and pathogen.

Our results might appear to contradict an earlier study (1) in which conidia of *P. pannosa* were not released at high RH (100%) but were released when RH was reduced independent of light or darkness (1), thereby indicating that change in RH and not light is the principle factor in liberation of conidia. However, in our experiments, RH was maintained at 80 ± 4%. Thus, fluctuations in RH were minimal and not directly comparable with treatments imposed in the foregoing study (1). *P. pannosa* forms conidia in chains, and release of conidia is dependent upon maturation of new conidia from the base of the chain. We found that no or few mature conidia were produced if detached leaves or whole plants were kept in darkness.

Adams et al. (1) suggested that liberation of conidia of *P. pannosa* involved an active release mechanism (1). However, others have proposed a passive process for *P. pannosa* (6), as well as for *Erysiphe graminis* in wheat (7,8), wherein conidial

release required minimum wind speeds of 0.6 to 2 m s^{-1}. In the present experiments, the wind speed in the canopy of the plants placed in the wind tunnels was not more than 0.06 m s^{-1} when passing through the plant canopy (i.e., one order of magnitude lower than the minimum wind speed previously reported to be necessary to release conidia). This supported the hypothesis that, if an active mechanism existed, discharge should occur in still air in response to environmental factors that trigger spore release (1).

There was a clear and significant reduction in percent germinated conidia when day length was increased from 18 to 24 h. However, the reduction was not of the magnitude observed for total spore production. Similar to reports for *P. aphanis* (syn. *Sphaerotheca macularis*; 18), the cause of strawberry powdery mildew, we found no significant reduction of conidial germination at shorter day lengths or in light or in darkness.

Fungi causing powdery mildew are obligate biotrophic pathogens, and it is difficult to distinguish between a direct effect of day length on powdery mildew or an effect of day length on physiological processes in the plant that subsequently affect the fungus. Experiments conducted in oat powdery mildew showed that length of day during the growth of the plant prior to inoculation was an important factor in determining the level of resistance. Plants grown in 16 h of day length showed higher level of adult (ontogenic) plant resistance compared with plants grown in 8 h of day length (9), and it was speculated that presence of light during the initial infection induces the host plant resistance. For barley powdery mildew (*E. graminis hordei*), nascent colonies formed significantly more haustoria at 12 h post inoculation in darkness than in light (5).

Increasing day length will increase photoassimilation in greenhouse-grown roses (20). In certain plant tissues, susceptibility to powdery mildew appears to be dependent on availability of excess carbohydrates that facilitates disease development (23). Thus, rose plants treated with continuous lighting may have a higher nutrient supply for fungal growth and development compared with the other treatments. It may then be postulated that plants treated with continuous lighting should develop more powdery mildew than at shorter day lengths. Our results gave the opposite result.

There is a constant pressure on the greenhouse industry to reduce pesticide usage. From our results and previous findings in Norway (17,20), we can now recommend increased day length as an important control measure to decrease powdery mildew in roses. This practice is currently being implemented among Norwegian rose growers. Furthermore, we have also found evidence that not only day length but also

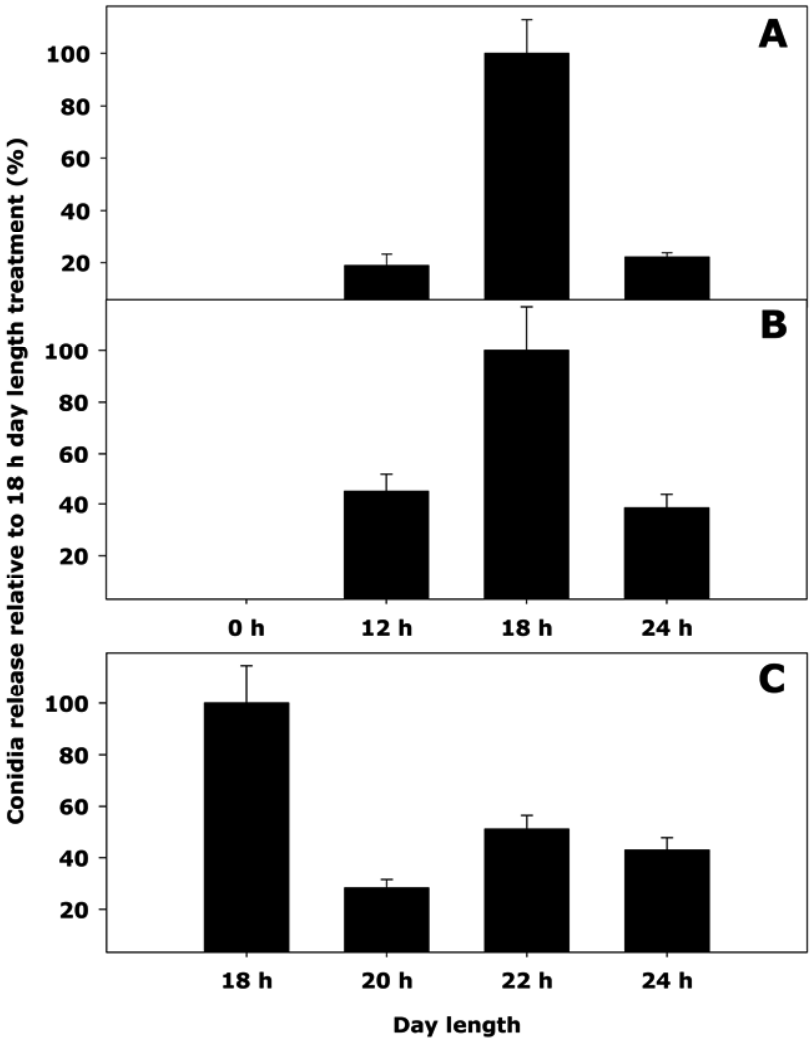

Fig. 4. Effect of day length on formation and release of *Podosphaera pannosa* conidia in whole plants of *Rosa* × *hybrida* cv. Mistral placed in a wind tunnel (photon flux density of light at 50 µmol m^{-2} s^{-2} with mercury lamps, 20°C, 80 ± 4% relative humidity). Host plants were spray inoculated and kept in either **A** and **C,** 18-h or **B,** 12-h day length for 12 days before the day-length treatments started. Values given are the percent conidia trapped on a microscope tape relative to the 18-h day length (set to 100%) during 1 week. Experiments were repeated **A,** three; **B,** four; or **C,** two times. Bars indicate standard error of the mean.

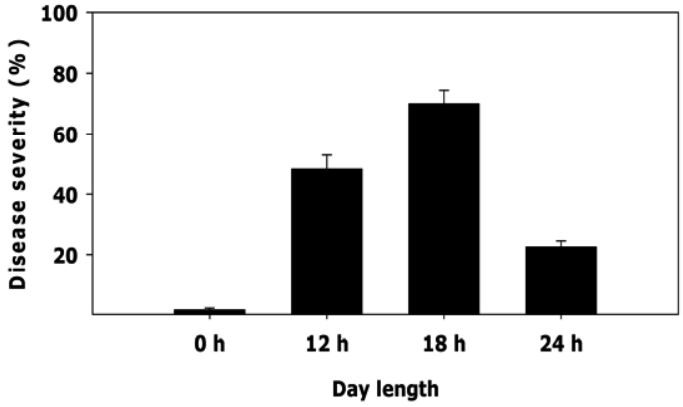

Fig. 5. Effect of day length on disease severity of powdery mildew caused by *Podosphaera pannosa* in whole plants of *Rosa* × *hybrida* cv. Mistral. Immediately after inoculation, plants were placed in growth chambers (photon flux density of light at 50 µmol m^{-2} s^{-2} with mercury lamps, 20°C, 80 ± 4% relative humidity) with 0-, 12-, 18-, or 24-h day lengths (three plants in each). Three leaves from each plants were detached 8 days after inoculation and percent diseased leaf area (severity) was scored based on visual comparison with an assessment key. Data presented are an average score of nine diseased leaves. Bars indicate standard error of the mean.

light quality strongly influence powdery mildew, and very low light intensities seem necessary to suppress the fungus (A. Suthaparan, *unpublished data*). Light-emitting diode (LED) technology is currently developing rapidly, and, in the future, it may be possible to manipulate day lengths and light qualities with low-energy LED lamps with the purpose of controlling or delaying powdery mildew epidemics. Finally, this information may also be transferred to other greenhouse crops where powdery mildew is problematic, such as cucumber and tomato.

