Epidemiological Investigations of Disease Complexes of Tomato under Protected Cultivation in the Humid Tropics

Von der Naturwissenschaftlichen Fakultät
der Gottfried Wilhelm Leibniz Universität Hannover
zur Erlangung des Grades einer

Doktorin der Gartenbauwissenschaften
- Dr. rer. hort. -

genehmigte
Dissertation
von

Dipl.-Ing. agr. Simone Anna Kandziora
geboren am 28.08.1973 in Kassel

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… dedicated to my parents

in love and gratitude

I Abstract

Surveys were conducted in several regions in Thailand in order to get an overview of the main tomato diseases. Pythium root rot (PRR), caused by *Pythium aphanidermatum*, was quickly identified as a major soil-borne disease. Symptoms of the foliar disease early blight, caused by *Alternaria solani*, were frequently found in tomato fields in different climatic regions of the country.

Isolates of *A. solani* were obtained from tomato leaves collected in four regions in Thailand and evaluated for pathogenicity under controlled conditions using detached leaflets and whole plants. The morphological characteristics of colony growth and sporulation of all *A. solani* isolates were determined and compared with those of representative isolates
A. solani could successfully infect tomato plants under controlled conditions in the growth chamber, and could be easily re-isolated. In experiments under greenhouse conditions (closed net greenhouse, Econet M, pore size 0.18 mm, 40 x 37 mesh (40-mesh), base area 10 x 20 m), the temperature regime inside (mean temperature > 30°C) was not suitable for early blight development. Under these conditions, black leaf mold (BLM), caused by *Pseudocercospora fuligena,* turned out as the major leaf disease limiting greenhouse production of tomato. This is the first report of this disease on the research site of the Asian Institute of Technology in Thailand.

The complex of two tomato (*Solanum lycopersicon* L.) diseases, composed of BLM and PRR, was investigated under the conditions of a closed net house. Four experiments were conducted in different seasons and with two different tomato varieties (´New King Kong´ and ´King Kong 2´).
The primary occurrence of PRR, inoculated in 2 different densities was monitored up to 14 days after positioning plants in the greenhouse. Tests of substrate samples from experimental pots with the potato-baiting-method confirmed a successful inoculation of *P. aphanidermatum* in all experiments. No other soil-borne pathogen than *P. aphanidermatum* occurred. Due to PRR, 30% to more than 40% of plants dropped out and were substituted, in the control treatment up to 64%. In order to observe BLM effects, *Trichoderma harzianum* was used as a biological antagonist in two subsequent experiments. BLM naturally occurred inside the greenhouse throughout the year without great differences among seasons. To keep the disease level in one part of the greenhouse low, the fungicide Maneb was applied.

In pre-experiments with artificial inoculation, BLM showed first symptoms roughly after 14 days. Under greenhouse conditions with natural infections, an incubation time of 7 to 28 days was found. The main experiments were conducted for 84 and 112 days. The disease progress curves of incidence of plants were S-shaped and the 100% infection level was reached approximately after 63 days. At the end of the experiments, a maximum disease severity of 30% was reached. The spatial disease patterns within rows at the beginning of the epidemics were analysed with the join-count statistics. In two experiments, the disease occurred in a random pattern, while in the two others an aggregation of diseased plants was indicated. The 3-D plots of the spatial disease distribution did not show a gradient. The data were also analysed for vertical disease distribution. The plants of the determinate variety ´New King Kong´ had a maximum number of 26 (± 1.11) leaves, those of the indeterminate variety ´King Kong 2´ of 51 (± 1.21). The highest leaf insertion with symptoms was at position 45 from the bottom. The leaf position with the maximum severity of 41.42 ± 4.46% was around leaf number 11.

Neither the different *Pythium*-levels nor the treatment with *Trichoderma* changed the overall trend of the disease severity progress curves of BLM.

The results of tomato growth and yield parameters are in general very heterogeneous. The marketable yield harvested was insignificant. The overall tendency of interactions between the two diseases was negative. For example, the fresh weight of plants of the P0-BLM0 treatment (i.e. without inoculation of *P. aphanidermatum*, but sprayed against BLM) in the first experiment was 1029.71 ± 69.56 g, while plants in the P2-BLM0 treatment (i.e. inoculated with high level of *P. aphanidermatum*, but fungicide sprayed against BLM) had roughly 340 g less weight. The plants of the P0-BLM1-treatment (i.e. without inoculation of *P. aphanidermatum*, and without fungicide use), in which only BLM developed symptoms, had a value of 660.00 ± 72.66 g, thus a difference of 365 g compared to the control plants

Keywords: *Pseudocercospora fuligena, Alternaria solani, Pythium aphanidermatum*, primary occurrence, spatial distribution, temporal progress, vertical distribution, disease interaction.

II Zusammenfassung

Zu Beginn der Studie wurden in Thailand Vorort-Informationen gesammelt, um einen Gesamtüberblick der hauptsächlich verbreiteten Krankheiten an der Tomate zu erhalten. Die Pythium-Wurzelfäule, verursacht durch den Erreger *Pythium aphanidermatum*, gehörte dabei zu den Hauptkrankheiten. Als Blattkrankheit wurden immer wieder Symptome der Dürrfleckenkrankheit, hervorgerufen durch *Alternaria solani,* gefunden.

In vier verschiedenen Regionen von Thailand wurden Tomatenblätter, die mit *A. solani* befallen waren, gesammelt, im Labor unter kontrollierten Bedingungen untersucht und die gewonnenen Isolate auf ihre Pathogenität getestet. Dabei wurden die morphologischen Charakteristika, wie z.B. die Form der Konidien, bestimmt und mit Hilfe von repräsentativen Isolaten verifiziert. Für diese Untersuchungen wurden sowohl einzelne Blätter als auch die gesamte Pflanze genutzt.

Unter kontrollierten Bedingungen, u.a. im Klimaschrank, gelang es, den Erreger zu inokulieren und auch wieder zu re-isolieren. Mehrere Krankheitszyklen konnten beobachtet werden. Die Temperatur in den Experimenten im Gewächshaus (Seitenwände mit Netz verkleidet, Econet M, Maschengröße 0,18 mm, Grundfläche 10 x 20 m) war allerdings zu hoch (> 30°C), sodass kein Krankheitszyklus vollendet wurde und die Ausbildung von Dürrfleckenkrankheits-Symptomen ausblieb.
Unter den klimatisch gegebenen Gewächshausbedingungen trat der schwarze Blattschimmel, verursacht durch den Erreger *Pseudocercospora fuligena,* erstmals in Erscheinung. Der schwarze Blattschimmel (black leaf mold = BLM) stellte sich als limitierender Faktor für die Tomatenproduktion in Gewächshäusern dar.
Die weiteren Versuche zur Interaktion von Pflanzenkrankheiten unter Gewächshaus-bedingungen wurden mit dem Krankheits-Komplex schwarzer Blattschimmel und Pythium-Wurzelfäule durchgeführt.
Dazu wurden vier Experimente zu verschiedenen Jahreszeiten mit zwei verschiedenen Tomatensorten (´New King Kong´ und ´King Kong 2´), durchgeführt. Das Auftreten der Pythium-Wurzelfäule, die in zwei Stärken appliziert wurde, wurde bis 14 Tage nach Inokulation beobachtet. Abgestorbene Pflanzen wurden in dieser Zeit mit überzähligen Pflanzen in der gleichen Pythium-Inokulum-Variante ersetzt.

Während des gesamten Versuchszeitraumes wurden Substratproben entnommen und mit Hilfe der Kartoffel-Köder-Methode der Erreger re-isoliert. Dabei bestätigte sich, dass *P. aphanidermatum* erfolgreich inokuliert wurde und keine weiteren Krankheiten auftraten. In dieser 14-tägigen Startphase des Experiments fielen 30 bis über 40% der Pflanzen aus, in den Kontrollvarianten sogar bis zu 64%.
Um ganzheitlich gesehen die Interaktionen der Pflanzenkrankheiten zu beobachten, nicht zu viele Verluste durch die Pythium-Wurzelfäule zu haben und mögliche Eingriffe in die Dynamik des schwarzen Blattschimmels zu vermeiden, wurde in den folgenden Experimenten der Antagonist *Trichoderma harzianum* eingesetzt.
Die Versuche waren für den Zeitraum von 84 – 112 Tage nach Positionierung der Pflanzen im Gewächshaus angelegt. BLM trat unabhängig von saisonalen Unterschieden auf. Während des ganzen Jahres wurden die Pflanzen im Gewächshaus auf natürliche Weise infiziert. Um in einem Teil des Gewächshauses das Befallsniveau möglichst niedrig zu halten, wurde eine Seite wöchentlich mit dem Fungizid Maneb behandelt.
In weiteren Experimenten mit künstlicher Inokulation zeigte BLM die ersten Symptome nach 14 Tagen (Erstauftreten). Unter Gewächshausbedingungen mit natürlicher Infektion wurden Inkubationszeiten von 7 bis 28 Tagen beobachtet.

Die Analyse der Befallskurven zeigte für das Erstauftreten einen S-förmigen Verlauf, wobei 100% Befall nach 63 Tagen erreicht wurde. Am Ende der Experimente, nach 84 bzw. 112 Tagen, betrug die Befallsstärke 30%.
Die räumliche Ausbreitung innerhalb der Reihen wurde am Anfang der Epidemie mit einer “join count“ Statistik analysiert. In zwei Experimenten war die Verteilung zufällig; in den zwei anderen Experimenten konnte eine Anhäufung von kranken Pflanzen festgestellt werden. Die 3-D Darstellung der räumlichen Verteilung zeigte keinen deutlichen Trend innerhalb der Versuche auf. Neben der horizontalen wurde auch die vertikale Krankheitsverteilung untersucht. Die Pflanzen der Sorte ´New King Kong´ hatten eine maximale Blattanzahl von 26 Blättern (±1,11), die der undeterminierten Sorte ´King Kong 2´ bis zu 51 Blätter (±1,21). Das höchste Blatt mit sichtbaren BLM Symptomen war das 45. Blatt (gezählt vom Boden zur Spitze). Das Blatt mit der größten Befallsstärke von 41,42 ± 4,46% wurde in Höhe des 11. Blattes gefunden.
Weder die verschiedenen *Pythium*-Inokulationsstärken noch die Behandlung mit *T. harzianum* änderten den Verlauf der BLM - Befallskurven signifikant.

Sowohl die Wachstumsparameter als auch die Ertragsergebnisse waren generell sehr uneinheitlich. Die vermarktungsfähigen Erträge fielen sehr gering aus.
Die genauere Analyse der Wachstumsparameter zeigte, dass sich die Krankheiten gegenseitig beeinflussten und es zu negativen Interaktionen kam. Zum Beispiel hatten die Pflanzen des ersten Experiments in der Variante P0-BLM0 (Kontrollpflanzen, ohne Pythium - Inokulation und mit Fungizid behandelt) ein Frischgewicht von 1029,71 ± 69,56 g, Pflanzen der P2-BLM0-Variante (inokuliert mit hoher *Pythium*-Stufe, mit Fungizid Behandlung) waren ca. 340 g leichter als die Kontrollpflanzen. Die Pflanzen der Varianten P0-BLM1 (ohne Pythium - Inokulation, ohne Fungizid Behandlung), in der nur Symptome von BLM auftraten, hatten ein Frischgewicht von 660,00 ± 72.66 g und waren somit im Verhältnis zu den Kontrollpflanzen 365 g leichter.

Schlüsselworte: *Pseudocercospora fuligena, Alternaria solani, Pythium aphanidermatum*, Erstes Auftreten, Räumliche Verteilung, Zeitliche Dynamik, Vertikale Verteilung, Interaktion.

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VI Abbreviations

A. solani	= *Alternaria solani*
a. i.	= active ingredient
AIT	= Asian Institute of Technology
ANOVA	= Analysis of Variance
AUDPC	= Area Under Disease Progress Curve
AUPC	= Area Under Progress Curve
AVRDC	= Asian Vegetable Research and Development Centre
BLM	= black leaf mold
CFU	= Colony Forming Units
cm^2	= square centimeter
dai	= days after inoculation
dap	= days after positioning
det	= determination number
EB	= early blight
f. sp.	= forma specialis
FAO	= Food Agricultural Organisation
h	= hour
hp	= horsepower
iA	= inoculation of *Alternaria solani*
L	= liter
lb	= pound
LSD	= last significant difference
min	= minute
mL	= milliliter
mm	= millimeter
m^2	= square meter
m^3	= cubic meter
n	= number
P. aphanidermatum	= *Pythium aphanidermatum*
P. fuligena	= *Pseudocercospora fuligena*
PDA	= Potato Dextrose Agar
pers. com.	= personal communication
PRR	= Pythium root rot

P0–BLM0	= 1 plain Petri dish, with control of *P. fuligena*
P0–BLM1	= 1 plain Petri dish, naturally infected with *P. fuligena*
$P0_T$–BLM0	= 1 plain Petri dish, with control of *P. fuligena,* with *T. harzianum*
$P0_T$–BLM1	= 1 plain Petri dish, naturally infected with *P. fuligena*, with *T. harzianum*
P1–BLM0	= 1 Petri dish with *P. aphanidermatum* per pot, with control of *P. fuligena*
P1–BLM1	= 1 Petri dish with *P. aphanidermatum* per pot, naturally infected with *P. fuligena*
P2–BLM0	= 3 Petri dishes with *P. aphanidermatum* per pot, with control of *P.fuligena*
P2–BLM1	= 3 Petri dishes with *P. aphanidermatum* per pot, naturally infected with *P. fuligena*
P0*–BLM0	= 0.2 g sterile inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*
P0*–BLM1	= 0.2 g sterile inoculum of *P. aphanidermatum* per pot, naturally infected with *P. fuligena*
P1*–BLM0	= 0.2 g inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*
P1*–BLM1	= 0.2 g inoculum of *P. aphanidermatum* per pot, naturally infected with *P. fuligena*
P2*–BLM0	= 1 g inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*
P2*–BLM1	= 1 g inoculum of *P. aphanidermatum* per pot, naturally infected with *P. fuligena*
ppm	= parts per million
RH	= relative humidity
rpm	= rounds per minute
s	= second
SE	= standard error
T. harzianum	*= Trichoderma harzianum*
TOA	= Tomato Oatmeal Agar
wA	= without *Alternaria solani*
µm	= micrometer

1 General Introduction

"The primary objective of epidemiological research is to increase our understanding of how disease develops in host crop populations and how other factors influence their development in order to develop sustainable and effective strategies for managing disease." (Xu, 2006).

Epidemiology is the study of diseases in populations (Van der Plank, 1963). The temporal course of an epidemic can be displayed by the disease progress of a population, usually estimated from a sample of a few plants. The entire epidemic is therefore a summation of disease progress on each individual component (Berger and Luke, 1979). Little attention has been given to interactions among plant diseases in epidemiological research, even though the occurrence of more than one disease at the same time on one host is the rule in the field (Campbell and Madden, 1990; Kranz and Jörg, 1989), especially in tropical regions (Intanoo, pers. com.; Waller and Bridge, 1984). Most articles in literature are focused on "one to one" host – pathogen interactions, but research on pathogen – pathogen interactions or multiple attacks are rare. Examples of studies on disease complexes include those of Latch and Potter (1977), Pieczarka and Zitter (1981), Johnson et al. (1987), Kranz and Jörg (1989), Weber et al. (1994), Ngugi et al. (2001) and Paula Junior (2002).

The definitions of Odum (1953) can be helpful to interpret interactions concerning the dynamics of pathogens. The author suggested the following classifications for associations between organisms: neutralism (neither population is affected by association), competition (each population adversely affects the other in the struggle for food, nutrients, living space, or other common need), mutualism (growth and survival of both populations is benefited and neither can survive under natural conditions without the other), protocooperation (both populations benefit by the association, but relations are not obligatory), commensalism (one population benefits, but the other is not affected), amensalism (one population is inhibited and the other not affected), parasitism (one population adversely affects the other by direct attack, but is dependent on the other) and predation (one population adversely affects the other by direct attack). Cook (1981) expanded the definitions of Odum and included terms like antibiosis, competition, hyperparasitism, and stimulation of active defence mechanisms in the host.

Regarding the internal disposition of hosts, Powell (1971b) considered three theoretical mechanisms of bio-predisposition involving interacting pathogens: (1) the primary pathogen may make the host more susceptible to the secondary pathogen; (2) the primary pathogen may

enhance the activity of the secondary pathogen; and (3) the secondary pathogen may even enhance the activity of the primary pathogen.
Zacheo (1993) differentiated between synergistic interaction in terms of combined effect of the pathogens and antagonistic interaction in terms of competitive exclusion. Antagonistic interactions are explained as direct effect of an organism on another or indirect effects through changes in the host physiology (Waller and Bridge, 1984).

Usually interacting pathogens affect the same plant organ (Powell 1971a), but aerial and soil-borne pathogens can simultaneously attack different parts of the plant. One pathogen may influence the resistance of a host to infection and colonization by another. Discerning the importance of effects of root and shoot diseases on the same plant, it is difficult to distinguish influences and effects even when one disease does not clearly affect the susceptibility of the plant to the other (Waller and Bridge, 1984). The aerial parts are more readily seen and damage is mostly attributed to them. However, root diseases can, for example, reduce the capacity of plants for compensatory growth (Waller and Bridge, 1984).
The changes of the nutritional status and the composition of the host cell (Powell, 1971b; Evans and Haydock, 1993) can explain increments of the host susceptibility and the enhancement of the activity of the interacting pathogens, in the case of interactions between aerial and soil-borne pathogens (Waller and Bridge, 1984; Paula Junior, 2002)
Foliar diseases caused by non-obligate pathogens seem to increase when the host is simultaneously infected with a destructive pathogen. Nicholson et al. (1985) found that corn plants infected by *Pratylenchus hexincisus* developed significantly more leaf blight, caused by *Colletotrichum graminicola*, and proposed that leaf senescence hastened by the nematode infection favoured anthracnose leaf blight. Bhowmik and Singh (1977) also observed that *Alternaria* leaf blight on sunflower was more severe on plants infected with *Rhizoctonia solani*. Similarly, Verticilium wilt reduced plant vigour and caused premature senescence on potato plants resulting in an increase of early blight severity, caused by *Alternaria solani* (Harrison, 1974).

With respect to yield production, the interactions are termed synergistic if the yield reduction caused by the interacting pathogens is greater than the sum of the reduction caused by the pathogens attacking the host individually. On the other hand, if the yield reduction is less, than the sum, the interaction is termed antagonistic. According to the simultaneous damage, which pathogens can cause to the host, Bassanezi et al. (1998) suggested a classification of

the interactions between pathogens. If the damage caused by two concurrently infecting pathogens is similar to the sum of damages caused by the pathogens attacking the host separately, the effect is additive; if the damage is less, there is a negative interaction; if it is greater, a positive interaction. Synergistic interactions are important because the economic damage threshold for each disease can be significantly lowered by the presence of an interacting disease. Vice versa, antagonistic interactions can increase the economic damage threshold of a disease in the presence of another (Johnson, 1990).
Interactions between diseases caused by aerial and soil-borne pathogens may have significant implications for assessing crop losses and selecting appropriate control strategies (Hau, 2001).

The major objective of this work, that was part of a larger project, was to develop an optimal strategy to control fungal disease complexes on tomatoes grown under protected cultivation in greenhouses in Bangkok, Thailand.
Tomato (*Solanum lycopersicon L.)* is after potato the most widely grown solanaceous vegetable (Rubatzky and Yamaguchi, 1999) and one of the most important crops in Thailand.
After the turn of the millennium, the production in Thailand increased, mainly for processed tomatoes. In 2000, more than 224 thousand tons were produced. In 2004, the production reached almost 266 thousand tons. The latest number available estimated by FAOSTAT is a production of around 197 thousand tons for 2006 (www.fao.org, 2008). Probably the production decreased because of the Tsunami in 2005. For processed tomato, the major growing area is the north and northeast of Thailand, while for table tomato the planting area is distributed in various parts of the country (Intanoo, pers. com.; Pongam, pers. com.). In the central part of Thailand, tomatoes are exclusively grown in open fields so that no production is possible during the rainy season. Producing tomatoes in protected cultivation in greenhouses could overcome this problem and increase the income of the farmers. Compared to processed tomatoes for approximately 3 c lb^{-1}, fresh market tomatoes have a current market value of 25-35 c lb^{-1}. In production systems under protected cultivation, high ambient temperature, increased relative humidity and heavy dew in greenhouses are of paramount importance for plant growth, but also for pests and diseases causing damage to the crop and subsequently reducing the income of the grower.

Out of the 51 pathogens, comprising bacteria, fungi, viruses and nematodes, which can attack tomatoes, twelve may be considered as major diseases under hot humid conditions (Villareal, 1987). These are bacterial wilt (*Ralstonia solanacearum*), virus diseases (TMV, CMV,

ToMV, PVY), leaf mold (*Cladiosporum fulvum*), grey leaf spot (*Stemphylium solani*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), Pythium root rot (*Pythium aphanidermatum*), southern blight (*Sclerotium rolfsii*), and root knot nematodes (*Meloidogyne incognita*) (Thi Bich Ha, 1992; Heine 2005). The "Pesticide Action Network UK" (Davis et al., 2002) proved late and early blight as the most important diseases, accompanied by bacterial leaf spots. Concerning diseases of soil-borne pathogens, bacterial wilt, southern blight, Fusarium wilt, and damping off caused by *Rhizoctonia* and *Pythium* ssp. were most significant.

In the fields, foliar diseases such as early blight, late blight and powdery mildew caused by *Alternaria solani*, *Phytophthora infestans* and *Leveillula taurica*, respectively, are limiting factors of tomato production in Thailand (Pongam, research proposal). Early blight (EB), caused by *Alternaria solani* (Ellis & Martin) Jones & Grout, is one of the most common and destructive diseases of tomato in areas of heavy dew, rainfall, and high relative humidity (Barksdale, 1971; Nash and Gardner, 1988). EB is also important in semiarid areas when nightly dew is sufficiently frequent to allow disease development (Rotem and Reichert, 1964). It was expected that EB would also be a problem in greenhouses in the Bangkok area, as this disease can develop in a wide temperature range (Rotem, 1994) varying from 10 to 35°C and even above. The germination of *A. solani* conidia occurred at temperatures as high as 34°C or under relative humidity as low as 90% (MacNab and Sherf, 1986). As few as 3 h of continuous leaf wetness between 21 and 25°C are sufficient for lesion formation of EB (Madden et al., 1978). *A. solani* can cause disease symptoms on foliage (leaf blight), stem (collar rot), and fruit, and can result in severe damage during all stages of plant development (Barksdale, 1971; Barksdale, 1977; Nash and Gardner, 1988; Jones et al., 1991; Spletzer and Enyedi, 1999).

