Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Fakulteten för landskapsplanering, trädgårds- och jordbruksvetenskap

Biological control of powdery mildew in greenhouse produced cucumber

– An evaluation of two microbiological control agents

Biologisk bekämpning av mjöldagg i växthusodlad gurka

– En utvärdering av två mikrobiologiska preparat

Anna-Carin Almqvist

Självständigt arbete vid LTJ-fakulteten, SLU
Examensarbete 30 hp, Hortonomprogrammet, Alnarp 2012

Sveriges lantbruksuniversitet
Swedish University of Agricultural Sciences

Biological control of powdery mildew in greenhouse produced cucumber – An evaluation of two microbiological control agents

Biologisk bekämpning av mjöldagg av i växthusodlad gurka – En utvärdering av två mikrobiologiska preparat

Anna-Carin Almqvist

Handledare: *Margareta Hökeberg,* SLU, Centre for Biological Control (CBC), Department of Forest Mycology and Plant Pathology

Btr handledare: *Erik Andreasson,* SLU, Department of Plant Protection Biology, & *Mira Rur*, SLU, Department of Plant Protection Biology

Examinator: *Birgitta Rämert*, SLU, Department of Plant Protection Biology

Kurstitel: Examensarbete inom hortonomprogrammet
Kurskod: EX0547
Omfattning: 30 hp
Nivå: E
Fördjupning: A2E

Program/utbildning: Hortonomprogrammet
Examen: Hortonomexamen
Ämne: Trädgårdsvetenskap

Serienamn: Självständigt arbete vid LTJ-fakulteten, SLU
Utgivningsort: Alnarp
Utgivningsmånad och -år: December 2012
Elektronisk publicering: http://stud.epsilon.slu.se

Omslagsbild: *Anna-Carin Almqvist*

Nyckelord: *Powdery mildew, cucumber, Golovinomyces cichoracearum, Podosphaera xanthii, biological control, Ampelomyces quisqualis, Pythium oligandrum*

Ansvarig institution/motsvarande:
SLU, Sveriges lantbruksuniversitet
Fakulteten för Landskapsplanering, trädgårds- och jordbruksvetenskap
Område Växtförädling och bioteknik

Medansvarig institution/motsvarande:
Område Växtskyddsbiologi
Område Hortikultur
Område Agrosystem
Område Arbetsvetenskap, ekonomi och miljöpsykologi

Acknowledgments

First of all I wish to thank my supervisor, Margareta Hökeberg, assistant supervisors, Erik Andreasson and Mira Rur, and examiner, Birgitta Rämert, for all help during this project. Special thanks to Tomas Isberg at Sånnagården, Kvidinge, for material and practical help. I also wish to thank Erland Liljeroth for help with identifications of the powdery mildew in the experiment and also Jan-Eric Englund for help with the statistical analysis. Finally, I wish to thank all further persons who helped me during this project. This study was performed as a part of the SLF financed project Bio-evaluation.

Anna-Carin Almqvist

Alnarp 2012

ABSTRACT

Powdery mildew is a serious problem in both field and greenhouse cucumber production and can cause serious infection and radically reduced yields. Today, powdery mildew is the main disease in greenhouse produced cucumber.

Disease control should preferably be both efficient and environmentally friendly, which is not easy to achieve at all times. Alternatives to chemical control of powdery mildews are biological control, physical control, non-fungicide control and cultivation of resistant or tolerant varieties. Biological control can be described as the suppression of damaging activities inflicted of a harmful organism by one or more other organisms, which are called antagonists or natural enemies. In Sweden, there are currently no registered biological control products available to control cucumber powdery mildew.

The aim of this study was to investigate the effect of two biological control products in order to evaluate their ability to prevent or reduce powdery mildew infection to an acceptable level in greenhouse produced cucumber. In the experiments, different application regimes were also studied. The active organisms in both tested products are fungi. In one of the products the active organism was *Ampelomyces quisqualis* and in the other *Pythium oligandrum.* The fungal species causing the powdery mildew in the experiments was characterized.

The present report consists of a literature study, an experimental part and a microscope study. Trials were performed in a greenhouse at a conventional cucumber grower's site at Sånnagården, Kvidinge, and in a chamber in one of the greenhouses at the Swedish University of Agricultural Sciences, Alnarp.

In this study, some of the biocontrol treatments could prevent and reduce powdery mildew attacks to an acceptable level in greenhouse produced cucumber. The effect of using the biocontrol agents prophylactically was significantly better with *P. oligandrum* compared to the untreated control but not with *A. quisqualis.* The effect of using *A. quisqualis* with an application interval of fourteen days was significantly better compared to a seven days application interval. For *P. oligandrum,* there was no difference between the two application intervals. The fungus causing the powdery mildew symptoms in this experimental study was most probably *Golovinomyces cichoracearum.*

SAMMANFATTNING

Både på friland och i växthus är mjöldagg ett stort problem i gurkodling. Mjöldagg kan skapa allvarliga sjukdomsangrepp och kraftigt reducera skörden. Idag är mjöldagg det huvudsakliga växtskyddsproblemet i växthusodlad gurka.

Det är viktigt att sjukdomsbekämpningen både är effektiv och miljövänlig även om de inte alltid går att kombinera. I gurkodling finns det flera alternativ till kemisk bekämpning för att bekämpa mjöldagg. Exempel på dessa är biologisk bekämpning, fysikalisk bekämpning, fungicid-fri bekämpning samt att odla resistenta eller toleranta sorter. Biologisk bekämpning kan beskrivas som den begränsning av en skadlig organisms aktivitet som orsakas av en eller flera andra organismer, där de senare benämns antagonister eller naturliga fiender. I Sverige finns det i dagsläget inga tillåtna biologiska bekämpningsmedel för att bekämpa mjöldagg i gurka.

Syftet med denna studie var att undersöka effekten av två biologiska preparat för att se om de kan förhindra eller motverka mjöldaggsangrepp och hålla angreppet på en acceptabel nivå i växthusodlad gurka. I försöken utvärderades även applicering av preparaten i olika intensiteter samt olika appliceringsmetoder. Syftet var också att identifiera mjöldaggssvamparna i de aktuella experimenten. De aktuella preparaten innehöll antingen svampen *Ampelomyces quisqualis* eller svampen *Pythium oligandrum*, vilka är godkända i andra delar av världen.

Rapporten består av en litteraturstudie, en experimentell del som utfördes i växthus och en mikroskopistudie. Försöken genomfördes i en konventionell gurkodling (Sånnagården, Kvidinge) och i en odlingskammare i växthusen vid Sveriges Lantbruksuniversitet, Alnarp.

Resultatet av försöken visade att vissa av behandlingarna med de studerade biologiska preparaten kunde förhindra och motverka mjöldaggsangrepp och hålla dem på en acceptabel nivå i växthusodlad gurka. Effekten av att använda de biologiska preparaten förebyggande jämfört med kurativt utvärderades. Förebyggande behandling med *P. oligandrum* gav en statistiskt signifikant förbättrad sjukdomshämmande effekt jämfört med obehandlad kontroll, men inte med *A. quisqualis*. Effekten av att applicera i olika appliceringsintervall med sju respektive fjorton dagar utvärderades. Fjorton dagars appliceringsintervall gav för *A. quisqualis* en signifikant bättre sjukdomsbekämpning jämfört med sju dagars intervall. Ingen signifikant skillnad vad gäller appliceringsintervall påvisades för *P. oligandrum.* Studien visade också att mjöldaggssvampen i växthusförsöken med största sannolikhet var *Golovinomyces cichoracearum.*

TABLE OF CONTENTS

1. INTRODUCTION

1.1 Background

Powdery mildew, caused by *Golovinomyces cichoracearum var. cichoracearum* (D.C.) V.P. Heluta (syn. *Erysiphe cichoracearum D.C.*) and *Podosphaera xanthii* (Castagne) U. Braun and N. Shishkoff (syn. *Sphaerotheca fuliginea*, formerly known as *Sphaeroteca fusca* Blumer), is a big problem in production of cucumber, *Cucumis sativus* L. and other cucurbits and can be found all around the world in both greenhouses and in field production (Robinson and Decker-Walters, 1997; Sitterly, 1978). Powdery mildews are common and can cause serious infections, yield reductions and quality impaired fruits, in cool or warm, humid areas (Agrios, 2005; Robinson and Decker Walters, 1997). In greenhouse culture, powdery mildew is the main disease on cucurbits (Sitterly, 1978).

Today, we want increased yields and at the same time keep low impacts on the environment using as little resources as possible (Wivstad, 2010). Pest control needs to be efficient, but environmentally friendly. To create a sustainable pest management system, it is necessary to increase the use of non-chemical methods (Wivstad, 2010).

Some biological control methods are used today, mainly in horticultural production (Wivstad, 2010). In some cases, an alternative is to use microbiological control. The microbiological products typically do not leave any residues in harvested crops, which means that no time of restraint is needed (Hökeberg, 2010). Applying crop rotation is another option to reduce the amount of chemical agents used, but it is not possible in monocultures (Wivstad, 2010). Growing resistant and tolerate varieties is also a possibility, but commonly other qualities in the crops are prioritized, such as high yield.

According to a list of plant protection products that are authorized by the Swedish Chemicals Agency (Kemikalieinspektionen) for use in Swedish production of greenhouse grown vegetables, there are no biological agents permitted so far for control of powdery mildew in cucumber production (Hansson and Jansson, 2012). In cucumber production there are only two chemical agents allowed for control of powdery mildew, Amistar, with the active substance azoxystrobin, and Fungazil, with the active substance imazalil. Today it is possible to find biological products for control of powdery mildew in cucumber production in other countries, but they need to be thoroughly investigated before they can be permitted for use in Sweden.

1.2 Aim and Disposition of the Report

The aim of this study was to investigate the effect of two biological control agents to see if they can prevent or reduce powdery mildew in greenhouse produced cucumber. An important part was also to identify the fungal species causing powdery mildew in the experiments in this study.

This report consists of a literature study, an experimental part and a microscope study. The aim of the literature study was to collect background information about cucumber production, about powdery mildew in cucumber production and control measures against this disease, including biological control. The literature study is focused on Swedish cucumber production and powdery mildew in greenhouse grown cucumber in Sweden. The aim of the microscope study was to identify the fungal species causing powdery mildew in the experiments by studying morphological characteristics.

The experimental study is based on three trials with two different experimental designs. The aim of the first experiment was to evaluate if the two investigated biocontrol agents can be used to prevent or reduce powdery mildew infection and compare it the effect of conventional methods. The aim was also to study the efficiency of the two biocontrol agents, by using different application intensities of these agents. The second and third experiments was aimed to compare the effect of prophylactic or curative application of the two biocontrol agents and evaluate if it made a difference in how well they could prevent or reduce powdery mildew infection.

The questions investigated in this study were:

- Which fungus/fungi caused powdery mildew infection in the experiments?
- Can the tested microbiological preparations be used to prevent or reduce powdery mildew infection in greenhouse produced cucumber and keep it at an acceptable level?
- What effect do the two microbiological agents tested have on powdery mildew in cucumber production?
- How important is the application method, prophylactic versus curative, for the plant protection efficacy?

2. THEORETICAL BACKGROUND

2.1 Cucumber Production in Sweden

2.1.1 Botany

Cucumber (*Cucumis sativus*) belongs to the family *Cucurbitaceae* (Heywood et al, 2007; Judd et al 2008). The family has two subfamilies and includes 118 genera and 825 species (Judd et al, 2008). Most species are climbing perennial herbs. The family is important for edible fruits and seeds and major food crops are produced in the tropical, subtropical and temperate regions (Heywood et al, 2007; Judd et al 2008).

When the seed germinates, a typical taproot is developed (Bjelland, 1988). After the first growing phase, the rot will be branched and develops a large root system. Even the stem of the cucumber will easily develop roots. The main stem grows with an unlimited height. Lateral shoots and stem tendrils grow auxiliary. Stem, shoots and leaves are hispid (Judd et al, 2008). The leaves have a long petiole and are alternate and spiral, palmate lobed, dentate and with palmate venation. The flowers are unisexual with bell shaped large yellow connate petals. Female flowers have a long spiny ovary (Bjelland, 1988). The seeds are flattened, pointed ovoid and grey-white. The viability of cucumber seeds is high, normally between 90-100% (Bjelland, 1988; Molén, 2007/2008).

From the beginning, there were only monoecious (the same plant have separate female and male flowers) cultivars of *C. sativus*, but through breeding both gynoecious (plants produce only female flowers) and andromonoecious (plants have both hermaphrodite and male units) cultivars were developed (Robinson and Decker-Walters, 1997). Today, only gynoecious cultivars are cultivated (Bjelland, 1988; Molén 2007/2008). The cultivated varieties are also parthenocarpic (fruit is produced without fertilization of the ovules), since fertilized flowers may result in deformed fruits (Bjelland, 1988).

2.1.2 History

The original cucumber species is found wild in Himalaya in northern India (Molén, 2007/2008). Cucumbers have been grown in India for more than 3000 years and around 2000 B.C. was brought to the area around the Mediterranean Sea and Egypt (Bjelland, 1988). It was later spread through Greece and the Roman Empire to Western Europe and France. In the 17th century the cucumber came to Holland and was first grown in field.

Since cucumber prefers warm climate to thrive, production did not succeed. Around the turn of the 19th to 20th century cucumber was grown in greenhouse in Holland and England (Molén, 2007/2008).

2.1.3 Cultivation in Greenhouse

Growth of the plant is an important part of cucumber cultivation (Molén, 2007/2008). A proper plant development is required for a healthy and well developed foliation and an active root system. Technical equipment needs to be well prepared and well adapted to support a successful plant growth (Molén, 2007/2008).

Plants are sown or grafted (Molén, 2007/2008). When grafting is used, it is to produce plants with roots that are resistant to some soil-borne diseases, caused by e.g. *Fusarium* spp., *Verticillum* spp. and *Pythium* spp., and to create plants with more robust root systems, which results in better vegetative growth. Seeds can be sown in different substrates (Molén, 2007/2008). If plants are grown in an inorganic substrate, seeds are also sown in inorganic substrate and if the plants will be grown in organic substrate, seeds are best sown in organic substrate (Bjelland, 1988). Cucumber plants can also be propagated by cutting propagation (Bjelland, 1988; Molén, 2007/2008).

Cucumber plants demand high temperatures during plant growth (Bjelland, 1988). The temperature affects the development of the plant, especially the development of flowers and ovaries. It is also important to have the right balance between light periods and temperature to not adversely affect plant growth (Molén, 2007/2008). The relationship between the root and leaf temperatures and the air humidity may also affect the plant growth. It is important with stimulated transpiration to avoid the roots becoming weak. Aerial circulation is necessary. Raising carbon dioxide levels above natural atmospheric levels in the greenhouse will result in increased productivity (Taiz and Zeiger, 2006). The additional carbon dioxide increases the photosynthesis and inhibits photorespiration. This will also result in increased plant growth with thicker leaves and better plant vigour (Molén, 2007/2008).

The water demand is strongly dependent of the plant size (Molén 2007/2008). Water and nutrients are given to the plant by watering with a complete nutrient solution (Bjelland, 1988). It is important to regularly control the added nutrient solution and to use a nutrient solution adapted to the substrate and the water quality (Bjelland, 1988; Molén, 2007/2008).

The cucumber plants are tied up with a string, which they can grow around until they reach the right size (Bjelland, 1988). The plants are pruned to regulate the relationship between vegetative growth and fruit-setting. Redundant leaves are removed to improve the air circulation around the plants. The most common training system for cucumber plants is the umbrella system (Molén, 2007/2008). The umbrella system works to distribute the plants in order to ensure that as much light as possible reaches the plant (Bjelland, 1988). The plants are topped at about two meters height and the two-three topmost laterals are left. Fruits and laterals at the lowest part of the plant are removed and in upper levels are only laterals removed. Today there are several alternative forms of this system. Another method is to, part by part, lay down the plants down while they are growing.

In conventional cucumber cultivation in Sweden, it is most common to have two cucumber cultures each year (Molén, 2007/2008). Seeds for the first culture are sown in January/February and for the second culture in mid-June to end July. There are companies growing three cultures per year, claiming that more cultures per year will give better fruit quality and better harvest in both summer and autumn (Molén, 2007/2008).