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Paper II

Specific Light-Emitting Diodes Can Suppress Sporulation of *Podosphaera pannosa* on Greenhouse Roses

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ABSTRACT

When rose plants bearing colonies of *Podosphaera pannosa* were placed in a wind tunnel, the number of conidia trapped was directly proportional to intensity of daylight-balanced (white) light from 5 to 150 μmol m^{-2} s^{-1}. Illumination of samples using blue (420-520 nm) light-emitting diodes (LEDs) increased the number of conidia trapped by a factor of approximately 2.7 over white light, but germination of conidia under blue light was reduced by approximately 16.5% compared to conidia germination under white light. The number of conidia trapped under far-red light (> 685 nm) LEDs was approximately 4.7 times higher than in white light, and 13.3 times higher than under red (620-720 nm) LEDs, and germination was not induced compared to white light. When mildewed plants were exposed to cycles of 18 h white light followed by 6 h of blue, red, far-red light, or darkness; light from the red LEDs reduced the number of conidia trapped by approximately 88 % compared to darkness or far-red light. Interrupting the above dark period with 1 h of light from red LEDs also reduced the

number of conidia trapped, while a 1 h period of light from far-red following the 1 hr of light from red LEDs nullified the suppressive effect of red light. Our results indicate that brief exposure to red light during the dark interval may be as effective as continuous illumination in suppressing powdery mildew in greenhouse roses.

Additional keywords: Day length, *Rosa* × *hybrida*

In Northern Europe, greenhouse rose (*Rosa* × *hybrida*) production requires supplemental lighting during winter months due to short day length and relatively low natural intensity (9). Powdery mildew caused by *Podosphaera pannosa* (Wallr.: Fr.) de Bary is one of the most serious diseases of greenhouse grown roses, reducing both plant vigour and esthetic value. Rose growers in Northern Europe generally rely upon evaporated sulfur or direct applications of synthetic fungicides to control powdery mildew. Although usually effective, use of either tactic can be problematic. Many fungicides, and particularly sulfur, may interfere with natural predators deployed to control insect and mite pests (1). Furthermore, resistance to both demethylation inhibitors and strobilurines; two of the most widely used groups of synthetic fungicides used for management of rose powdery mildew, has recently been reported for *P. pannosa* (5).

We recently demonstrated that increasing the length of the day light period to 20-24 h using supplemental lighting significantly reduced both sporulation of *P. pannosa* and severity of powdery mildew in greenhouse roses (17, 18). However, cost of energy required to provide such supplemental lighting through the fixtures customarily used for plant growth could make the application of this strategy prohibitively expensive. We therefore sought to maximize the efficiency of this approach to disease management by quantifying the effects of light intensity and quality during supplemental lighting upon *P. pannosa* upon suppression of

disease, and by providing specifically suppressive wavelengths of light using low-energy light-emitting diodes (LEDs). Preliminary accounts of this work have been published (19).

MATERIALS AND METHODS

Production of host plant material. Mildew free potted miniature rose plants (*Rosa* × *hybrida* cv. Mistral) were grown in limed and fertilized peat (Floralux, Nittedal torv industri A/S, Norway) and Perlite (25 % by volume) in a greenhouse compartment to produce plant material for the experiments. Mercury lamps (Powerstar HQI-BT 400W/D day light, OSRAM GmbH, Augsburg, Germany) at photon flux densities (PFD) (400-700) of 200 µmol m^{-2} s^{-1} recorded at top of the plants, were used as supplementary light source (18 h per day). The lights were automatically turned off when natural light exceeded 200 µmol m^{-2} s^{-1}. Temperature was set at 20 ºC and RH at 80 %. Plants were exposed to evaporated sulphur for 4 h per day during night and watered with complete nutrient solution when needed. The plants were pruned to induce young active shoot growth to ensure sufficient supply of uniformly-aged mildew free leaflets**.**

Production of inoculum. Mildewed rose leaves were obtained from a commercial greenhouse located in south-eastern Norway, and the pathogen was transferred to newly unfolded detached rose leaflets as follows. Leaflets were surface sterilized as previously described (18) and were placed in Petri dishes containing water agar. The leaflets were then inoculated by touching them gently with mildewed leaves obtained from a commercial greenhouse. Detached leaflets were subsequently incubated for 7 days as described (18). Isolates were subsequently sub-cultured and transferred twice more to reduce presence of contaminating fungi (18), and were maintained by periodic transfer to disease-free plants in an isolated growth chamber. Environmental conditions in the growth chamber were as described above for production of host plant material. Inoculum for all experiments consisted

of conidia from 7-day-old cultures of powdery mildew grown on detached leaves prepared as described previously (18).

Influence of light quality on germination and formation of conidia on detached leaflets. Studies of the effect of light quality on conidial germination and sporulation *in vitro* took place in four growth chambers with 18 h day length. Chambers were equipped with light emitting diodes (LEDs) of either blue (Model- QB-2001-A), red (Model- QB-2200-A), far-red (Model- QB-2200-A) (Quantum devices, Inc., Barneveld, WI, USA) or white (full spectrum) mercury lamps (Powerstar HQI-BT 400W/D day light, OSRAM GmbH, Augsburg, Germany) with light spectrum of blue (420-520 nm, with peak of 465 nm), red (620-720 nm, with peak of 675 nm), far-red (685-780 nm, with peak of 755 nm) and full spectrum white light (Fig. 2). The PFD's were 3.6 μmol m^{-2} s^{-1} for the LED lamps and 5 μmol m^{-2} s^{-1} for the mercury lamps. Wire mesh was used to reduce the white light level from 50 to 5 μmol m^{-2} s^{-1}. Temperature was kept at 20 ± 2 ºC. Detached leaflets (abaxial surface uppermost) on water agar were kept in upper part of the dual-chamber boxes with lid. Saturated KCl salt solution in the lower part of the dual–chamber boxes provided a constant humidity in the upper chamber containing the leaflets (96 ± 2 % RH) (18).

For germination tests, three of the above dual-chamber boxes, each containing six healthy leaflets were placed in each of four growth chambers. Each chamber provided a different light quality. Immediately before they were illuminated, the leaflets were inoculated by touching them gently with source inoculum leaflets, with a separate sporulating leaflet used to apply conidia to each healthy leaflet. Four leaflets from each treatment were removed 24 h after inoculation and frozen at -2 °C until further examination. The leaflets were then prepared for microscopic observation, and germination was assessed (18). Germination was assessed for 50 conidia from each leaflet. Conidia were counted as germinated if the length of the germ tube was greater than or equal to one-half the length of the conidium.

For conidial production tests, leaflets in the above-described boxes were inoculated by placing one drop (15 µl) of a spore suspension on each leaflet. Immediately after inoculation, the humidity boxes were first kept for 4 d at 18 h day length provided by mercury lamps (same type as described above) with PFD of 50 µmol m^{-2} s^{-1}. Temperature was kept at 20 ± 2 ºC. Then three boxes were transferred to each of the four growth chambers where they were exposed to either blue, red, far red, or white light. Six leaflets with sporulating powdery mildew colonies from each treatment were randomly removed 9 d after inoculation, placed in 50 ml centrifuge tubes containing 5 ml of distilled water, and shaken by hand for 2 min. The leaflets were removed from the centrifuge tube, and conidia in the resultant suspensions were enumerated by examining three subsamples of spore suspension per tube using a haemocytometer at 200 X. The experiment was conducted two times, and the factors of lighting treatment, experimental repeat, and replicates were included in the data analysis.

Influence of light quality on formation and release of conidia on whole plants. Studies of the effects of light quality and light intensity on conidial productivity (combined effect of conidia formation and release, recorded as number of conidia trapped) took place in small wind tunnels (18) placed within four growth chambers with RH 80 ± 5 % and temperature 20 ± 2 ºC. Mercury lamps (as described above) were used as white light source. LED lamps (Affinium string kit IP 66, Philips, The Netherlands) were used as blue and red light sources. Two incandescent 60 W lamps (OSRAM, GmbH, Augsburg, Germany) with dark green filters (number 124) (Lee filters, Burbank, CA, USA) were used as far-red light source. These light sources provided the spectrum of blue (420-520 nm, with peak of 465 nm), red (575-675 nm, with peak of 630 nm), far-red (> 685 nm) and full spectrum for white light (Fig. 1). The PFD light levels of all lamps were 5 µmol m^{-2} s^{-1}. Wire mesh was used to reduce the white light level from 50 to 5 µmol m^{-2} s^{-1}.

Plants of uniform size were inoculated with one week old powdery mildew inoculum in a conidial suspension as previously described (18) at the rate of 2.5 ml for each plant. These inoculated plants were kept at 18 h day length provided by mercury lamps at 200 µmol m^{-2} s^{-1}, 20 °C and 80 % RH for 12 d. For each run of an experiment, two plants were placed in each of the wind tunnels. Conidia released from the plants in the wind tunnel were trapped on a plastic tape attached to a 24 h rotating clock cylinder with a circumference of 288 mm (rotating 4 times = 4 days), providing continuous readings of conidial formation and release (18). Lacto fuchsin was used to stain the conidia trapped on the plastic tape. The tape was scanned perpendicular to the direction of rotation at 2 mm (10 min) intervals at 200 X magnification under a compound light microscope (total of 144 transects per day). The counts from these transects were further pooled within eight 3-h periods (i.e., 0 to 0300 h, 0300 to 0600 h, 0600 to 0900 h, etc) and the factors of lighting treatment, experimental repeat, and time period were included in the data analysis.