Black leaf mold (BLM), also formerly known as *Cercospora* leaf mold, is caused by *Pseudocercospora fuligena* (Roldan) Deighton (= *Cercospora fuligena* (Roldan)) that belongs to the family of *Mycosphaerella* (Crous and Braun, 2003). The fungus is widespread in warmer regions or greenhouses around the world, especially in tropical and subtropical Asia (Hsieh and Goh, 1990; Crous and Braun, 2003). It was first reported on tomato in 1938 in the Philippines (Roldan, 1938), in 1951 in Japan, in 1955 in India, in 1974 in southern USA, in 1990 in Taiwan, in 1995 in Malaysia (Wang et al., 1995) and recently in Brazil (Halfeld-Vieira et al., 2006). In Thailand it was first detected in 1979 in the Nongkham District, Amphoe Pasrijarern, Bangkok (Saranark and Chandrasrikul, 1980).

The disease symptoms of BLM are irregularly shaped pale yellow to light green lesions on leaves, which are initially covered with white mycelium on the lower leaf surface that turns grey to black as the fungus starts to sporulate. The occurrence of infections or symptoms on fruits has never been reported (Hartman et al., 1991). In the field, initial symptoms were recorded on lower leaves from which the disease advanced up the canopy to newly developed leaves. Infected leaves wilt and sometimes drop prematurely, but also after heavy infection, leaves curl upwards and die but remain on the plant (Wang et al., 1995). The total number of leaves formed may not be affected by the disease. Without fungicide control, the disease can reach quite high disease levels of up to 81% disease severity (Mersha, 2008).

In greenhouse experiments at the AIT Campus at Bangkok, Thailand, BLM naturally occurred on tomatoes and it turned out to be the most serious leaf disease of tomato. In addition, *Pythium aphanidermatum* was identified as the most important soil-borne pathogen causing damage in open fields in Thailand.

Pythium aphanidermatum (Edson) is one of the worldwide 87 *Pythium* species recognized by Waterhouse (1968). It belongs to the species most frequently associated with root diseases, also named damping-off. As the species is a typical plant pathogen of warm regions (Van der Plaats-Niterink, 1981), the occurrence in temperate climates is confined to greenhouses (Raffin and Tirilly, 1995). For long time survival, *P. aphanidermatum* forms thick-walled oospores that remain slumbering in the soil until germination is triggered by external stimuli like moisture or root exudates (Hoppe, 1966; Kraft and Erwin, 1968). For short term survival, asexually formed sporangia germinate, either directly or indirectly by formation of zoospores. The zoospores, which are initially wall-less and mobile in water are responsible for the dispersion in moist environments (Jones et al., 1991).

P. aphanidermatum, like other soil-borne pathogens, is difficult to control (Runia, 1995). Widely used management practices are soil sterilization by chemicals, solar radiation and fumigation (MacNab and Sherf, 1986; Jayaraj and Radhakrishnan, 2008).

Fungal pathogens, which are highly favoured by confined warmth and humidity, are gaining more and more economic importance in the past years especially under greenhouse conditions, and the use of fungicides remains the sole option to maintain optimal productivity. This tendency of heavy fungicide reliance for the production of vegetables and fruits is documented mainly in Asian countries. Only the fungicide sales in Western Europe are higher because of the dominant position of the cereal crop production (Kuck and Gisi, 2007). Thailand has faced environmental problems caused by an increasing use of pesticides over the past decade. Large amounts of chemicals are imported to the country in order to improve the

effectiveness of agricultural production. Unfortunately, most of the farmers and agricultural workers use pesticides without considering their potentially adverse effects on human health and on the environment (Thapinta and Hudak, 1998). Thus, there are increasing societal concerns about the environmental and health effects of chemicals so that a pesticide-free vegetable or floral product may give greenhouse growers a market advantage (Paulitz and Belanger, 2001; Jacobsen, 1997). Biological control using *Trichoderma* species can be an option for pesticide-free vegetable production by reducing the activities of soil-borne pathogens like *Rhizoctonia solani* (Harman et al., 1980; Chet and Barker, 1981; Lewis et al., 1995; Kok et al., 1996). *Trichoderma* species are particularly prevalent in humid environments and are relatively intolerant to low moisture levels; however, they can be isolated from all climatic zones including desert soils (Klein and Eveleigh, 1998). Especially *T. harzianum* Rifai is one of the more intensively investigated biological control agents (Henis et al., 1978; Hadar et al., 1979; Elad et al., 1980, 1981a, 1981b; Chet et al., 1982; Marshall, 1982; Wu, 1982; Dal Soglio et al., 1998).

T. harzianum has multiple mechanisms of actions, including mycoparasitism via production of chitinases, ß-1-3 glucanases and ß-1-4 glucanases (Lorito et al., 1996), antibiotics (Sivasithanparam and Ghisalberti, 1998), competition (Elad et al., 1999), solubilization of inorganic plant nutritions (Altomare et al., 1999), induced resistance (Bailey and Lumsden, 1998) and inactivation of the pathogen's enzymes involved in the infection process (Elad et al., 1999; Elad and Kapat, 1999). The control provided is equal to that by fungicides (Harman, 2000), with which it is mostly compatible, but it must be applied as a preventative before disease occurs. The bio-control agent can be directly applied in the growing substrate (Chamswarng and Intanoo, 2002). The integration of biological agents with additional strategies is increasingly recommended to enhance disease control (Sweetingham, 1996).

The first aim of the following studies was to identify the most important tomato diseases in Thailand, especially under greenhouse conditions. Then experiments with naturally occurring epidemics were conducted under protected cultivation and relevant disease complexes were investigated with special focus on disease dynamics. Spatial and temporal distributions of the diseases were monitored. Such information is needed to fully understand disease dynamics, to develop more accurate sampling plans, to make better assessments of crop loss in relation to disease intensity, and to design and analyse experiments more efficiently (Xu and Madden, 2004).

The outcome of our studies may help to identify seasons of high and low epidemics and to choose the right control strategy based on forecasting disease developments. Several scientists (Kranz, 2003; Strange, 2003; Cooke, 2006; Madden et al., 2007) pointed out that the measurement of plant diseases and their effects on crop yield, quality and value are important for control mechanism and the right choice of action.

2 Is early blight and Pythium root rot an important disease complex on tomato in greenhouses in Thailand?

2.1 Abstract

Surveys were conducted in four regions in Thailand in order to get an overview of the main tomato diseases. *Pythium aphanidermatum* was identified as a major soil-borne pathogen. Symptoms of the aerial disease early blight, caused by *Alternaria solani*, were frequently found in tomato fields in different climatic regions of the country.

During the survey, isolates of *A. solani* were obtained from tomato leaves and evaluated for pathogenicity under controlled conditions using detached leaflets and whole plants. The morphological characteristics of colony growth and sporulation of all *A. solani* isolates were determined and compared with those of representative isolates. All isolates could be grouped into the species of *A. solani*. (Ellis & Martin) Jones & Grout.

The effects of early blight and Pythium root rot and their interactions on plant growth and yield were investigated in greenhouse experiments with two different tomato varieties (´New King Kong´ and ´King Kong 2´). The experimental unit was a closed net greenhouse (Econet M, pore size 0.18 mm, 40 x 37 mesh (40-mesh), with the base area of 10 x 20 m, Ludvig Swensson, Netherlands) on the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand. The experiments included the effect of artificial inoculation on host data (yield, biomass, and root weight) and defoliation.

Plants inoculated with *Pythium* showed symptoms of browning leaves resulting in a significant reduction in growth parameters, e.g. shoot and root weights. However, the disease complex of early blight and Pythium root rot could hardly be established under the given greenhouse conditions.

A. solani could successfully infect tomato plants under controlled conditions in the growth chamber but the temperature regime in the greenhouse (mean temperature > 30°C) was not suitable for disease development, most likely due to too low RH and high temperature. Under these conditions, black leaf mold caused by *Pseudocercospora fuligena* turned out as the major leaf disease limiting greenhouse production of tomato. This is the first report of BLM on the research site of the Asian Institute of Technology in Thailand. Since BLM is favoured by the prevailing environmental conditions in the greenhouses, more research on this disease is needed.

2.2 Introduction

Tomato (*Solanum lycopersicon* L.) is one of the most important vegetable crops in Thailand. For processed tomato, the major growing areas are located in the north and the northeast of Thailand, while the production of table tomatoes is distributed over various parts of the country (Intanoo, pers. com.; Pongam, pers. com.). In the central part of Thailand, tomatoes are exclusively grown in open land so that the production during the rainy season is not possible due to heavy rain falls. This restriction can be overcome by the production of tomatoes under protected cultivation. However, high ambient temperature and relative humidity as well as heavy dew in greenhouses might not only stimulate plant growth but also favour pests and diseases causing damage to the crop. The major objective of this work within a larger project was to develop an optimal strategy to control fungal diseases on tomatoes grown under protected cultivation in greenhouses in central Thailand.

Out of the 51 pathogens, comprising bacteria, fungi, viruses and nematodes, which can attack tomato, twelve are so far considered as major diseases under the climatic conditions of central Thailand (Villareal, 1987), namely bacterial wilt (*Ralstonia solanacearum*), virus diseases TMV, CMV, ToMV, PVY, the fungal diseases leaf mold (*Cladiosporum fulvum*), grey leaf spot (*Stemphylium solani*), early blight (*Alternaria solani*), late blight (*Phytophthora infestans*), Pythium root rot (*Pythium aphanidermatum*), southern blight (*Sclerotium rolfsii*) and the root knot nematodes *Meloidogyne incognita* (Thi Bich Ha, 1992). The “Pesticide Action Network UK” proved late and early blight as the most important diseases, accompanied by bacterial leaf spots in tropical und subtropical regions (Davis et al., 2002). With regard to soil-borne pathogens, bacterial wilt, southern blight, fusarium wilt and damping off diseases caused by *Rhizoctonia* and *Pythium* ssp. are most significant in Thailand (Heine, 2005; Intanoo, pers. com.; Pongam, pers. com).

In open land production, foliar diseases such as early blight, late blight and powdery mildew caused by *Alternaria solani*, *Phytophthora infestans* and *Leveillula taurica*, respectively, are the most limiting fungal diseases of tomato production in Thailand (Pongam, pers. com.). Especially early blight (EB), caused by *Alternaria solani* (Ellis & Martin) Jones & Grout, is one of the most common and destructive diseases of tomato in areas of heavy dew, rainfall, and high relative humidity (Barksdale, 1971; Nash and Gardner, 1988). EB is also important in semiarid areas when nightly dew is sufficient to allow disease development and it can develop in a wide temperature range between 10 to 35°C and even above (Rotem & Reichert, 1964; Rotem, 1994). Therefore, EB was expected to be a problem in greenhouse production

of tomato in central Thailand. *A. solani* causes disease symptoms on foliage (leaf blight), stem (collar rot), and fruit during all stages of plant development (Barksdale, 1971; Barksdale and Stoner, 1977; Nash and Gardner, 1988; Jones et al., 1991; Spletzer and Enyedi, 1999). The conidiophores of *A. solani* are arising singly or in small groups. Conidia are usually solitary, and consist of a simple or branched chain; the conidium is dark, olive coloured, brown or mid pale golden. It is 150-300 µm long and 15-19 µm thick with 7 to 11 transverse and no or a few longitudinal septa (von Arx, 1974; Streets, 1979; Ellis, 1971).

In field surveys, *P. aphanidermatum* was identified as an important pathogen causing diseases like seed rot, damping-off, root rot, and soft rot (Agrios, 2005, Intanoo, pers. com). *P. aphanidermatum* occurs worldwide causing root and stem lesions as well as root rots. Although the pathogen seldom kills older plants, it can considerably retard plant growth and drastically reduce yield (Heine, 2005; Agrios, 2005).

The purpose of this study was to determine possible effects of interactions between the aerial disease EB and the soil-borne disease Pythium root rot (PRR). As both fungi are adapted to the warm climate in Thailand and can cause severe damage on tomato (Intanoo, pers. com.), the effects of both pathogens and their interactions were investigated in greenhouse experiments conducted on the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand. In our studies, we investigated plant parameters, yield and dynamics of both diseases occurring simultaneously under greenhouse conditions in Thailand.

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2.3 Materials and Methods

2.3.1 Surveys

Surveys were conducted to get information about the most important tomato diseases in the main growing areas in Thailand. Tomatoes are mainly grown in the north (region Chiang Mai), in the northeast (district Ampher) and in the south (district Chan). Randomly selected fields were observed, 11 in the north, 9 in the northeast and 2 in the south. The average annual temperature in the north is 25.1°C, in the south 27.3°C and in the northeast 26°C. The mean annual temperature for the greater Bangkok area is 28.1°C (Loose et al., 2000; Baedecker, 1993). Field plants were visually inspected and the main diseases and pests identified. Affected plant material was collected in order to isolate the relevant pathogens. In addition to the collection of fungal isolates, information on crop cultivation was obtained by conducting interviews with the tomato growers.

2.3.2 Collecting isolates of *Alternaria solani*

Isolates of *A. solani* were collected from tomato leaves in fields in four regions in Thailand. These regions were the area around Chiang Mai in the north of Thailand, the area along the border to Laos in the northeast, the area around Cha Am in the south and the central region around Bangkok. The samples were taken from open land production areas since production under protected cultivation is still uncommon in Thailand. Plants were visually inspected and diseased plant material was taken, ten randomly selected leaves per field. To obtain isolates from active early blight lesions, leaves that had clearly delineated lesions were chosen. Sections of 5 mm^2 were removed from the leading margin lesions, washed in pure sodiumhyperchloride for 10 s, shortly air dried and plated onto Petri dishes (100 x 15 mm) containing potato dextrose agar (PDA; Merck, Bangkok, Thailand). The Petri dishes were incubated at 25°C under cool white fluorescent lights for 12 h dark and 12 h light. Putative colonies of *A. solani* were randomly selected from the Petri dishes and single spores were transferred to new PDA plates to obtain pure cultures.

2.3.3 Morphological characterization of *Alternaria solani*

Following the method of Pryor and Michailides (2002), the morphological characteristics of colony growth and sporulation apparatus of all samples were determined using single-spore colonies. To obtain these colonies, single-spores were transferred to Petri plates containing a special medium for the sporulation of *Alternaria* (SSA) consisting of 30 g of $CaCO_3$, 20 g of

sucrose and 20 g of agar per litre of distilled water. Subsequently, isolates were incubated for 7 to 10 days at 25°C. To ensure consistent sporulation, dishes were kept below a cool white fluorescent bulb and illuminated with 12 h / 12 h periods of light / dark. After incubation, conidial suspensions were obtained by flooding dishes with 10 mL of sterile water. Conidia were extracted with a pipette tip and transferred on a microscope slide. The conidia were examined at 40 to 100 magnifications with a dissecting microscope (Nikon, Eclipse E 200) and transmitted light for morphological characteristics.

2.3.4 Selection of isolates of *Alternaria solani*

The three most vigorous isolates of *A. solani* were chosen for the further experiments: As-1 from the area around Chiang Mai, As-2 from the area near the borderline of Laos and As-3 from the province of Cha Am. In addition, three reference isolates were used: An isolate (As-4) from the area of Chiang Rai, Thailand was provided by Dr. Patchara Pongam, Kasetsart University, Thailand. Two isolates originating from the United States (As-5) and Greece (As-6) were made available by Mrs. Maendy Fritz (Biometry and Population Genetics, University Giessen, Germany).

2.3.5 Inoculum preparation

The cultures were grown on SSA in Petri dishes and incubated at 25°C under a cool-white fluorescent diurnal light with a 12 h photoperiod. After 10 to 14 days, conidia were collected by flooding the plates with 50 mL of sterile distilled water containing 0.01% Tween 20. The colonies were than gently brushed and the suspension was filtered through two layers of cheesecloth to remove mycelia fragments.

The spore density in the suspension was counted using a haemocytometer and adjusted to a density of 5 $x10^4$ conidia mL^{-1}. Spray inoculation was done with an atomizer.

2.3.6 Pathogenicity tests

All tests were carried out with detached leaves or plants of the tomato variety ´King Kong2´.

2.3.6.1 *Tests with detached leaflets*

Five selected isolates of *A. solani* (As-1, As-2, As-3, As-5 and As-6) were tested for pathogenicity on detached leaves. For each isolate tested, ten randomly selected leaves from 2 months old tomato plants grown in the greenhouse were chosen. Tests were conducted on

unwounded (n = 5) and wounded (n = 5) leaves. For wounded leaf assays, each leaf was slightly scratched on the surface with a sponge prior to inoculation. Petioles of tomato leaves were immersed into microcentrifuge tubes filled with water and the tops of the tubes were wrapped with parafilm to hold the leaves in upright and to prevent evaporation of water. After spraying the leaves with conidial suspension, the tubes with leaves were covered with clear plastic bags to maintain the relative humidity (RH) near 100%. The inoculated leaflets were placed in racks inside a plastic chamber (size 30 x 23 x 10 cm) and incubated at ~28°C with 12 h day/night. Leaves were observed daily until first lesions appeared. The experiment was carried out twice.

2.3.6.2 *Tests with plants under laboratory conditions*

Plants were sown, cultivated and stored in a net greenhouse with a mean temperature of 30°C (for further greenhouse information see 2.3.6) and transplanted to 2-L-pots after four weeks.

The experiment was conducted with 6 weeks (A1), 8 weeks (A2) and 10 weeks (A3) old plants having 6 to 8, 10 to 12, and 16 to 18 leaves, respectively. Inoculation was done by spraying the whole plant with 25 mL of inoculum solution of the isolates As-1 and As-5. Sterile water was used for control plants. All plants were individually covered in plastic bags for 24 h to increase the RH and to facilitate infection and were incubated with additional light at a constant temperature of 25°C in the laboratory. Plants were observed for 14 days after inoculation (dai) and diseased leaves were estimated daily by using a rating scale (see 2.3.7.1).

For each age class, 13 plants were used, 10 inoculated and 3 as control plants without inoculation. The experiment was repeated once.

2.3.6.3 *Tests with plants under greenhouse conditions*

The experiment under greenhouse conditions was carried out in the same way as the experiment under laboratory conditions, except that the inoculated plants were placed in rows in the net greenhouse with an average temperature of 30°C (for detailed greenhouse description see 2.3.6). The plants were observed for 14 dai and disease severity was daily rated as described below.

2.3.7 Greenhouse experiments

The trials were conducted in a greenhouse, located at the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand. The greenhouse (size 200 m^2) was a closed net-house (Econet M, pore size 0.18 mm, Ludvig Swensson, Netherlands) equipped with two exhaust fans (550 m^3 min^{-1}, 1.5 HP, 960 rpm, Sriroz Company, India) at the front side of the net-house (Figure 2.1)

Figure 2.1: Closed net-house located at the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand.

The fans were operated by a computerized control system that automatically switched on one fan when the temperature inside the net-house exceeded 25°C, and the second one at a temperature > 30°C. The climate in the greenhouse was monitored using a data logging system (ITG data logger, Leibniz Universität, Hannover, Germany). During the trials, mean temperature and relative humidity was 28-30ºC and 70-80%, respectively. The total planting area of the greenhouse was 160 m^2 .The greenhouse was lengthways subdivided with a net (Econet M, pore size 0.18 mm, Ludvig Swensson, Netherlands) in two halves, each with a separate entrance door. In each half, plants were arranged in 3 rows with a distance of 160 cm between the rows and 55 cm from the outer rows to the sidewall.

Tomato seedlings in the required age for the experiments were planted in plastic pots (30 x 25 cm) filled with a commercial growing substrate composed of clay, sand, and silt in proportions of 31, 30 and 39%, respectively, and 29% of organic matter. The pots were placed on a black ground plastic cover (Chaisiri Nylon Canvas Factory Ltd., Bangkok, Thailand) and arranged in rows with no inter-pot distance within a row (Figure 2.2).

Figure 2.2: Inside view of one half of a closed net-house at the campus of the AIT in Bangkok, Thailand.

Plants were fertigated 7-9 times per day (2.5 L day^{-1}) with a drip irrigation system controlled by solar light integral. The fertilizers [Hakaphos® (N-P-K) (2.5 kg 100 L^{-1}), COMPO Austria, GmbH, and Bai-plus (calcium) (1.8 kg 100 L^{-1}), Bayer Ltd., Thailand] were injected into the irrigation system with mechanical injectors (DI 16, Dosatron®, France). Tomato plants were supported by ropes, which were fixed to the ceiling of the greenhouse, and cultivated in a single-stem system. Pruning, layering and removing of side branches and up binding was done weekly.

2.3.7.1 *First Experiment*

Two fungal pathogens, *A. solani* and *P. aphanidermatum,* were investigated in this experiment, which was carried out with the tomato variety ´King Kong 2´. The plants were arranged in a split-block design (with 4 replications) and 3 time harvests. The total number of plants in the greenhouse was 360 arranged in 6 rows. For data collection, 216 plants were used, the boarder plants of each split-block were not taken into account.

Tomato seeds were sown on 22 May 2002. After four weeks, tomato seedlings were planted in plastic pots as described above. The inoculation with *A. solani* and *P. aphanidermatum* was done one month later (on 23 July 2002) when the plants were in the 10 to 12 leaves stage.

Plants were inoculated with *A. solani* in one half of the greenhouse only, the other was sprayed with water for control. Inoculum suspension was prepared as a mix of the As-1, As-2, and As-3 isolates. Three leaves per plant (number 5, 6 and 7, counted from the bottom) were wounded by scratching the leaf surface and then sprayed with 100 mL of inoculum solution. Tomato plants were than individually packed in plastic bags for 24 h to increase the relative humidity and to favour infection. To avoid the spread of early blight into the control half,

Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}) was sprayed once a week, starting one week after inoculation.

In addition, in both parts, *P. aphanidermatum* was inoculated with agar pieces containing the pathogen in 3 different densities in the soil.

The treatments were:

1 plain Petri dish per pot	*P. aphanidermatum* without *A. solani*	(P0–wA)
1 Petri dish per pot	*P. aphanidermatum* without *A. solani*	(P1–wA)
3 Petri dishes per pot	*P. aphanidermatum* without *A. solani*	(P2–wA)
1 plain Petri dish per pot	*P. aphanidermatum* inoculated with *A. solani*	(P0–iA)
1 Petri dish per pot	*P. aphanidermatum* inoculated with *A. solani*	(P1–iA)
3 Petri dishes per pot	*P. aphanidermatum* inoculated with *A. solani*	(P2–iA)

For the inoculation of *P. aphanidermatum,* the isolate kindly provided by Dr. Wanwilai Intanoo, Kasetsart University, Bangkok, Thailand, was grown for 4 days on PDA medium. Thereafter, Petri dishes containing the mycelium were cut in 1 cm^2 pieces and 1 or 3 dishes per pot were mixed into the soil. For control treatments, one Petri dish containing plain PDA medium was cut in pieces and mixed into the soil.