2.1.4 Cucumber Greenhouse Cultivation in Sweden

Cucumber, together with tomatoes and herbs is the largest greenhouse culture in Sweden (Jordbruksverket, 2011). In 2011 there were 160 hectares with greenhouse cucumber production and 99% of this area was harvested (Jordbruksverket, 2012).The mean harvest was 44 tonnes cucumbers per 1000 square meters.

Since 1990, the production area of greenhouse grown cucumbers has almost doubled, with 605671 square meters in 2011 (Jordbruksverket, 2012). During this period, the harvest increased from 18129 tonnes to 26802 tonnes, an increase of 48 %, the cucumber yield per 1000 square meters has increased with 29 %, from 34 tonnes to 44 tonnes. The number of greenhouse companies growing cucumbers was during the same period reduced with 49 %, from 309 to 159 (Jordbruksverket, 2012).

In 2007, about 2% of the Swedish cucumber cultivation consisted of organic production (Molén, 2007/2008).

2.2 Powdery Mildew in Cucumber

2.2.1 Taxonomy

In the family of *Cucurbitaceae*, the two major species found of cucumber powdery mildew are; *Golovinomyces cichoracearum var. cichoracearum* (D.C.) V.P. Heluta (syn. *Erysiphe cichoracearum D.C.*) and *Podosphaera xanthii* (Castagne) U. Braun and N. Shishkoff (syn. *Sphaerotheca fuliginea*, formerly known as *Sphaeroteca fusca* Blumer) (Sitterly, 1978; Miazzi et al, 2011). There is also a third species of powdery mildew, *Leveillula taurica* (Lev.) Arn., rather frequently reported (El-Ammari and Wajid Khan, 1983). El-Ammari and Wajid Khan, 1983, also mention that there are in total six species of powdery mildew fungi recorded on cucurbits in different parts of the world, but it is difficult to separate the species because some species have many similarities. *Leveillula* sp. is usually considered a synonym of *Golovinomyces* sp. (Sitterly, 1978).

Since *G. cichoracearum* and *P. xanthii* are the major powdery mildew species in cucumber other powdery mildew species have been excluded in this thesis.

2.2.2 Disease Symptoms

Powdery mildew can appear in most parts of the cucumber plant, but is most common in young tissues on the upper side of the leaves (Agrios, 2005) (Picture 1). The root is not infected and fruits are free of visible infection (Sitterly, 1978). The first signs of infection are circular white spots, in both the upper and lower surfaces of the leaf (Robinson and Decker-Walters, 1997).

Picture 1. Cucumber leaves with powdery mildew infection. Photo: Anna-Carin Almqvist

The white lesions increase in number, until they cover both leaf surfaces and stems (Sitterly, 1978). Leaves that are seriously affected will become brown and shrunken. When young

leaves are infected it can result in chlorosis (Picture 2). When conditions are ideal the powdery mildew can cover the whole leaf, cause leaves to die, which results in premature defoliation. Powdery mildew may also cause reduced yields with failed maturity and small and deformed fruits (Sitterly, 1978).

Picture 2. Chlorosis of cucumber leaf infected by powdery mildew. Photo: Anna-Carin Almqvist

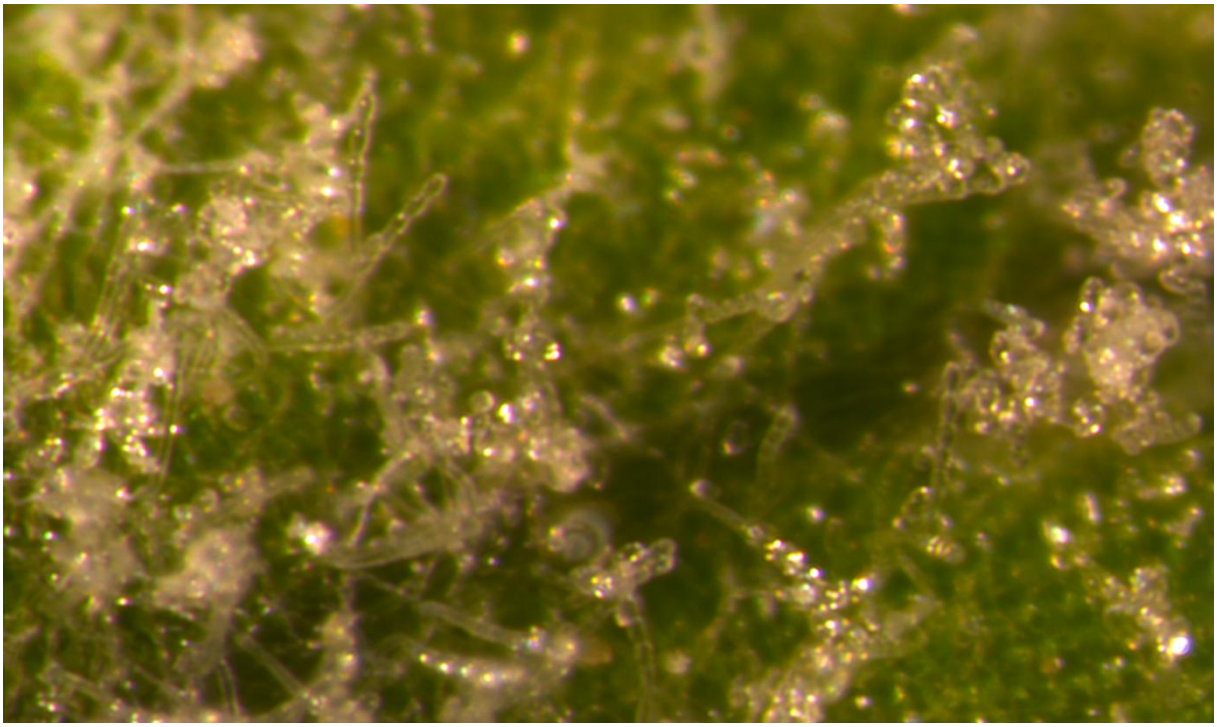
2.2.3 Epidemiology

Powdery mildew affects all kind of plants, except gymnosperms, and is one of the most common and widespread plant diseases (Agrios, 2005). Fungi that cause powdery mildew are obligate parasites, which mean that they cannot be cultured on artificial media (Agrios, 2005).

The disease is more common in warm and dry climates (Agrios, 2005). *Podosphaera xanthii* is predominant in many countries and *Golovinomyces cichoracearum* is common in temperate zones (Bardin et al, 1999). Dry climate favours dispersal of spores, while humid climate favour spore germination (Agrios, 2005). As long as the relative humidity is high, the spores can be released, germinate and cause an infection even if there is no water film on the plant surface (Agrios, 2005). Powdery mildews not often kill their hosts. However, the yield will be reduced, sometimes by as much as 20 to 40 %, due to reduced nutrient utilization, reduced photosynthesis, impaired growth, increased respiration and transpiration caused by the pathogen. Generally, powdery mildew is not only favoured by dry atmospheric and soil conditions. Moderate temperatures, reduced light intensity, fertile soil and succulent plant tissues also promote the disease development (Sitterly, 1978). Powdery mildews do also develop better in shade than in full light.

In Sweden, *G. cichoracearum* is most common during early season and develops favourably when the climate is dry, while *P. xanthii* dominates during summer when the humidity is higher (Jordbruksverket, n.d.).

The mycelia of powdery mildew are completely external and grow only on the surface of plant tissues (Picture 3). Only haustoria penetrate the leaves (Robinson and Decker-Walters, 1997; Agrios, 2005). Because of the external mycelia these fungi are sensitive for environmental factors such as wind and heavy rains (Agrios, 2005). Therefore the disease development is favoured by hot and dry weather (Agrios, 2005).



Picture 3. Mycelium of cucumber powdery mildew. Photo: Anna-Carin Almqvist

On the plant surface, the fungal mycelium produces short conidiophores and each conidiophore produces chains of conidia (Agrios, 2005). The conidia are round, ovoid or rectangular (see Table 1 for the common cucumber powdery mildews). The powdery mildew fungi may produce cleistothecia, containing asci, when environmental conditions become unfavourable or nutrients are lacking. The cleistothecia are tiny, pinhead-sized and spherical. At first they are white, but will later turn into yellow-brown and finally into black (Agrios, 2005). The fungus causing powdery mildew can overwinter in crop residues and perennial weeds (Seebold, 2010).

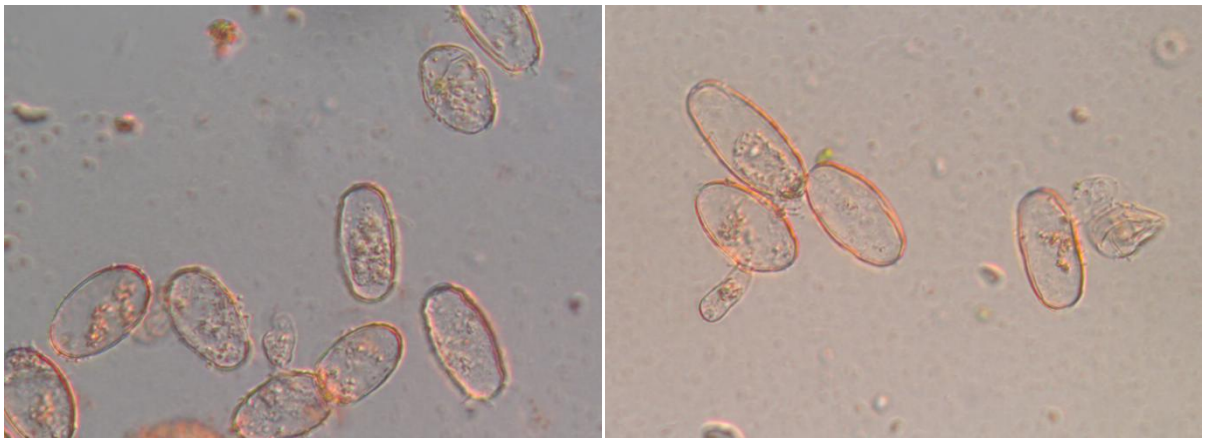
Ascospore germination is favoured by absence of water and relative humidity below 20% (Sitterly, 1978). For conidia to germinate water is needed. Because of their high water content and extremely efficient water conservation system they use internal water from the vacuoles instead of water from an external source. Because of reduced air circulation and light intensities, higher temperatures and continuous cropping, powdery mildews are more serious in greenhouses than in field. Severe powdery mildew infections in greenhouse can result in that the earlier formed chlorotic spots later become necrotic (Sitterly, 1978).

2.2.4 Etiology

The life cycle of cucumber powdery mildew is initiated by conidia or ascospores (Sitterly, 1978). Germination can commence within two hours if the light intensity is reduced, the temperature is between 22 and 31 °C and there is an absence of moisture, when the pathogen spores makes contact with the host. The first germ tube is commonly short and forms a convoluted appressorium. The penetration tube grows into the centre of the cell lumen. A haustorium is established and more germ tubes forms from the same spore. From the primary appressorium hyphae are sent along the leaf surface. Appressoria forms laterally on all hyphae after the first germ tube formation. The mother spore does not collapse after the establishment on the host. Conidiophores begin to form about four days after infection. If cleistothecia are formed, they occur several weeks after the infection (Sitterly, 1978). A normal life cycle takes five to six days (Sitterly, 1978).

2.2.5 Morphology

There are three criteria to identify fungi causing cucumber powdery mildew by anamorphs from the conidial stage; i) type of conidiophores, ii) presence or absence of well-developed fibrosin bodies and iii) morphology of the conidial germ tube with reserved appressoria (Sitterly, 1978). This disease is best known to the growers in the asexual phase of the causing fungus, when the conidial stage is present. Morphological characteristics of *Golovinomyces cichoracearum* and *Podosphaera xanthii* are listed in Table 1. Within each species, the conidia differ in shape. In Picture 4 the conidium shape for *G. cichoracearum* is shown.



Picture 4. Powdery mildew conidia (most probably *Golovinomyces cichoracearum*). Photo: Anna-Carin Almqvist

G. cichoracearum has ellipsoid to barrel-shaped conidia (Kapoor, 1998). Fibrosin bodies are absent and the germ tube is single (Sitterly, 1978). The mycelium is hyaline to dark brown, commonly well developed but evanescent (Kapoor, 1998). Kapoor, 1998, also mentions that the mycelium sometimes is persistent and effused.

P.xanthii has got an epiphyllous mycelium and thin-walled and superficial hyphae (Liberato et al, 2006). The mycelium is branched with a septum. Conidiophores are produced, commonly one conidia per conidiophores, from the external mycelium but sometimes two per hyphal cell. *P. xanthii* has ovoid conidia and an absence of appressoria (Sitterly, 1978; Chen et al., 2007). The conidia contain fibrosin bodies and are formed in chains with up to four conidia (Sitterly, 1978; Liberato et al, 2006; Chen et al., 2007). Unlike *G. cichoracearum* the germ tube is usually short and broadened and sometimes forked with up to three germ tubes per conidia (Sitterly, 1978).

Table 1. Morphological characteristics of *Golovinomyces cichoracearum* and *Podosphaera xanthii* (Sitterly, 1978; Boesewinkel, 1980; Kapoor, 1998; Liberato et al, 2006; Chen et al., 2007; Miazzi et al, 2011).

Characteristics:	*Golovinomyces cichoracearum*	*Podosphaera xanthii*
Conidiophores	• Long • 75-130(-230) µm	• (32-)80-100 x 10-13 µm
Foot-cell	• Straight • Slightly swollen • 55-80 x10-13 µm	• Slightly swollen • 12.5 µm wide at the base
Conidia	• Cylindrical-ovoid, barrel-shaped • 32-40 x15-18 µm	• Ovoid • 28-31 x 15-18 µm
Germ tube	• Straight or flexuous	• Long or short • Broadened • Forked
Fibrosin bodies	• Absent	• Present
Mycelium	• Conspicuous • Dense • Amphigenous • Hyaline to dark brown	• Dense • Amphigenous • White-grey-yellow • Flexous hyphae
Plant host families	• *Asteraceae* • *Cucurbitaceae* • *Solanaceae*	• *Asteraceae* • *Brassicaceae* • *Cistaceae* • *Coriariaceae* • *Cucurbitaceae* • *Dipsaceae* • *Fabaceae* • *Gesneriaceae* • *Malvaceae* • *Plantaginaceae* • *Scrophulariaceae* • *Solanaceae*

2.3 Current Methods for Control of Powdery Mildew in Cucumber Production

2.3.1 Chemical Control

In 2012, only two chemical agents, for control of powdery mildew in cucumber production, are allowed in Sweden, Amistar, with the active substance azoxystrobin, and Fungazil, with the active substance imazalil (Hansson and Jansson, 2012). Both Amistar and Fungazil are off-label, which means that they are used to control powdery mildew even if that is not their primary recommended use (Dahlqvist, 2008).

Amistar is a systemic fungicide, which means that the active substance penetrates through the plant tissues and can both prevent and disrupt infections (Hansson and Jansson, 2012). The effiency of Amistar is low, since it has been used for a long time and resistance has developed in the cucumber powdery mildews (Dahlqvist, 2008). Today it is allowed to use Amistar three times per culture (KEMI, 2010).

Fungazil, containing the active substance imazalil, is a fungicide with a repressive effect (Dahlqvist, 2008). A benefit when using it in umbrella cultivation systems is that Fungazil can be used with both dimmer and spraying equipment. Fungazil is only allowed to be used twice per year (KEMI, 2010).

Even if the use of chemical control has been reduced in greenhouse production it is sometimes necessary to use chemicals to control pests and diseases (Jönsson, 2001). It is important that the chemicals used to control insect pests do not negatively affect the natural enemies, which are used for insect pest control (Jönsson, 2011).

2.3.2 Biological Control

Biological control can be described as the suppression of damaging activities inflicted of a harmful organism by one or more other organisms (Pal and McSpadden Gardener, 2006). These suppressive activities are often conducted by natural enemies, when it concerns insect or arachnid control. In plant pathology the term biological control commonly applies to the phenomenon of microbial antagonism (Kiss, 2003). In some crops, such as tomato, biological control is used as an alternative to chemical control to prevent or suppress powdery mildews. Greenhouse environments are favourable for biological control, since conditions in the greenhouse can be optimized for the biocontrol agent (Paulitz and Bélanger, 2001). Crops have a high value and when there are a limited number of registered fungicides this will result in a unique niche of using biocontrol agents to control plant diseases. The use of biocontrol agents is an increasing research area (Paulitz and Bélanger, 2001).