Plants in wind tunnels were exposed to the following lighting treatments: i) all plants were provided 18 h of either blue, red, far-red or white light with PFD of 5 µmol m^{-2} s^{-1}; ii) all plants were provided 18 h white light by mercury lamps (PFD of 50 µmol m^{-2} s^{-1}) followed by 6 h of either blue, red, or far-red light at PFD of 5 µmol m^{-2} s^{-1}, or darkness; iii) all plants were exposed to 18 h white light (50 µmol m^{-2} s^{-1}) followed by 6 h darkness or with 1 h night breaks with either red, far-red, or red + far-red light at PFD of 5 µmol m^{-2} s^{-1} (Fig. 2); iv) all plants were given 18 h white light with mercury lamps, but PFDs for the different treatments were set to either 5, 50, 100 and 150 µmol m^{-2} s^{-1}.

Recording of environmental parameters. Light intensity was recorded under the top (facing the light source) of the Petri dishes or dual-chamber humidity boxes in the detached leaf experiments and on top of the plants in the whole plant experiments, and temperature and RH was recorded in the same compartments, all with instrumentation as described previously

(18). Light quality was recorded inside (at same height as for light intensity) and outside (on the top facing the light source) the Petri dish, dual-chamber humidity boxes and wind tunnels with a Spectra Wiz spectrometer Model EPP 2000 Fiberoptic (Stellarnet Inc., Tampa, FL, USA).

Statistical analysis. Minitab (version 14) was used to conduct the analysis of variance (GLM procedure) and comparisons of means (Bonferroni test at P = 0.05).

RESULTS

Influence of light quality on germination and formation of conidia on detached leaflets. The mean percentage of conidia that germinated was significantly reduced (P = 0.001) in blue light (51.75%) relative to the white light control (62 %) (Fig. 3). Germination of conidia was not reduced by red or far red light relative to the white light control (Fig. 3).

The mean number of conidia enumerated in suspension was approximately 1.3 x 10^4 per ml under white light. Far red light strongly stimulated production of conidia. The number of conidia formed on detached leaflets was 3 x 10^4 per ml when exposed to 18 h far-red light, and it was approximately 2.3 times higher (significant at P < 0.0001) compared to 18 h white, red, or blue light (Fig. 4)). No significant differences in the production of conidia were found among leaflets exposed to 18 h blue (1.1 x 10^4), red (1.3 x 10^4) and white light (Fig. 4).

Influence of light quality on formation and release of conidia on whole plants. When whole plants were exposed in wind tunnels to diurnal cycles incorporating 18 h of low intensity (PFD 5 μmol m^{-2} s^{-1}) blue, red, far-red or white light followed by 6 h of darkness, blue and far-red light significantly (P = 0.029) stimulated productivity (formation and release) of conidia compared to the white light control. Conidial productivity in red light was significantly (P <0.0001) reduced compared to the white light control (Fig. 5).

When whole plants were exposed in wind tunnels to diurnal cycles of 18 h high intensity (PFD 50 µmol m^{-2} s^{-1}) white light followed by 6 h low intensity (PFD 5 µmol m^{-2} s^{-1}) light of either blue, red, far-red, or darkness; the number of conidia trapped was significantly ($P < 0.0001$) reduced only by red light (Fig. 6). The total number of conidia trapped under 18 h high intensity white light followed by 6 h of low intensity red light treatment were 306 (mean of three experiments). There was no significant suppression of conidial productivity by darkness, far-red, or blue light, where a mean of 2429, 2518 and 1915 conidia were enumerated, respectively. A 1 h exposure to red light during the dark period was as effective as continuous light (18) in suppressing productivity of conidia (Fig. 7, $P < 0.0001$). When plants exposed to 18 h white light followed by 6 h of darkness, the total number of conidia trapped during the 4 days was 6253 (mean of three experiments), compared to a mean of 2042 conidia trapped when the night period was interrupted with 1 h of low intensity red light. Furthermore, the suppressive effect of red light was reversed by 1 h far-red light (Fig. 7), after which a mean of 5403 conidia were trapped.

When plants were supplied with 18 h of white light followed by a 6 h dark period, the number of conidia trapped in the wind tunnels was directly proportional to light intensity 5 to 150 µmol m^{-2} s^{-1} (Fig. 8). The total number of conidia trapped under a light intensity of 5 µmol m^{-2} s^{-1} was 239 (mean of three experiments) compared to 1486, 2384 and 5275 conidia trapped at respective light intensities of 50, 100 and 150 µmol m^{-2} s^{-1}. Linear regression of natural log transformed numbers of conidia trapped during 4 d against light intensity (Fig. 8) yielded an R^2 of 0.71 with a slope coefficient that was significant at $P < 0.0001$ (Fig. 8).

DISCUSSION

Rose powdery mildew is a polycyclic disease with the potential to spread rapidly, especially within greenhouse production systems. Spread of the disease depends on abundant

and nearly continuous production of conidia. Our previous research showed that rose powdery mildew could be suppressed by management of lighting in greenhouse production; specifically that sporulation of the pathogen was suppressed under continuous light (18). In the present study we have further clarified the quantity and quality of light required for suppression of sporulation, and has also illustrated which components of the visible spectrum actually stimulate sporulation.

Low energy light emitting diodes (LEDs) may provide a means of supplying mildew-suppressive lighting to roses without the potentially prohibitive cost of continuous lighting used in our earlier studies (18, 19). LED technology (4, 11) makes it possible to provide the specific spectrum of red light required to suppress sporulation, and to do so at an intensity far below the energy levels required for plant growth. Thus apart from energy costs, day extension using low energy red LEDs may be more desirable for its lower potential to interfere with plant development. While red light is important for the development of the photosynthetic apparatus, it can also induce thicker leaves and promotes axillary bud growth (10, 15), and far-red light can result in thin leaf formation, elongation of inter-nodes, and reduced axillary bud growth (2, 10). Reduced post harvest keeping quality in roses due to stomatal malfunction is also a potential side effect of continuous exposure to white light if relative humidity remains high during exposure (12). At low PFD's (*e.g.,* below 15 μ mol m^{-2} s^{-1}) red light has not affected stomatal opening or resulted in reduced stomatal conductance in previous studies (7, 21).

Reduced lesion growth and conidial formation in response to manipulation of light exposure have been reported for other powdery mildews. Reduced severity of cucumber powdery mildew caused by *Podosphaera fusca* (syn. *Sphaerotheca fusca*) was observed under polythene films that blocked far-red and UV light (6). Cucumber plants inoculated with *Podosphaera xanthii* (syn. *Sphaerotheca fuliginea*) and grown under light of different spectral

composition developed the highest number of powdery mildew colonies per leaf under broad spectrum metal halide lamps, an intermediate number of colonies under red to far-red light, or red to blue, and the lowest number of colonies under red light (16). Colonies on leaves grown under red light furthermore exhibited sparse mycelium and reduced conidiation compared to the other light treatments (16). Because powdery mildews are biotrophic, it is not possible from studies conducted thus far to separate direct effects of light upon the pathogen from those exerted through the host. Exposure to red light has been associated with increased plant resistance in other pathosystems, *e.g.* leaf spot disease of broad bean (*Vicia faba* L) caused by *Alternaria tenuissina* (14) and chocolate spot disease of broad bean caused by *Botrytis cinerea* (8). Recently it has been reported that red light induced resistance of cucumber plants against powdery mildew via higher levels of H_2O_2 and salicylic acid, and stronger expression of defense genes (20). In continued work, we are investigating pre-inoculation exposure of host plants to red light to reveal possible stimulation of host defence responses in the suppressive impact of red light. Direct effects of such lighting on fungi would indicate the presence and operation of photoreceptors. Our results wherein far-red light stimulated conidial formation and negated the effect of red light may indicate the operation of a photoreceptor system in *P. pannosa*, such as that reported for *Aspergillus nidulans* (3, 13).

Powdery mildews are among the most problematic pathogens to manage using chemical fungicides, particularly in greenhouse production systems. Resistance to benzimidazoles, demethylation inhibiting, and strobilurine fungicides were reported in several powdery mildew pathosystems within 2 to 5 years after commercial deployment of these fungicide classes (5). While the degree of disease suppression we have obtained in our preliminary studies is not comparable to the performance of the most effective fungicides in the absence of resistance, the continued availability of highly effective fungicides cannot be guaranteed. Our results provide an additional means of disease suppression that could

potentially be exploited to reduce selection for resistance to fungicides, or to use in combination with partial disease resistance and cultural practices to produce roses in systems where deployment of conventional fungicides is not an option. Manipulation of day length and light quality using modern lighting technology (4, 11, 17, 18) might also be useful to other greenhouse crops where powdery mildew may be problematic (*e.g.,* tomato, cucumber, and several ornamentals), and perhaps within selected field-grown crops.