During the experiment, the plant height, number of leaves per plant and the number of ripe tomatoes were recorded on a weekly base. Disease severity on each leaf was estimated once a week. These observations were done for 3 months after inoculation.

A destructive sampling (3 altogether) of 20 plants per treatment was done every 4^{th} week and 12 plants were used for data collection. The plants for data collection were the middle plants out of a group of five plants.

The leaf area was measured with a leaf area meter (LI-COR; Model Li-3100 AREA meter, left-Cor. Inc. Lincoln, Nebraska, USA), roots were washed free of substrate, air dried and weighed. At each sampling date, one third of the plants, starting at one side of the greenhouse was removed.

2.3.7.2 *Second Experiment*

The second experiment was conducted similar to the first one (see above), except that the variety ´New King Kong´, a determinate, early flowering variety, was used and that the isolates As-3 and As-4 were used for inoculation of *A. solani*. Seeds were sown on 14 August 2002 and transplanted 4 weeks later to 10-L-pots. Inoculation was done on 22 October 2002. Again three leaves (number 5, 6 and 7) were scratched, but in the second experiment, the agar

plates with mycelium were directly pressed for 5 seconds smoothly onto the leaves. Thereafter, the leaves were moistened by spraying tap water and covered separately with plastic bags for 24 h in order to increase the relative humidity. Control plants were treated with sterile agar plates.

The inoculation of *P. aphanidermatum* in the second experiment was identical to that in the first one (see above). The data collection was done weekly and three time harvests were made.

2.3.8 Disease assessment.

2.3.8.1 *Early blight*

For disease assessment of early blight, the leaves of all plants were visually rated for percent diseased foliage using a modified Beaumont rating scheme (Beaumont, 1954) with 0% indicating no visible symptoms of *A. solani* infection and 100% indicating completely diseased foliage. To avoid errors in disease assessment, the same person did the assessment at all times. The rating scale was modified as followed:

no recognizable lesions : 0%
0.5 – 2.5% diseased foliage : 1%
2.5 – 7.5% diseased foliage : 5%
7.5 – 15% diseased foliage : 10%
15 – 25% diseased foliage : 20%
25 – 35% diseased foliage : 30%
35 – 45% diseased foliage : 40%
45 – 62% diseased foliage : 50%
63 – 82% diseased foliage : 75%
83 – 100% diseased foliage : 100%

2.3.8.2 *Pythium aphanidermatum*

The root pathogen *P. aphanidermatum* rarely shows lesions on the upper part of plants. To prove the presence or absence of the fungus, a potato baiting method was used for detection and re-isolation (Stanghellini and Kronland, 1985). Every week, soil samples (about 50 mL) from 10 randomly selected pots were taken out of the rootstock section, placed in a Petri dish and saturated with deionised water. A slice of potato (0.25 cm^2 and 3 mm thick) with a piece of water agar on top (same size as potato slice) was used as bait and placed onto the saturated soil surface (Figure 2.3). After incubating for 48 hours at 30°C, the water agar slice was removed and placed onto a selective medium for *P. aphanidermatum*, containing PDA with

100 ppm Pimaridin + 100 ppm Streptomycin. Petri dishes were evaluated after incubating for 24 hours at 32°C in the dark. Cotton wool pad-like mycelium indicated a positive soil sample.

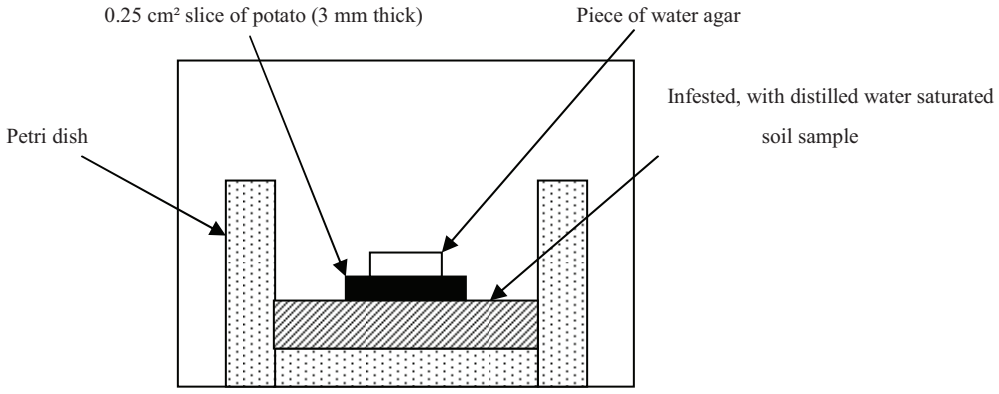


Figure 2.3: Schematic description (cross-sectioned) of potato baiting method (after Stanghellini and Kronland, 1985).

2.3.9 Statistical analyses

The quantitative data of the plants, i.e. total leaf number, plant height, yield, root weight, dry weight, leaf area etc., were subjected to two-way analyses of variance (ANOVA) using the PROC GLM procedure of the SAS software package (SAS Users Guide, SAS Institute, Cary, NC). The two factors investigated were the influence of EB (marked with capital letters A and B) and PRR (characterized with small letters a and b) and their interaction (marked with ***). Mean comparisons were conducted using Tukey's t-test ($p \leq 0.05$).

2.4 Results

2.4.1 Surveys and collection of isolates

From the surveys it became clear that tomatoes in Thailand are mainly cultivated for 2-3 months in the so-called short cultures in open land. The main cultivation time for tomatoes in Thailand is the dry season from November to March. The average frequency of pesticide application is every 3rd day. Tomatoes are mainly used for industrial processing.

During the survey, foliar diseases such as early blight (EB), late blight and powdery mildew caused by *Alternaria solani*, *Phytophthora infestans* and *Leveillula taurica* were found. The leaf diseases with the highest frequency were EB (found in 15 fields) and late blight (observed in 7 fields), the latter mainly in relatively cooler regions in the north. In the central area of Thailand, no symptoms of EB were detected. Major pests found were leaf miners, fruit borers and thrips. Bacterial wilt occurred as well frequently.

Soil samples were taken to detect soil-borne pathogens and analysed using the potato-baiting method. In the soil samples showing positive infestation, *Pythium* spp. were identified. *P. aphanidermatum* was the most relevant soil-borne pathogen in warmer regions.

In addition to the surveys in the central region, where the greenhouses for the experiments were located, young tomato plants were used as trap plants to collect wind-borne spores of tomato pathogens at the AIT ground. However, no disease symptoms on the trap plants appeared after incubation.

2.4.2 Morphological characterization of *Alternaria solani*

About 150 *Alternaria* leaf samples were taken from fields and analysed based on the characteristics described by von Arx (1974), Streets (1979) and Ellis (1971). The conidiophores of *A. solani* are arising singly or in small groups. Conidia are usually solitary and consist of a simple or branched chain; the conidium is dark, olive coloured, brown or mid pale golden. It is 150-300 µm long and 15-19 µm thick with 7 to 11 transverses and no or a few longitudinal septa. All isolates could be grouped into the species of *A. solani* (Figure 2.4).

Figure 2.4: Conidia (x 40) of an *A. solani* isolate from the area around Chiang Mai, Thailand.

2.4.3 Pathogenicity tests

For all experiments, the results in the repetitions were similar. Therefore, only the data from the first run of experiments are presented.

2.4.3.1 *Tests with detached leaflets*

Symptoms of early blight appeared on all inoculated leaves 6 days after inoculation and *A. solani* could successfully be re-isolated from all diseased plants. The pathogenicity test conducted on wounded and unwounded leaves showed no significant difference and the isolates tested had no influence on disease severity.

2.4.3.2 *Tests with plants under laboratory conditions*

In the laboratory with the mean temperature of 25 °C, first symptoms of EB were visible after 3 days when plants in the age of 8 weeks (A2) and 10 weeks (A3) were inoculated (Fig. 2.5). Subsequently, the disease developed more rapidly in the A2 treatment than in the A3 treatment and 20 % of the leaf area was covered with lesions at 14 days after inoculation (dai) in comparison to 15 % in the A2 treatment. In contrast, first symptoms were found in the A1 treatment after 11 days and there was only a slight increase until 14 dai.

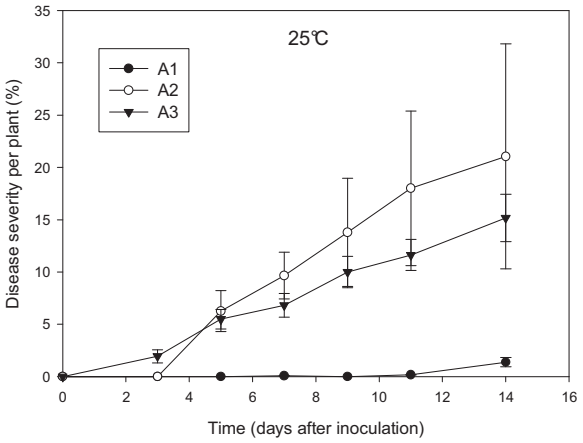


Figure 2.5: Disease progress curves of early blight on leaves of tomato plants inoculated at different plant ages (6 (A1), 8 (A2) and 10 (A3) weeks) and incubated at 25°C, n = 10.

2.4.3.3 *Tests with plants under greenhouse conditions*

No characteristic symptoms of *A. solani* could be identified up to 14 dai in the greenhouse with an average temperature of 35°C,

2.4.4 Greenhouse experiments

During the second experiment, a second foliar disease occurred, namely black leaf mold (BLM) caused by the fungus *Pseudocercospora fuligena*. As in experiment 1, BLM symptoms were not noticeable the data of this experiment were analysed according to the different early blight treatments. In the second experiment, BLM was influenced by chemical treatments, which were targeted against early blight. Thus the results were grouped according to the different chemical treatments (with fungicide and without fungicide).

2.4.4.1 *First Experiment*

Despite of the inoculation of *A. solani*, no symptoms were found on inoculated plants. In contrast, both Pythium treatments (P1 – with low level of *P. aphanidermatum*; P2 – with high level of *P. aphanidermatum*) showed symptoms of browning leaves starting from the bottom of the plants which were not found in the PRR free control treatments. Randomly chosen soil samples were analysed and *P. aphanidermatum* could be re-isolated from substrate of visibly damaged plants, proving *P. aphanidermatum* as the causal agent of the symptoms. No Pythium was re-isolated from non-inoculated control pots.

In this experiment, plants of all treatments died rapidly. After three weeks, one third of the plants in the greenhouse was already dead. In addition, over 50% of the plants had lost more

than 50% of the healthy tissue. Because of these damages of the plants, the experiment was stopped two months after inoculation (on 17 September 2002) so that only two time harvests were done (28 dai and 56 dai). In both assessments, 18 plants per treatment were used.

The shoot dry weight of tomato plants at harvest is shown in Fig. 2.6. At 28 dai, the shoot dry weight was significantly reduced in the *A. solani* inoculated plants, compared to the non-inoculated and fungicide treated control plants. However, this effect could not be seen at 56 dai. At 28 and 56 dai, the plants of both *Pythium*-treatments (P1 and P2) were significantly reduced in dry weight.

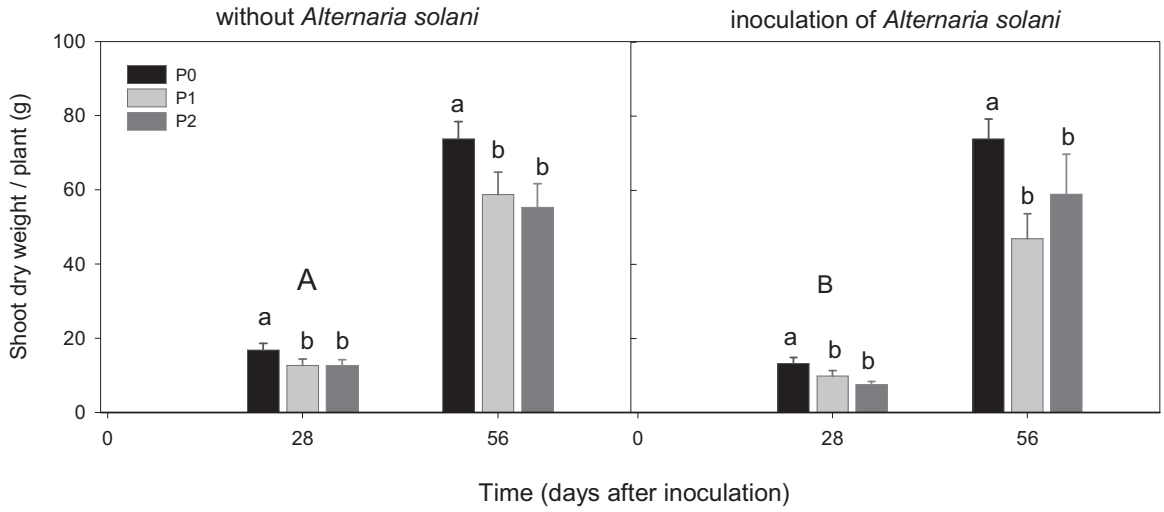


Figure 2.6: Means (± SE) of shoot dry weight per plant (g) in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 18. The treatments without *A. solani* were weekly sprayed with fungicide to avoid undesired infection.

Root fresh weight (Figure 2.7) and shoot dry weight showed a similar response to the treatments. All fungal treatments, *A. solani* and both *Pythium* levels, significantly reduced the root fresh weight at 28 dai. At 56 dai, there was no effect of the *A. solani* -treatment and of the low *Pythium* level (P1), but the high *Pythium* level significantly reduced the root dry weight. On average the difference between the *Pythium*-treatments was 10 g.

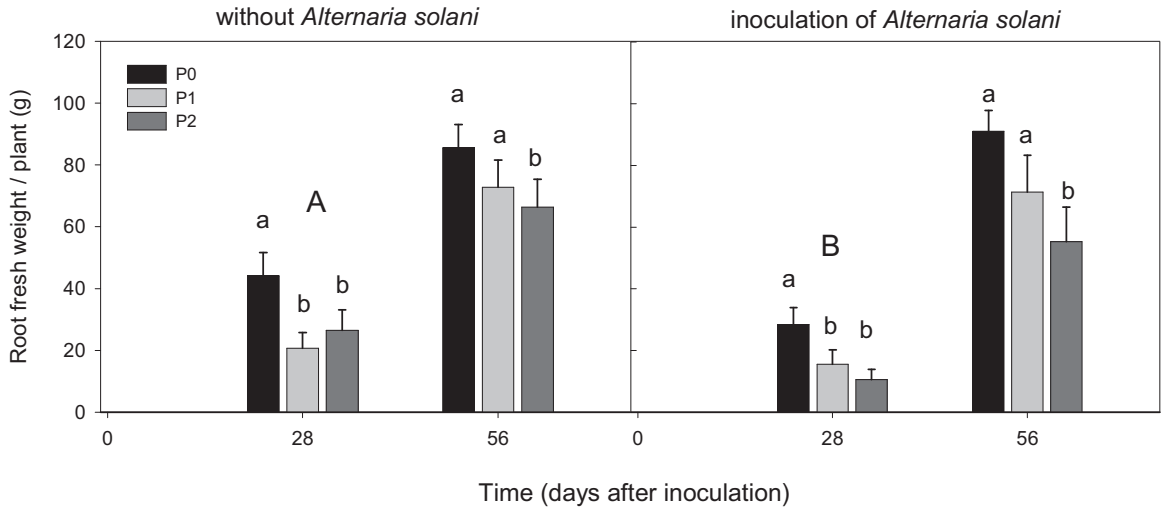


Figure 2.7: Means (± SE) of root fresh weight per plant (g) in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1-with low level of *Pythium* and P2- with high level of *Pythium*), n = 18. The treatments without *A. solani* were weekly sprayed with fungicide to avoid undesired infection.

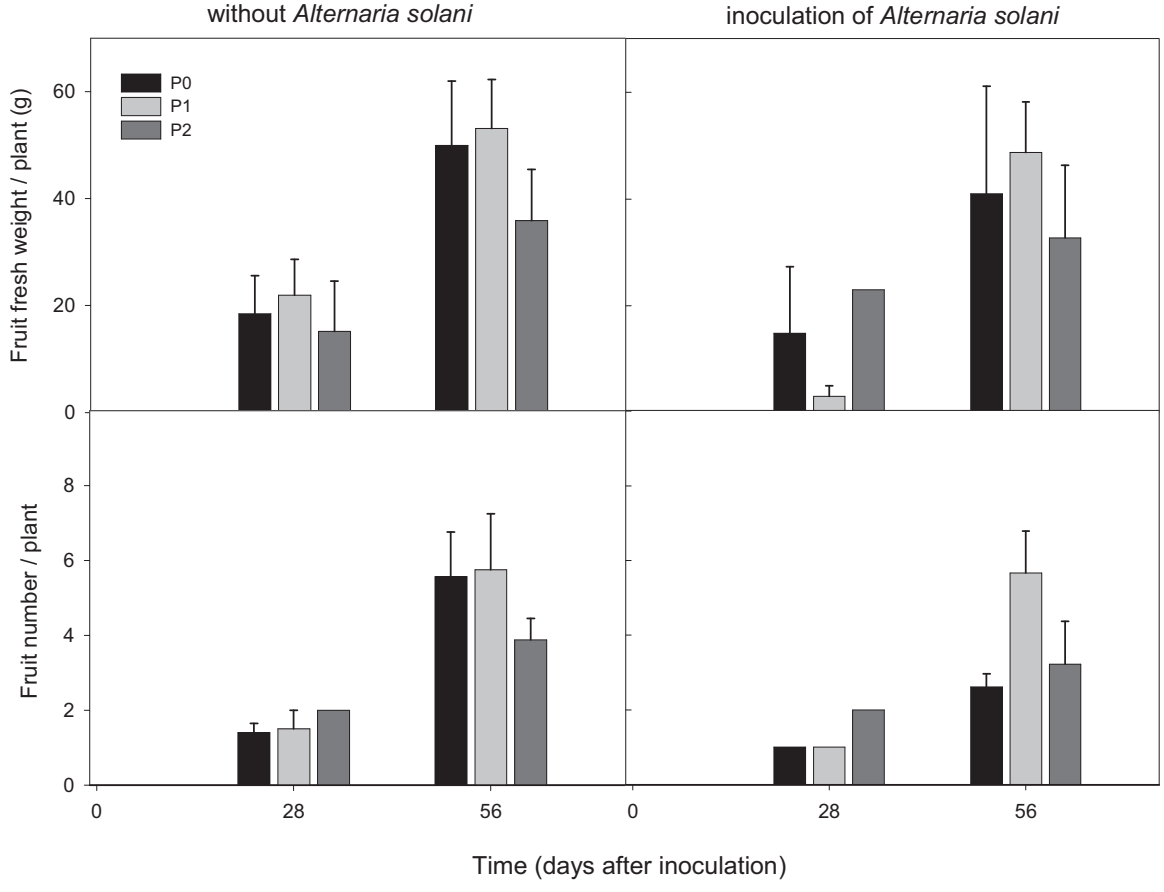


Figure 2.8: Means (± SE) of fruit fresh weight (g) and of the total number of fruits per plant in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 18. The treatments without *A. solani* were weekly sprayed with fungicide to avoid undesired infection

Yield per plant, given as fruit fresh weight and number of fruits per plant are shown in Figure 2.8. There were no significant differences in the treatments, neither in the weight nor in the number of fruits. It should be considered that at 28 dai only 1 fruit could be harvested in the inoculated treatments P0-iA and P1-iA. In the other treatments, no fruits were yielded.

The dynamics of plant height is shown in Figure 2.9. Plant growth did not significantly differ among the treatments. However, there was a tendency of tomato plants in the P0-treatment having a slightly faster growth as compared to *Pythium*-inoculated plants.

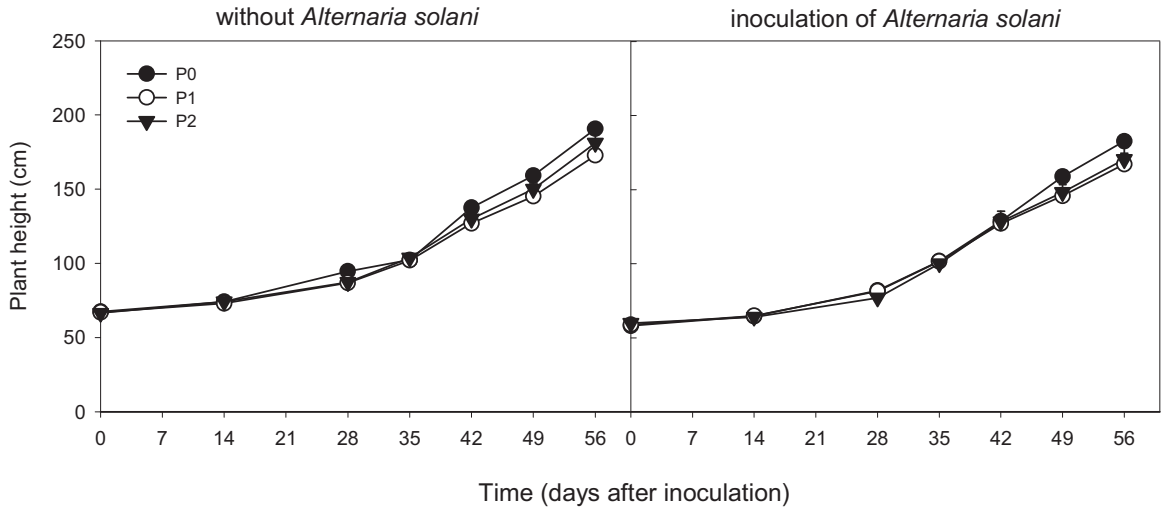


Figure 2.9: Dynamics of plant height (± SE) (cm) under the influence of both inoculations in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 18. The treatments without *A. solani* were weekly sprayed with fungicide to avoid undesired infection.

The number of leaves (Figure 2.10) was significantly reduced in the non-sprayed treatments (iA) compare to the sprayed ones, at 56 dai. In the P0-treatment at 56 dai, plants had a mean leaf number of 29 leaves whereas in the P1- and P2-treatments the leaves were significantly reduced to 27. The number of shed leaves, which are displayed with the second line in Figure 2.10, did not show a significant difference between the sprayed and non-sprayed-treatments. The loss of leaves was significantly higher when plants were inoculated with *Pythium*. The high inoculation level of *Pythium* (P2) led to the highest number of dead leaves (approx. 9 leaves) while in the P0-treatment only an average of 6 leaves were lost.