For treatment of aerial plant diseases, the biocontrol agents need specific environmental conditions (Romero et al, 2007). High relative humidity is one example of environmental conditions that optimizes the activity of the biocontrol agent. Efficacy may also differ between different seasons and cultivars (Dik et al, 1998). Romero et al, 2007, also stated that biocontrol agents perform better under greenhouse conditions than under field conditions. Furthermore, the climate conditions in the greenhouse can affect the efficacy of the biocontrol agent (Dik et

al, 1998). Most of the biocontrol agents require above 70% relative humidity (Dik et al, 1998).

The most explored agents for biocontrol of powdery mildew in cucumber are the mycoparasites *Ampelomyces quisqualis* Ces. and *Lecanicillum lecanii* (Zimm.) Zare and Gams (Romero et al, 2007). Commonly when using mycoparasites, a certain level of disease has to be tolerated by the plant, because mycoparasites can only attack an already established infection (Kiss, 2003). Mycoparasites cannot usually stop the spread of powdery mildew, but it can follow the spread and reduce the damages in the infected plants (Kiss, 2003).

One of the best known mechanisms of fungal antagonism is mycoparasitism of powdery mildew by *Ampelomyces* spp (Kiss, 2003). The *Ampelomyces* spp. hyphae penetrate the powdery mildew hyphae and continue their growth inside the hyphae. The attacked powdery mildew mycelia are averted in its sporulation by the intracellular mycoparasitism (Kiss, 2003). *A. quisqualis* occurs naturally and requires free water to infect powdery mildew colonies (Falk et al, 1995). *A. quisqualis* is a hyperparasite and host by different powdery mildews e.g. cucurbit powdery mildew (*Golovinomyces cichoracearum* and *Podosphaera xanthii*), apple powdery mildew (*Podosphaera leucotricha* (Ell. And Ev.) Salm.), grapevine powdery mildew (*Uncinula necator* (Schw.) Burr.) and rose powdery mildew (*Spaerotheca pannosa* (Wallr.: Fr.) Lév).

Pythium spp. is a broadly operative genus of oomycetes and has more than 200 described species pathogenic or saprophytic on plants, mammals and fish (Al-Sa'di et al, 2007). *Pythium oligandrum* Drechs. has shown antagonistic activities in several species of pathogenic fungi (Mohammed et al, 2006). This antagonist produces oligandrin, which is a plant defence elicitor. In the rhizosphere, *P. oligandrum* can indirectly affect pathogen control (Kaewchai et al, 2009). The fungus can also induce plant resistance and induces plants to respond more rapidly and efficiently to pathogen infections. To reduce damping-off diseases *P.oligandrum* oospores have been used as seed treatment (Kaewchai et al, 2009).

2.3.3 Physical Control

Physical control agents are for example vegetable oils and soap (Borg Ohlsson and Jansson, 2011). Oils and soaps are commonly used to control insects but can also be used to control powdery mildews. Both oils and soaps have contact action, which means that the fungi will directly be affected only if it comes in contact with the solution. In Sweden there are special

oil agents available, like Zence 40 (fatty acid potassium salt), Bioglans (paraffin oil) and Reniderm (fatty acid potassium salt). Common soap from the supermarket can also be used. In-house oil suspensions can be made from cold-form rapeseed oil. An alginate, Agri-50E, have been shown to have effect on powdery mildews (Borg Ohlsson and Jansson, 2011).

Du et al, 2010, tested the control effect of different vegetable oils on cucumber powdery mildew e.g. soybean oil, sun flower oil and corn oil in both indoor protective test and field efficacy tests. They were able to show that they had a significant controlling effect on cucumber powdery mildews compared to a fungicide containing triadimefon. The oils used in the experiment had a protective effect between 87.2 and 100 % and a control effect between 59.0 and 69.7 % (Du et al, 2010).

2.3.4 Other Non-Fungicide Products

Non-fungicide products can induce resistance in powdery mildew infected plants (Kiss, 2003). They can also act as prophylactic or curative factors. This commonly has an effect in greenhouse production. Common non-fungicide products are soluble silicon, milk enzyme and garlic extracts (Borg Ohlsson and Jansson, 2011).

Silicon treatments can be used as growth improvement, mixed in the plant nutrient solution and can increase the resistance to powdery mildews in cucumber (Borg Ohlsson and Jansson, 2011). Shuerger and Hammer, 2003, and Liang et al, 2005, showed in their experiments that silicon can suppress powdery mildew (*Podosphaera xanthii*) in cucumber plants.

Enzicur is a relatively new non-chemical method to control powdery mildew using milk enzymes (Borg Ohlsson and Jansson, 2011). It has shown good results and can be used by exemption in Sweden. Enzicur will not imply any risk for resistance problems (Dahlqvist, 2008).

Garlic extracts have some effect against powdery mildew in cucumber (Borg Ohlsson and Jansson, 2011). Experiments, evaluating if powdery mildew can be controlled in cucumber production by using garlic extracts were made by Qvarnström, 1992 and Qvarnström et al, 1995. The results showed that this method can be used with a good result if the treatment is started at an early phase of the attack (Qvarnström, 1992; Qvarnström et al, 1995).

2.3.5 Tolerance and Resistance

There are cucumber varieties ranging from low tolerance to total resistance (Molén, 2007/2008). Commonly powdery mildew tolerant varieties give reduced or delayed yield (Dahlqvist, 2008). Other problems with tolerant varieties are susceptibility to grey mould (*Botrytis cinerea* Pers.: Fr) and gummy stem blight (*Didymella bryoniae* (Auersw.) Rehm.) (Tomas Isberg 2012-05-29). These are all reasons why tolerant varieties are currently not commonly used by Swedish conventional cucumber growers.

Opinions differ considering where the first resistant variety was observed. Morishita et al, 2003 believe that the first resistant variety was observed in Puerto Rico in USA and Velkov, 2007 believe that the first resistant variety was found in India. Sakata et al, 2006 showed in an experiment that they developed a cucumber variety with resistance to powdery mildew. They tested the variety with various powdery mildew isolates and populations, but breakdown of resistance was observed.

2.3.6 Common Preventative Methods

Greenhouse cultures are ideal environments for pests and diseases (Jönsson, 2001). A well-made plan for hygiene and sanitation in greenhouse production is fundamental for successful cucumber production (Åkesson and Jansson, 2011). Common preventative methods are used to protect the culture from several pests and diseases, including powdery mildew. Good hygiene during the whole cultivation period will reduce the need to sanitize between cultures (Åkesson and Jansson, 2011).

The first step to prevent powdery mildew infection is to keep good hygiene and an even climate in the greenhouse by avoiding draught from doors and windows (Molén, 2007/2008). When the climate is changing between warm and cold, it will favour powdery mildew infections (Dahlqvist, 2008). Infections commonly start close to doors and ventilation windows. Dry climate supports dispersal of powdery mildew spores and humid climate is favourable for sporeal/conidial germination. This is also why moist, mist and shading should be avoided during daytime. Even guttation should be avoided to prevent spore germination.

Important sanitation and hygiene measures in a greenhouse can be summarized in five steps (Åkesson and Jansson, 2011). The first step is to make a plan of how to clean, wash and sanitize the greenhouse. It is important to find suspect pests and diseases, note the time of their appearance and find methods to prevent them. The second step is to prevent the infesta-

tion/infection by pests and diseases by avoiding getting them into the greenhouse. It is necessary to have healthy plants with optimal growing conditions. The third step is to keep the greenhouse clean from infection sources by controlling infected plants and locating the infection in the greenhouse. It is also important to perform weeding around the greenhouse since weeds can act as hosts for different pests and diseases. The fourth step is to choose methods for disinfection. It is more important to make a reasonable disinfection plan than to choose the right disinfectant. The fifth and last step is to make a schedule for disinfection for different situations that may occur during the time of cultivation (Åkesson and Jansson, 2011).

3. MATERIALS AND METHODS

3.1 Biocontrol Agents

During the experiments, two biocontrol agents have been used. Since they are not yet permitted for use in Sweden, the product names have been coded during the experiments by desideratum from the manufacturers. The products have been coded with A for the active organism *Ampelomyces quisqualis* and B for the active organism *Pythium oligandrum.*

3.1.1 Biocontrol Agent A: *Ampelomyces quisqualis*

The product is formulated as water dispersible granules and contains at least 5.0 x 10^3 viable spores of *Ampelomyces quisqualis* per gram of the product. The recommended application dosage is between 35 and 70 grams per hectare according to the crop. The recommended application instructions includes that treatments must be initiated at low powdery mildew infection levels and that the best time of application is when the relative humidity is high. According to the manufacturer this biocontrol agent should be applied every 6-8 day. It will take 3-5 days for the antagonist to colonize the host.

3.1.2 Biocontrol Agent B: *Pythium oligandrum*

The product is formulated as a water dispersible powder containing 1 x 10^6 germinal oospores of *Pythium oligandrum* per gram of the product. The recommended dosage during application in fruiting vegetables is 0.10-0.20 kg product per hectare. The product is also recommended to be applied every 7-14 days after planting.

3.2 Experiments

In this study, the experiments have been made with two different experimental plans.

Experiment 1 was designed to analyze if the biocontrol agents A and B could be used to prevent or reduce powdery mildew attacks compared to the standard chemical control at a conventional greenhouse company. The efficacy of the biocontrol agents was studied using two different application intensities; treatment every 7^{th} or 14^{th} day. Experiment 1 was performed once and took place in a greenhouse at Sånnagården in Kvidinge.

Experiment 2 and 3 were designed to analyze if biocontrol agents A and B could prevent or reduce powdery mildew attacks in greenhouse produced cucumber. The effect by using the biocontrol agents prophylactically or curatively was studied. The experimental design was partial based on previous similar experiments made by Qvarnström, 1989 and 1992 and

Qvarnström et al., 1995. For the inoculation method the experiment was also based on a model by Verhaar et al., 1996. Experiments 2 and 3 took place in a greenhouse chamber at the Swedish University for Agricultural Sciences in Alnarp.

3.2.1 Experimental Plan 1 – In Farmers Greenhouse

3.2.1.1 Treatments and Design

Experiment 1 started the 7th of May. Applications of the biocontrol agents were made by hand with a 5 litres pressure sprayer (Gardena, 822). Spraying application was made according to the schedule in Table 2. The application resulted in five treatments: 1. biocontrol agent A high intensity, 2. biocontrol agent A low intensity, 3. biocontrol agent B high intensity, 4. biocontrol agent B low intensity and 5. standard chemical treatment, normally used for this cultivation. The treatments are shown in Table 2. The high intensity applications were made the 7th, 14th and 21st of May and the low intensity applications were made the 7th and 21st of May. The last disease assessment was made the 28th of May. The biocontrol solutions sprayed on the treated plants until run-off. A timeline, for the different activities in Experiment 1, can be seen in Figure 1.

Table 2. Treatments in Experiment 1 – In Farmers Greenhouse. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.*

Treatment	
A7d	Biocontrol agent A, application every seventh day
A14d	Biocontrol agent A, application every fourteenth day
B7d	Biocontrol agent B, application every seventh day
B14d	Biocontrol agent B, application every fourteenth day
Standard	For this cultivation standard control During the time for the experiment: - Fungazil - Nissorun

Before the experiment started, the powdery mildew infection in the plants used in the experiment was controlled with the chemical fungicide Fungazil (Nordisk Alkali). The plants were also treated with Nissorun (Nordisk Alkali), with the active substance hexytiazox to control spider mites. Earlier in the cultivation the fungicide Previcur (Bayer CropScience), with the active substance propamocarb, was used one day after planting to control *Pythium* sp. and the

insecticide Vertimec (Syngenta), with the active substance abamektin, have been used to control thrips in the first flowers. At the start of harvesting, *Amblyseius cucumeris* (Oudemans) was used to control thrips and *Amblyseius californicus* (McGregor) to control spider mites.

Experimental plan 1 resulted in one experiment, Experiment 1. In total there were 84 plants in the experiment, divided into groups with 25 plants for the standard treatment, 16 plants for treatment A 7 days, 14 plants for treatment A 14 days, 14 plants for treatment B 7 days and 15 plants for treatment B 14 days. The treatments were not randomized and the plants in the same treatment were placed together.

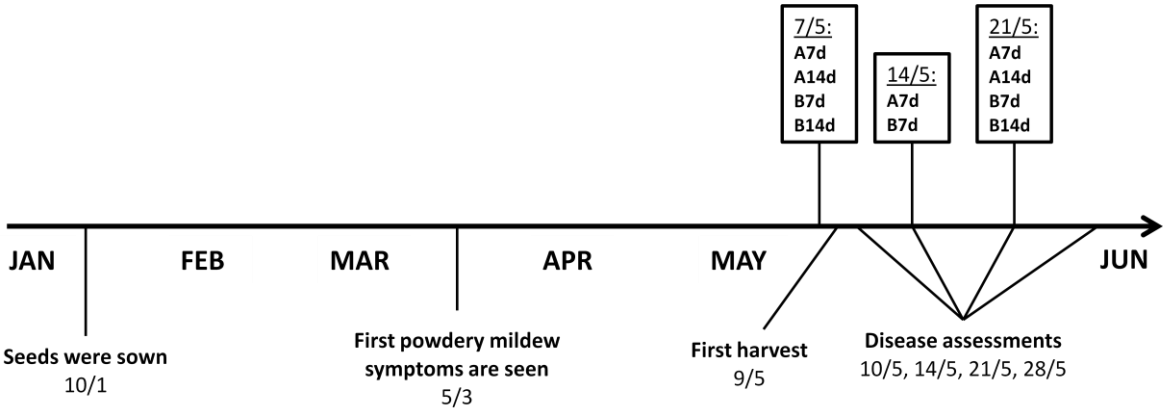


Figure 1. Timeline of the activities in Experiment 1 – In Farmers Greenhouse. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.*A7d = Biocontrol agent A, application every 7th day; A14d = Biocontrol agent A, application every 14th day; B7d = Biocontrol agent B, application every 7th day; B14d = Biocontrol agent A, application every 14th day.

3.2.1.2 Plant Material

The plant material in Experiment 1 was *Cucumis sativus* 'Euphoria'. Seeds were sown the 10th of January directly in rock wool cubes (100x100x70 mm). The rock wool cubes were put two and two in 11 liters containers (400 mm height x 200 mm wide x 150 mm deep) filled with perlite. The cucumber plants were grown using the umbrella system and with a density of 1.7 plants per square meter.

3.2.1.3 Climate Regime

The climate in the greenhouse was set to 20-21°C during day and night and 75-80% relative humidity. During the experiment no cover screens were used. Plants have been exposed to natural day length and no extra carbon dioxide was supplemented during the cultivation.

3.2.1.4 Supply of Water and Nutrients

The plants were irrigated by drip irrigation and controlled by a computer system. Irrigation was regulated by light radiation, drainage and balance. The amount of water per portion was always 65 milliliters per plant. During daytime the plants were irrigated every 7-60 minutes and during night time in total 2-5 times. The conductivity was between 2.5 and 3.5 depending on the absorption in the plants.

Nutrients were added through irrigation. At normal fruit production levels the nutrient solution was composed of about 21.7 % N; 4.0 % P; 30.2 % K; 15.2 % Ca; 5.5 % Mg; 5.8 % S; 0.3 % Fe; 0.2 % Mn; 0.046 % B; 0.009 % Cu; 0.0048 % Zn and 0.004 % Mo. The conductivity was 3 to 3.5 in the drainage.

3.2.1.5 Preparations of Suspensions

Since there was an on-going powdery mildew infection in the greenhouse the highest recommended dose of the biocontrol agents was used. Biocontrol agent A was used with 0.07 gram per litre water and biocontrol agent B was used with 1.00 gram per litre water.

3.2.1.6 Inoculation with Powdery Mildew

During Experiment 1 the powdery mildew infection was naturally initiated. The first powdery mildew infection in the greenhouse was seen between the 5th and 9th of March.

3.2.1.7 Yield

During the experiment cucumbers were harvested every other day. The first harvest was made the 9th of May. Data about amount and weights of harvested cucumbers were collected.

3.2.2 Experimental Plan 2 – In Experimental Greenhouse

3.2.2.1 Treatment and Design

Experimental plan 2 resulted in two experiments, Experiment 2 and Experiment 3. Experiment 3 is a repetition of Experiment 2. In Experiment 2, there were six replicate plants in every treatment and in Experiment 3 there were seven replicate plants in every treatment. For practical application reasons, the plants were not randomized and plants in the same treatment were placed together.