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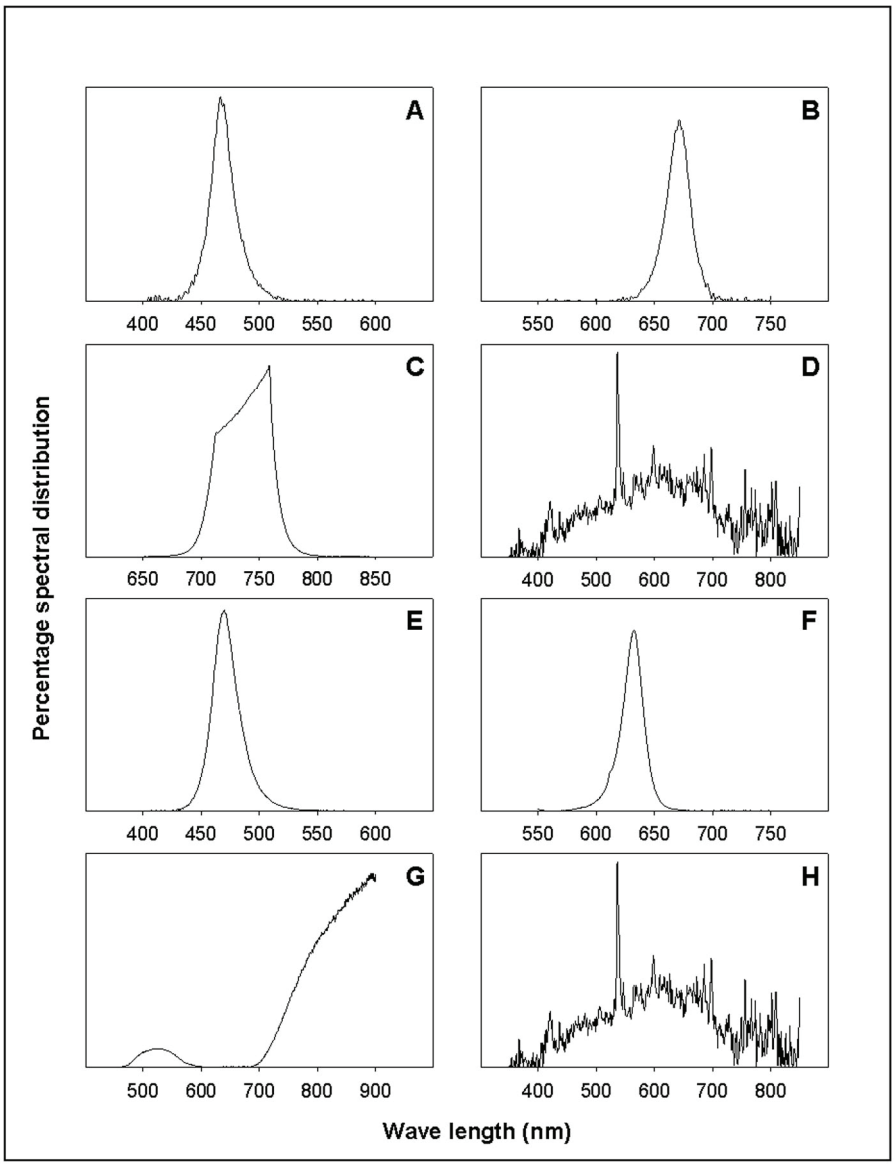

Fig. 1. Spectral distribution of blue (A), red (B), far red (C) and white (D) light sources that were used in conidia germination and formation experiments in conjunction with dual - chamber humidity boxes; and spectral distribution of blue (E), red (F), far red (G) and white (H) light sources that were used in wind tunnel experiments.

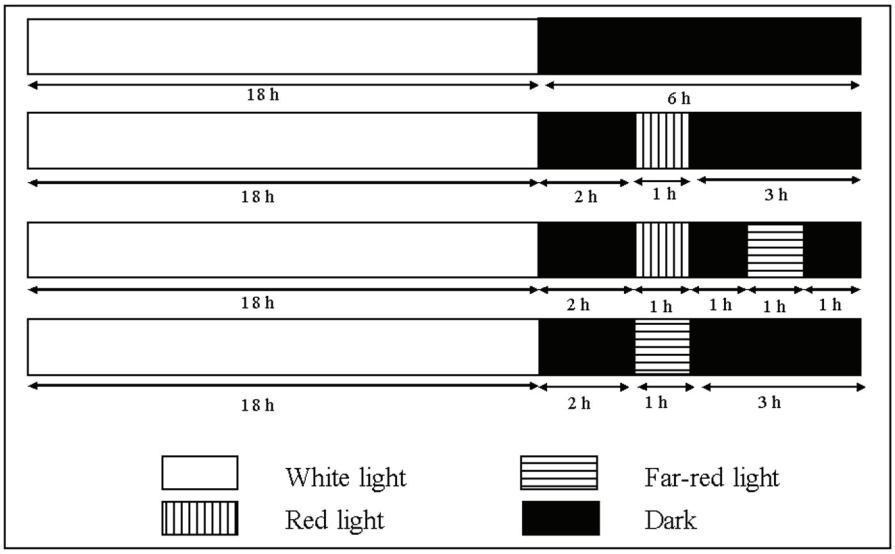

Fig. 2. Schematic illustration of short night break treatments imposed in wind tunnel studies. Plants were exposed to white light for 18 h followed by a 6 h period in which the plants were in darkness for the entire 6 h, or the dark period was interrupted by red light, red light followed by far red light, or far red light.

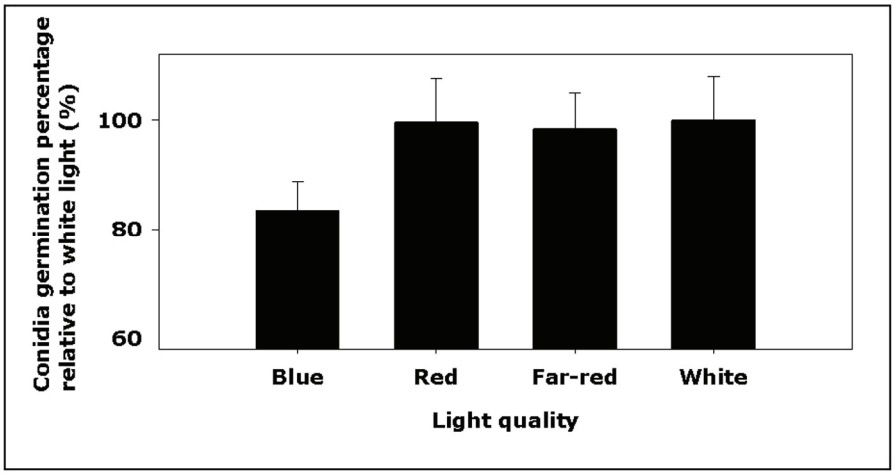

Fig. 3. Effect of light quality on germination of conidia of *Podosphaera pannosa* on detached leaflets of *Rosa* × *hybrida* cv. Mistral. Leaflets were exposed to 18 h of blue, red, far-red or white light followed by 6 h darkness (mean of 2 repeated experiments and 4 leaflets in each). Treatment means are reported as percentage of germination relative to the white light control. Bars indicate one standard error of the mean.

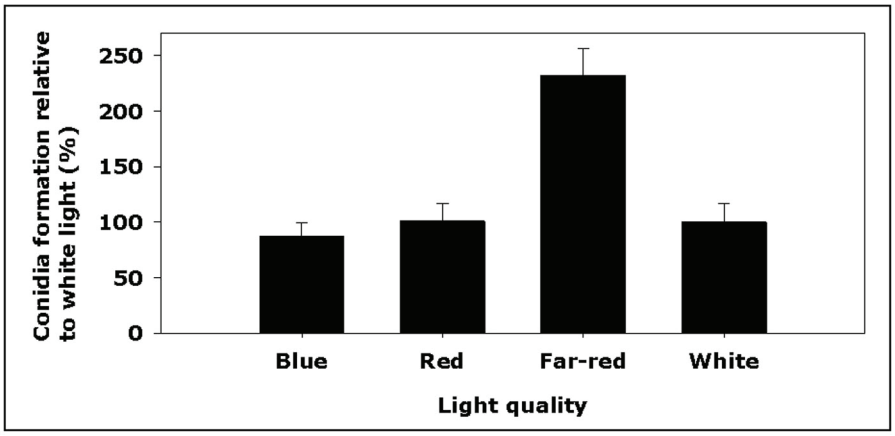

Fig. 4. Effect of light quality on formation of conidia by *Podosphaera pannosa* on detached leaflets of *Rosa* × *hybrida* cv. Mistral. Leaflets were inoculated and then exposed to 18 h of white light per 24 h for 4 d, and were then transferred to cycles of 18 h with either blue, red, far-red or white light followed by 6 h darkness. Conidia were innumerated 9 d after inoculation (mean of two repeated experiments). Treatment means are reported as percentages relative to white light control. Bars indicate one standard error of the mean.