The numbers of leaves were analysed by calculating the area under the progress curve (AUPC, Table 2.1.), e.g. the actual value of the AUPC in the treatment P0–wA was 810.83 number of leaves * days, in P2-iA it was reduced by 267.93 to 542.91.

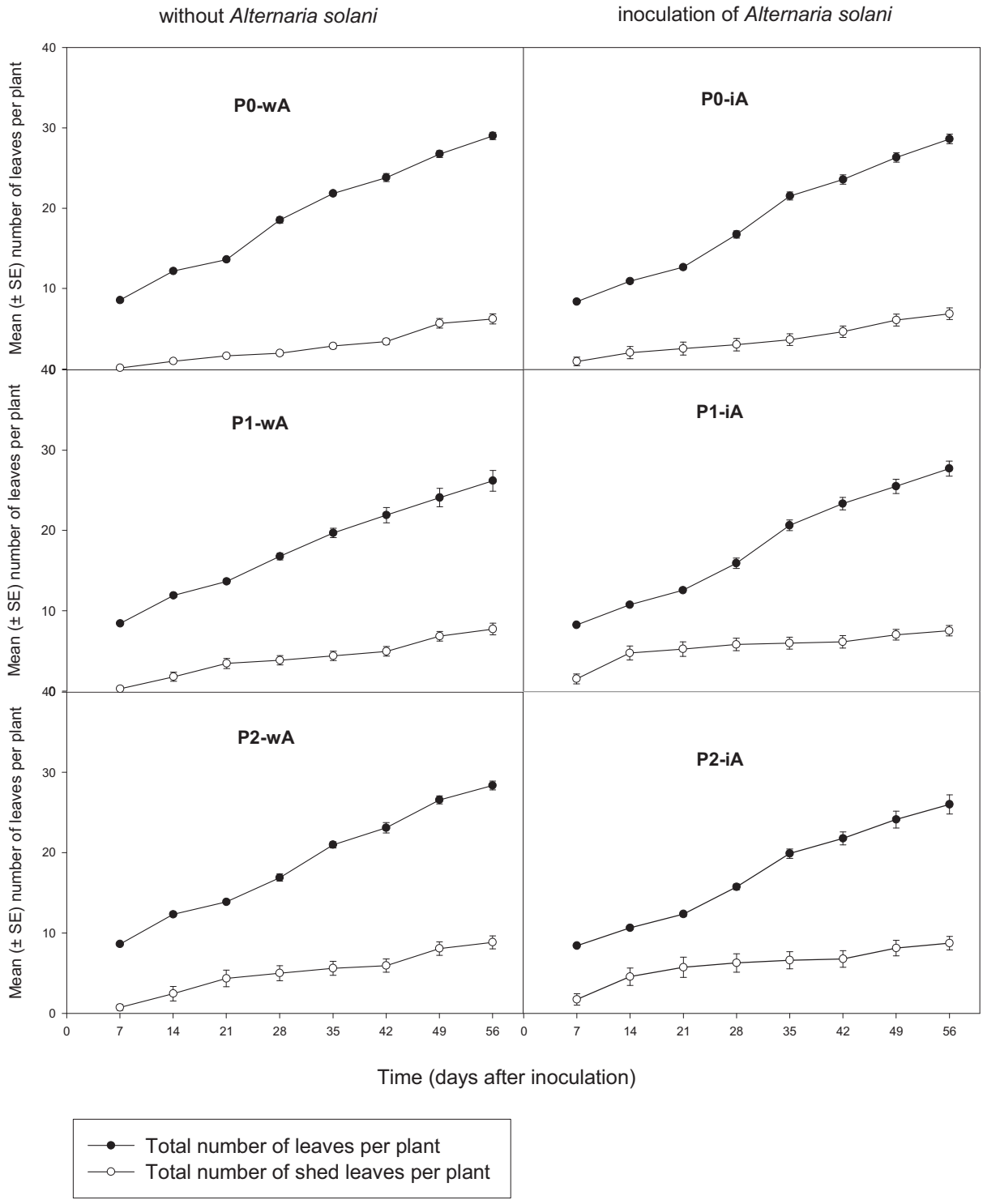


Figure 2.10: Means (± SE) of the total number of leaves formed and of leaves shed in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 18. The treatments without *A. solani* were weekly sprayed with fungicide to avoid undesired infection.

Table 2.1 Values of area under progress curve (AUPC), measured in leaf number * days, for total number of leaves and number of shed leaves in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 18 The treatments without *A. solani* were weekly sprayed with fungicide to avoid undesired infection.

Treatment		Without *A. solani* (wA)	Inoculation of *A. solani* (iA)	Statistic for PRR
AUPC for total number of leaves	P0	948.11	910.35	a[1]
	P1	877.09	885.61	b
	P2	925.23	851.88	b
Statistic for EB		A	B	
AUPC for number of shed leaves	P0	137.28	182.58	a
	P1	205.53	280.20	ab
	P2	255.70	308.97	b
Statistic for EB		A	A	

[1] Data were subjected of a two-way analysis of variance (ANOVA) and means separated by LSD ($p < 0.05$). The two factors investigated are the influence of PRR within column (characterized with small letters) and EB within rows (marked with capital letters). Means followed by the same letter are not significantly different (P = 0.05).

2.4.4.2 *Second Experiment*

This experiment was carried out with the indeterminate tomato variety ´New King Kong´. Like in the first experiment, *P. aphanidermatum* was inoculated and could successfully be re-isolated from the substrate of *Pythium*-inoculated plants. Leaves inoculated with *A. solani* showed marginal necrosis or wilting, but no lesions of early blight could be observed.

One week after the inoculation (28 October 2002) of *A. solani* and *P. aphanidermatum*, symptoms of another leaf disease were detected (Fig. 2.11). The disease was identified as black leaf mold (BLM) by Dr. Grunewaldt-Stöcker, (Institute of Plant Diseases and Plant Protection, Leibniz Universität, Hannover, Germany, pers. com.) and Prof Dr. Braun (Martin-Luther-University, Halle-Wittenberg, Germany, pers. com.). The fungal pathogen was subsequently identified as *Pseudocercospora fuligena* (Centraalbureau voor Schimmelkultures, Utrecht, Netherlands, ‘det 321-2003’). In Thailand, this disease was first detected in 1979 in the Nongkham District, Amphoe Pasrijarern, Bangkok (Saranark and Chandrasrikul, 1980).

Figure 2.11: Tomato leaves with natural infestation of black leaf mold, caused by *Pseudocercospora fuligena* (front, backside and at the crop).

The disease severity of BLM was estimated in the same way as described above for *A. solani*. The progress of BLM incidence of plants of the two greenhouse sides (inoculated with *A. solani* and without *A. solani*) followed an S-shaped curve (Figure 2.12). The disease incidence nearly doubled between 35 and 42 dai and reached 100% at 63 dai.

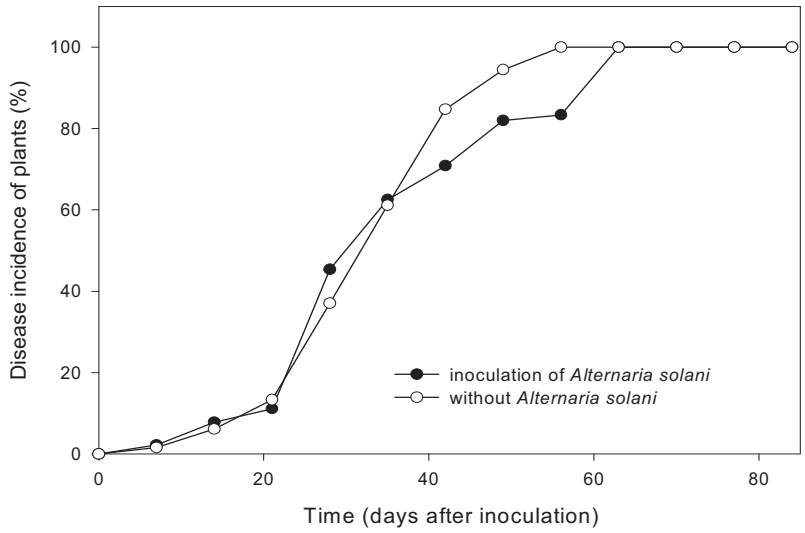


Figure 2.12: Disease progress curve of black leaf mold, expressed as disease incidence of plants, in two *A. solani* treatments, n= 36.

As the disease incidence was recorded on individual plants, the spread of the disease from plant to plant could be followed. Maps of the diseased plants were generated showing the positions of these plants. As diseased plant, plants with at least one visible spot of BLM were counted. The spatial patterns of BLM distribution at three observation dates (7, 14 and 21 dai) are shown in Figure 2.13.

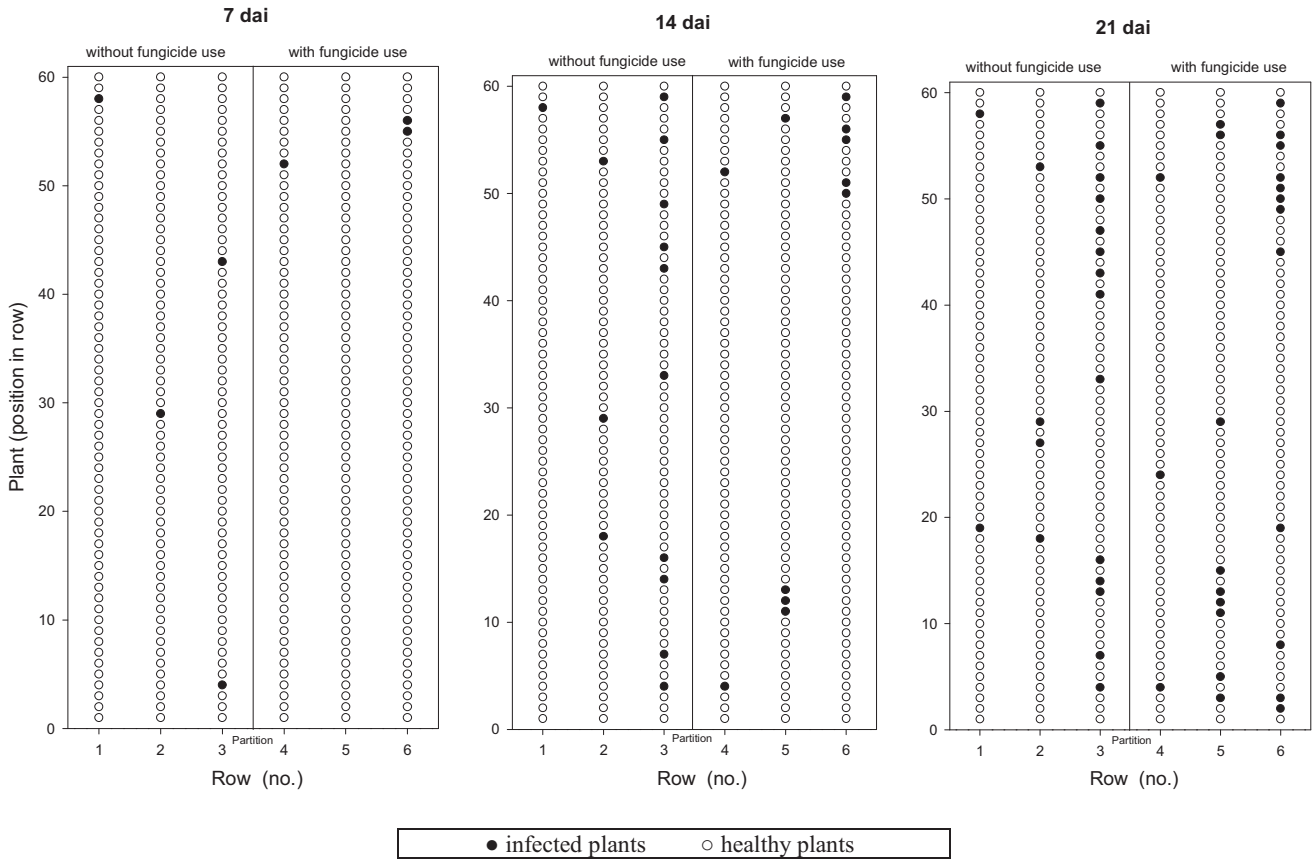


Figure 2.13: Spatial pattern of *Pseudocercospora fuligena* infected plants in the greenhouse at three disease assessment dates (7, 14, and 21 dai).

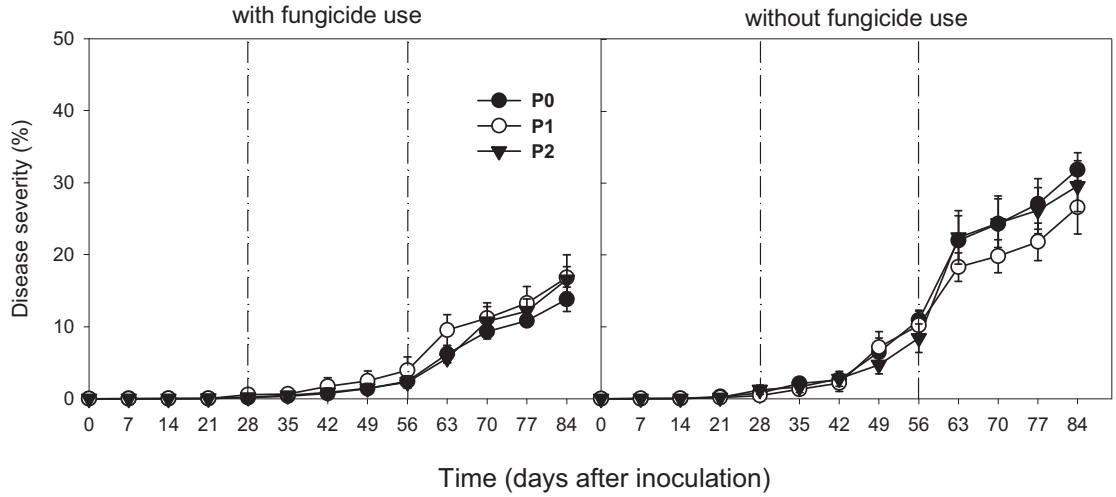


Figure 2.14: Disease severity (± SE) of *Pseudocercospora fuligena*, in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*). Vertical lines show the times when part of the plants were removed, resulting in a reduced sample size

During the primary investigations, related to *A. solani*, we observed that BLM responded to the weekly fungicide application with Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}). Therefore, data were separating the sprayed and non-sprayed treatments.

First symptoms of BLM were detected at 7 dai. These leaf spots were counted, and the disease severity was estimated in the same way as described before for *A. solani* (Figure 2.14). On the plants inoculated with *Alternaria* the disease progressed slowly until 28 dai but a strong increase in disease severity was recorded subsequently. Disease severity in the non-sprayed treatments reached 30%. On plants treated with fungicides, the disease severity at 84 dai was 18% (in P1-wA and P2-wA) and 12% in P0-wA.

As plant development was normal, the second experiment was monitored for 84 days. At three times (28, 56 and 84 dai), 20 plants per treatment were harvested and 12 plants were used for data collection.

For the second experiment, only shoot dry weight is shown (Fig. 2.15). However, due to the positive correlation between shoot dry weight and other parameters of above ground plant growth, e.g. shoot fresh weight to shoot dry weight were correlated with $r > 0.96$ ($P < 0.01$), it can be assumed that the results are similar.

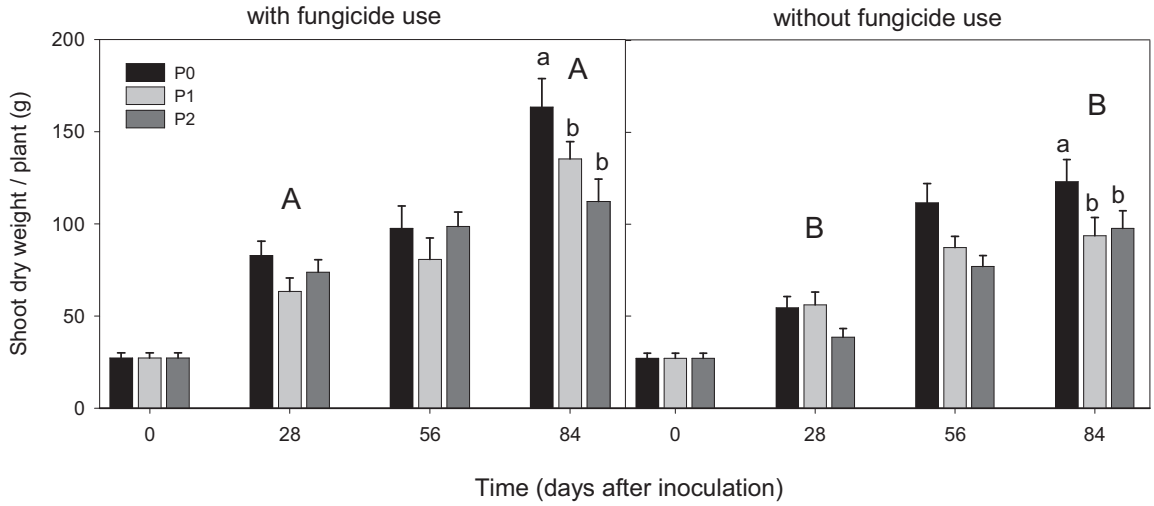


Figure 2.15: Means (± SE) of shoot dry weight (g) per plant in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 12.

At 0 dai, plants were 6 weeks old and had an average shoot dry weight of 27 g per plant. At 28 dai, the dry weight was significantly reduced by 20 g in the fungicide-treatment compared to the non-fungicide-treatment. At 56 dai, no statistical significance were detectable. At 84

dai, both treatments (*P. aphanidermatum*-inoculations and without fungicide) had a significant negative effect on shoot dry weight.

There was a strong tendency that plants in the *Pythium* inoculated treatments (P1 and P2) had lower root fresh weight at different sampling times compared to the P0-treatment (Fig. 2.16). At 84 dai, root fresh weight was significantly reduced in the plants non-sprayed. At the other dates, no significant differences were noticed.

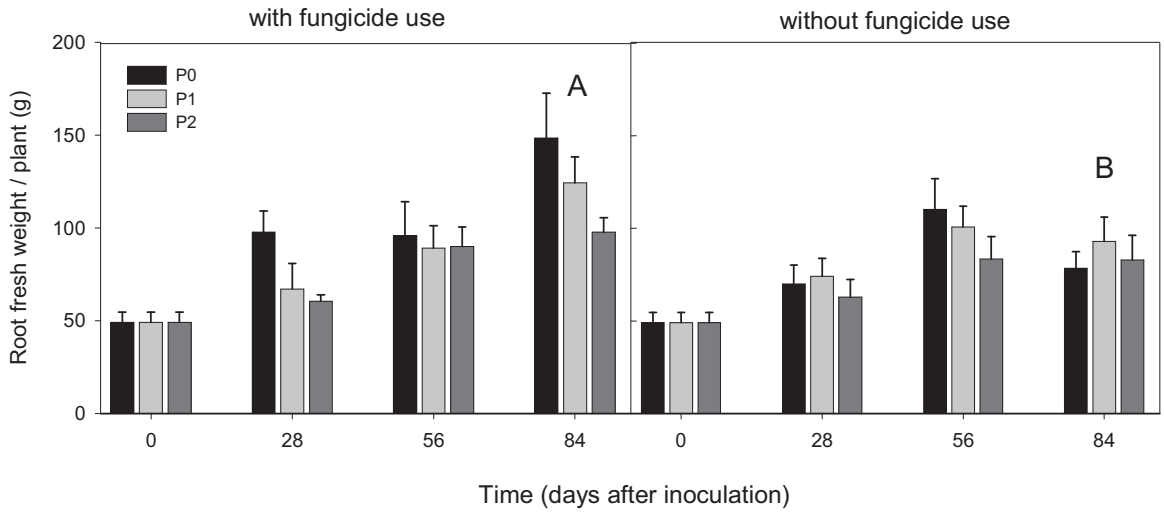


Figure 2.16: Means (± SE) of root fresh weight per plant (g) in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 12.

The leaf area of tomato plants is shown in Figure 2.17. The leaf area was about 2200 cm^2 per plant at the beginning of the experiment. In the treatment with fungicide spraying, the leaf area reached up to 3400 cm^2, at 28 dai. In the non-sprayed treatments, the leaf area was significantly reduced at 28 to 84 dai. In the *Pythium* variants, no significant differences were found.

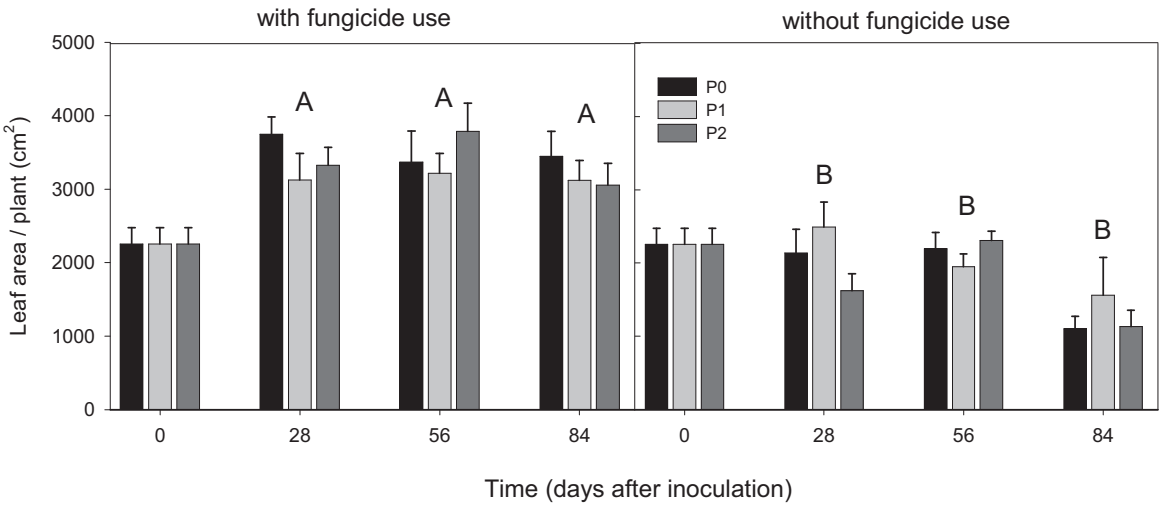


Figure 2.17: Means (± SE) of leaf area per plant (cm^2) in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 12.