In Experiment 2, prophylactic application of the biocontrol agents was made the 21st and 28th of May, until powdery mildew infection first was seen in the experimental plants 1st of June. The treatment continued with curative application the 1st, 8th and 15th of June. The experiment

was finished the 19th of June, when the last disease assessment was made. The different treatments can be shown in Table 3.

In Experiment 3, prophylactic application of the biocontrol agents was made the 13th and 20th of June and continued with curative application 25th of June, when the first infection was seen, and continued the 2nd and 9th of July. The last disease assessment was made the 16th of July when the experiment was finished.

In both Experiment 2 and Experiment 3, the application of the biocontrol agents were made by hand with a 5 litres pressure sprayer (Gardena, 822).

Table 3. Treatments in Experiment 2 and Experiment 3. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.*

Treatment	
A prophylactic	Plants were treated prophylactically with biocontrol agent A every seventh day until the first powdery mildew infection was seen in the experiment. The treatment was then made curatively every seventh day.
A curative	First treatment was made curatively with biocontrol agent A when the first powdery mildew infection was seen in the experiment. The treatment was made every seventh day.
B prophylactic	Plants were treated prophylactically with biocontrol agent B every seventh day until first powdery mildew infection was seen in the experiment. The treatment was then made curatively every seventh day.
B curative	First treatment was made curatively with biocontrol agent B when the first powdery mildew infection was seen in the experiment. The treatment was made every seventh day.
Non-treated	All plants were untreated

3.2.2.1.1 Experiment 2

Experiment 2 started the 21st of May and ended 19th of June. It lasted in total 29 days. Each treatment contained six replicate plants. The plants were about one meter high when the experiment started. A timeline for the different activities in Experiment 2 can be seen in Figure 2.

The 29th of May Western Flower Thrips (*Frankliniella occidentalis* (Pergande)) were found in some plants. Biological control (*Amblyseius swirskii* Athias-Henriot) was administered to the plants on the 14th of June.

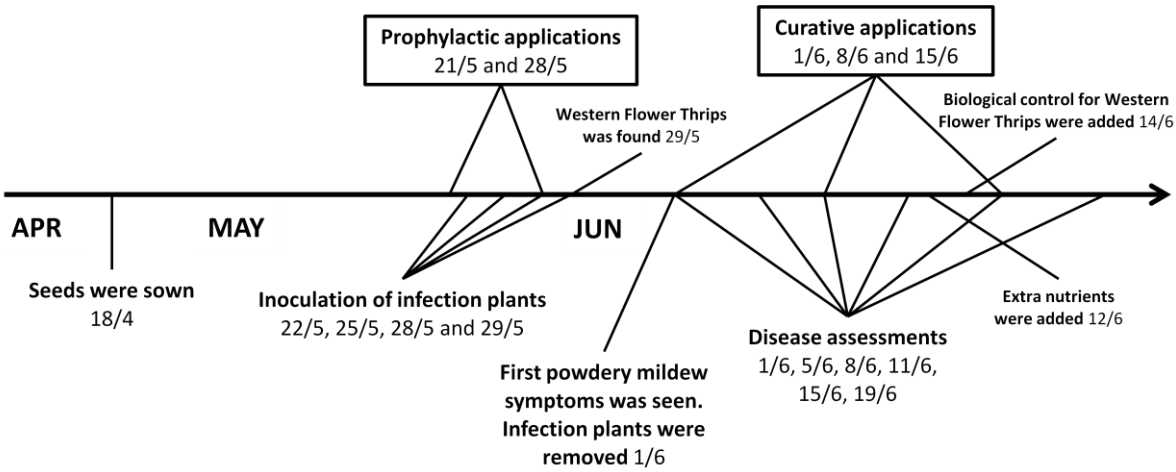


Figure 2. Timeline of the activities in Experiment 2 – In Experimental Greenhouse. The prophylactic and curative treatments are made with Biocontrol agent A, *Ampelomyces quisqualis* and Biocontrol agent B, *Pythium oligandrum.* In the experiment there were five different treatments: Non-t. = all plants were untreated; A cur = First treatment was made curative with biocontrol agent A when the first powdery mildew infection was seen in the experiment; A pro = Plants were treated prophylactic with biocontrol agent A every seventh day until first powdery mildew infection was seen in the experiment; B cur; First treatment was made curative with biocontrol agent B when the first powdery mildew infection was seen in the experiment; B pro = Plants were treated prophylactic with biocontrol agent B every seventh day until first powdery mildew infection was seen in the experiment. All applications of the biocontrol agents were made every 7th day. The inoculation plants were removed when the first powdery mildew symptom was seen in the experimental plants.

3.2.2.1.2 Experiment 3

Experiment 3 started 13th of June and ended 16th of July. It lasted in total 33 days. Each treatment contained seven replicate plants. The plants were about one meter high when the experiment started. A timeline for the different activities in Experiment 3 can be seen in Figure 3.

To avoid the Western Flower Thrips (*Frankliniella occidentalis*), biological control (*Amblyseius swirskii*) was used from the beginning of the cultivation period.

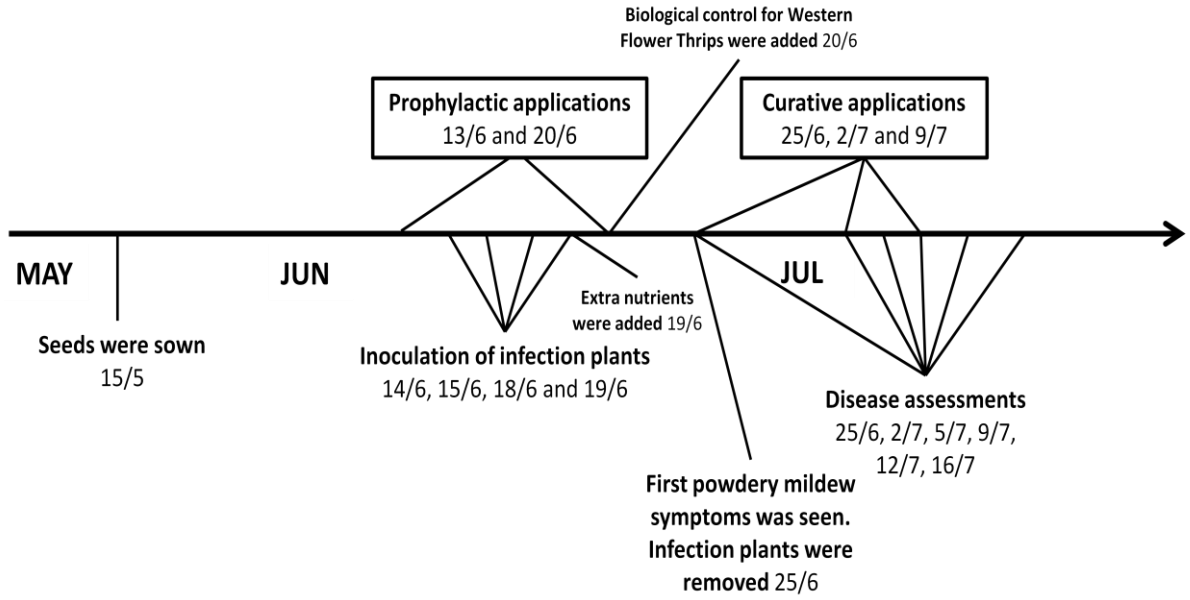


Figure 3. Timeline of the activities in Experiment 3 – In Experimental Greenhouse. The prophylactic and curative treatments are made with Biocontrol agent A, *Ampelomyces quisqualis* and Biocontrol agent B, *Pythium oligandrum.* In the experiment there were five different treatments: Non-t. = all plants were untreated; A cur = First treatment was made curative with biocontrol agent A when the first powdery mildew infection was seen in the experiment; A pro = Plants were treated prophylactic with biocontrol agent A every seventh day until first powdery mildew infection was seen in the experiment; B cur; First treatment was made curative with biocontrol agent B when the first powdery mildew infection was seen in the experiment; B pro = Plants were treated prophylactic with biocontrol agent B every seventh day until first powdery mildew infection was seen in the experiment. All applications of the biocontrol agents were made every 7th day. The inoculation plants were removed when the first powdery mildew symptom was seen in the experimental plants.

3.2.2.2 Plant Material

The plant material used in both experiments was *Cucumis sativus* 'Euphoria'. The seeds were sown one by one in 5 liter pots (Ø 230 mm x height 180 mm). For Experiment 2, the seeds were sown the 18th of April and for Experiment 3 the seeds were sown 15th of May. The growing medium contained a peat soil mixture with expanded clay beads (Weibulls krukväxtjord med leca). The plants were grown according to the umbrella system.

3.2.2.3 Climate Regime

The climate in the growth chamber was set to 22°C in day and night temperature and 75% relative humidity. Since windows in the roof were automatically opened for ventilation, the floors were wetted to increase humidity in the growth chamber during both experiments. Nozzles for irrigation in the growth chamber were closed not to impact the infection and the application of the biocontrol agents. The plants were exposed to natural day length and no cover screens were used during night time.

3.2.2.4 Supply of Water and Nutrients

In both experiments, plants were irrigated by hand when needed. To prevent impact on the powdery mildew infection watering on the leaves was avoided.

When sowing the cucumber seeds, a teaspoon of long lasting nutrient granulates (Osmocote® Pro) was added. The basic composition of the nutrient solution was 17% N; 4.8% P; 8.3% K; 1.2% Mg; 0.01 % B; 0.042% Cu; 0,30% Fe; 0.04% Mn;0.015% Mo and 0.010% Zn.

In Experiment 2, the plants started to get chlorosis and the 12th of June extra nutrient solution (Blomstra), with the composition 5.1% N; 1.0% P; 4.3% K; 0.4% S; 0.3% Ca; 0.4% Mg; 0.035% Fe; 0.020% Mn; 0.010% B; 0.003% Zn; 0.0015% Cu and 0.0004% Mo, was added when irrigating. To avoid shortage of nutrients in Experiment 3, extra nutrient solution was added from the beginning when the experiment started and was used every to every other watering.

3.2.2.5 Preparations of Suspensions

For both biocontrol agents, the recommended doses for prophylactic and curative treatments, respectively, were used. For the prophylactic treatments suspensions with 0.035 gram per litre water of biocontrol agent A and 0.50 gram per litre water of biocontrol agent B were prepared and used. When powdery mildew symptoms were seen in the treatment plants the curative treatment started with suspensions with 0.070 gram per litre water of biocontrol agent A and 1.00 gram per litre water of biocontrol agent B. The spraying of the biocontrol solutions was made until run-off.

3.2.2.6 Inoculation with Powdery Mildew

In both experiments, infected plants (inoculation plants) were used as inoculums to create an infection of the experimental plants as natural as possible. In Experiment 2, the inoculation plants were inoculated with powdery mildew suspension 22nd, 25th, 28th and 29th of May until the first powdery mildew symptom was seen. In Experiment 3, the inoculation plants were inoculated with powdery mildew suspension the 14th, 15th, 18th and 19th of June until the first powdery mildew was seen. In both experiments, it took eight days from the first inoculation until the first symptom was seen on the inoculation plants. In both experiments, 3-4 leaves in every infection plant were rubbed with powdery mildew infected leaves to increase the possibility of the disease to migrate. The infected leaves used for rubbing, came from Sånnagården, Kvidinge.

The powdery mildew suspension was made by putting a piece of an infected leaf into a tube with 10 ml tap water. In Experiment 2, these leaves came from Sånnagården, Kvidinge and in Experiment 3 the leaves came from an extra plant in Experiment 2. The tube was shaken until a spore suspension was formed. The spore concentration was determined in a Bürker chamber and later diluted into about 10^5 spores per millilitre and used for inoculation of the inoculation plants.

Until the first powdery mildew symptoms developed, the inoculation plants were kept away from the greenhouse where the experimental plants were kept to avoid infection of these plants earlier than planned. Experiment 2 had five inoculation plants and Experiment 3 had six inoculation plants. Inoculation plants were evenly distributed and rotated in the experiment area. In Experiment 2, it took four days from when the inoculation plants were put in the experiment until the first infection symptom was seen in the experimental plants. In Experiment 3, it took five days from when the inoculation plants were put in the experiment until the first infection symptom was seen in the treatment plants. The inoculation plants were removed when the first powdery mildew symptom was seen in the experimental plants.

3.2.2.7 Yield

Cucumbers were harvested when they had a marketable size or were too big to be left in the plant. In both Experiment 2 and Experiment 3, the number of harvested cucumbers was recorded.

3.2.3 Disease Assessment in all Experiments

To estimate the severity of the powdery mildew attack, leaves from the cucumber plants were graded. The disease symptoms were estimated by a six-degree scale based on the nine-degree scale by Jasinski et al, 2010, shown in Table 4. In each plant, 10 leaves were graded, 5 in the lowest level of the plant and 5 in about 1.5 meters height in the plant. By grading disease infection at different levels of the plant it was possible to study differences at different parts of the plant and also to see if there were any differences in the result according to factors such as leaf age and microclimate.

In Experiment 1, the disease severity was graded once every week and in Experiments 2 and 3 the severity was graded twice every week.

Table 4. Scale for grading of disease symptom, disease severity index (DSI), in the experiments.

Degree	% attacked leaf area
0	0
1	0.10-0.90
2	1.0-3.9
3	4.0-15.9
4	16-63.9
5	>64.0

3.2.4 Statistical Analysis in all Experiments

The statistical analysis was made in Minitab 16. The disease measurements were analyzed with one-way ANOVA and Tukey's multiple comparison test. Differences were considered significant at $p<0.050$. Diagrams were made in Microsoft Excel.

3.3 Identification of Powdery Mildews in the Experiments

To identify the powdery mildew in the experiments, different methods were used. Since it is possible to distinguish *Podosphaera xanthii* from *Golovinomyces cichoracearum* morphologically, no genetic identification was made in this investigation. It is possible to identify the powdery mildew species by studying the shape of the conidia and the germ tube and also by studying the presence of well-defined fibrosin bodies (Miazzi et al., 2011). For identification a key by Boesewinkel (1980) was used.

Infected leaf samples were collected at Sånnagården, Kvidinge, in the same greenhouse as Experiment 1 was performed, but not in the same area, the 7th, 10th, 14th, 21st, 28th and 29th of May. Identifications in light microscope were repeated several times to ensure the result.

There was also identifications made later during the season with samples from Sånnagården collected the 18th and 25th of July. These identifications were made to compare with the previous identification results and to see if there were any differences.

To prepare for the microscope study, small pieces of infected leaf areas were suspended in a tube with 10 ml tap water until a spore suspension was formed. Droplets of the spore suspension were put on microscope slides. The spore size was measured and the spore shape was studied under the microscope (Leica DMLB100T). Attempts to observe fibrosin bodies were also made using a 3% KOH-solution. A droplet of the 3 % KOH-solution was added to a droplet of spore suspension on a microscope slide and studied under the microscope.