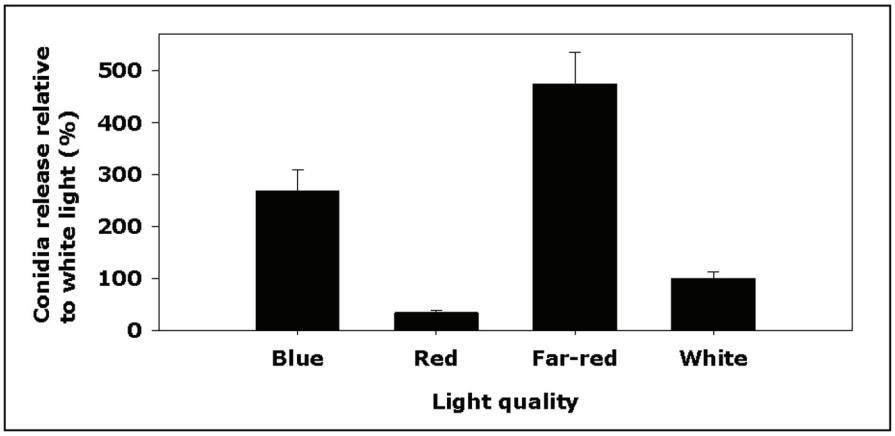

Fig. 5. Effect of light quality on conidia productivity (formation and release) of *Podosphaera pannosa* on whole plants of *Rosa* × *hybrida* cv. Mistral in wind tunnels. Inoculated plants (kept at 18 h day length) bearing 12-day-old colonies were exposed to 18 h of blue, red, far-red or white light followed by 6 h darkness for 4 d (experiment was repeated three times). Treatment means are reported as percentages relative to the white light control. Bars indicate one standard error of the mean.

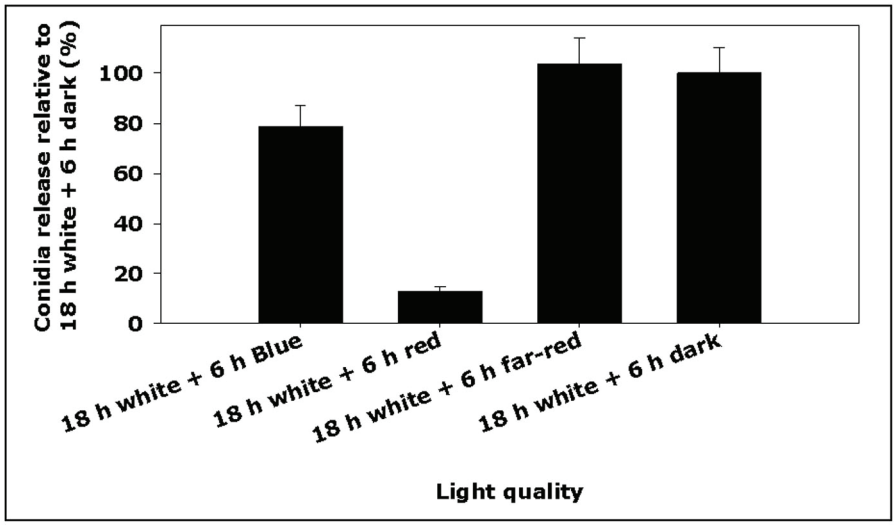

Fig. 6. Effect of extension of day-length using different light qualities on productivity (formation and release) of conidia by *Podosphaera pannosa* on whole plants of *Rosa* × *hybrida*. Inoculated plants (kept at 18 h day length) bearing 12-day-old colonies were exposed to 4 cycles (days) of 18 h white light followed by 6 h of either blue, red, far-red light or darkness in wind tunnels (experiment was repeated three times). Treatment means are reported as percentages relative to 18 h white light + 6 h darkness control. Bars indicate one standard error of the mean.

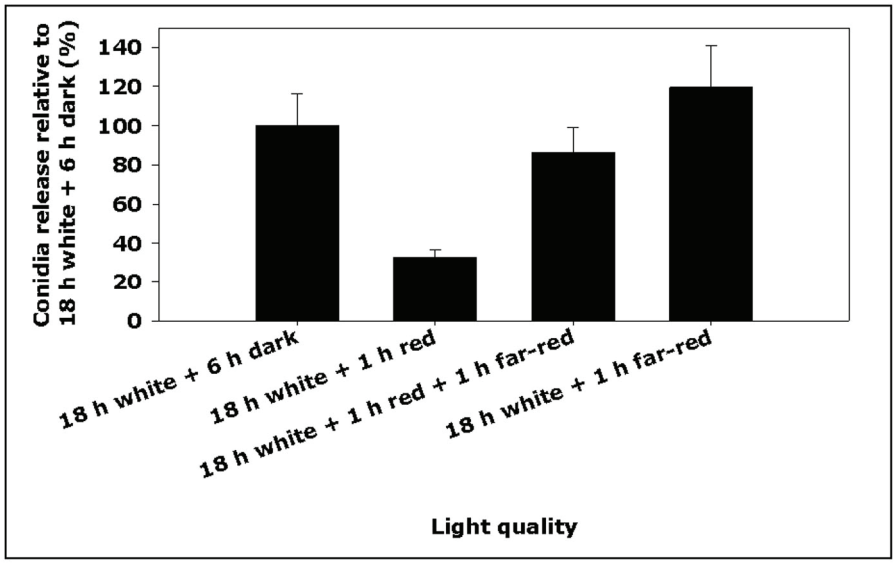

Fig. 7. Effect of brief illumination during a dark period on productivity of conidia by *Podosphaera pannosa* on whole plants of *Rosa* × *hybrida.* Plants (kept at 18 h day length) bearing 12-day-old mildew colonies were exposed to white light in a wind tunnel for 18 h per day followed by a 6 h dark period for 4 days. The dark period was interrupted by either 1 h of red light, 1 h of far- red light, or 1 h of red light followed by 1 h of far- red light (experiment was repeated three times). Treatment means are reported as percentages relative to the 18 h white light + 6 h dark control. Bars indicate one standard error of the mean.

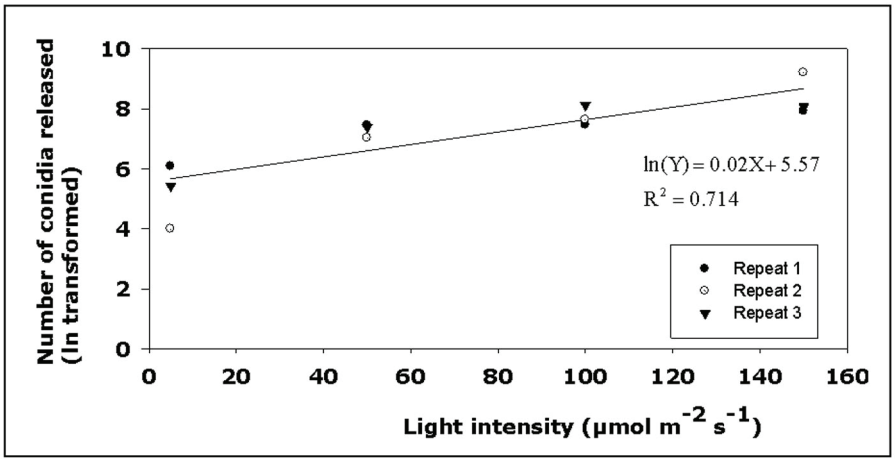

Fig. 8. Effect of light intensity on productivity of conidia by *Podosphaera pannosa* on whole plants of *Rosa* × *hybrida* in a wind tunnel. Plants bearing 12-day-old mildew colonies were exposed to white light at various intensities for 18 h per day followed by 6 h of darkness for 4 days (experiment was repeated three times). Treatment means were transformed to natural logs before regression.

Paper III

A Molecular Genetic Approach to Identify Possible Phytochrome-like Photoreceptors in *Podosphaera pannosa*

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ABSTRACT

A molecular genetic investigation was performed to possibly identify phytochrome-like genes in *Podosphaera pannosa*, the causal agent of rose powdery mildew. Genomic DNA was extracted, and the target gene(s) were tried amplified using degenerate primers. DNA sequences of cloned PCR products were analysed, and existing sequences available at The National Centre for Biotechnology Information (NCBI) searched for similarities to the translated nucleotide sequence using the software tblastx. There were no similarities of the amplified gene sequences with phytochrome protein sequences available in NCBI. Use of more specific primers with gradient annealing temperatures for PCR amplification, proteomic analysis, or genome or transcriptome sequence analysis may give better outcomes.

Light is an important environmental cue for living organisms, providing sensory information to adapt changing environmental conditions. This light signal transduction process is mediated by single or various combinations of sensory photoreceptor molecules. In higher plants, three classes of signal transducing photoreceptors have been well characterized with molecular details and its functional analysis (12). They include phytochromes (red / far red), cryptochromes (blue / UV- A), phototropins (blue / UV- A) and UV-B photoreceptors (1, 8).

Phytochrome was the first photo sensory receptor (sensor pigment) family identified nearly 50 years ago in higher plants (12). It consists of protein and pigment. Molecular genetics have revealed the existence of several genes encoding the protein portion of the

phytochrome and one particular organism may have more than one phytochrome. Five members of phytochrome genes (*PHYA, PHYB, PHYC, PHYD* and *PHYE*) have been reported in the dicot *Arabidopsis* and three members of phytochrome genes (*PHYA, PHYB* and *PHYC*) are found in the monocot rice (6).

Initially phytochromes were thought to be confined to photosynthetic organisms including cyanobacteria. More recent studies have proved the presence of phytochrome related genes in representative organisms from all forms of life on earth except Archaea (7).