Fresh fruit weight and number of fruits per plant at the three assessment dates is displayed in Figure 2.18. The ripening of fruits started 28 dai. At this time, the fruit weight and the fruit number were significantly increased in the fungicide sprayed treatments. For instance, the fruit number per plant was 14 in the sprayed treatment compared to 8 fruits per plant in the non-sprayed treatments. The mean fruit weight per plant was 440 g per plant and 180 g for sprayed and non-sprayed plants, respectively.

At 56 dai, a significant disease interaction within the treatments was found. The P0–iA-treatment (non-sprayed) had a higher yield and fruit number than for example the P0-wA-treatment (sprayed). For both fungicide treatments, at 84 dai, the fruit fresh weight per plant in P0 was significantly higher than in P1 and P2. The spraying of fungicide also significantly increased the fruit fresh weight at 84 dai.

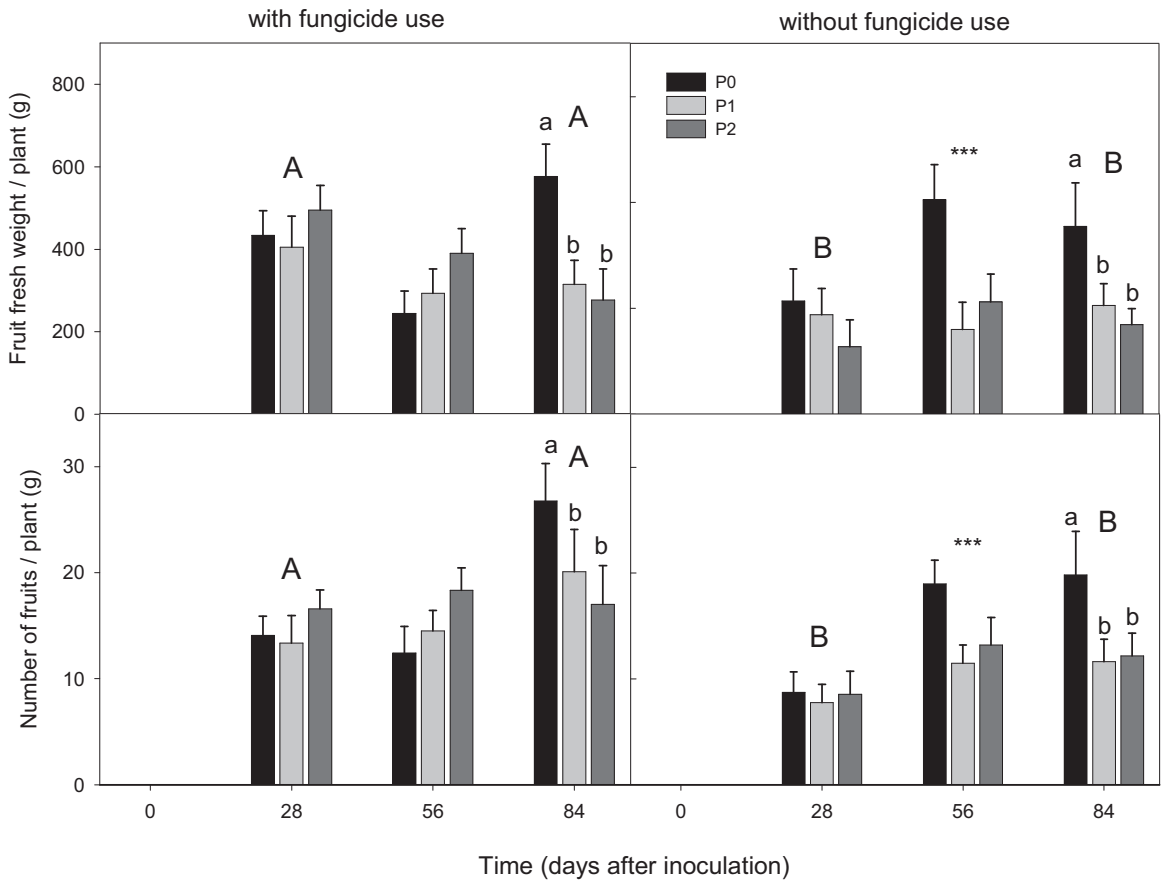


Figure 2.18: Fruit weight (± SE) (g) and number of fruits per plant in the six treatments (combined inoculations of *P. aphanidermatum* and *A. solani*; P0- without *Pythium,* P1- with low level of *Pythium* and P2- with high level of *Pythium*), n = 12.

2.5 Discussion

Sustainable crop production systems are readily available, but not widely disseminated in developing countries (Davis et al., 2002). For tomato production, the high temperature throughout the year and heavy rainfalls in the rainy season, which typify the lowland tropics, exert their most striking loss by reducing fruit set and therefore yield (Tunggim and Ruch, 1979). To avoid these negative effects, tomatoes could be produced under protected cultivation. Even though outside and inside temperatures of greenhouses are only slightly different (Ajwang, 2004), little is known about greenhouse crops, sustainable production, and upcoming diseases, although diseases are the major limiting factors in vegetable production in the tropics (Villareal, 1980). Thus, before investigations in greenhouses could be carried out, surveys about the important diseases on tomatoes in Thailand were conducted.

2.5.1 Disease surveys

The climatic conditions in Thailand are very favourable for many pathogens, especially fungal diseases (Villareal, 1987; Thi Bich Ha, 1992; Davis et al., 2002). The observations made in the surveys in Thailand confirmed this statement. The most important leaf diseases found under field production were early and late blight, the latter was mainly found in the relatively cooler regions in the north of Thailand. No symptoms of early or late blight were detected in the central area of Thailand. This might be related to the fact that tomato productions as well as other solanaceous host plants are rare in the central region. Main growing areas for tomato are the northeast and northwest of Thailand.

Late blight, caused by *Phytophthora infestans,* spreads very fast under cool and humid conditions (Davis et al., 2002; Campell and Madden, 1990; Jones et al., 1991). For some *Phytophthora* spp., for example *P. meadii*, which occurs in Sri Lanka and India on *Hevea*-rubber trees, only one day of bright sunlight and warm weather inhibits fungal spread (Kranz et al., 1979). These facts exclude late blight as relevant disease in the central region, and especially under greenhouse conditions.

Kranz et al. (1979) emphasized that EB is distributed world-wide and specially in warm climates. Jones et al. (1991) mentioned that primary infections of EB occurred during periods of mild (24-29°C) and rainy weather. In addition, Rotem (1994) confirmed that *Alternaria* species developed best in warm and moist environment, but they are also very tolerant to both low and extremely high temperatures, including 35°C. According to Rotem (1994), wetting periods, inoculation density and temperature determine the level of infection. Even at 35°C, with a wetting period of 24 h, he received a disease severity of 80% with an inoculum

concentration of $1*10^4$ spores cm^{-2}, and of 62% with a concentration of $1*10^3$ spores cm^{-2}. Therefore, EB seemed a model disease to be investigated under protected cultivation in the humid tropics.

Soil samples proved the occurrence of *Pythium* spp. in tomato fields and supported the finding that *P. aphanidermatum* is the most relevant soil-borne pathogen in warmer regions of Thailand (Heine, 2005; Wanwilai, pers. com.).

2.5.2 Pathogenicity tests

Strains of *A. solani* from leaf samples of the surveys and the reference strains were analysed microscopically. Although it is recognized, for example for *A. alternata,* that conidia formed in natural habitats are usually larger, have longer beaks, and are more uniform in size than those produced *in vitro* on common agar media (Misaghi et al., 1977), no differences in morphology existed between samples originating from the field and from *in-vitro* cultures. To exclude difficulties in the maintenance of *A. solani*, the temperature was chosen as 25°C under laboratory conditions. Misaghi et al. (1977) reported that *A. alternata* isolates grew at temperatures of 6-33°C but did not grow at 36°C or above. 27°C was the most favourable temperature of this fungus.

Bhatia et al. (2002) discovered in experiments with *Alternaria* spp. that a significant proportion of isolates were not pathogenic. In the current experiments, pathogenicity tests conducted on detached leaflets proved the pathogenicity of all collected isolates.

After inoculation of *A. solani*, no differences were found between wounded and unwounded leaves under laboratory conditions. This finding contradicts reports of Pryor and Michailides (2002) and Rotem (1994) that wounded leaves are more susceptible to infection. The temperature chosen for the pathogenicity tests was 25°C. According to Canhios et al. (1999), the highest levels of infection occurred at a temperature of 23 to 27°C and 24 h of leaf wetness. Other authors reported a wider temperature range for infection. For instance, the optimum for sporulation of *A. solani* was 26-28°C in experiments of Rands (1917) and 20°C according to McCallan and Chan (1944). Douglas (1972) found an optimum temperature of 25°C for sporulation of *A. solani* in light and 20°C in a light-dark photoperiod. In more recent studies in Israel, the optimum temperature for lesion expansion was determined to be 20 to 25°C (Solel and Kimchi, 1998). Rotem (1994) assumed that the optimum temperature of most species of *Alternaria* is 25°C or above at a wetting period of 24 h. The packing of leaves (like done in the experiments) in clear plastic bags had the aim to prolong the period of wetness and to increase relative humidity. However, wetting periods necessary for infection in nature

are usually shorter than those in the laboratory (Rotem, 1994). In the field, infections can occur during short wetting periods in night time interrupted by dry days. In such an interrupted wetting period regime, germination of spores starts during the first wet night, stops on the following dry day, and resumes during the next wet night. This process is repeated until the germ tubes penetrate the host (Bashi and Rotem, 1975). Based on results of a recent study (Vloutoglou and Kalogerakis, 2000), it appears that for a single disease cycle a wetness period of 24 h and 25°C would allow almost maximum infection.

A. solani is able to attack tomato in different growth stages, e.g. seeds, seedlings or mature plants (Agrios, 2005). In the current experiment, investigations were mainly done at the seedling stage where *A. solani* causes collar rot. It was reported that after the susceptible seedling stage, tomato plants became increasingly resistant to EB. However, susceptibility increased again when plants entered the senescent stage (Moore, 1942; Moore and Thomas, 1943; Rotem, 1994). In contrast, Jones et al. (1991) reported that tomato plants were susceptible to *A. solani* infection in all growth stages. We focussed on the infection of plants in the vegetative (6-week old) and reproductive (8-week old) growth stages, because this is a crucial time when plants were transplanted, e.g. in the greenhouse. Vloutoglou and Kalogerakis (2000) reported that the susceptibility of tomato to EB was greatest in plants at the reproductive stage. These results could not be confirmed in our experiment in which plants in the age of 10 weeks were 15% less susceptible to EB than plants in the age of 6 weeks and 8 weeks. Rotem (1994) showed similar results as Vloutoglou and Kalogerakis (2000) in their experiments with 12 h wetting period: disease severity increased with enhanced plant age. Increased susceptibility to infection with increasing host age has been reported in many *Alternaria*-host systems, such as *A. porri* on onions (Gupta and Pathak, 1986), *A. macrospora* on cotton (Rotem et. al., 1990), and *A. brassicae* and *A. brassicola* on brassica crops (Babadoost and Gabrielson, 1979).

Under laboratory conditions, infections of tomato with *A. solani* occurred. Within the repetition, under greenhouse conditions, no visual disease symptoms of EB appeared, possibly due to the fact that the disease needs a longer development time under field conditions as compared to controlled conditions. Rotem (1978) obtained similar results when he reported that young potato plants were successfully infected by *A. solani* in the laboratory whereas young plants in the field remained disease-free for a relatively long period, He accounted this phenomenon to an immune reaction of the plants in the field. Such an “immune” response may result from a scarcity of inoculum, an unfavourable microclimate in the young crop, or

from the effectiveness of a fungicide treatment, which in the nearly open stand covers the foliage more easily. Another important factor could be the climatic conditions. Temperatures up to 35°C in the early summer season (April - May) were measured inside the greenhouse. As mentioned above, EB is suitable for temperatures around 35°C, but Rotem (1994) didn't point out how long EB was able to withstand these conditions. According to Canhios et al. (1999), 32°C was the upper limit for infection with *A. solani* and significantly higher infection was observed at 27°C, especially when the leaves were constantly wet. At temperatures higher than the optimum, infection decreased rapidly resulting in a low disease severity at 32°C, even with extended wetness periods (Canhios et al., 1999). It seems that EB can sustain high temperatures for a short time period only, so that experiments should be conducted in lower-temperature seasons.

2.5.3 Greenhouse experiments

The purpose of these experiments was to study the effects of co-inoculation of the soil-borne pathogen *P. aphanidermatum* and the foliar pathogen *A. solani*. Since information about the combined effects of two pathogens and their interactions on plant health is rare (Waller and Bridge, 1984), an experimental approach with co-inoculation of *P. aphanidermatum* and *A. solani* was chosen. This combination of a foliar and a soil-borne pathogen could be of particular importance for the assessment of crop losses and the selection of appropriate management strategies. In addition, it can be expected that infection rates, maximum disease levels and the shapes of the disease progress curves are changed when two diseases are interacting as compared to a single disease (Hau, 2001). For example, Bhowmik and Singh (1977) found that *Alternaria* leaf blight of sunflower was more severe on plants infected with *Macrophomina phaseolina* root rot. *Verticilium* wilt reduced plant vitality and caused premature senescence on potato plants, which increased EB severity, caused by *A. solani* (Harrison, 1974). In order to answer the question if there were differences in the disease dynamics and in the growth of tomato plants under greenhouse conditions, two different inoculum levels were used, one with low density (P1) and one with higher density (P2) of *P. aphanidermatum*.

Even though both experiments were carried out with different varieties, the results of both experiments are comparable. Recent results of Vloutoglou and Kalogerakis (2000) showed that there were no significant differences between host cultivars with respect to disease severity of *A. solani* and it was proven that both cultivars used are susceptible to *A. solani* and *P. aphanidermatum* (Kandziora, unpublished).

In both experiments, it seems that even without visible disease symptoms of EB, the fungus had a significant negative influence on the plant growth (shoot dry weight, leaf area) at the first sampling time (28 dai), in the second experiment also on yield. In the first experiment, a significant negative influence of *P. aphanidermatum* on growth parameters was also found. However, neither the dominance of one of the pathogens nor the interaction between both pathogens was noticeable. This finding was also confirmed for the parameters root fresh weight and plant height. Due to the low yield in the first experiment, no reliable information about yield can be given. The low number of tomatoes harvested in some treatments excluded a statistical analysis.

In both experiments, at the second sampling date (56 dai) the main effects was related to the different inoculum levels of *Pythium* (P0, P1 and P2). The shoot dry weight was significantly reduced by both *Pythium* inoculation levels. The same observations were made for the root fresh weight in the first experiment. In the second experiment, the leaf area of the non-sprayed treatments was significantly reduced at 56 dai. To subsume a trend, in the first experiment, *Pythium* root rot had mainly a significant negative influence on plant growth parameters and in the second experiment it seems possibly related to the different fungicide treatments. This trend could be observed for all parameters in the third sampling date (84 dai) of the second experiment.

The significant influence was mainly related to EB, the foliar disease. However, as indicated above, the observed effects were more likely linked either to the fungicide treatment or to black leaf mold (BLM), caused by *Pseudocercospora fuligena*. This observation suggested that in both greenhouse experiments, a primary disease cycle of EB occurred, initiated through inoculation. However, no secondary cycle followed as no symptoms of EB were visible. Hillocks and Bridge (unpublished results cited in: Waller and Bridge, 1984) observed in a nematode-fungus complex (*Meloidogyne incognita* and *Fusarium oxysporum* f. sp. *vasinfectum*) that even without showing symptoms, *F. oxysporum* diseases were able to develop.

Recent experiments from Mersha (2008), carried out under nearly similar greenhouse conditions like in the current experiment, supported the assumption that EB can establish in the greenhouses with different net systems. Mersha (2008) conducted experiments in four different greenhouses types and at three different seasons at the AIT research facility. In his experiments, EB naturally appeared in greenhouses on plants transplanted in May. Plants in the greenhouse with pad cooling system were frequently affected by *A. solani* and had the highest disease severity of EB compared to plants of the other experimental greenhouses. In

the 40-mesh greenhouse BioNet ™, plants showed only a marginal EB severity. Thus it can be concluded that EB can occur in greenhouses, but only in the dry season and at a low level under the given greenhouse conditions. In addition, EB seemed to be important only during the early phase of plant growth (3 to 6 weeks after transplanting), while in the later stages, BLM was the more important leaf disease (Mersha, 2008).

The soil-borne pathogen *P. aphanidermatum* is known mainly as the causal agent of damping-off diseases (Agrios, 2005). On matured plants, disease symptoms like wilting or browning of lower leaves (or finally dying of the entire plant) are expressed. Even though the shedding of senescent leaves (Figure 2.10) is a normal process, a significantly increased number of shed leaves was observed that could be assigned to *P. aphanidermatum*. This assumption is supported by the results of the re-isolation.

In general, it is difficult to identify the cause of plant damage separating between root and shoot diseases on the same plant, especially if one pathogen does not obviously affect the susceptibility of the plant to the other. Usually more damage is credited to the more obvious disease (i.e. that on the aerial plant parts). On the other hand, Waller and Bridge (1984) mentioned that a root disease can reduce the plant's capacity for compensatory growth. This should be investigated further in general because this aspect seems to have received very little attention so far.

While the second experiment was running, symptoms of another foliar disease appeared identified as BLM caused by *Pseudocercospora fuligena* (Roldan). This fungus belongs to the family of *Mycosphaerella* (Crous and Braun, 2003) and is widely distributed in tropical regions all over the world. BLM develops small lesions on young leaflets as indistinct discolorations without definite margins (Jones et al., 1991). Disease development is favoured by warm (27°C), wet weather. The presence of moisture on the foliage from dew, rainfall and fog provides good conditions for infection (AVRDC, 2004). Increasing periods of leaf wetness are associated with increasing disease severity. Consequently, the disease may become more serious during the rainy season when high temperatures prevail.

Based on the results of the current study, the need for more detailed studies on *Pseudocercospora fuligena* can be emphasised. The fungus has the potential to be a serious problem for tomato production under protected cultivation in the humid tropics. Investigations under greenhouse conditions on BLM development and yield loss followed by studies on adequate management strategies should be done. Therefore, *P. fuligena* will be explored more thoroughly in subsequent papers.

3 Temporal progress and spatial distribution of black leaf mold in greenhouses in Thailand

3.1 Abstract

Investigations of black leaf mold (BLM) on tomato, caused by *Pseudocercospora fuligena*, were conducted under greenhouse conditions in Thailand (closed net house, Econet M, base area 10 x 20 m). Four experiments in different weather seasons and with two different tomato varieties (´New King Kong´ and ´King Kong 2´) were carried out to determine the spatial distribution and temporal progress of BLM. In addition, two treatments with *Pythium aphanidermatum* inoculation were included in the study in order to observe interactions of a disease complex and its influence on the disease dynamics of BLM. However, no influence of *P. aphanidermatum* on the dynamics of BLM was found. In some experiments, *Trichoderma harzianum* was used as a biological antagonist of *Pythium*.

BLM occurred naturally under greenhouse conditions. In pre-experiments with artificial inoculation, BLM showed first symptoms roughly after 14 days. Under greenhouse conditions with natural infestation, an incubation time was 7 to 28 days long. The main experiments were conducted for 84 and 112 days. The disease progress curves of incidence of plants were S-shaped and the 100% infection level was reached approximately after 63 days. At the end of the experiments, the maximum disease severity was 30%. The spatial disease patterns within rows at the beginning of the epidemics were analysed with the join-count statistics. In two experiments, the disease occurred in a random pattern, while in the two others an aggregation of diseased plants was indicated. The 3-D plots of spatial distribution did not show a gradient. The data were also analysed for vertical distribution. The maximum number of leaves per plant was 26 (± 1.11) for the determined variety ´New King Kong´ and around 50.54 (± 1.21) for the indeterminate variety ´King Kong 2´. The highest leaf insertion with BLM symptoms was at position 45 from the bottom. The leaf position with the maximum severity of 41.42 ± 4.46% was around leaf number 11.

3.2 Introduction

Black leaf mold (BLM), also formerly known as *Cercospora* leaf mold, is caused by *Pseudocercospora fuligena* (Roldan) Deighton (= *Cercospora fuligena* (Roldan)) that belongs to the family of *Mycosphaerella* (Crous and Braun, 2003). The fungus is widespread in warmer regions and greenhouses around the world, especially in tropical and subtropical Asia (Hsieh and Goh, 1990; Crous and Braun, 2003). It was first reported on tomato (*Solanum lycopersicon* L.) in 1938 in the Philippines (Roldan, 1938) and in 1951 also found in Japan, in 1955 in India, in 1974 in southern USA, in 1990 in Taiwan, in 1995 in Malaysia (Wang et al., 1995) and recently in Brazil (Halfeld-Vieira et al., 2006). In Thailand, it was first detected in 1979 in the Nongkham District, Amphoe Pasrijarern, Bangkok (Saranark and Chandrasrikul, 1980).

Observations of the disease in sub-tropical regions suggest that this disease has become a major problem in tomato production areas in recent years (Hartman et. al., 1991; Hartman and Wang, 1992; Hartman and Wang, 1993; Wang et al., 1995; Wang et al., 1996; AVRDC, 2004; Braun, pers. com.). In Japan, the disease is widely distributed and causes severe reductions in yield (Hartman et al., 1991). In Taiwan, BLM caused extensive damage on naturally infected hybrid tomatoes, reaching disease severities of 60% (Hartman and Wang, 1992). In experimental plots with four commercial cultivars, yield losses of 32 to 40% were recorded, caused by a reduction in fruit number and fruit weight (Hartman and Wang, 1992; Wang et al., 1995). Wang et al. (1994) reported that the yield of tomato grown in inoculated field plots without fungicidal protection amounted to 63% compared to yields from non-inoculated plots receiving fungicidal protection.

Several reports noted that most tomato cultivars are highly susceptible to BLM (AVRDC, 2004; Blazquez and Alfieri, 1973; Hartman and Wang, 1992). However, resistant and tolerant cultivars were identified in Florida, USA. Investigations regarding the reduction of yield losses with resistant cultivars were conducted in Taiwan (Blazquez and Alfieri, 1973; Jones et al., 1991; Hartman and Wang, 1993).

Disease development of BLM is favoured by warm (27°C), wet weather and occurs throughout the year. Conditions that favour disease development include high humidity (more than 85%) and moisture caused by dew or rainfall (Jones et al., 1991; AVRDC, 2004). In Taiwan, BLM severity was high when moderate to warm day and cool night temperatures resulted in extended periods of leaf wetness (Wang et al., 1996).