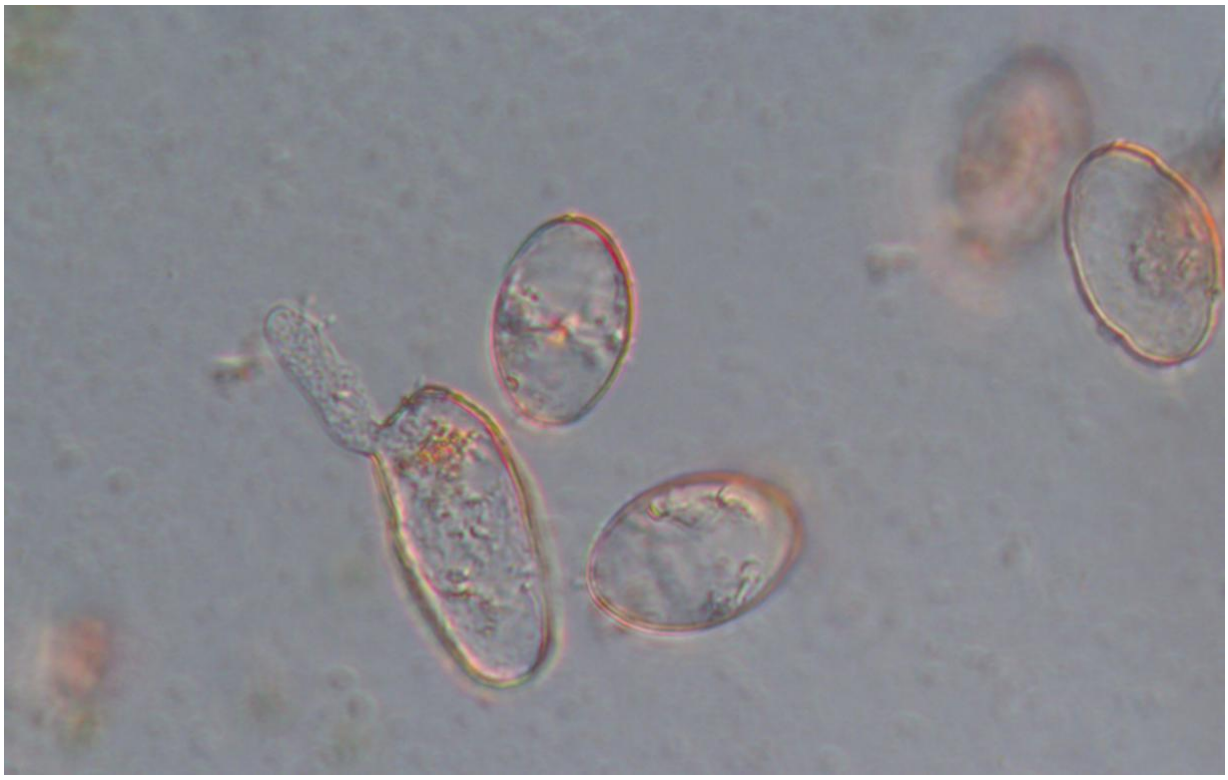
To induce germination for assessment of germ tube characteristics, a drop of the spore suspension was put on a microscope slide and put in a moist chamber in room temperature for 4-7 days before the spores were studied again in the light microscope.

Pictures were taken with a camera (Leica DFC450 C) connected to the light microscope.

4. RESULTS

4.1 Identification of Powdery Mildew in the Experiments

The majority of the conidia were cylindrical-ovoid and barrel-shaped. A minority of the conidia were more ovoid in their shape. After germination in a moist chamber, most of the conidia had a straight germ tube while the other conidia had an absence of germ tubes. Characteristic conidia of the samples are shown in Picture 5. When 3% KOH solution was added to the samples there was still an absence of fibrosin bodies. By measuring the conidia, size was determined to be between 27-38 x 15-26 µm, which means these conidia had the expected sizes for both *Golovinomyces cichoracearum* and *Podosphaera xanthii* according to Table 1.



Picture 5. Characteristic cucumber powdery mildew conidia sample in the experiments (most probably *Golovinomyces cichoracearum*). Photo: Anna-Carin Almqvist.

This means that by identifying morphological characteristics the powdery mildew species in the experiments are most comparable with *Golovinomyces cichoracearum*.

4.2 Experiment 1 – In Farmers Greenhouse

In the beginning of the experiment, there were no significant differences between the treatments of both in lower and upper parts of the plant. There was a small infection in the standard treated plants in the upper parts of the plant, but these disease symptoms did not make any significant difference in the disease severity index (DSI). Mean DSI of the powdery mildew leaf infections can be seen in Table 5. Mean values for powdery mildew symptoms graded as DSI, in lower and upper parts of the plant, over time can be seen in Appendix 1.

In lower parts of the plant during the two last disease assessments, the mean DSI for the treatments B7d and B14d had at this time a significantly higher mean DSI compared to the standard treatment and a significantly lower mean DSI compared to the treatments A7d and A14d. The treatments A7d and A14d were significantly higher compared the other treatments. At the last time for disease assessment, the standard treatment had the significantly lowest mean DSI.

At the last time for disease assessment, in upper parts of the plant, there were no significant differences between the standard treatment and the treatments B7d and B14d. The plants with the treatments B7d and B14d showed the lowest mean DSI in upper parts of the plant. The treatments A7d and A14d had a significant higher mean DSI compared to the other treatments. At the two last times for disease assessment, both in upper and lower parts of the plant, both treatments with biocontrol agent B, *Pythium oligandrum*, shows significantly lower mean DSI compared to both treatments with biocontrol agent A, *Ampelomyces quisqualis* (Table 5).

To conclude, the disease assessment in Experiment 1 shows that for biocontrol agent A, it was better to apply every 14^{th} day compared to every 7^{th} day. For biocontrol agent B there was no difference in the different application intensities. Application with biocontrol agent A, showed the significantly highest mean DSI.

Table 5. Disease severity index (DSI) of powdery mildew leaf infections, Experiment 1. The DSI is graded 0-5, where 5 is the highest grade of infection. Standard = for this cultivation standard control. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.*A7d = Biocontrol agent A, application every 7th day; A14d = Biocontrol agent A, application every 14th day; B7d = Biocontrol agent B, application every 7th day; B14d = Biocontrol agent A, application every 14th day. Means of DSI that do not share a grouping letter are significant different according to Tukey's test.

	Lower parts of the plant				Upper parts of the plant			
	Treatment	**N**	**Mean DSI**	**Grouping**	**Treatment**	**N**	**Mean DSI**	**Grouping**
10th of May	Standard	125	0.0	A	Standard	125	0.048	A
	A7d	80	0.0	A	A7d	80	0.0	A
	A14d	70	0.0	A	A14d	70	0.0	A
	B7d	70	0.0	A	B7d	70	0.0	A
	B14d	75	0.0	A	B14d	75	0.0	A
14th of May	Standard	125	0.0080	A	Standard	125	0.14	A
	A7d	80	0.013	A	A7d	80	0.075	A B
	A14d	70	0.014	A	A14d	70	0.029	B
	B7d	70	0.0	A	B7d	70	0.014	B
	B14d	75	0.0	A	B14d	75	0.013	B
21st of May	Standard	125	1.2	C	Standard	125	1.54	B
	A7d	80	2.2	A	A7d	80	2.1	A
	A14d	70	1.7	B	A14d	70	1.5	B
	B7d	70	0.91	C	B7d	70	0.91	C
	B14d	75	1.0	C	B14d	75	0.70	C
28th of May	Standard	125	2.5	C	Standard	125	3.4	C
	A7d	80	4.7	A	A7d	80	4.8	A
	A14d	70	4.5	A	A14d	70	4.4	B
	B7d	70	3.5	B	B7d	70	3.3	C
	B14d	75	3.3	B	B14d	75	3.3	C

In Experiment 1, there was a small difference in total number of cucumbers (data not shown). The standard treatment had the highest number of harvested cucumbers and the treatment A14d had the lowest number. The total harvested weight per treatment showed a small difference between the treatments. The treatment B14d had the highest total weight with 60 kg, which was 24 % more compared to the standard treatment. The standard treatment had the lowest total weight per treatment (Figure 4). By comparing the average weight per cucumber between the treatments, it was shown that the standard treatment had the lowest average weight per cucumber and the treatment B14d had the highest average weight per cucumber. The average weight per cucumber for the treatment B14d was 27 % more than the standard treatment (Figure 5).

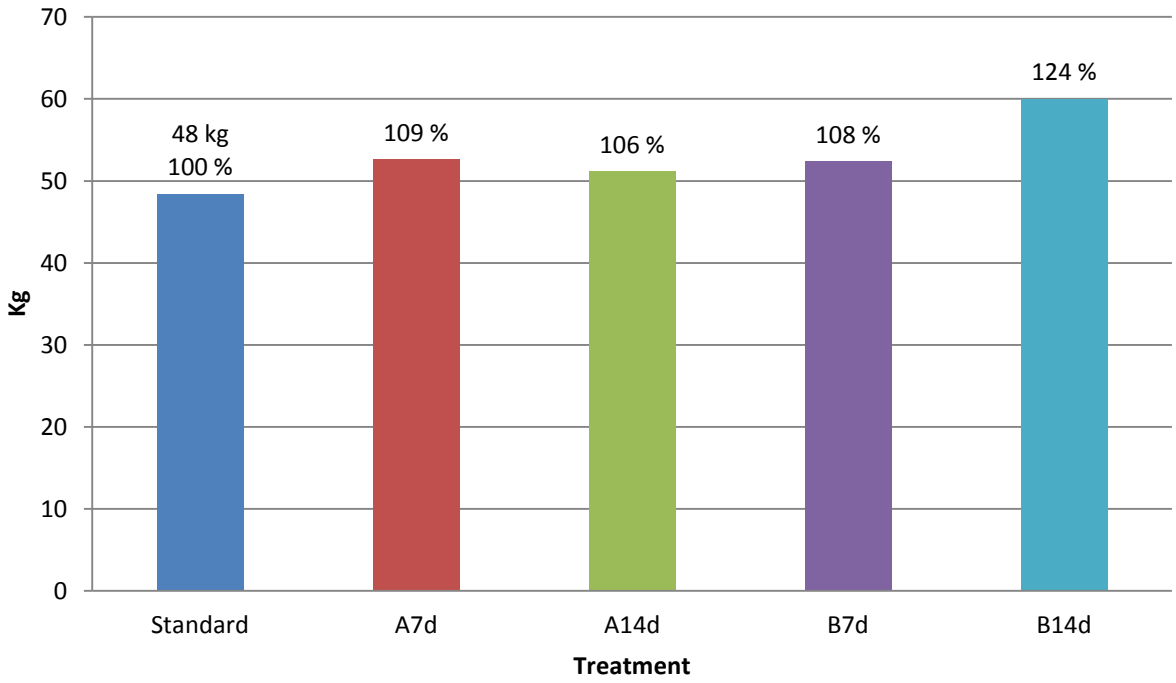


Figure 4. Total weight per treatment (Kg) in Experiment 1. Every treatment represents the same area of cucumber production. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.* A7d = Biocontrol agent A, application every 7th day; A14d = Biocontrol agent A, application every 14th day; B7d = Biocontrol agent B, application every 7th day; B14d = Biocontrol agent A, application every 14th day.

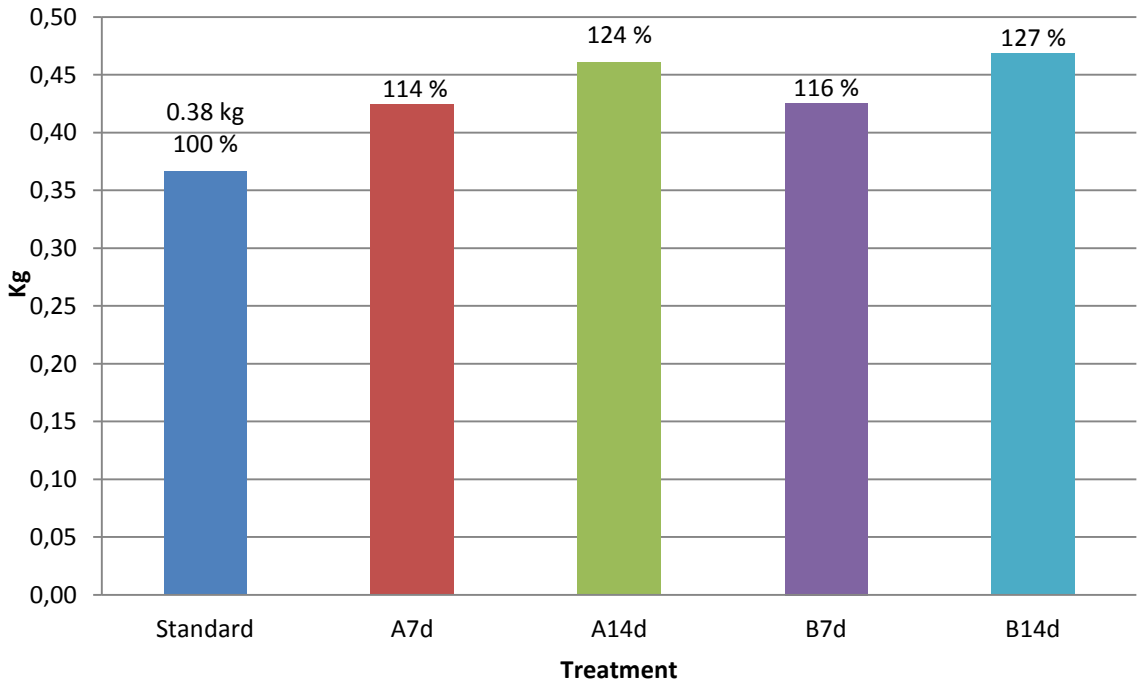


Figure 5. Average weight per cucumber (Kg) in Experiment 1. Every treatment represents the same area of cucumber production. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.*A7d = Biocontrol agent A, application every 7th day; A14d = Biocontrol agent A, application every 14th day; B7d = Biocontrol agent B, application every 7th day; B14d = Biocontrol agent A, application every 14th day.

4.3 Experiment 2 – In Experimental Greenhouse

In lower parts of the plant, it was shown that the treatment B prophylactic had significantly lower mean DSI (disease severity index) compared to the non-treated plants. This was shown at five of six times for disease assessments. The non-treated control had the highest mean DSI at most of the times for disease assessment.

In lower parts of the plant, the result shows that prophylactic application was significantly better for biocontrol agent B, *Pythium oligandrum,* at three out of six times for disease assessment, compared to curative application. Prophylactic application with biocontrol agent A, *Ampelomyces quisqualis,* was only significantly better once during the disease assessments.

In upper parts of the plant, the treatment B prophylactic had the lowest mean at the disease assessments, and had a significantly lower man DSI than all other treatments at the two last assessments. Mean DSI of the powdery mildew leaf infections can be seen in Table 6. Mean values for powdery mildew symptoms over time, in lower and upper parts of the plant, can be seen in Appendix 2.

In upper parts of the plant, prophylactic application of biocontrol agent B was significantly better compared to curative application at the two latest disease assessments. Even in upper parts of the plant prophylactic application with biocontrol agent A shows significant better results only once compared to curative application.

The total number of harvested cucumbers showed that there was a small difference between the treatments (data not shown). The treatment B prophylactic had the highest yield and the treatment B curative gave the lowest yield.

Table 6. Disease severity index (DSI) of powdery mildew leaf infections, Experiment 2. The DSI is graded 0-5, where 5 is the highest grade of infection. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.* Non-t. = all plants were untreated; A cur = curative treatment with biocontrol agent A; A pro = prophylactic treatment with biocontrol agent A; B cur= curative treatment with biocontrol agent B; B pro = prophylactic treatment with biocontrol agent B. All applications of the biocontrol agents were made every 7th day. Means of DSI that do not share a grouping letter are significant different according to Tukey's test.

	Lower parts of the plant				Upper parts of the plant			
	Treatment	**N**	**Mean DSI**	**Grouping**	**Treatment**	**N**	**Mean DSI**	**Grouping**
1st of June	Non-t.	30	0.33	A	Non-t.	30	0.0	A
	A cur	30	0.30	A B	A cur	30	0.0	A
	A pro	30	0.13	A B	A pro	30	0.0	A
	B cur	30	0.13	A B	B cur	30	0.0	A
	B pro	30	0.033	B	B pro	30	0.0	A
5th of June	Non-t.	30	0.90	A	Non-t.	30	0.0	B
	A cur	30	0.87	A	A cur	30	0.0	B
	A pro	30	0.53	B C	A pro	30	0.10	A
	B cur	30	0.60	A B	B cur	30	0.0	B
	B pro	30	0.23	C	B pro	30	0.0	B
8th of June	Non-t.	30	1.8	A	Non-t.	30	0.47	A
	A cur	30	1.3	A B	A cur	30	0.47	A
	A pro	30	1.6	A	A pro	30	0.47	A
	B cur	30	1.5	A	B cur	30	0.10	B
	B pro	30	1.0	B	B pro	30	0.23	A B
11th of June	Non-t.	30	3.0	A	Non-t.	30	2.5	A
	A cur	30	3.0	A	A cur	30	2.0	B
	A pro	30	3.0	A	A pro	30	1.6	B
	B cur	30	3.0	A	B cur	30	0.67	C
	B pro	30	2.0	B	B pro	30	0.67	C
15th of June	Non-t.	30	3.2	A B	Non-t.	30	2.7	A
	A cur	30	3.0	A B	A cur	30	2.0	B
	A pro	30	3.4	A	A pro	30	1.3	C
	B cur	30	3.0	B	B cur	30	1.0	C
	B pro	30	2.8	B	B pro	30	0.40	D
19th of June	Non-t.	30	4.2	A	Non-t.	30	2.5	A
	A cur	30	4.5	A	A cur	30	1.8	B
	A pro	30	4.4	A	A pro	30	1.5	B
	B cur	30	3.3	B	B cur	30	0.87	C
	B pro	30	3.1	B	B pro	30	0.20	D

4.4 Experiment 3 – In Experimental Greenhouse

In lower parts of the plant, the treatment B prophylactic had the lowest mean DSI (disease severity index). This was significantly lower compared to the non-treated control at five out of six times for disease assessment. Both treatments B curative and A prophylactic had significantly lower mean DSI compared to the non-treated control at four out of six disease assessment occasions.