The extended phytochrome super family can be categorized to five families, i.e. plant phytochromes (Phys), cyanonobacterial phytochromes (Cphs), bacteriophytochrome photo receptors (BphPs), the fungal phytochromes (Fphs) and a collection of phytochrome-like sequences without apparent relationships (5).

Most of the work on the phytochrome system in fungi has been related to *Aspergillus* and *Neurospora* spp. Two phytochrome coding genes, *PHY-1* and *PHY-2* were identified in *N. crassa*, but the function of these phytochromes remains unclear (4). Conidiation of *A. nidulans* was less in either blue or red light compared to white light, but the combination of red and blue restored the conidia production similar to white light (10). Deletion of the single phytochrome gene *FphA* of *A. nidulans*, resulted in loss of red light inhibition of cleistothecium formation, and this was the first function assigned to a specific fungal phytochrome (2).

Fungi causing powdery mildew in a wide range of plant species belong to the same phyllum (Ascomycota) as *A. nidulans* and *N. crassa*. Light regulation of powdery mildew biology is however rarely reported. Powdery mildew in cucumber caused by *Podosphaera xanthii* was low in red light, high under red to blue light, or red to far-red light, and highest under full spectrum white light (11). *Podosphaera pannosa*, the causal agent of rose powdery mildew showed clear day length response on conidia formation and release (13, 14). In addition, far-red light induced while red light greatly reduced conidia formation and release (15). It was also observed that, red followed by far-red light, almost neutralized the effect of red light on conidial release (15). From this, it was speculated that the *P. pannosa* may have photoreceptors that regulate conidia formation and release process. However, very little is known about photoreceptors, especially regarding phytochrome function in fungi which belong to the family Erysiphaceae. In this study, we initiated a molecular genetic investigation of phytochrome function in *P. pannosa*.

MATERIALS AND METHODS

DNA extraction, PCR sequencing. Genomic (whole cell) DNA was extracted from *P. pannosa*. Pot roses of cv. Mistral were grown and inoculated with powdery mildew as described earlier (14). Rose leaflets with powdery mildew were placed in 50 ml centrifuge tubes, with 25 ml of distilled water added, followed by gentle hand shaking for 1 min. This process was repeated several times to get enough fungal tissue into the water, without contaminating with plant tissue. The leaflets were removed carefully and resultant suspension that contained conidia and mycelium were transferred to 1.5 ml micro centrifuge tubes. These tubes were centrifuged for 6 min. at 6000 rpm. The supernatant was removed carefully by pipeting, and samples were mixed together in one single tube. The sample was used to extract genomic DNA using the DNeasy plant mini kit (Qiagen, Valencia, CA, USA) according to the manufacturer's description.

For amplification of target gene, five pairs of degenerate primers (Table 1) were designed based on the most conserved regions of known fungal phytochrome protein sequences (NCBI), aligned by ClustalW (MegAlign version 5.05, Lasergene Sequence Analysis Software, DNASTAR, Madison, WI, USA) (Fig. 1). For this, phytochrome protein sequence data published in NCBI database was retrieved. The selected conserved protein regions were decoded as nucleotides by using the standard nucleotide symbol (Table 3). Various combinations (twenty) of primers were used in the attempt to amplify the gene of interest. Polymerase Chain Reaction (PCR) was conducted in 50 µl volumes with Taq DNA polymerase of 1 µl (New England BioLabs; Ipswich, England). The reaction mixture contained 39 µl of Milli Q water, 5 µl of ThermoPol reaction buffer (10 x) supplied with Taq DNA polymerase, 1 µl of dNTPs (10 mM), forward and reverse primers of 1 µl in each (10 µM) and 2 µl of DNA template. PCR thermal cycling conditions were as follows. An initial denaturing step for 1 min. at 94 °C followed by 40 sec. at 50 °C for annealing and 1 min. at 72 °C for extension. This cycle was repeated 39 more times followed by a final extension of 72 °C for 10 min.

PCR products were separated in 1.5 % agarose gel electrophoresis in 0.05 % TBE buffer. The concentrated TBE (x 10) stock solution contained Tris base 108 g, boric acid 55 g, 40 ml of 0.5 M EDTA (pH 8), 8.3 g of $Na_2EDTA.2\ H_2O$ per liter. PCR products stained with Ethidium bromide were visualized under UV illumination on GelDoc EQ (Bio-Rad Laboratories Inc., Hercules, CA, USA). Size of the PCR products (band size) were determined using 1 kb DNA ladder (New England BioLabs; Ipswich, England) as a marker.

Based on this, four primer pairs (Table 2) were chosen, and PCR was performed again as mentioned above.

Fresh PCR products were placed on ice and 1 µl of Taq DNA polymerase were added to each tube and mixed well. Then tubes were incubated at 72 °C for 10 min. PCR products were then cloned using TA TOPO cloning kit (Invitrogen, Carlsbad, CA, USA) according to the manufacturer's instruction. Each transformation reaction was spread on pre-warmed selective plates. Plates were incubated overnight at 37 °C. From each transformation reaction, 8 white colonies were picked by tooth picks, resuspended in 10 µl TE buffer and 2 µl of which used directly as template in a new PCR to amplify the cloned inserts with the M 13 forward and M 13 reverse primer pair.

Two µl of PCR samples were used for gel electrophoresis as mentioned above and bands with the desired size for sequencing were identified when compared to the 100 bp DNA ladder. Using this strategy, twelve samples were chosen (Fig. 2), cleaned with ExoSAP-IT (USB Corporation, Cleveland, Ohio, USA) according to the manufacturer's protocol, and sequenced.

Sequence analysis. DNA sequences were analysed with the SeqMan TM II 4.03 (Lasergene Sequence Analysis Software, DNASTAR). Sequence data were used to search for homologies to sequences present in the NCBI database (http://www.ncbi.nlm.nih.gov/) using the program tblastx, and the Pfam database (pfam.sanger.ac.uk) was also searched for homologies.

RESULTS AND DISCUSSION

The gene fragments that were amplified by PCR had no similarities with any phytochrome protein sequences available in NCBI or the Pfam database. We used degenerate primers in our attempt to amplify the target gene. It was difficult to find the most conserved regions from known fungal phytochrome nucleotide sequences alignment. Use of degenerate primers can greatly reduce the specificity of the PCR amplification. Design of even more specific primers and amplification of the target region with gradient annealing temperatures may give better outcomes. However, sequence information on phytochromes is limited and most phytochrome proteins will also share structures with other types of proteins, making them nontrivial to detect. The Phytochrome domain (PF00360) in the Pfam database is one of the few characteristics of phytochromes known. This domain has been found in 1467 proteins, divided between 957 species. Only 11 of these proteins are from fungi, while 133 are from bacteria and the rest from plants. Hence, very little is known about the DNA sequences of

phytochrome encoding genes in fungi, especially none from powdery mildew causing fungi. The above mentioned 11 proteins having the Pfam phytochrome domain, all give rather poor (but significant) hits, when aligned to the Pfam Hidden Markov Model for this domain, indicating that, phytochrome in fungi is slightly different from phytochrome in plants. Designing primers suitable to detect phytochrome DNA in fungi by PCR is correspondingly difficult. An alternative option for unraveling the secret behind the light-response in this fungus lies in the sequencing of its genome. Currently available low cost sequencing technologies - e.g. 454 technology from Roche (http://www.454.com/) or Solexa technology from Illumina (http://www.solexa.com/), could make this feasible (3).

Another strategy to characterize the phytochrome function in powdery mildew fungi are through assessment of mRNA produced in fungi under different light quality treatments. This mRNA could be used to construct subtractive expression libraries and the sequences differentially expressed clones could be compared with phytochrome sequences available in NCBI and other sequence databases. This strategy has previously successfully been used to isolate genes expressed during known growth conditions or certain developmental stages (9). Proteomic analysis using mass spectrometry would be third alternative. These alternative strategies might help to confirm the presence of phytochrome in fungi.

On the other hand the response of fungi to red and far-red light could be due to the different level of resistance induced in host plants by light. So identifying induced resistance and its level might be an alternative option to better understand the role of light in rose powdery mildew.

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Table. 1. Degenerate primer pairs used for the initial test PCR amplification of the phytochrome gene in *P. pannosa* DNA.

Primer	Sequence (5'→ 3')
Phy 540 F1	TAY YTD CGB GCA ATG TCN CC
Phy 540 F2	TAY YTD CGB GCC ATG TCN CC
Phy 490 F1	TTY CCN GCH TCN GAC ATH CC
Phy 490 F2	TTY CCN GCH TCN GAT ATH CC
Phy 830 R1	YTC BAG DGC HAT TTC NAG
Phy 830 R2	YTC BAG DGC HAT CTC NAG
Phy 850 R1	ACR TAD ATS ARD GAT TTN GA
Phy 850 R2	ACR TAD ATS ARD GAC TTN GA
Phy 540 R1	GGN GAC ATK GCV CGH ARA TA
Phy 540 R2	GGN GAC ATK GCV CGH ARG TA

Table. 2. Degenerate primer pairs used for final test PCR amplification of the gene of interest from genomic DNA of *P. pannosa*.