The lesions caused by *P. fuligena* develop slowly. Small lesions on young leaflets show indistinct discolorations without definite margins (Jones et al., 1991). As the size of lesions

increases, a faint halo appears at the lesion margin. The tissue inside that margin turns brown and collapses on both, the upper and lower side of the leaflet. Under humid conditions, heavy conidial production can be observed on the lower leaf surface. Nearly senescent leaves roll upward, but generally remain hanging on the plant with a soot-covered appearance. Spores are disseminated by wind-driven rain, water, or human transmission over shorter distances and by wind over long distances (Hsieh and Goh, 1990; Wang et al., 1995; AVRDC, 2004). No petiole, stem, or fruit symptoms have been observed.

Tomato (*Solanum lycopersicon* L.) is one of the most important crops in Thailand. For processed tomato, the major growing areas are the north and northeast of Thailand, while for table tomato the planting areas are distributed in various parts of the country (Intanoo, pers. com; Pongam, pers. com.). In the central part of Thailand, tomato is exclusively grown in open fields so that no production during the rainy season is possible, because of the heavy driving rain. Producing tomatoes in protected cultivation in greenhouses could overcome this problem. Therefore, sustainable tomato production systems in Bangkok area were investigated under protected cultivation where temperature, relative humidity and heavy dew in greenhouses are of paramount importance for plant growth as well as for pests and diseases causing damages to the crop. The major objective of this work within a larger project was to develop an optimal strategy to control fungal diseases on tomatoes grown under protected cultivation in greenhouses in Bangkok, Thailand.

The purpose of this study was specifically to investigate the spatial distribution and the temporal progress of BLM in greenhouses. In addition, the interaction of BLM with the soil-borne pathogen *P. aphanidermatum* was studied.

3.3 Materials and Methods

3.3.1 Collection and selection of isolates of *Pseudocercospora fuligena*

The tomato plants were not artificially inoculated but plants were exposed to natural infection by *P. fuligena* in the greenhouses on the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand.

To obtain active isolates from *P. fuligena*, leaves were collected that had clearly delineated lesions of BLM. Ten leaves were randomly selected from each greenhouse sampled. Sections of 5 mm^2 were removed from the edge of lesions, washed in pure sodiumhyperchloride for 10 s, air dried for 4 s and then plated onto Petri dishes (100 x 15 mm) containing potato dextrose agar (PDA; Merck). The Petri dishes were incubated under cool white fluorescent lights at 25°C for 12 h dark and 12 h light. Putative *P. fuligena* colonies were randomly selected from these Petri dishes and subcultured on tomato oatmeal agar (TOA) produced according to Hartmann et al. (1992) until pure cultures were obtained. For TOA, 50 g of shredded tomato leaves and 15 g of oatmeal agar were boiled separately. The tomato-leaves suspension was sieved through two layers of cheesecloth, mixed, and 25 g agar per litre of water was added. The suspension was autoclaved at 121°C for 15 min with 2 bar (Hartman and Wang, 1992). After positive verification as *P. fuligena* by the Centraalbureau voor Schimmelcultures (reference det 321-2003), Utrecht, Netherlands, and by Prof. Dr. Uwe Braun, Martin-Luther-University, Halle-Wittenberg, Germany, the most vigorous isolates were chosen for further experiments. To maintain isolates, the fungus was transferred monthly to new Petri dishes containing TOA via mycelia discs. The infested Petri dishes were incubated at 25°C under cool white fluorescent lights for 12 h dark and 12 h light.

3.3.2 Inoculum preparation of *Pseudocercospora fuligena*

TOA plates containing isolates of *P. fuligena* were incubated for 10 to 14 days and conidia were collected by flooding the plates with 50 mL of sterile distilled water (containing 0.01% Tween 20). Colonies were gently brushed and the suspension was filtered through two layers of cheesecloth to remove mycelia fragments.

The spore density in the suspension was counted using a haemocytometer and adjusted to a density of 10^4 conidia mL^{-1}. An atomizer was used for spray inoculation.

3.3.3 Effects of temperature and plant age on disease development

The following experiments were conducted under greenhouse condition. For further greenhouse information, see 3.3.4.

3.3.3.1 *Effects of temperature and plant age*

The experiments were conducted with the tomato variety ´King Kong 2´. They were set up as two-factor experiments with plants of four ages (2, 3, 4 and 6 weeks) and 3 temperature regimes (25, 30 and 35°C). Seeds were sequentially sown at required dates and plants were raised in pots in a greenhouse with an average temperature of 30°C. At the start of the experiment, six plants per age group were inoculated by spraying 20 mL of inoculum solution on the leaves inside the experimental greenhouse. Additionally, as control plants, three plants per age group were sprayed with sterile water. All plants were individually covered in plastic bags for 24 h to increase the relative humidity (nearly until saturated) in order to facilitate infection. Disease severity of leaves was estimated every second day for a period of 30 days by using a rating scale (see 3.3.5). The experiment was repeated three times.

3.3.3.2 *Effects of plant age and inoculation method*

The experiments were conducted with the tomato variety ´King Kong 2´ under greenhouse conditions. Plants were raised and cultivated as described above. The experiments were set up as two-factor experiments with plants of three ages (6, 8 and 10 weeks) and with two inoculation methods. Plants were inoculated either by spray inoculation as mentioned above or by blowing the spores from diseased plants with a pocket ventilator (commercially available, with 15 cm diameter, circulation time 1 min) onto the leaves. In both cases, plants were then individually covered in plastic bags for 24 h to facilitate infection. Each variant contained 10 plants. The plants were observed for 12 days after inoculation (dai); first occurrence and disease severity were rated every second day as described below. Disease severity of leaves was estimated every second day by using a rating scale (see 3.3.5) for a period of 12 days.

3.3.4 Disease dynamics under greenhouse conditions

3.3.4.1 *Experimental set up*

The experiments were conducted in a greenhouse, located at the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand. The greenhouse with the base area of 10 x 20 m was a closed net-house (Econet M, pore size 0.18 mm, 40 x 37 mesh (40-mesh), Ludvig

Swensson, Netherlands) equipped with two exhaust fans (550 m^3 min^{-1}, 1.5 hp, 960 rpm, Sriroz Company, India) at the front side of the net-house (Figure 3.1).

Figure 3.1: Main entrance side of the net-house located at the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand.

The fans were operated by a computerized control system that automatically switched on one fan when temperature inside the net-house exceeded 25°C, and the second one at a temperature above 30°C. The climate in the greenhouse was monitored using a data logging system (ITG data logger, Leibniz Universität, Hannover, Germany). During the experiments, the mean temperature was between 25-30ºC and relative humidity between 70-80%. The total planting area of the greenhouse was 160 m^2. The greenhouse was lengthwise subdivided with a net (Econet M, pore size 0.18 mm, Ludvig Swensson, Netherlands) in two halves, each with 3 rows and a separate entrance door. The main entrance was equipped with a sanitation sluice and a disinfectant tray. The second entrance was a normal single door exit at the opposite side of the main entrance.

Tomato seedlings were raised in a nursery under the same conditions as in the experimental greenhouse. In the age required for the experiments, they were planted in plastic pots (30 x 25 cm) filled with a commercial growing substrate composed of clay (31%), sand (30%) and silt (39%) and 29% of organic matter. The pots were placed on a black ground plastic cover (Chaisiri Nylon Canvas Factory Ltd., Bangkok, Thailand) and arranged in 6 rows with 60 pots each. There was no inter-pot distance in the rows. The distance between rows was 160 cm and from the sidewall to the outer row 55 cm (Figure 3.2).

Figure 3.2: Inside view of one part of the closed net-house at the AIT campus in Bangkok, Thailand.

Plants were fertigated 7 to 9 times per day (2.5 L day^{-1}) with a drip irrigation system controlled by solar light integral. The fertilizers [Hakaphos® (2.5 kg 100 L^{-1}), COMPO GmbH, Austria, and Bai-plus (1.8 kg 100 L^{-1}), Bayer Ltd., Thailand] were injected into the irrigation system with mechanical injectors (DI 16, Dosatron®, France). Tomato plants were supported by ropes, which were fixed to the ceiling structure of the greenhouse. The plants were cultivated in a single-stem system. Pruning and layering of plants was done weekly.

To control leaf diseases, one part of the greenhouse was weekly sprayed with Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}), beginning at the day after positioning (dap) plants in the greenhouse. The sprayed part will be referred to as "with control of".

Altogether four experiments were conducted between October 2002 and March 2004. The dates of each experiment and the prevailing environmental conditions are summarized in Table 3.1. Prior to each experiment, the greenhouse was cleaned and disinfected.

Table 3.1: Duration of experiments under greenhouse conditions and the climatic conditions

Exp No.	Start	End	Duration (dap)	Mean Temp (°C)	Min Temp (°C)	Max Temp (°C)	Mean RH (%)
1	21 Oct`02	13 Jan`03	84	28.3 ± 0.3*	20.9 ± 0.9 *	35.2 ± 0.1 *	**
2	19 May`03	11 Aug`03	84	29.1 ± 0.1	25.4 ± 0.1	35.2 ± 0.3	77.28 ± 0.78
3	21 Oct`03	10 Feb`04	112	26.6 ± 0.2	21.9 ± 0.2	33.4 ± 0.2	73.54 ± 0.53
4	26 Nov`03	17 March`04	112	25.9 ± 0.2	21.0 ± 0.2	33.0 ± 0.2	72.98 ± 0.65

* values from the Deutsche Wetterdienst databank of the measuring point Bangkok, Data logger were not yet already in function at that time;
** no values available

3.3.4.2 *First Experiment*

This experiment was carried out with the tomato variety ´New King Kong´, an early flowering determinate variety. The seeds were sown on the 26th August 2002 and the seedlings transplanted eight weeks later. At 21 October 2002, in the 10- to 12- leaf stage,

plants were placed in the greenhouse. At the same day, the substrate was inoculated with the pathogenic isolate of *P. aphanidermatum* (confirmed by the Centraalbureau voor Schimmelcultures, Netherlands; reference det 273-2002) at 3 different densities (P0, P1 and P2). Inoculation was carried out by mixing 1-cm^2 pieces of potato dextrose agar containing actively growing mycelium into the planting substrate. For the lower (P1) and higher (P2) inoculum level, 1 and 3 Petri dishes per pot were used, respectively. Plain agar medium was applied for control plants.

Plants were arranged in a split-block design with 3 replications. Every split-block was subdivided by 3 levels of *P. aphanidermatum* (P0/P1/P2) as sub-plot factor. The sub-plot factor was repeated 4 times and one sub-plot factor contained 5 plants in a row. The total number of plants in the greenhouse was 360 (270 were used for data collection), arranged in 6 rows (Figure 3.3).

In one-half of the greenhouse, plants were inoculated with *A. solani*. Three leaves (number 5, 6 and 7 counted from bottom to top) were scratched, the agar plates with mycelium of *A. solani* were smoothly pressed for 5 seconds onto the leaves. Thereafter, the leaves were moistened by spraying tap water and covered separately with plastic bags for 24 h in order to increase the relative humidity. Control plants were treated with sterile agar plates.

Because the inoculation with early blight was not successful (see chapter 2), the main focus was on the observation of the interaction of Pythium root rot (PRR) and BLM.

No artificial inoculation with *P. fuligena* was necessary due to natural infection pressure. To avoid the spread of black leaf mold and early blight, the side of the greenhouse without *A. solani* inoculation was weekly treated with Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}), beginning with the day after positioning (dap).

The treatments in the first experiment were:

P0–BLM0: 1 plain Petri dish per pot, with control of *P. fuligena*

P1–BLM0: 1 Petri dish with *P. aphanidermatum* per pot, with control of *P. fuligena*

P2–BLM0: 3 Petri dishes with *P. aphanidermatum* per pot, with control of *P. fuligena*

P0–BLM1: 1 plain Petri dish per pot, naturally infected with *P. fuligena*

P1–BLM1: 1 Petri dish with *P. aphanidermatum* per pot, naturally infected with *P. fuligena*

P2–BLM1: 3 Petri dishes with *P. aphanidermatum* per pot, naturally infected with *P. fuligena*

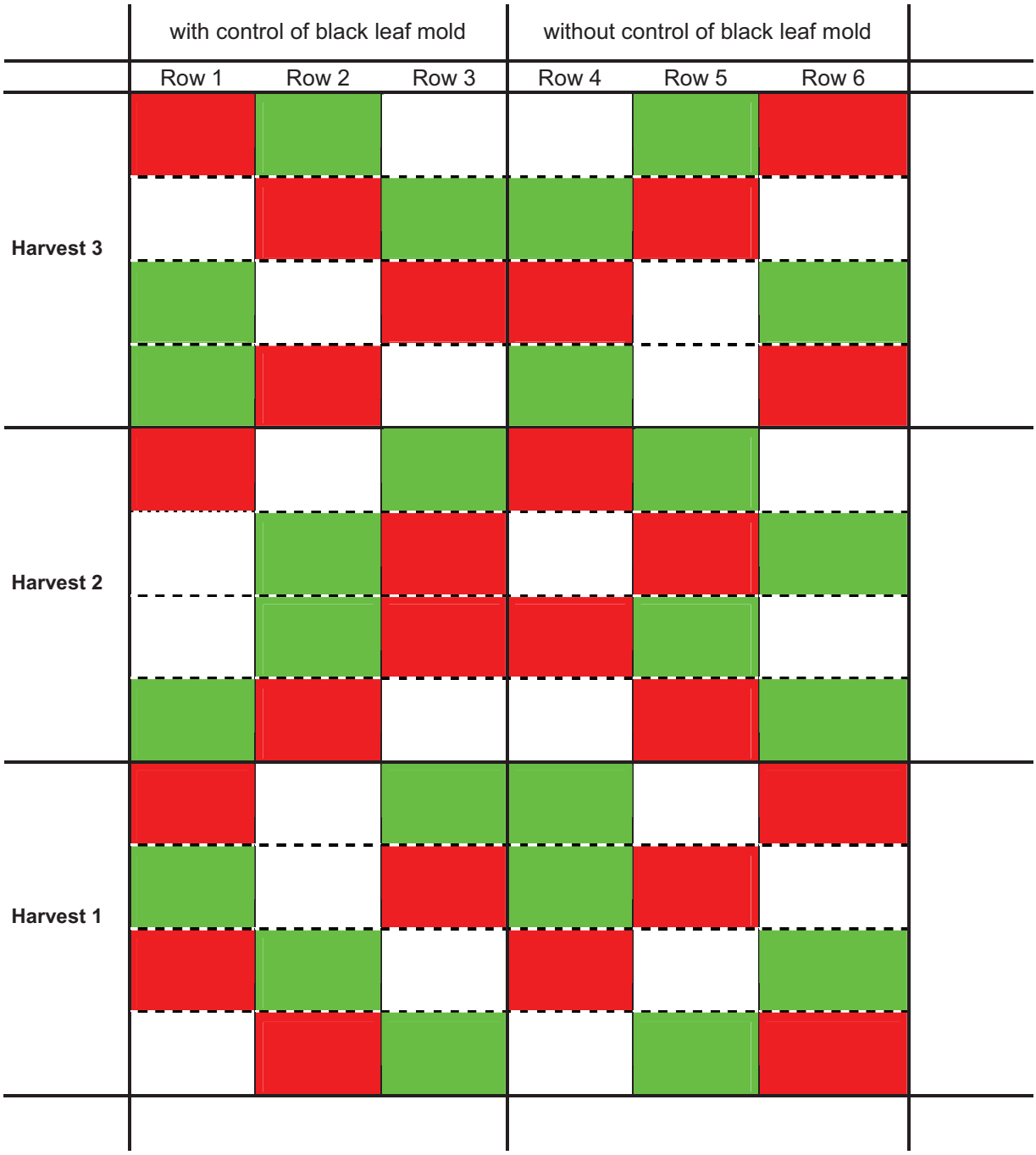


Figure 3.3: Greenhouse plan for experiment 1 laid out in a split-block design (with 3 replications). Control of BLM as main factor and 3 levels of *P. aphanidermatum* (P0/P1/P2) as sub-plot factor. Each rectangle represents 5 plants.
Colour Code: White: P0 = 1 plain Petri dish with agar per pot
Red: P1 = 1 Petri dish with *P. aphanidermatum* per pot
Green: P2 = 3 Petri dishes with *P. aphanidermatum* per pot

For a period of 3 months after inoculation, growing parameters of individual plants were recorded. The disease incidences of plants and leaves were determined and the disease severity was visually estimated for each individual leaf on a weekly base (see 3.3.5). Three destructive harvests were done in order to specify biomass production of plants, starting one month after beginning of the experiment on the opposite side of the ventilation.

3.3.4.3 *Second Experiment*

The experimental setup of the second experiment was similar to the first one (see above), except that the indeterminate variety ´King Kong 2´ was used. This variety was considered to be more susceptible than ´New King Kong´. Seeds were sown on the 21 April 2002. After one month (on 19 May 2002) when plants had reached the 10- to 12- leaf stage, tomato seedlings were transferred to plastic pots as described above.

The inoculation of *P. aphanidermatum* was carried out using a modified technique of Chaengchaiyasakulthai and Chamswarng (1986). A stock culture containing sand, soil, and maize flour (3/1/1) was infested with mycelium plugs from four days old cultures of *P. aphanidermatum* grown on PDA and incubated in darkness at 35°C. After two weeks, the potting substrate was mixed with 5% of the stock culture and 1.5% maize flower. This mixture was incubated overnight at 25°C and then used as inoculum (P1* = 0.2 g and P2* = 1 g of inoculum per pot). The experimental control plants received 0.2 g per pot of sterilized inoculum.

Additional plants with the same treatments were designated as reserve in order to replace dying plants and were kept at the edge of the greenhouse

The treatments in the second experiment were:

P0*–BLM0: 0.2 g sterile inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*

P1*–BLM0: 0.2 g inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*

P2*–BLM0: 1 g inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*

P0*–BLM1: 0.2 g sterile inoculum of *P. aphanidermatum* per pot, naturally infected with *P. fuligena*

P1*–BLM1: 0.2 g inoculum of *P. aphanidermatum* per pot, naturally infected with *P. fuligena*

P2*–BLM1: 1 g inoculum of *P. aphanidermatum* per pot, naturally infected with *P. fuligena*

At 6 dap, all plants were monitored for disease symptoms related to *P. aphanidermatum*. To enable an undisturbed monitoring of leaf disease development, dead plants were replaced by healthy plants of the same inoculation density. If more plants dropped out than pots were available for replacement, new plants from the nursery of the same age were transplanted to the same pots. In the control treatment, the substrate was also changed. This procedure was repeated at 13 dap. An overview over the replacement strategy is shown in Tab. 3.2. More details are given in the appendix (Figure 6.1).

Table 3.2: Replaced plants in the different *Pythium* – treatments of experiment 2 at 6 and 13 dap

Treatments	Total number of plants	Replaced plants at 6 dap	Replaced plants at 13 dap
P0*-BLM0	60	40	29
P1*-BLM0	60	13	14
P2*-BLM0	60	13	17
P0*-BLM1	60	37	24
P1*-BLM1	60	28	23
P2*-BLM1	60	28	18

For the treatment with *P. fuligena*, no artificial inoculation was done but plants were subjected to natural infestation with the pathogen. The control treatment was kept free of the disease by spraying the respective half of the greenhouse with Maneb (see above). However, by mistake the wrong half of the greenhouse was sprayed from 28 to 49 dap.
Data collection including three destructive harvests were done as described for experiment 1 (see 3.3.4.2).

3.3.4.4 *Third Experiment*

The experimental setup of the third experiment was similar to the second experiment. Again, the tomato variety ´King Kong 2´ was used. Seeds were sown on 20 September 2003. One month later (on 21 October 2003), seedlings were transplanted to the greenhouse and inoculated with *P. aphanidermatum* as described for the first experiment (3.3.4.2). In addition, the pots of the treatment without Pythium inoculation (P0) were supplemented with the biological control organism *Trichoderma harzianum* in order to control possible contamination with soil-borne pathogens. According to the method of Chamswarng (1995), 0.2 % inoculum of *T. harzianum* was added to the substrate prior to transplanting.

Again, all plants were exposed to natural infection by *P. fuligena,* but the half of the greenhouse designated for the control treatments of *P. fuligena* was sprayed with Maneb.

The treatments in the third experiment were:

$P0_T$–BLM0: 1 plain Petri dish per pot, with control of *P. fuligena,* with *T. harzianum*

P1–BLM0: 1 Petri dish with *P. aphanidermatum* per pot, with control of *P. fuligena*

P2–BLM0: 3 Petri dishes with *P. aphanidermatum* per pot, with control of *P. fuligena*

$P0_T$–BLM1: 1 plain Petri dish per pot, naturally infected with *P. fuligena,* with *T. harzianum*

P1–BLM1: 1 Petri dish with *P. aphanidermatum* per pot, naturally infected with *P. fuligena*

P2–BLM1: 3 Petri dishes with *P. aphanidermatum* per pot, naturally infected with *P. fuligena*

At 2, 3, 5, 10, 13, and 15 dap, all plants were monitored for disease symptoms related to *P. aphanidermatum.* At 5 dap, pots with dead plants were replaced by spare pots with healthy plants of the same inoculation density. If more plants died than spare plants were available for replacement, no further replacement was done. A detailed plan of the replacement strategy is given in the appendix (Figure 6.1).

The experiment was planned for 4 months with 4 destructive samplings. Disease observations were done weekly as described above. A destructive sampling of 15 plants per treatment was done every month, 9 plants were used for data collection. At each sampling, one fourth of the greenhouse plants was removed.

At 84 dap, all leaves from the bottom up to leaf number 10 were removed.

3.3.4.5 *Fourth Experiment*

The experiment was carried out with the tomato variety ´King Kong 2´. Seeds were sown on 25 October 2003 and emerging plants transplanted to the greenhouse on 26 November 2003. Half of the pots were inoculated with *T. harzianum* as described for the third experiment (see 3.3.4.4). In contrast to the other experiments, no *P. aphanidermatum* treatment was included. Infection by *P. fuligena* occurred naturally. The *P. fuligena* control was weekly sprayed with Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}), beginning with the day after positioning.

The treatments in the fourth experiment were:

P0–BLM0: with control of *P. fuligena*

$P0_T$–BLM0: with control of *P. fuligena*, inoculated with *T. harzianum*

P0–BLM1: naturally infected with *P. fuligena*

$P0_T$–BLM1: naturally infected with *P. fuligena,* inoculated with *T. harzianum*

Disease incidences and disease severity of plants and leaves were recorded on a weekly base. At 77 dap, all leaves from the bottom up to leaf number 10 were removed.

In the fourth experiment, no intermediate harvests were done. After 4 months of observation, all plants were harvested.