In upper parts of the plant, the plants applied with biocontrol agent A, *Ampelomyces quisqualis,* had the highest mean DSI at most of the disease assessment occasions. Mean DSI of the powdery mildew leaf infections can be seen in Table 7. Mean values for powdery mildew symptoms, in lower and upper parts of the plant, over time can be seen in Appendix 3.

When comparing prophylactic and curative treatments in lower parts of the plant, the result showed that prophylactic application was significantly better for biocontrol agent B, *Pythium oligandrum* at five out of six disease assessment occasions. Prophylactic application with biocontrol agent A, *Ampelomyces quisqualis,* was only significant better once of the disease assessments.

In upper parts of the plant, prophylactic application of both of the biocontrol agents were only significantly better compared to curative application once for each biocontrol agent.

The total number of harvested cucumbers showed that there was a small difference between the treatments (data not shown). The treatment B curative had the highest number of harvested cucumbers and the treatment B prophylactic had the lowest number.

Table 7. Disease severity index (DSI) of powdery mildew leaf infections, Experiment 3. The DSI is graded 0-5, where 5 is the highest grade of infection. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.* Non-t. = all plants were untreated; A cur = curative treatment with biocontrol agent A; A pro = prophylactic treatment with biocontrol agent A; B cur= curative treatment with biocontrol agent B; B pro = prophylactic treatment with biocontrol agent B. All applications of the biocontrol agents were made every 7th day. Means of DSI that do not share a grouping letter are significant different according to Tukey's test.

	Lower parts of the plant				Upper parts of the plant			
	Treatment	**N**	**Mean DSI**	**Grouping**	**Treatment**	**N**	**Mean DSI**	**Grouping**
25th of June	Non-t.	35	0.086	A	Non-t.	35	0.0	A
	A cur	35	0.0	A	A cur	35	0.0	A
	A pro	35	0.11	A	A pro	35	0.0	A
	B cur	35	0.0	A	B cur	35	0.0	A
	B pro	35	0.057	A	B pro	35	0.0	A
2nd of July	Non-t.	35	2.8	A	Non-t.	35	0.0	B
	A cur	35	1.9	B	A cur	35	0.17	A B
	A pro	35	2.1	B	A pro	35	0.29	A
	B cur	35	2.0	B	B cur	35	0.0	B
	B pro	35	1.1	C	B pro	35	0.17	A B
5th of July	Non-t.	35	3.6	A	Non-t.	35	0.11	B C
	A cur	35	3.4	A	A cur	35	0.51	B
	A pro	35	3.3	A B	A pro	35	0.94	A
	B cur	35	3.0	B	B cur	35	0.0	C
	B pro	35	2.2	C	B pro	35	0.17	B C
9th of July	Non-t.	35	4.3	A	Non-t.	35	0.57	B
	A cur	35	4.1	A B	A cur	35	1.3	A
	A pro	35	3.7	B C	A pro	35	1.4	A
	B cur	35	3.6	C	B cur	35	0.43	B
	B pro	35	2.6	D	B pro	35	0.60	B
12th of July	Non-t.	35	4.7	A	Non-t.	35	1.4	B
	A cur	35	4.7	A B	A cur	35	2.1	A
	A pro	35	4.0	C	A pro	35	1.9	A B
	B cur	35	4.3	B C	B cur	35	1.8	A B
	B pro	35	3.5	D	B pro	35	1.4	B
16th of July	Non-t.	35	4.9	A	Non-t.	35	3.7	B
	A cur	35	5.0	A	A cur	35	4.2	A
	A pro	35	4.9	A	A pro	35	3.2	C
	B cur	35	5.0	A	B cur	35	3.7	B
	B pro	35	4.6	B	B pro	35	2.7	D

5. DISCUSSION

5.1 Identification of Powdery Mildew in the Experiments

The identification was not as easy as expected, since the conidia sometimes had morphological characteristics that are similar for both *Golovinomyces cichoracearum* and *Podospaera xanthii.* Even when identifications were made later during the season, these showed the same identification results as earlier during the season. The later identifications were made as an attempt to find *P. xanthii* to compare with the earlier results, since this species commonly appears later during the season (Jordbruksverket, n.d.).

It is most likely that the identified species in the experiments is *G. cichoracearum.* The absence of fibrosin bodies and presence of broadened and forked germ tubes made the result quite certain. The shape of the conidia was cylindrical-ovoid which also indicated that it was in fact *G. cichoracearum.*

However, no genetic identification was made to confirm this on a molecular level. There is also a possibility that there were more than one powdery mildew species present during the experiments, since some characteristics of both species common in cucumber were found.

As the powdery mildew was studied during a limited time of the season, there is probably only one powdery mildew species tested, since the two powdery mildew species commonly are present during different times of the season (Jordbruksverket, n.d.). This also means that biocontrol agents A, *Ampelomyces quisqualis,* and B, *Pythium oligandrum,* only have been tested against this species.

5.2 Experiments

5.2.1 Experiment 1 – In Farmers Greenhouse

In the beginning of Experiment 1, the result showed that there was almost no difference between the treatments, neither in lower nor in upper parts of the plant. After four days and until the end of the experiment, there were differences in the amount of powdery mildew infection between the different treatments. When the experiment started, there was an ongoing infection in the greenhouse and there were also some infected leaves in the standard treated plants (chemical control). It takes a only a few days for powdery mildew to spread and to infect, which could explain why the disease increased rapidly some days after the experiment started.

According to the Swedish Meteorological and Hydrological Institute (SMHI, 2012a), the mean day temperature was even during the first two weeks of the experiment. In the last week of Experiment 1 there was a high temperature increase with a temperature above average. The temperature increase may partly explain the heavy increase of the powdery mildew infection.

Another reason could be that the plants were treated with Fungazil three days before the first application with the tested biocontrol agents was made. It is difficult to know if the previous treatments with Fungazil have had an impact on the viability of the biocontrol agents A, and B since the degradation of the active substance in Fungazil, imazalil, only has been analysed in the cucumber fruits and not in/on the leaves (Helena Nylund, Nordisk Alkali, 2012-08-13). The degradation of imazalil can vary in relation to factors like plant growth, temperature and light intensity.

In Experiment 1, the standard treatment had the significantly lowest mean disease incidence, measured disease severity index (DSI) at the end of the experiment in lower parts of the plant. Treatment A7d (Biocontrol agent A, application every seventh day) had the significantly highest mean DSI followed by treatment A14d (Biocontrol agent A, application every fourteenth day). Even in the upper parts of the plant, treatments A7d and A14d had the highest means of the mean disease incidence value. The standard treatment had the significantly lowest disease symptom in lower parts of the plant. In lower parts of the plant, the disease incidence value for plants treated with biocontrol agent B were commonly significantly lower than plants treated with biocontrol agent A.

It was shown that there were no significant differences for using biocontrol agent B at different intensities, but it was significantly better to use biocontrol agent A every fourteenth day compared to every seventh day.

The total yield with numbers of harvested cucumbers was not much affected by using the biocontrol agents. The standard treatment had the highest number of harvested cucumbers and even if the biocontrol treatments resulted in a lower number of harvested cucumbers the numbers were still at an acceptable level according to the grower.

The total weight per treatment differed between the treatments as for the average weight per cucumber, which also showed that the treatment B14d (Biocontrol agent B, application every fourteenth day) had both the highest total weight per treatment and the highest average weight

per cucumber. The standard treatment showed the lowest mean DSI, but showed an indication to give lowest yield numbers. The low yield numbers might be a result of the pesticide use.

In Experiment 1, the standard treatment was the best option followed by the treatment with the biocontrol agent B with a 14 day interval.

5.2.2 Experiment 2 – In Experimental Greenhouse

The inoculum used for the inoculation plants in Experiment 2 and 3, came from the cucumber culture in Experiment 1. This means that biocontrol agents A, *Ampelomyces quisqualis,* and B, *Pythium oligandrum,* only have been tested for control of this species. It also means that it is a relevant powdery mildew species for commercial cucumber production in Sweden.

In both lower and upper parts of the plant, the disease assessment showed that both treatments with biocontrol agent B, gave significantly lower mean DSI by powdery mildew than both control plants and treatments with biocontrol agent A. This means that the biocontrol agent B had a better ability to control powdery mildew than the biocontrol agent A. This experiment also showed that the prophylactic application of biocontrol agent B had a significantly positive effect. In these plants, there was a stronger reduction of the powdery mildew infection, compared to the plants with other treatments and the non-treated control. The powdery mildew infection was almost totally controlled in upper plant parts in plants controlled prophylactically with biocontrol agent B.

In this experiment, the plants were attacked by Western Flower Thrips. Most of the plants had a shortage of nutrients after some weeks and the plants also started to get necrotic spots caused by the powdery mildew. Even if biological control was used to control the Western Flower Thrips and extra nutrients were added, the attack may have weakened the plants and increased their susceptibility towards powdery mildew. The Western Flower Thrips may also have assisted pathogen dispersal.

According to the Swedish Meteorological and Hydrological Institute (SMHI, 2012b), the mean day temperature was even during the time of the experiment. The mean day temperature has been lower than normal for this time of the year. This means that the temperature did not have any distinct increases, which could have increased the powdery mildew infection.

In Experiment 2, the non-treated control plants could have increased the powdery mildew infections in the plants with the other treatments. The control plants acted as extra inoculation plants during the experiment, since they were totally untreated.

There was not a big difference between the treatments regarding harvested cucumbers, which mean that in this experiment the different treatments did not have an impact on this yield parameter.

5.2.3 Experiment 3 – In Experimental Greenhouse

As in Experiment 2, the non-treated control plants could have increased the powdery mildew infections in the plants with the other treatments. The control plants acted as extra inoculation plants during the experiment, since they were totally untreated.

When the disease symptoms started to increase the mean DSI of treatment B, *Pythium oligandrum,* with prophylactic application was significantly smaller than for the other treatments in lower parts of the plant. This shows that the prophylactic treatment had a clear disease controlling effect. In upper parts of the plant, the result was not that clear all the way through the experiment, even if treatment with prophylactic application of biocontrol agent B had the significantly lowest mean DSI of the treatment at the end of the experiment.

Since Experiment 3 is based on the same experimental plan as Experiment 2, it was possible to build on the experiences from the previous experiment, Experiment 2. Biological insect control was applied from the start and extra nutrient solution added when watering, to avoid nutrient shortage and Western Flower Thrips. Except for the powdery mildew infection, the plants looked healthy during the whole experiment, which might be a result of these activities.

According to the Swedish Meteorological and Hydrological Institute (SMHI, 2012b and 2012c), the mean day temperature was higher than normal in the beginning of the experiment and consistent but colder than normal in the end of the experiment. This means that the temperature might have had an effect on the rapid increase of the disease symptoms in the beginning of the experiment.

Experiments 2 and 3 showed that there were only small differences between the different biocontrol agents when applied prophylactically compared to curatively. It was shown that it was significantly better to use biocontrol agent B prophylactic compared to curative. Biocontrol agent B showed a more positive result than biocontrol agent A, *Ampelomyces quisqualis*, compared to the disease incidence. For biocontrol agent A, no significant differences were found in these experiments, when comparing prophylactic and curative uses.

5.3 Limitations of the Study

In all of the three experiments, the efficacy of the biological control agents was only tested in one cucumber cultivar. The used cultivar was not tolerant to cucumber powdery mildew.

Experiment 1 started when powdery mildew infection was already in the greenhouse. In the experiment, the biocontrol products have only been tested against the powdery mildew species present during the time for the experiments. In Experiment 1, the plants had previously been treated with a fungicide, which can have affected the result of this study.

Experiments 2 and 3 have been conducted with the same powdery mildew species as in Experiment 1, since the inoculum came from the cucumber culture in that experiment.

In Experiments 2 and Experiment 3, the weight of the harvested cucumbers has not been recorded.

5.4 General

Similar experiments have been made with *Ampelomyces quisqualis* to study the control effect of powdery mildew, caused by *Podosphora xanthii*. Dik et al (1998) showed that in both their experiments *A. quisqualis* did not control powdery mildew (*P. xanthii*) compared to its control treatments with Tween 80, which is a polyethylene ester with surfactant activity. Shishkoff and McGrath (2002) showed that application of *A. quisqualis* did not significantly reduce powdery mildew (*P. xanthii*) colony sizes. However they showed also that *A. quisqualis* reduced the amount of inocuclum produced by each colony. Sundheim (1982) showed in his experiments by comparing the effect *A. quisqualis* to fungicides that the cucumber yields was higher when a reduced rate of fungicides was used. The yield difference between the treated plants and the control plants sprayed with water was significantly less for the water sprayed plants compared to the treated plants. For the other treatments there were no significant differences in yield. In these experiments both *G. cichoracearum* and *P. xanthii* were present.

Falk et al (1995) showed that *A. quisqualis* only moderately controlled *P. xanthii*. They explained that this might be a result of profuse and rapid sporulation of *P. xanthii*. Since *A. quisqualis* parasites on different types of powdery mildew, e.g. grape powdery mildew (*Unicula necator* (Schw.) Burr.) and strawberry powdery mildew (*Podosphaera macularis* (Wallr.) U. Braun & S. Takam) they also suggested that the different powdery mildew in their experiment reacted differently to parasitism. The results in this study do agree with the results

found in literature. The study by Sundheim, 1982, is the only study found, evaluating the effects of *A. quisqualis* controlling *G. cichoracearum* and *P. xanthii.*

It is known that the biological control agents tested so far cannot control powdery mildews within the same efficacy as chemical agents (Gilardi et al., 2008). Therefore, it is important to find methods and control strategies possible to combine with biological control and at the same time reduce the use of chemicals to control powdery mildew.

Greenhouse conditions are favourable for cucumber powdery mildew and provide good conditions for both infection and parasitism by hyperparasites (Sundheim, 1982). This means that there are good possibilities to use hyperparasites as biological control, as long as the right hyperparasite is used. A Master thesis by G. Andersson (2003), about climate dependence of powdery mildew on cucumber in greenhouse production, showed that climate has a great impact on cucumber powdery mildew infections.

There is limited information about the biocontrol agents, *A. quisqualis* and *Pythium oligandrum,* and their effect against both *Golovinomyces cichoracearum* and *P. xanthii*. Most of the articles and research reports found discuss the effects of using *A. quisqualis* as a method to control *P. xanthii.* There is also a lack of information about the control mechanisms and effects of using *A. quisqualis* to control *G. cichoracearum* and of *P. oligandrum* to control both *G. cichoracearum* and *P. xanthii.*

5.5 Further Studies

To clarify if these biocontrol agents are possible to use in Sweden, more studies needs to be done. This study is only an initial part of all studies needed to make a final evaluation of the biocontrol agents for use under Swedish conditions.

For further studies, a genetic analysis is necessary to be sure what powdery mildew species the biocontrol agents are tested against. Genetic analysis can also provide a better basis for studies of the different biocontrol agents antagonize.

In order to see how the biocontrol agents act after application, a possibility might be to try to make an isolation of the active substance. This can be done to investigate how long the biocontrol agent will be active after application. It might also be possible to study the sporulation of the powdery mildew after application of the biocontrol agents. Reduced sporulation will decrease the pathogen spread and slow down the disease development. The investigations

can be made both by using a magnifying glass, and study the disease development directly on the leaf surface, and also by making samples to study in a light microscope.

In this study, each biocontrol agent is tested alone. It might be a possibility to study application of these biocontrol agents together with an adhesion agent. According to the manufacturers it is important not to use these products together with other products containing other microorganisms in a short time interval, since other microorganisms may affect the effect the active organism in these products.

To provide a decision basis regarding the suitability of these biocontrol agents for Swedish conditions, more investigations need to be done. Further studies will make it possible to see the effect of the biocontrol agents over a longer time period. The presented studies have been limited and only lasted a few weeks. Therefore, it is necessary to make further studies to see the effect of the biocontrol agents over a full season.