Phy 540 F2 + Phy 850 R2
Phy 490 F1 + Phy 540 R1
Phy 540 F1 + Phy 830 R1
Phy 540 F1 + Phy 850 R1

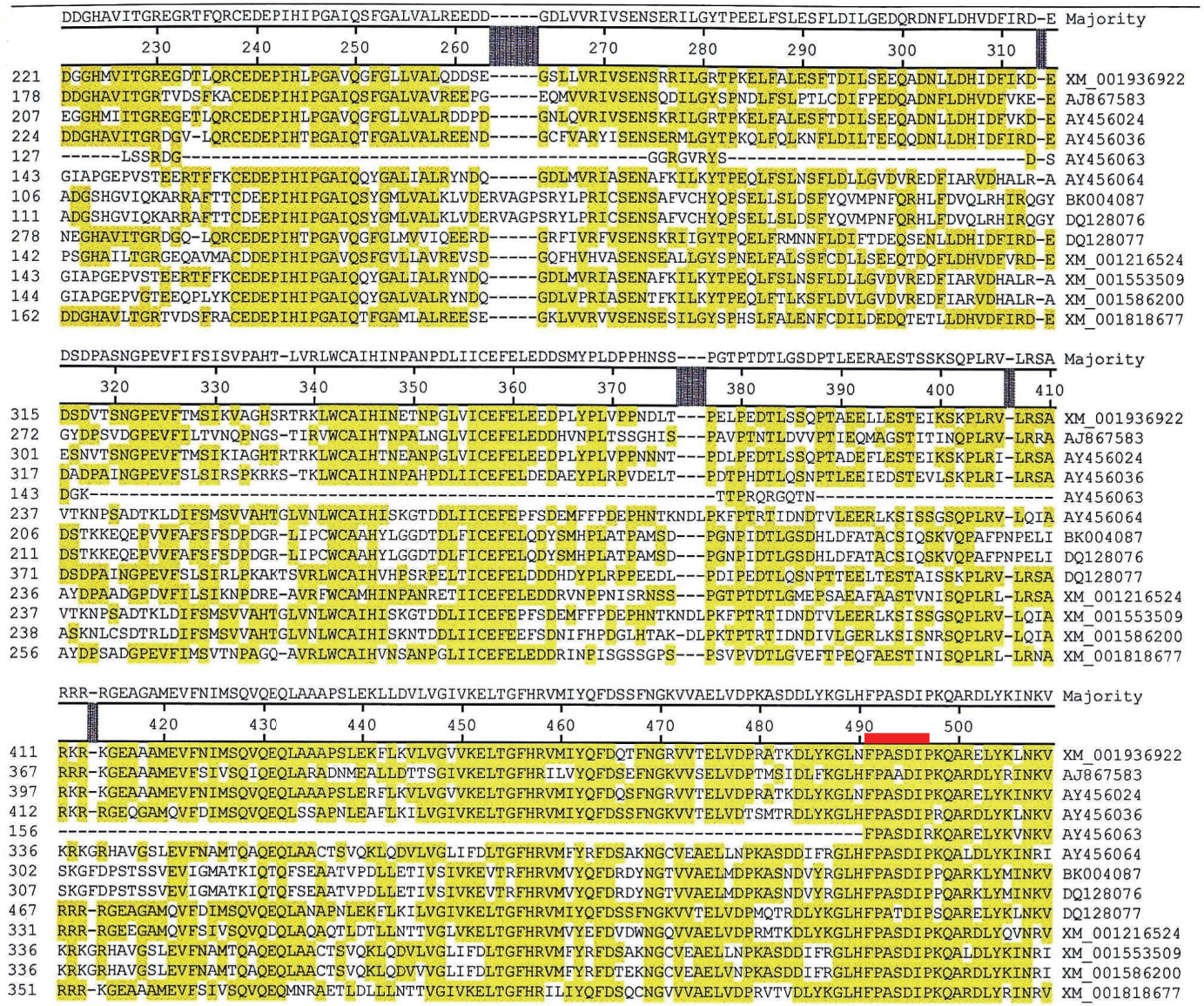
Table. 3. The standard nucleotide symbols used to design the degenerate primers.

Symbol	Specification
A	Adenine
C	Cytocine
G	Guanine
T	Thymine
M	A + C
R	A + G
W	A + T
S	C + G