3.3.5 Disease assessment

The 3 middle plants from the 5 plants of each sub-plot were sampled for disease assessment. Results of the 3 plants analysed were pooled. All plants were visually rated for percent diseased foliage using a modified Beaumont rating scheme (Beaumont, 1954) with 0% indicating no visible symptoms of *P. fuligena* infection and 100% indicating completely diseased foliage. The rating scale was modified as followed:

no recognizable lesions : 0%
0.5 – 2.5% diseased foliage : 1%
2.5 – 7.5% diseased foliage : 5%
7.5 – 15% diseased foliage : 10%
15 – 25% diseased foliage : 20%
25 – 35% diseased foliage : 30%
35 – 45% diseased foliage : 40%
45 – 62% diseased foliage : 50%
63 – 82% diseased foliage : 75%
83 – 100% diseased foliage : 100%

3.3.6 Spatial pattern analyses

The spatial disease patterns within rows at the beginning of the epidemics were analysed using the join-count statistics (Madden et al., 2007) considering joins only along rows. The joins analysed were pairs of diseased plants as well as pairs of diseased and healthy plants. In both cases, the tests were carried out using a standard normal distribution test statistics. For instance, with a z-value greater than 1.64 for the pairs of diseased plants, the null hypothesis of randomness was rejected ($P \leq 0.05$) and the hypothesis of clustering accepted (Madden et al., 2007). In the analyses, the missing data of plants due to virus infection, thrips attack or wilting were taken into consideration, especially in experiment 3.

For further spatial analyses, the 3 middle plants from groups of 5 plants (sub-plot factor, Figure 3.3) were pooled.

3.3.7 Temporal analyses

Comparisons of the disease progress curves were done using the Area Under Disease Progress Curve (AUDPC), calculated as described by Campbell and Madden (1990). The output values are presented as %-days.

Data were subjected of two-way analyses of variance (ANOVA) using the PROC GLM procedure of the SAS software package (SAS 9.1., Users Guide, SAS Institute, Cary, NC) and means separated by LSD ($p < 0.05$). The two factors investigated were the influence of *P. fuligena* (marked with capital letters A and B) and of *P. aphanidermatum* (characterized with small letters a and b). Interactions between these factors are marked with ***. Mean comparisons were conducted using the Tukey's t-test ($p \leq 0.05$).

3.4 Results

3.4.1 Effects of temperature and plant age on disease development

Under laboratory condition, inoculated plants of all age groups expressed symptoms of BLM at 25 and 30, but not at 35°C. The disease symptoms developed faster on older than on younger plants, except for the 6-week-old plants at 25°C (Table 3.3). At 25°C, first lesions of 6-week-old plants were observed at 12 dai as compared to an incubation time of 18 days for 2-week-old plants. At 30 dai, the disease severity was highest for 4-week- (16.1 %) and 3-week- (15.3 %) old plants. Plants with an age of 2 weeks and 6 weeks showed a disease severity of 9.4 %, respectively.

Table 3.3: Effects of temperature (25 and 30°C) and plant age on disease severity (%) and first occurrence (dai) of BLM

Plant age	25°C				30°C			
	First occurrence (dai)	Disease severity* (%)	Statistics for		First occurrence (dai)	Disease severity[1] (%)	Statistics for	
			temp	age			temp	age
2 weeks	18	9.4 ± 4.3	B**	b	17	34.1 ± 11.9	A	a
3 weeks	17	15.3 ± 2.0	A	a	16	23.4 ± 5.5	A	ab
4 weeks	15	16.1 ± 2.0	A	a	13	21.2 ± 9.5	A	ab
6 weeks	12	9.4 ± 5.1	A	b	7	12.5 ± 3.3	A	b

*Maximum values at the end of the experiment, 30 days after inoculation (dai).
** Data were subjected to a two-way analysis of variance (ANOVA) and means separated by LSD ($p < 0.05$). Means followed by the same capital letters within a row refer to non-significant differences (P=0.05) between the different inoculation methods and the day of first occurrence. Same small letters within a column refer to non-significant differences (P=0.05) between the different plant ages within a temperature.

Also at 30°C, symptoms of BLM were expressed earlier on older plants. The first symptoms appeared 7 days after inoculation on 6-week-old plants as compared to 17 days on plants 2 weeks old. The 2- and 3-week-old plants showed interactions in terms of disease severity and increasing temperature.

Under greenhouse conditions, both inoculation methods (spraying and blowing of conidia) resulted in the formation of BLM symptoms on the inoculated plants. After spray inoculation with a conidia suspension, first symptoms appeared 4 dai on older plants (8- and 10-week-old) and 6 dai on younger plants (6-week-old) (Table 3.4).

Table 3.4: Effects of different inoculation methods (spraying and blowing) and plant age on disease onset (days) and disease severity (%) under greenhouse conditions

Plant age	spray inoculation				blow inoculation			
	First occurrence (dai)	Disease severity* (%)	Statistics for method	age	First occurrence (dai)	Disease severity[1] (%)	Statistics for method	age
6 weeks	6	0.1 ± 0.0	A**	c	6	5.6 ± 1.9	B	b
8 weeks	4	18.6 ± 2.1	A	a	8	13.1 ± 1.4	B	a
10 weeks	4	11.1 ± 1.3	A	b	4	7.6 ± 0.9	A	b

* Maximum values at the end of the experiment, 12 days after inoculation (dai).
** Data were subjected to a two-way analysis of variance (ANOVA) and means separated by LSD ($p < 0.05$). Means followed by the same capital letters within a row refer to non-significant differences (P=0.05) between the different inoculation methods and the day of first occurrence. Same small letters within a column refer to non-significant differences (P=0.05) between the different plant ages within a temperature.

Following spray inoculation, the disease severity at 12 dai was 18%, 11% and 0.10% for 8-, 10-, and 6-week-old plants, respectively. Blow inoculation resulted in a disease severity of 13% for 8-week-old plants, 8% for the 10-week-old plants, and 6% for the 6-week-old plants. There were different trends depending on plant age and the inoculation method for the 6- and 8-week-old plants, which led to significant differences.

3.4.2 Disease dynamics under greenhouse conditions

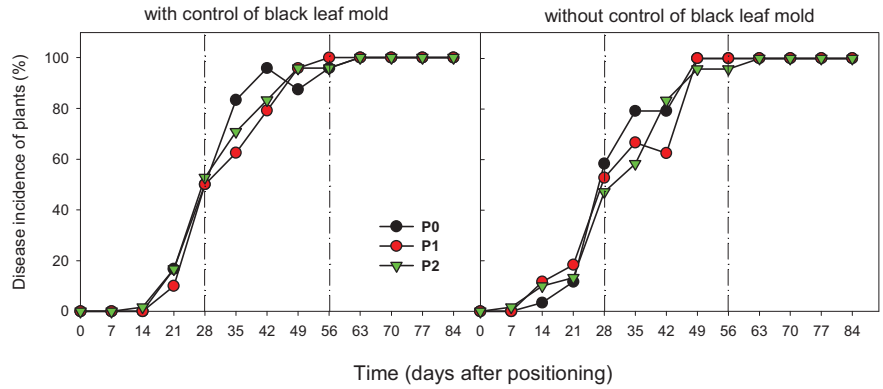
3.4.2.1 *Temporal progress*

Inside the greenhouse, BLM naturally occurred throughout the year without great differences between seasons. Both tested cultivars, ´New King Kong´ and ´King Kong 2´, were susceptible. No other foliar diseases occurred at damaging levels during the study.
In the P0*-treatment of experiment 2, symptoms of a soil-borne disease occurred. By re-isolation it could be proved that the only soil-borne disease occurring in all experiments was *P. aphanidermatum.*

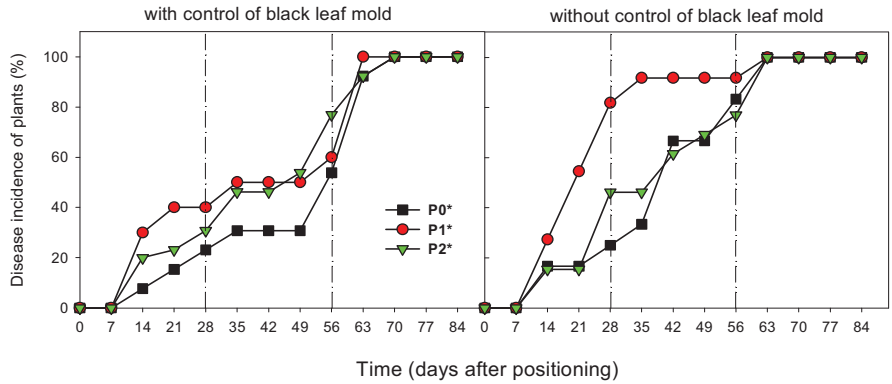
3.4.2.1.1 Disease incidence of plants

The observation period of the four experiments differed from 84 days in the first and second experiment to 112 days in the third and fourth experiment (Figure 3.4).

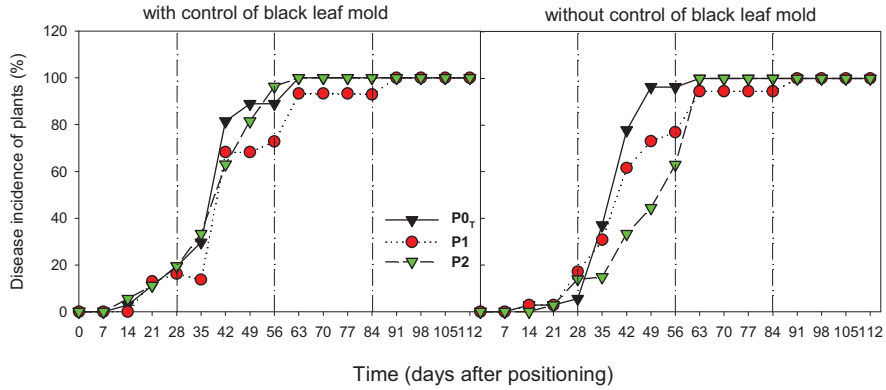
Experiment 1: *October 2002 – January 2003*



Experiment 2: *May – August 2003*



Experiment 3: *October 2003 – February 2004*



Experiment 4: *November 2003 – March 2004*

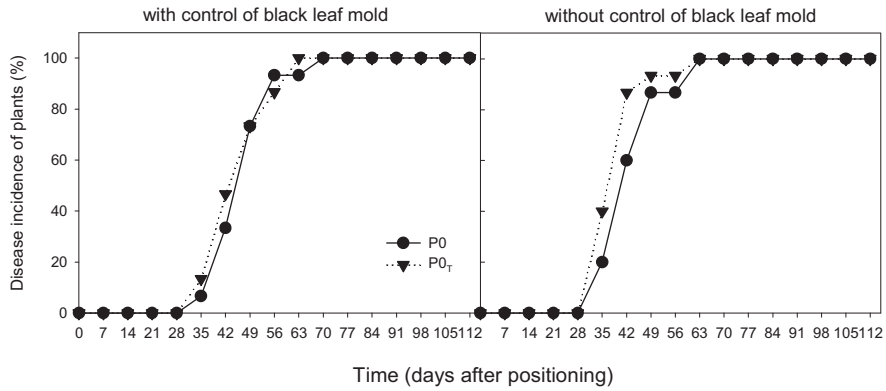


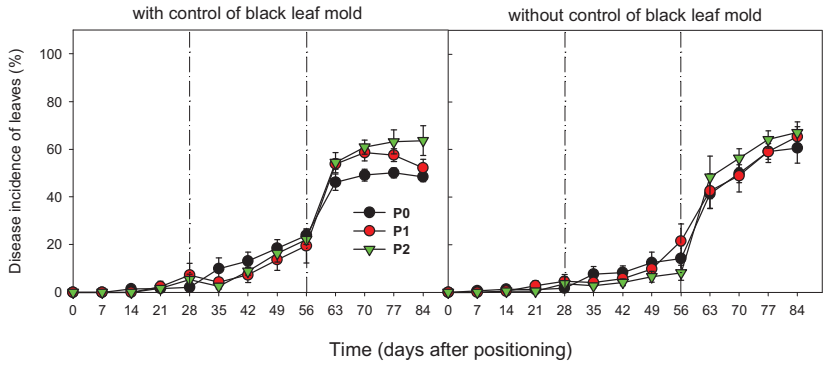
Figure 3.4: BLM progress curves given as incidence of plants (%) in four experiments with different *P. aphanidermatum* treatments: P0 – non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1 - low level and P2 - high level of *Pythium*. P0*, P1*- and P2*-treatments refer to another inoculation method (see 3.3.4.3). Vertical lines show the times when part of the plants were removed, resulting in a reduced sample size.

The progress curves in all experiments were S-shaped, except in experiment 2 where the progress curves had a plateau between 35 and 49 dap in the sprayed part of the greenhouse.
The first diseased plants in experiment 2 were observed already 7 dap; this might be due to a pre-infection problem in the nursery. In experiment 1 and 3, symptoms appeared 14 days after positioning. In experiment 4, it took 28 days to detect BLM symptoms. The 50% level of the progress curves was reached between 28 and 42 days in the first and fourth experiment, respectively. After 63 days nearly all plants in all treatments showed at least one lesion except in the third experiment, in which it took 91 days for the *Pythium* inoculated treatment P1 (sprayed and non-sprayed version) to reach the 100% level.
Neither the different *Pythium*-levels nor the treatment with *Trichoderma* changed the overall trend of the progress curves.

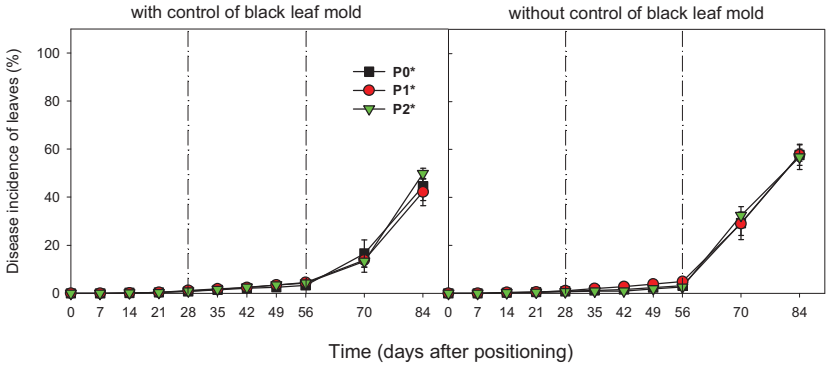
3.4.2.1.2 Disease incidence of leaves

Although the disease incidence of plants reached 100% after approximately 63 days, not all leaves of the tomato plants were diseased (Figure 3.5).
After symptom appearance, the curves in all experiments progressed very similar up to 56 days with an ascending trend. At 56 dap, the incidence of leaves was less than 10%, with exception of the first experiment, where it reached already 20%. After day 56, the progress curves differed because the first experiment was conducted with a determinate tomato variety. As no removal of lower leaves was necessary, disease incidence continued to increase reaching on average 60%. The other three experiments were conducted with an indeterminate variety. The incidence increased to a maximum level, especially in the non-controlled treatments, for example to 70% in experiment 4.
The fungicide controlled plants in experiments 1 and 2 showed on average 10% less BLM incidence of leaves than the non-controlled plants. In the experiments 3 and 4, the differences were 40% compared to the non-sprayed version.
The AUDPC values within the different *Pythium*-levels, even with or without *Trichoderma*, showed no general deviating trend (Table 3.5). For example, in experiment 1 the average AUDPC (in %-days) was roughly 1700 for the BLM0-treatments and around 1600 for the BLM1-treatments. The only exception was experiment 3 in the $P0_T$-BLM0-treatment: this value was about 300%-days higher than in the other BLM0-treatments.

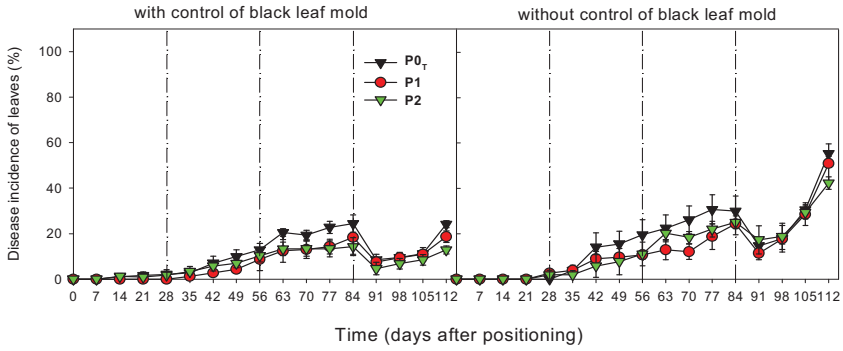
Experiment 1: *October 2002 – January 2003*



Experiment 2: *May – August 2003*



Experiment 3: *October 2003 – February 2004*



Experiment 4: *November 2003 – March 2004*

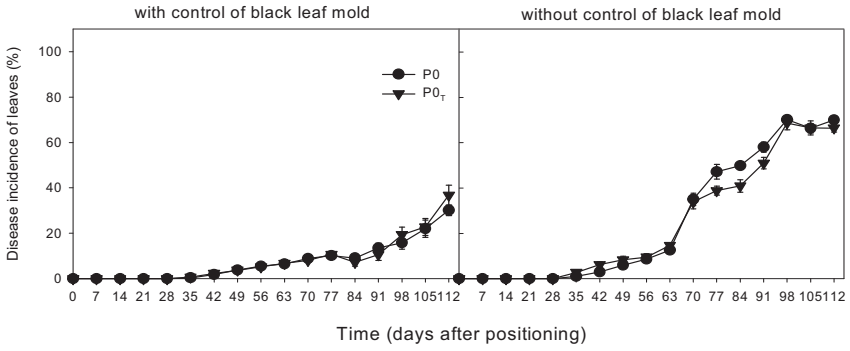


Figure 3.5: BLM progress curves given as incidence of leaves (%) in four experiments with different *P. aphanidermatum* treatments: P0 – non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1 - low level and P2 - high level of *Pythium*. P0*, P1*- and P2*-treatments refer to another inoculation method (see 3.3.4.3). Vertical lines show the times when part of the plants were removed, resulting in a reduced sample size. In experiments 1 to 3 the continuously monitored plants were displayed. Because of cultural practice, lower leaves (up to leaf no. 10) were removed in experiments 3 (at 84 dap) and 4 (after 77 dap).

Table 3.5: AUPDC values of incidence of leaves in %-days of BLM in four different greenhouse experiments: P0– non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1- low level and P2- high level of *Pythium*, BLM0 – with control of BLM and BLM1 – without control of BLM. P0*, P1*- and P2* -treatments refer to another inoculation method (see 3.3.4.3)

Experiment 1:	**BLM0**	**BLM1**
P0	1556 ± 101 a[1]	1645 ± 179 a
P1	1868 ± 145 a	1715 ± 208 a
P2	2052 ± 151 a	1993 ± 183 a
Statistic for BLM	A	A

Experiment 2:	**BLM0***	**BLM1***
P0*	698 ± 60 a	722 ± 117 a
P1*	720 ± 117 a	910 ± 85 a
P2*	797 ± 68 a	766 ± 76 a
Statistic for BLM	A	A

Experiment 3:	**BLM0**	**BLM1**
$P0_T$	1149 ± 100 a	1764 ± 147 a
P1	693 ± 139 b	1182 ± 203 a
P2	772 ± 86 ab	1396 ± 155 a
Statistic for BLM	B	A

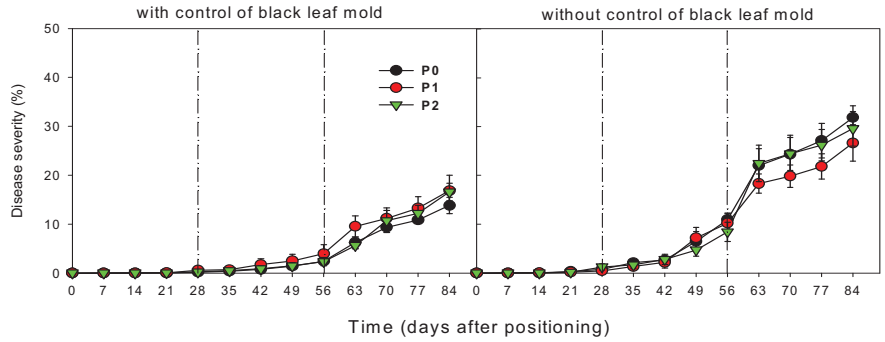
Experiment 4:	**BLM0**	**BLM1**
P0	936 ± 62 a	3034 ± 49 a
$P0_T$	972 ± 58 a	2923 ± 77 a
Statistic for BLM	B	A

[1] Data were subjected to a two-way analysis of variance (ANOVA) and means separated by LSD ($p < 0.05$). Means followed by the same letter are not significantly different ($P = 0.05$). The two factors investigated were the influence of *PRR* within column (characterized with small letters) and of BLM within rows (marked with capital letters).

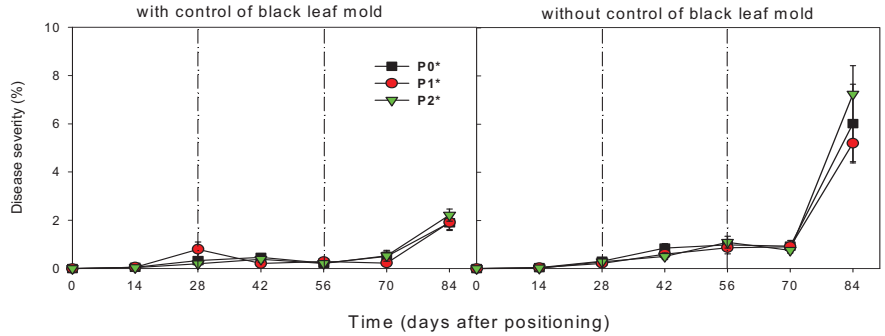
3.4.2.1.3 Disease severity

Disease severity (Figure 3.6) showed the same tendency as disease incidence of leaves (Figure 3.5).