Future studies might also include that each biocontrol agent is tested separately to not have an impact on other treatments. Commonly in cucumber productions, the whole greenhouse is treated instead of just a small part where the infection can be seen, to find out if an agent works or not (Tomas Isberg, 2012-05-29). Another suggestion for further studies might be to look at how the efficacy of the biocontrol agents are affected by climatic conditions and to study if there are possibilities to use different biocontrol agents during different parts of the season. This also needs to be studied over a longer time period and with separately tested agents.

In both Experiments 2 and Experiment 3, there have been non-treated control plants. This might have had impact on the result, since the non-treated control plants created an extra infection pressure on the other experimental plants. At the growers, the whole culture is treated upon an infection, which results in no plants left un-treated to increase the infection risk. In smaller experiments this might be difficult to avoid when there is not enough space keep treatments separate in different greenhouses or chambers. If there are possibilities, one option may be to have the different treatments in different greenhouses or chambers to avoid treatments impact on each other. The climate conditions may still be the same and the treatments may be separated far enough to avoid impact from each other. Another alternative is like in the experiments by Sundheim (1982), where there was no untreated control to minimize the spread of powdery mildew. The obvious down-side is that the treatment efficacy cannot be compared to an untreated control.

6. CONCLUSION

The causative agent of the powdery mildew infection in the above performed experiments was most probably *Golovinomyces cichoracearum.*

The experiments in this study showed that the biocontrol agents did reduce powdery mildew infection in greenhouse produced cucumber. The application method by using prophylactic application of bioncontrol agent B, *Pythium oligandrum,* showed significantly better result. The application of biocontrol agent A, *Ampelomyces quisqualis*, prophylactic or curative did not show any significant difference.

By studying the application intensity, it was shown that biocontrol agent A, *A. quisqualis*, was significantly better when applied every 14^{th} day compared to every 7^{th} day. For biocontrol agent B, *P. oligandrum*, the application intensity did not make any significant difference for the result.

There was also an indication of increased yield, even if it was not statistically significant, by using the biocontrol agents compared to standard (chemical) control in a conventional green-house cucumber production.

The biocontrol agents used in this study needs to be further investigated, but might be used in future integrated pest management programs.

REFERENCES

Agrios, G.N. (2005) *Plant Pathology*. Fifth edition. Burlington: Elsevier Academic Press. In English

Al-Sa'di, A.M., Drenth, A., Deadman, M.L., de Cook, A.W.A.M., and Aitken, E.A.B. (2007) Molecular characterization and pathogenicity of *Pythium* species associated with damping-off in greenhouse cucumber (*Cucumis sativus*) in Oman. *Plant pathology* 56. 140-149. In English

Andersson, G. (2003) Klimatberoende hos gurkmjöldagg i växthuskulturer. Swedish University of Agricultural Sciences, Alnarp. *Examensarbete inom Hortonomprogrammet.* 2003:1. In Swedish

Bardin, M., Carlier, J. and Nicot, P.C. (1999) Genetic differentiation in the French population of *Erysiphe cichoracearum*, a causal agent of powdery mildew of cucurbits. *Plant Pathology* 48. 531-540. In English

Bjelland, O. (1988) *Grönsaksodling i växthus*. Stockholm: LTs förlag. In Swedish

Boesewinkel, H. J. (1980) The morphology of the Imperfect States of Powdery Mildews (Erysiphaceae). *The Botanical Review* Vol. 46, No 2. 167-238. In English

Borg Ohlsson, M. and Jansson, J. (2011) *Bekämpning av trädgårdsväxternas skadegörare 2011/2012.* Jönköping: Publikationsservice Jordbruksverket. In Swedish

Chen, R-S., Chu, C., Cheng, C-W, Chen, W-Y and Tsay (2007) Differentiation of two powdery mildews of sunflower (*Helianthus annuus*) by a PCR-mediated method based on ITS sequences. *Eur J Pathol (2008)* 121:1-8. In English

Dahlqvist, M. (2008) Möjligt att bekämpa mjöldagg i gurkodling? *Bondeföretagaren* 2. In Swedish

Dik, A.J., Verhaar, M.A. and Bélanger, R.R. (1998) Comparison of three biological control agents against cucumber powdery mildew (*Spaerotheca fuliginea*) in semi-commercial-scale glasshouse trials. *European Journal of Plant Pathology* 104. 413-423. In English

Du, X-l., Xing, G-y., Ren, A-z. and Zhao, P-b. (2010) Control Effect of Vegetable Oil on Cucumber Powdery Mildew. *Plant Diseases and Pests* 1(5). 43-46. In English

El-Ammari, S.S. and Wajid Khan, M. (1983) *Leveillula taurica* Powdery Mildew on Greenhouse Cucumbers in Libya. *Plant disease* May. 553-555. In English

Falk, S.P., Gadoury, D.M., Pearson, R.C. and Seem, R.C. (1995) Partial Control of Grape Powdery Mildew by the Mycoparasite *Ampelomyces quisqualis. Plant disease* May. 483-490. In English

Gilardi, G., Manker, D.C., Garibaldi, A. and Gullino, M.L. (2008) Efficacy of the biocontrol agents *Bacillus subtilis* and *Ampelomyces quisqualis* applied in combination with fungicides against powdery mildew of zucchini. *Journal of Plant Diseases and Protection,* 115. 208-213. In English

Hansson, T. and Jansson, J. (2012) *Växtskyddsmedel 2012 – växthusgrönsaker*. Jönköping: Jordbruksverket. In Swedish

Heywood, V.H., Brummitt, R.K., Culham, A. and Seberg, O. (2007) *Flowering Plant Families of the World.* Ontario: Firefly Books. In English

Hökeberg, M. (2010) Goda mikrober räddar grödorna. In: *Jordbruk som håller i längden*. 159-172. Stockholm: Formas Fokuserar. In Swedish

Jasinski, J., Precheur, B., Miller, S., Lewis Ivey, M., Rhodes, L. and Riedel, M. (2010) *Conventional and Alternative Fungicides to Control Powdery Mildew on Pumpkin – Final report to Ohio Vegetable and Small Fruit Research and Development Program.* In English

Jordbruksverket. (2011) Homepage [online] Available from: http://www.sjv.se/amnesomraden/odling/tradgardsodling/gronsakerivaxthus/statistik.4.32b12c7f12940112a7c800036048.html [2012-08-01] In Swedish

Jordbruksverket. (2012) *Trädgårdsproduktion 2011.* Sveriges officiella statistik. In Swedish

Jordbruksverket. (n.d.) Homepage [online] Available from: http://www.sjv.se/etjanster/etjanster/vaxtskyddsinfotradgard.4.42c619a8136b9f2109d800067.html [2012-07-25] In Swedish

Judd, W.S., Campbell, C.S., Kellogg, E.A., Stevens, P.F. and Donoghue, M.J. (2008) *Plant Systematics – A Phylogenetic Approach* Third Edition. Sunderland, Massachusetts: Sinauer Associates, Inc. In English

Jönsson, B. (2001) *Trädgårdsnäringens växtskyddsförhållanden.* Rapport 2001:7A. Jordbruksverket. In Swedish

Kaewchai, S., Soytong, K. and Hyde, K.D. (2009) Mycofungicides and fungal biofertilizers. *Fungal Diversity Press.* 25-50. In English

Kapoor, J.N. (1998) *Erysiphe cichoracearum. C.M.I. Descriptions of Pathogenic Fungi and Bacteria No. 152.* In English

KEMI. (2010) Homepage [online] Available from: http://www.kemi.se/Documents/Forfattningar/KIFS/K10_1.pdf [2012-08-05] In Swedish

Kiss, L. (2003) A review of fungal antagonists of powdery mildews and their potential as biocontrol agents. *Pest Management Science* 59. 475–483. In English

Liang, Y.C., Sun, W.C. and Römheld, V. (2005) Effects of foliar- and root-applied silicon on the enhancement of induced resistance to powdery mildew in *Cucumis sativus*. *Plant pathology* 54. 678-685. In English

Liberato, J.R., Shivas, R.G. and Cunnington J.H. (2006) *Podosphaera xanthii* on *Euryops chrysanthemoides* in Australia. *Australasian Plant Pathology*, 35. 739-741. In English

Miazzi, M., Laguardia, C. and Faretra, F. (2011) Variation in *Podosphaera xanthii* on Cucurbits in Southern Italy. *J Phytopatol* 159, 538-545. In English

Mohammed, N., Lherminier, J., Farmer, M-J., Fromentin, J., Béno, N., Houot, V., Milat, M-L., and Blein, J-P. (2006) Defense Responses in Grapevine Leaves Against *Botrytis cinerea* Induced by Application of a *Pythium oligandrum*. *The American Phytopathological Society.* 611-620. In English

Molén, S. A. (2007/2008) Ekologisk odling av växthusgurka. In: *Ekologisk odling i växthus.* Page 1-30. Jordbruksverket. In Swedish

Morishita, M., Sugiyama, K., Saito, T. and Sakata, Y. (2003) Powdery Mildew Resistance in Cucumber. *JARQ* 37(1). 7-14. In English

Pal, K.K. and McSpadden Gardener, B. (2006) Biological Control of Plant Pathogens. *The Plant Health Instructor* 2006. 1-25. In English

Paulitz, T.C. and Bélanger, R.R. (2001) Biological control in Greenhouse Systems. *Annual Reviews Phytopathology* 39:103-133. In English

Qvarnström, K. (1989) Bekämpning av mjöldagg (*Erysiphe cichoracearum*) på gurkplantor. *Växtskyddsnotiser* 3:1989, 54-57. In Swedish

Qvarnström, K. (1992) Behandling mot mjöldagg (*Erysiphe cichoracearum*) på gurkplantor med låggiftiga medel. *Växtskyddsnotiser* 1:1992, 17-20. In Swedish

Qvarnström, K., Johnsson, L. and Olofsson, B. (1995) Bekämpning av gurkmjöldagg, *Erysiphe cichoracearum*, med låggiftiga medel. *Växtskyddsnotiser* 3:1995, 80-84. In Swedish

Robinson, R.W. and Decker-Walters, D.S. (1997) *Cucurbits*. Wallingford: CAB International. In English

Romero, D., de Vicente, A., Zeriouh, H., Carzorla, F.M., Fernández-Ortuño, D., Torés, J.A. and Pérez-García, A. (2007) Evaluation of biological control agents for managing cucurbit powdery mildew on greenhouse-grown melon. *Plant Pathology* 56. 976-986. In English.

Sakata, Y., Sugiyama, M. and Ohara, T. (2006) Development of a powdery mildew resistant cucumber. *Cucurbitaceae* 2006. 193-196. In English

Schuerger, A.C. and Hammer, W. (2003) Suppression of Powdery Mildew on Greenhouse-Grown Cucumber by Addition of Silicon to Hydroponic Nutrient Solution Is Inhibited at Higher Temperature. *Plant Disease* February 2003. 177-185. In English

Seebold, K. (2010) Foliar Diseases of Cucurbits. *Plant Pathology Fact Sheet.* University of Kentucky. In English

Shishkoff, N. and McGrath, M.T. (2002) AQ10 Biofungicide Combined with Chemical Fungicides or AddQ Spray Adjuvant for control of Cucurbit Powdery Mildew in Detached Leaf Culture. *Plant Disease* August 2002. 915-918. In English

Sitterly, W. R. (1978) Powdery Mildews of Cucurbits. In: Spencer, D.M.: *The Powdery Mildews,* 359-379. London: Academic Press Inc. Ltd. In English

SMHI. (2012a) Homepage [online] Available from: http://www.smhi.se/klimatdata/meteorologi/2.1353/dailyTable.php?par=tmpAvvDay&yr=2012&mon=5 [2012-08-14] In Swedish

SMHI. (2012b) Homepage [online] Available from: http://www.smhi.se/klimatdata/meteorologi/2.1353/dailyTable.php?par=tmpAvvDay&yr=2012&mon=6 [2012-08-14] In Swedish

SMHI. (2012c) Homepage [online] Available from: http://www.smhi.se/klimatdata/meteorologi/2.1353/dailyTable.php?par=tmpAvvDay&yr=2012&mon=7 [2012-08-14] In Swedish

Sundheim, L. (1982) Control of cucumber powdery mildew by the hyperparasite *Ampelomyces quisqualis* and fungicides. *Plant Pathology* 31. 209-214. In English

Taiz, L. and Zeiger, E. (2006) *Plant physiology.* Fourth Edition. Sunderland, Massachusetts: Sinauer Associates, Inc. In English

Velkov, N. (2007) *Cucumber powdery mildew, resistance and tolerance.* Research people and actual tasks on multidisciplinary sciences, 6-8 June 2007, Lozenec, Bulgaria. In English

Verhaar, M.A., Hijwegen, T. and Zadoks, J.C. (1996) Glasshouse Experiments on Biocontrol of Cucumber Powdery Mildew (*Spaerotheca fuliginea*) by the Mycoparasites *Verticillum lecanii* and *Sporothrix rugulosa. Biological control 6.* 353-360. In English

Wivstad, M. (2010) Ogräs, sjukdomar och skadeinsekter – hur ska de bekämpas? In: *Jordbruk som håller i längden*. 147-158. Stockholm: Formas Fokuserar. In Swedish

Åkesson, I. and Jansson, J. (2011) Sanering och hygien i växthus. *Faktablad om växtskydd. Trädgård.* 4T. In Swedish

Personal Communication

Isberg, T, Sånnagården, Kvidinge, oral information, 2012-05-29

Nylund, H. (helena.nylund@nordiskalkali.se), 2012-08-13. Fråga om Fungazil. E-mail to Anna-Carin Almqvist (acal0011@stud.slu.se)

Appendix 1: Experiment 1 – In farmers greenhouse

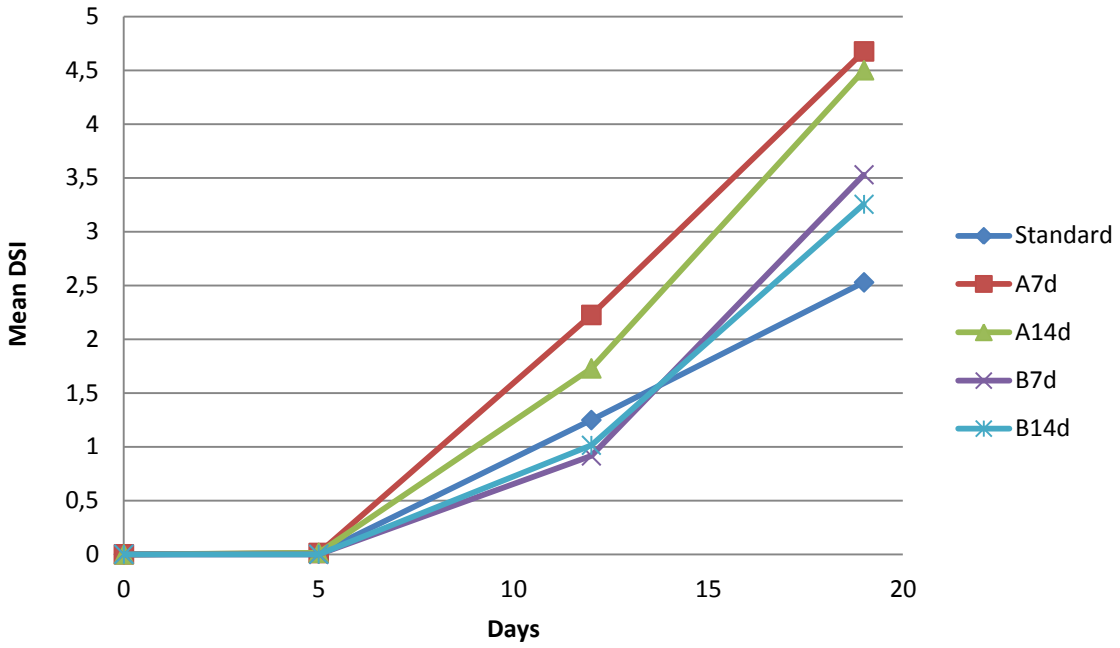


Figure 6. Means of disease severity index (DSI) of powdery mildew infections in lower parts of the plant in Experiment 1 – In farmers greenhouse, Sånnagården, Kvidinge. The figure shows the disease development in days from the start of the experiment. The DSI is graded 0-5, where 5 is the highest grade of infection. Standard = for this cultivation standard control. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.*A7d = Biocontrol agent A, application every 7th day; A14d = Biocontrol agent A, application every 14th day; B7d = Biocontrol agent B, application every 7th day; B14d = Biocontrol agent A, application every 14th day.