Y	C + T
K	G + T
V	A + C +G
H	A + C + T
D	A + G + T
B	C + G + T
N	A + C + G + T

Source. http://www.medprobe.no/no/default.plshowArticle=241&pageId=172

Fig. 1. Alignment report of the known fungal phytochrome (like) protein sequences of *Pyrenophora tritici* (XM_001936922), *Emericella nidulans* (AJ867583), *Cochliobolus heterostrophus* (AY456024), *Gibberella moniliformis* (AY456036), *Botryotinia fuckeliana* (AY456063), *B. fuckeliana* (AY456064), *Neurospora crassa* (BK004087), *N. crassa* (DQ128076), *N. crassa* (DQ128077), *Aspergillus terreus* (XM_001216524), *B. fuckeliana* (XM_001553509), *Sclerotinia sclerotiorum* (XM_001586200), *A. oryzae* (XM_001818677). Yellow shade shows the exact match between the sequences. Red lines indicate the conserved regions used to design degenerate primers in Table. 1.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230 240 250 260 270 280 290 300 310
221 DGGHMVITGREGDTLQRCEDEPIHLPGAVQGFGLLVALQDDSE-----GSLLVRIVSENSRRILGRTPKELFALESFTDILSEEQADNLLDHIDFIKD-E XM_001936922
178 DDGHAVITGRTVDSFKACEDEPIHIPGAIQSFGALVAVREEPG-----EQMVVRIVSENSQDILGYSPNDLFSLPTLCDIFPEDQADNFLDHVDFVKE-E AJ867583
207 EGGHMIITGREGETLQRCEDEPIHLPGAVQGFGLLVALRDDPD-----GNLQVRIVSENSKRILGRTPKELFALESFTDILSEEQADNLLDHIDFVKD-E AY456024
224 DDGHAVITGRDGV-LQRCEDEPIHTPGAIQTFGALVALREEND-----GCFVARYISENSERMLGYTPKQLFQLKNFLDILTEEQQDNLLDHIDFIRD-E AY456036
127 ------LSSRDG----------GGRGVRYS----------D-S AY456063
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111 ADGSHGVIQKARRAFTTCDEEPIHIPGAIQSYGMLVALKLVDERVAGPSRYLPRICSENSAFVCHYQPSELLSLDSFYQVMPNFQRHLFDVQLRHIRQGY DQ128076
278 NEGHAVITGRDGQ-LQRCEDEPIHTPGAVQGFGLMVVIQEERD-----GRFIVRFVSENSKRIIGYTPQELFRMNNFLDIFTDEQSENLLDHIDFIRD-E DQ128077
142 PSGHAILTGRGEQAVMACDDEPIHIPGAVQSFGVLLAVREVSD-----GQFHVHVASENSEALLGYSPNELFALSSFCDLLSEEQTDQFLDHVDFVRD-E XM_001216524
143 GIAPGEPVSTEERTFFKCEDEPIHIPGAIQQYGALIALRYNDQ-----GDLMVRIASENAFKILKYTPEQLFSLNSFLDLLGVDVREDFIARVDHALR-A XM_001553509
144 GIAPGEPVGTEEQPLYKCEDEPIHIPGAIQQYGALVALRYNDQ-----GDLVPRIASENTFKILKYTPEQLFTLKSFLDVLGVDVREDFIARVDHALR-A XM_001586200
162 DDGHAVLTGRTVDSFRACEDEPIHIPGAIQTFGAMLALREESE-----GKLVVRVVSENSESILGYSPHSLFALENFCDILDEDQTETLLDHVDFIRD-E XM_001818677
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320 330 340 350 360 370 380 390 400 410
315 DSDVTSNGPEVFTMSIKVAGHSRTRKLWCAIHINETNPGLVICEFELEEDPLYPLVPPNDLT---PELPEDTLSSQPTAEELLESTEIKSKPLRV-LRSA XM_001936922
272 GYDPSVDGPEVFILTVNQPNGS-TIRVWCAIHTNPALNGLVICEFELEDDHVNPLTSSGHIS---PAVPTNTLDVVPTIEQMAGSTITINQPLRV-LRRA AJ867583
301 ESNVTSNGPEVFTMSIKIAGHTRTRKLWCAIHTNEANPGLVICEFELEEDPLYPLVPPNNNT---PDLPEDTLSSQPTADEFLESTEIKSKPLRI-LRSA AY456024
317 DADPAINGPEVFSLSIRSPKRKS-TKLWCAIHINPAHPDLIICEFELDEDAEYPLRPVDELT---PDTPHDTLQSNPTLEEIEDSTEVLSKPLRI-LRSA AY456036
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371 DSDPAINGPEVFSLSIRLPKAKTSVRLWCAIHVHPSRPELTICEFELDDDHDYPLRPPEEDL---PDIPEDTLQSNPTTEELTESTAISSKPLRV-LRSA DQ128077
236 AYDPAADGPDVFILSIKNPDRE-AVRFWCAMHINPANRETIICEFELEDDRVNPPNISRNSS---PGTPTDTLGMEPSAEAFAASTVNISQPLRL-LRSA XM_001216524
237 VTKNPSADTKLDIFSMSVVAHTGLVNLWCAIHISKGTDDLIICEFEPFSDEMFFPDEPHNTKNDLPKFPTRTIDNDTVLEERLKSISSGSQPLRV-LQIA XM_001553509
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256 AYDPSADGPEVFIMSVTNPAGQ-AVRLWCAIHVNSANPGLIICEFELEDDRINPISGSSGPS---PSVPVDTLGVEFTPEQFAESTINISQPLRL-LRNA XM_001818677
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420 430 440 450 460 470 480 490 500
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RLLYDRDQETARLVCRTAEDLEVPLDLTHAYLRAMSPIHLKYLANMGVRSSMSISINAFGDLWGLIACHSYGPHGMRVSFPIRKMCRLIGDTASRNIERL Majority
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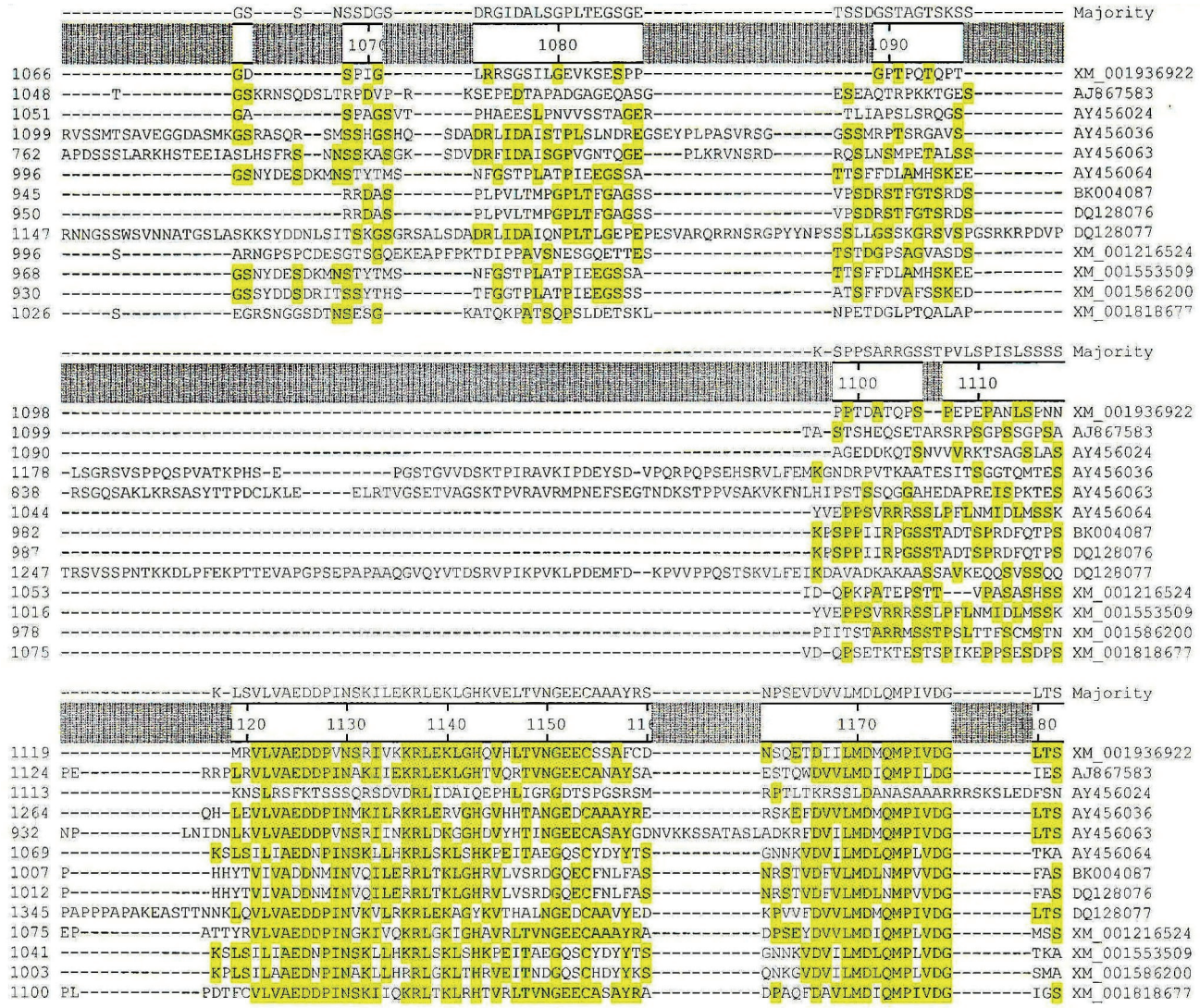
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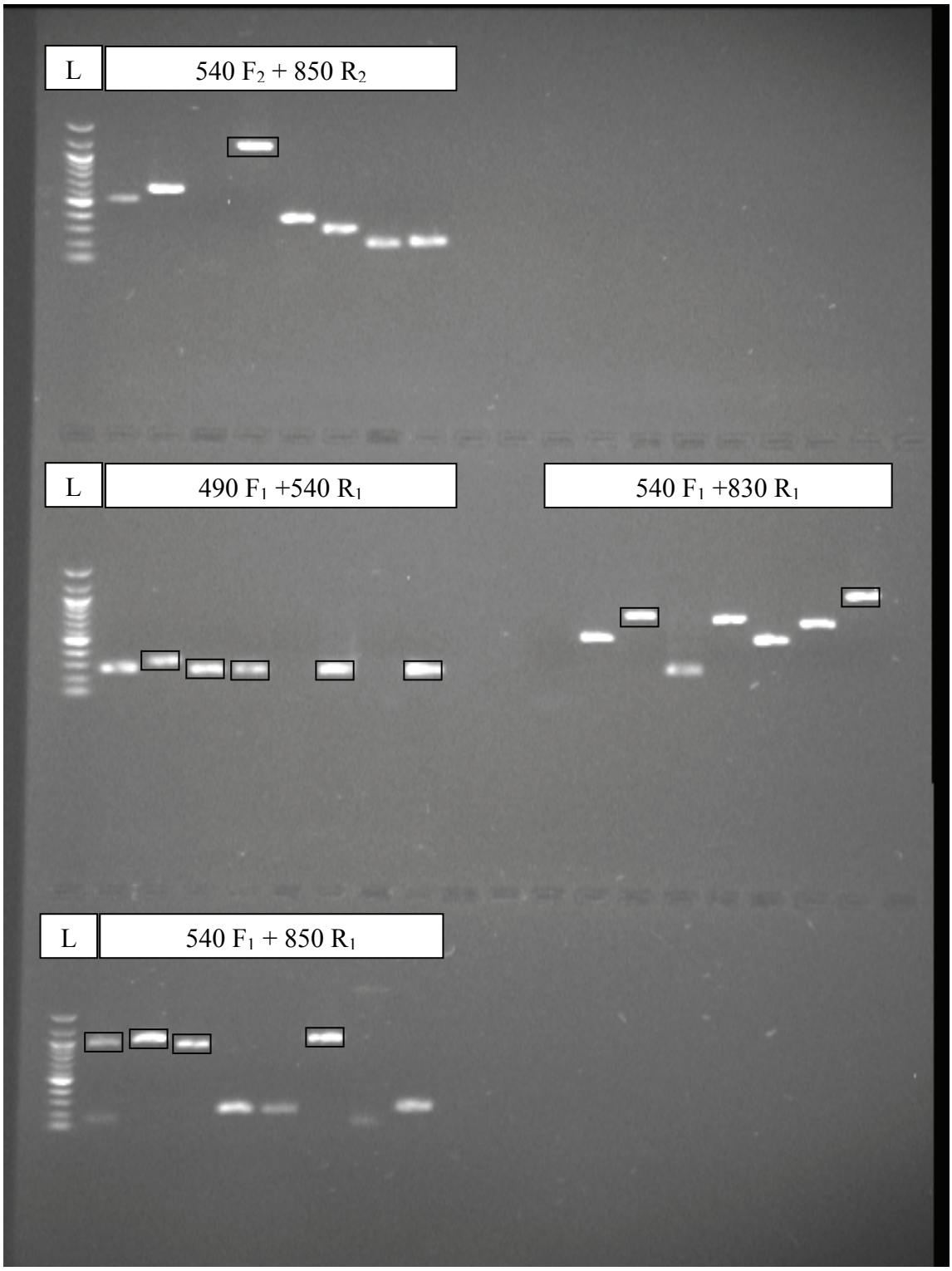

Fig. 2. Gel picture illustrating the cloned gene of interest from the plasmid vector, which were PCR amplified with M 13 F and M 13 R primers. Band size was detected by 100 bp DNA ladder (L) and twelve bands of interest were chosen for sequencing.

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