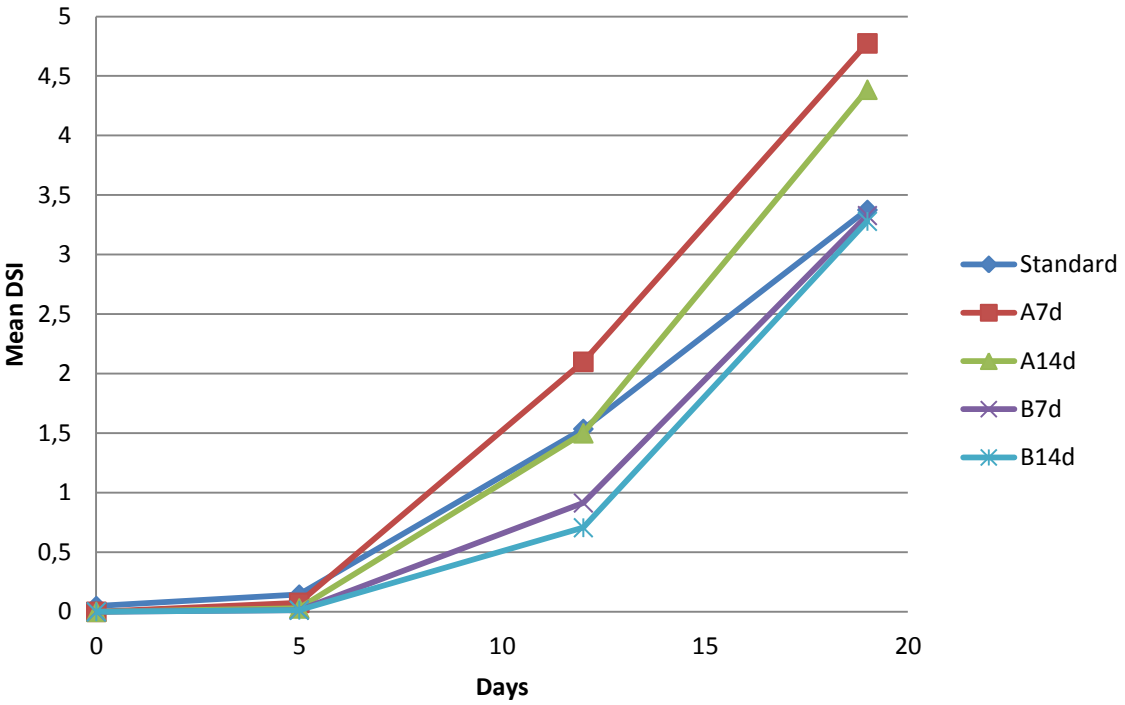


Figure 7. Means of disease severity index (DSI) of powdery mildew infections in upper parts of the plant in Experiment 1 - In farmers greenhouse, Sånnagården, Kvidinge. The figure shows the disease development in days from the start of the experiment. The DSI is graded 0-5, where 5 is the highest grade of infection. Standard = for this cultivation standard control. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.*A7d = Biocontrol agent A, application every 7th day; A14d = Biocontrol agent A, application every 14th day; B7d = Biocontrol agent B, application every 7th day; B14d = Biocontrol agent A, application every 14th day.

Appendix 2: Experiment 2 – In experimental greenhouse

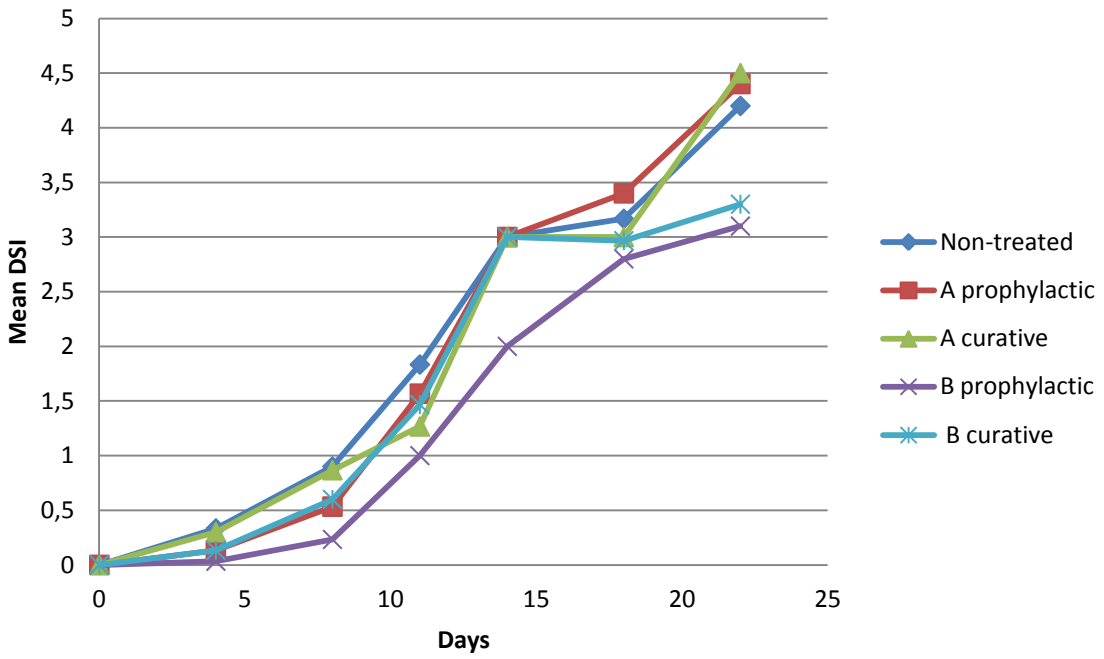


Figure 8. Means of disease severity index (DSI) of powdery mildew infections in lower parts of the plant in Experiment 2 – In experimental greenhouse, Alnarp. The figure shows the disease development in days from the start of the experiment. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.* Non-t. = all plants were untreated; A cur = First treatment was made curative with biocontrol agent A when the first powdery mildew infection was seen in the experiment; A pro = Plants were treated prophylactic with biocontrol agent A every seventh day until first powdery mildew infection was seen in the experiment; B cur; First treatment was made curative with biocontrol agent B when the first powdery mildew infection was seen in the experiment; B pro = Plants were treated prophylactic with biocontrol agent B every seventh day until first powdery mildew infection was seen in the experiment. All applications of the biocontrol agents were made every 7^{th} day.

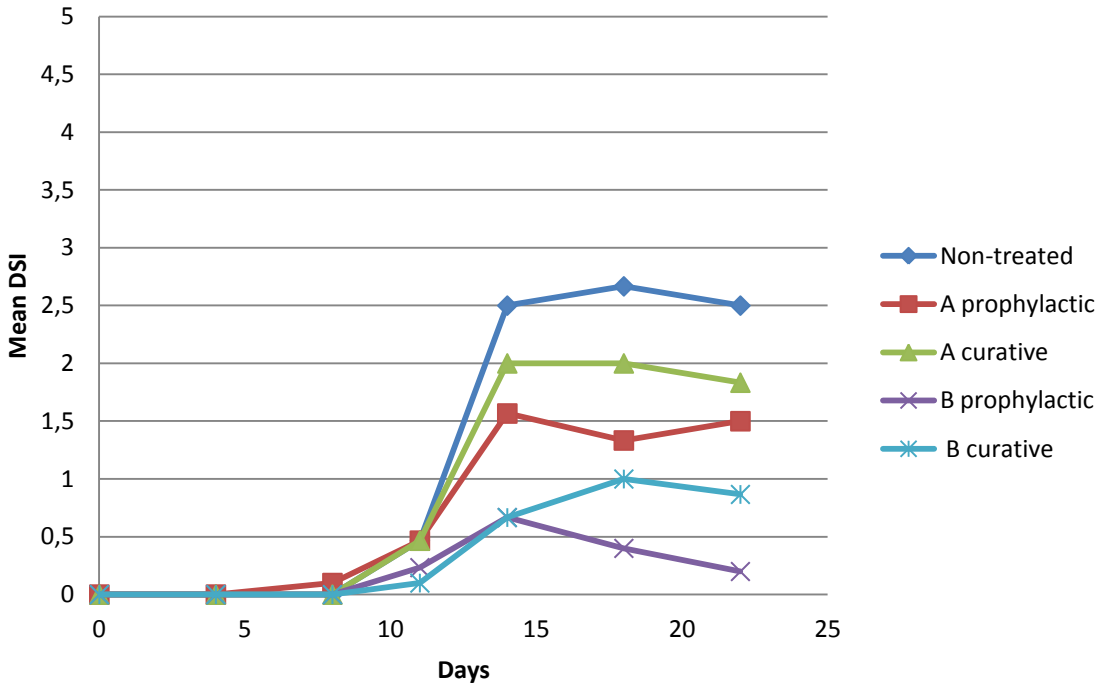


Figure 9. Means of disease severity index (DSI) of powdery mildew infections in upper parts of the plant in Experiment 2 – In experimental greenhouse, Alnarp. The figure shows the disease development in days from the start of the experiment. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.* Non-t. = all plants were untreated; A cur = First treatment was made curative with biocontrol agent A when the first powdery mildew infection was seen in the experiment; A pro = Plants were treated prophylactic with biocontrol agent A every seventh day until first powdery mildew infection was seen in the experiment; B cur; First treatment was made curative with biocontrol agent B when the first powdery mildew infection was seen in the experiment; B pro = Plants were treated prophylactic with biocontrol agent B every seventh day until first powdery mildew infection was seen in the experiment. All applications of the biocontrol agents were made every 7^{th} day.

Appendix 3: Experiment 3 – In experimental greenhouse

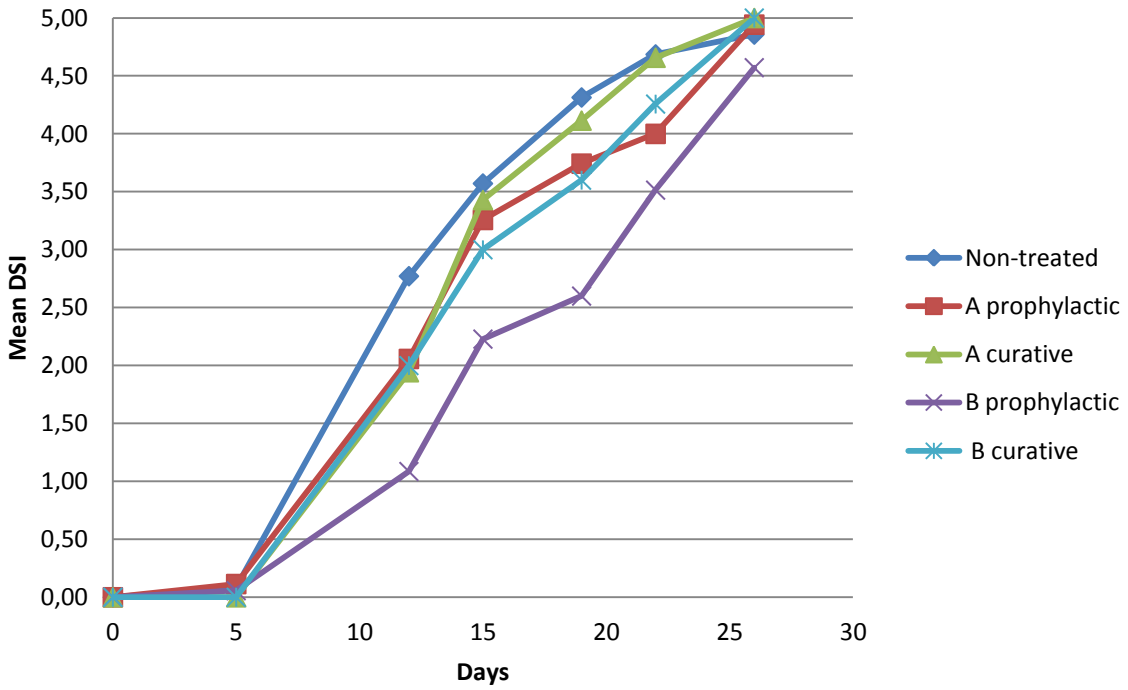


Figure 10. Means of disease severity index (DSI) of powdery mildew infections in lower parts of the plant in Experiment 3 – In experimental greenhouse, Alnarp. The figure shows the disease development in days from the start of the experiment. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.* Non-t. = all plants were untreated; A cur = First treatment was made curative with biocontrol agent A when the first powdery mildew infection was seen in the experiment; A pro = Plants were treated prophylactic with biocontrol agent A every seventh day until first powdery mildew infection was seen in the experiment; B cur; First treatment was made curative with biocontrol agent B when the first powdery mildew infection was seen in the experiment; B pro = Plants were treated prophylactic with biocontrol agent B every seventh day until first powdery mildew infection was seen in the experiment. All applications of the biocontrol agents were made every 7^{th} day.

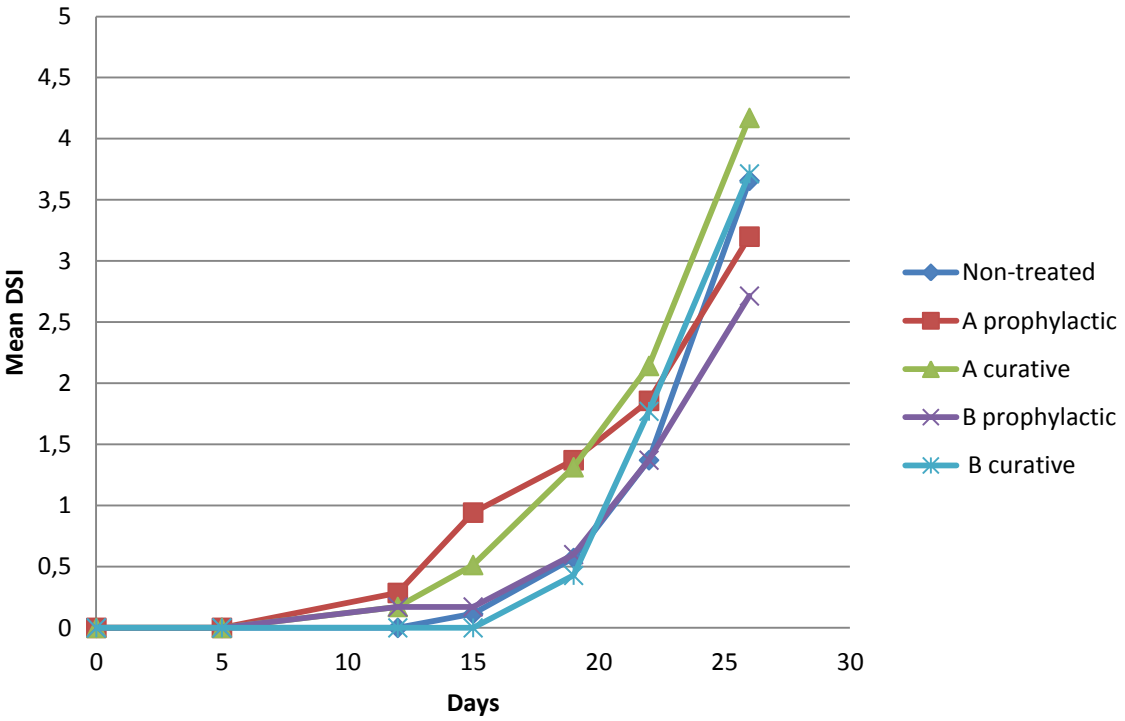


Figure 11. Means of disease severity index (DSI) of powdery mildew infections in upper parts of the plant in Experiment 3 – In experimental greenhouse, Alnarp. The figure shows the disease development in days from the start of the experiment. Biocontrol agent A = *Ampelomyces quisqualis* and Biocontrol agent B = *Pythium oligandrum.* Non-t. = all plants were untreated; A cur = First treatment was made curative with biocontrol agent A when the first powdery mildew infection was seen in the experiment; A pro = Plants were treated prophylactic with biocontrol agent A every seventh day until first powdery mildew infection was seen in the experiment; B cur; First treatment was made curative with biocontrol agent B when the first powdery mildew infection was seen in the experiment; B pro = Plants were treated prophylactic with biocontrol agent B every seventh day until first powdery mildew infection was seen in the experiment. All applications of the biocontrol agents were made every 7^{th} day.