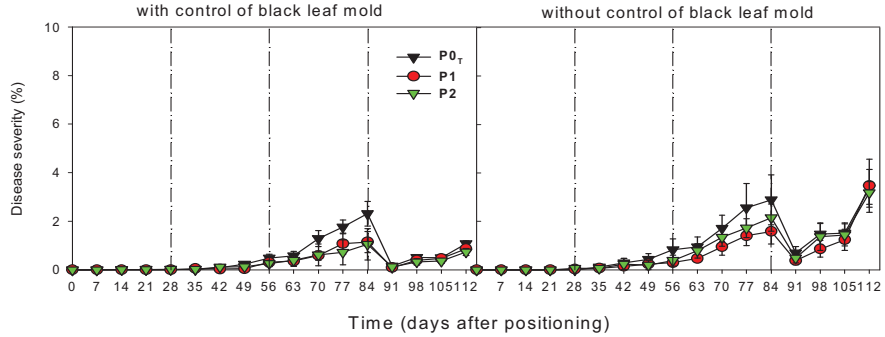
Experiment 1: *October 2002 – January 2003*



Experiment 2: *May – August 2003*



Experiment 3: *October 2003 – February 2004*



Experiment 4: *November 2003 – March 2004*

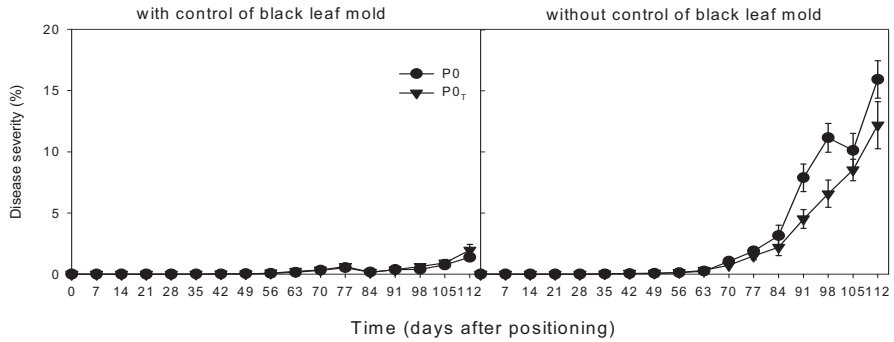


Figure 3.6: BLM progress curves given as disease severity (%) in four experiments with different *P. aphanidermatum* treatments: P0 – non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1 - low level and P2 - high level of *Pythium*. P0*, P1*- and P2*-treatments refer to another inoculation method (see 3.3.4.3). Vertical lines show the times when part of the plants were removed, resulting in a reduced sample size. Because of cultural practice, lower leaves (up to leaf no. 10) were removed in experiments 3 (at 84 dap) and 4 (after 77 dap).

The progress curves in all experiments inclined gently. In experiment 3, a descent occurred after 84 days due to the stripping-off of leaves. The highest severity was reached in experiment 1. Plants in the non-controlled part of the greenhouse reached approximately 30% disease severity. Plants in experiments 2 and 3 had a disease severity level below 10% in the non-sprayed parts of the greenhouse, while in the P0-treatment in experiment 4, BLM severity reached 15%.

A significant reduction in diseased severity, displayed in AUDPC values, in the sprayed compared to the non-sprayed treatments was recognizable (Table 3.6), for example in experiment 1 in which the BLM0-treatments had an average of 300%-days and the BLM1-treatments showed an average of 700%-days.

Within the different *Pythium*-levels of all experiments no general deviating trend was detected. Neither the different *Pythium*-levels nor the treatment with *Trichoderma* changed the overall trend of the disease severity progress curves.

Table 3.6: AUPDC values of disease severity in %-days of BLM in four different greenhouse experiments: P0– non-inoculated, $P0_T$ – no *Pythium* with *T. harzianum*, P1– low level and P2– high level of *Pythium*, BLM0 – with control of BLM and BLM1 – without control of BLM. P0*, P1*- and P2*-treatments refer to another inoculation method (see 3.3.4.3)

Experiment 1:	**BLM0**	**BLM1**
P0	268 ± 28 a[1]	776 ± 103 a
P1	362 ± 86 a	613 ± 58 a
P2	294 ± 51 a	747 ± 62 a
Statistic for BLM	B	A

Experiment 2:	**BLM0**	**BLM1**
P0*	42 ± 5 a	109 ± 24 a
P1*	34 ± 5 a	108 ± 17 a
P2*	39 ± 5 a	130 ± 16 a
Statistic for BLM	B	A

Experiment 3:	**BLM0**	**BLM1**
$P0_T$	59 ± 6 a	106 ± 17 a
P1	32 ± 7 b	56 ± 13 a
P2	31 ± 7 b	82 ± 15 a
Statistic for BLM	B	A

Experiment 4	**BLM0**	**BLM1**
P0	24 ± 2 a	293 ± 25 a
$P0_T$	30 ± 3 a	215 ± 23 b**
Statistic for BLM	B	A

[1] Data were subjected to a two-way analysis of variance (ANOVA) and means separated by LSD ($p < 0.05$). Means followed by the same letter are not significantly different ($P = 0.05$). The two factors investigated were the influence of *PRR* within column (characterized with small letters) and BLM within rows (marked with capital letters).

3.4.2.2 *Spatial distribution of BLM*

For experiments 1, 2 and 3, the greenhouse was subdivided in two parts. Rows 1 to 3 were treated with fungicide and rows 4 to 6 remained untreated. In experiment 4, rows 1 and 2 were untreated and rows 3 and 4 treated with fungicides.
In experiments 1 and 2, one third of the plants was removed and harvested for quantitative analyses at 28 dap, another third at 56 dap and the final third at 84 dap. In experiment 3, one fourth of the greenhouse was harvested at 28, 56, 84 and finally at 112 dap. The fourth experiment was conducted without early harvests.

3.4.2.2.1 Primary appearance
Plants were weekly monitored and spatial maps were diagrammed. In experiment 1, single diseased plants were already detected at 7 dap. In experiments 2 and 3, the disease started at 14 dap, in experiment 4 at 28 dap. The spatial disease patterns in the experiments were similar, so that only the results of experiment 4 are presented. In Figure 3.7, the disease patterns, given by the binary status of the disease per plant (healthy or diseased), at 28, 35, 42, 49, 56 and 63 dap were depicted. The progress of BLM is reflected by the increasing number of dark points representing diseased plants. Spatial disease maps of experiments 1, 2 and 3 are given in the appendix (Figures 6.2 - 6.4).
The spatial disease patterns within rows at the beginning of the epidemics were analysed with the join-count statistics (Madden et al., 2007). In experiment 1, the standard normal test statistics for joins of diseased plants at the first 3 dates indicated an aggregated pattern of diseased plants, while the joins of healthy and diseased plants still indicated randomness. At 28 dap, the statistics for the latter joins also supported the aggregation of the disease. In experiment 2, all tests showed that the diseased plants were randomly distributed within the rows. The results of the tests calculated for the disease patterns in experiment 3 were similar to those of experiment 1 indicating an aggregation. However, as the disease incidences of plants at the first two dates in experiments 1 and 3 were rather low (2 and 7 % and 2 and 8 %, respectively), the test results based on the normal statistics should be interpreted with care. In experiment 4, like in experiment 2, all tests supported the hypothesis of a random distribution of diseased plants. Thus in two experiments the disease occurred in a random pattern, while in the two others an aggregation of diseased plants was indicated.

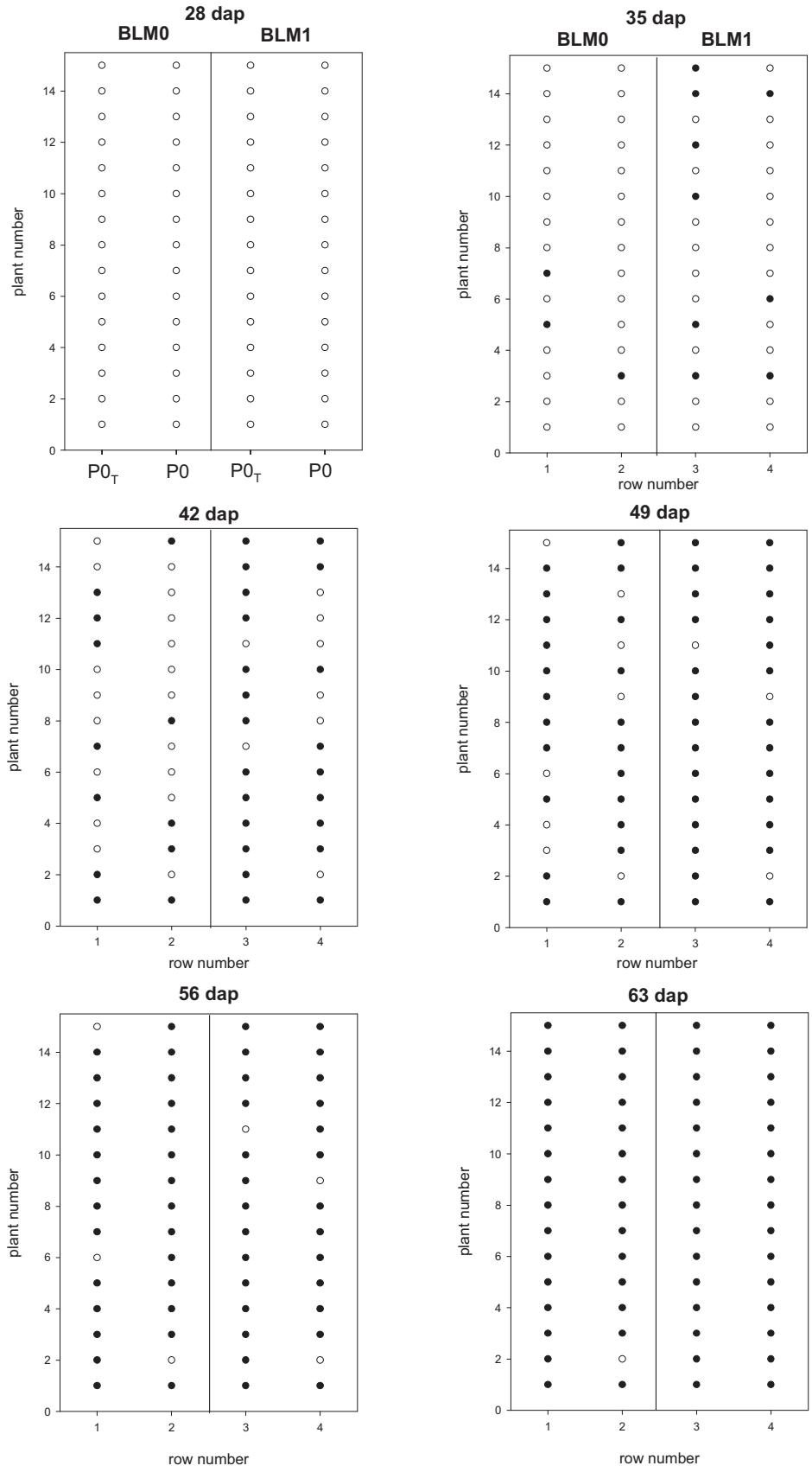
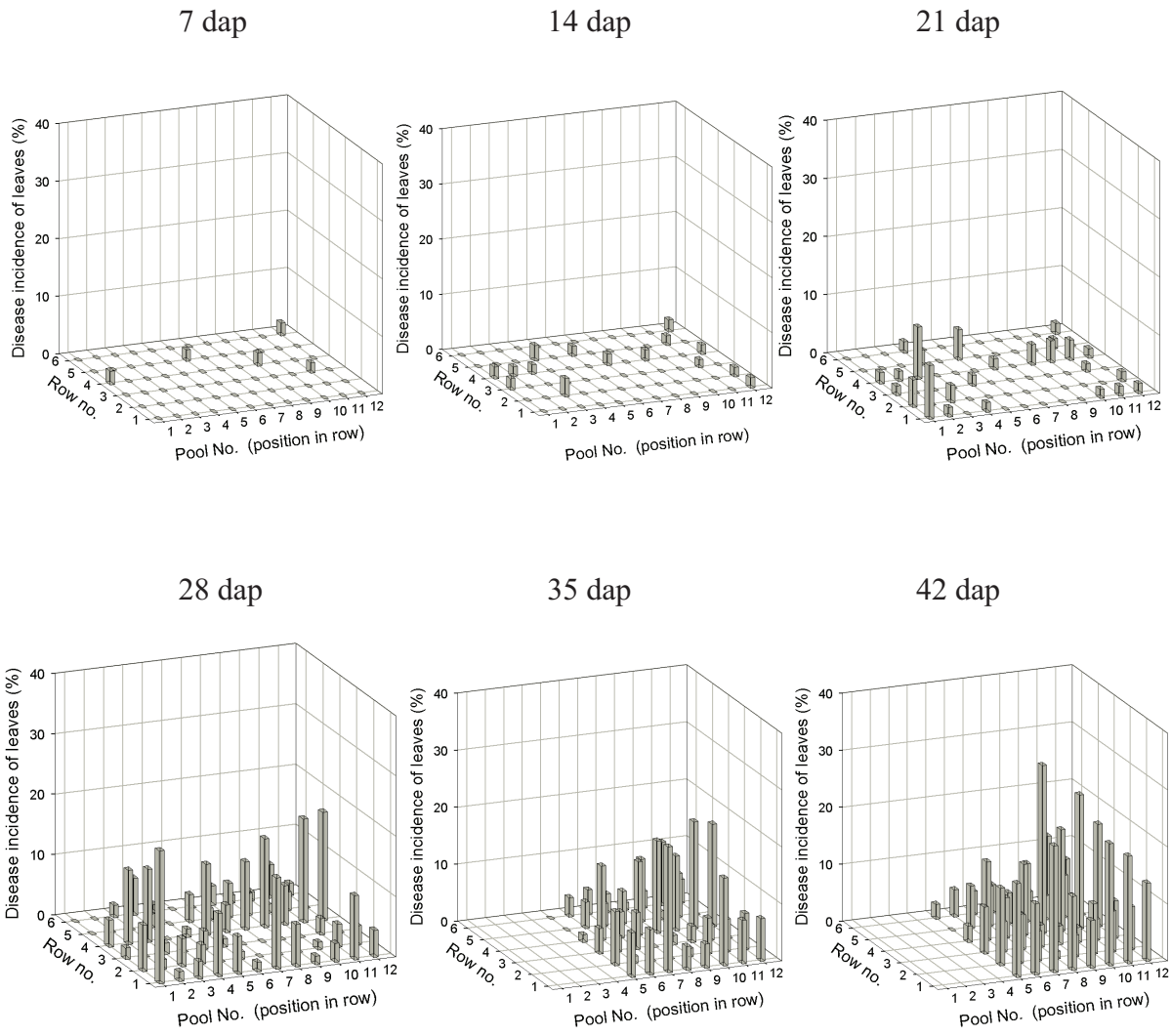


Figure 3.7: Spatial maps of BLM diseased plants in the greenhouse at 6 assessment dates (28, 35, 42, 49, 56 and 63 dap) in experiment 4 conducted from November 2003 till March 2004 ($P0_T$ – inoculated with *T. harzianum,* P0– non-inoculated, BLM0 – with control of BLM and BLM1 – without control of BLM). Black coloured dots refer to diseased and white dots to symptomless plants.

3.4.2.2.2 *Spatial analysis of disease incidence of leaves*

The temporal change of the spatial pattern will be analysed starting with the disease incidence of leaves, calculated for groups of three plants.

The second experiment is an example for a continuous progression of disease incidence of leaves measured in %, (Figure 3.8). Further experimental results are presented in the appendix (Figures 6.5 and 6.6).



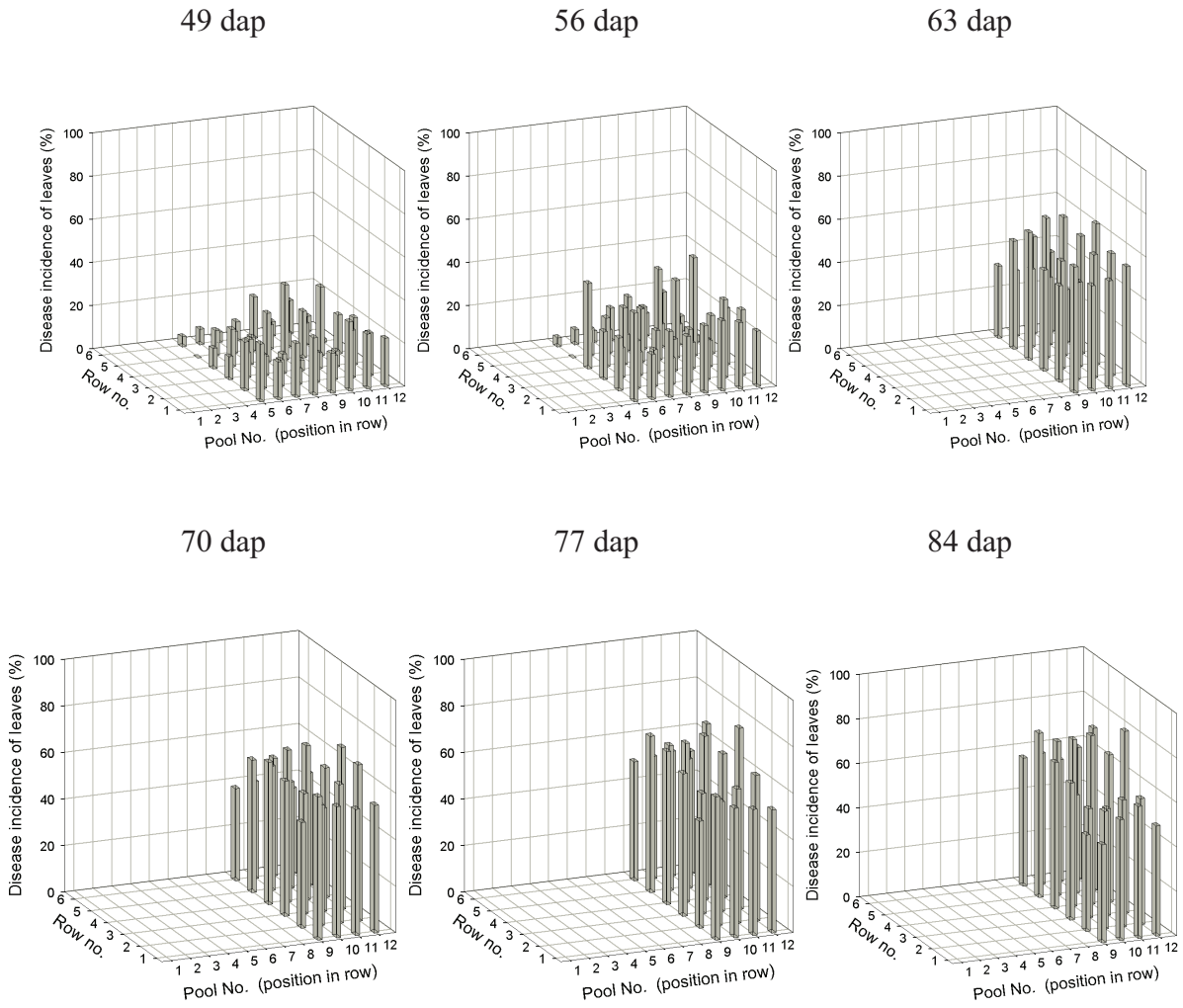


Figure 3.8: BLM incidence of leaves in the greenhouse at 12 assessment dates (7 till 84 dap) in experiment 2. Incidence was determined for pools of three plants. Notice the change of the disease scale at 49 dap (from 40 to 100%).

The epidemic started randomly at 7 dap. In the course of the experiment from 14 up to 28 dap, a potential aggregation of diseased leaves close to the wall of the main entrance door (row no.1, pool no. 1) and the wall of the side-door (row no. 6, pool no. 12) was detectable.

At the end of the experiment, a significant difference between rows 1 to 3 (sprayed) and 4 to 6 (non-sprayed) existed. The disease incidence of leaves in the non-sprayed treatments was 64 ± 2.81%, and in the sprayed treatments 55 ± 2.97%, but no clear spatial trend was detectable.

To compare experiments, the disease situations at 28 dap are displayed in Figure 3.9, for experiments 1, 2 and 3. In experiment 4, the epidemic started later (Figure 3.10).

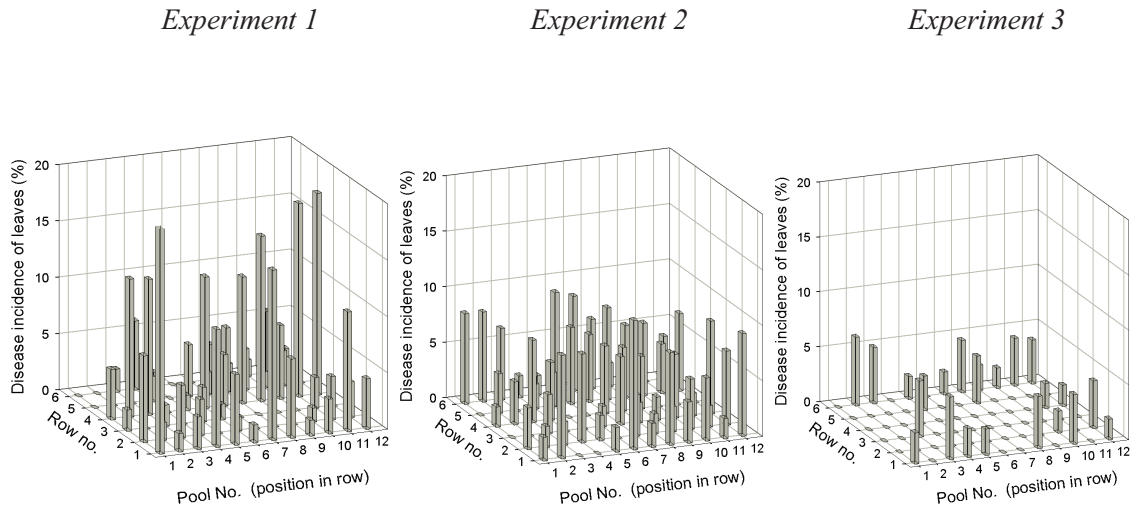


Figure 3.9: BLM incidence of leaves in the greenhouse at 28 dap in experiments 1, 2 and 3. Incidence is calculated as the average of three plants.

The disease levels in the three experiments differed: the first experiment showed a maximum percentage of diseased leaves of 22% (mean value 3.94 ± 0.58%), the second of 11% (mean value 3.79 ± 0.36%) and the third of 6.25% (mean value 1.12 ± 0.21%). The spatial distribution of the disease in experiment 1 was random with a non-significant difference between the sprayed (row 1 to 3) and the non-sprayed (row 4 to 6) treatments. The distribution of experiment 2 was also random. In contrast, the pattern of the third experiment revealed a tendency of a higher number of diseased leaves at the edge rows close to the sidewalls, but no differences with respect to the sprayed and non-sprayed treatments in the greenhouse existed at 28 dap.

In experiment 4 (Figure 3.10) at 42 dap, the edge rows seemed to have more disease than the inner ones. At 56 dap, the distribution was more regular. Within experiment 4, there was a clear difference between the sprayed and non-sprayed part of the greenhouse. Rows 3 and 4, naturally infected without protection, showed a maximum incidence of diseased leaves of nearly 81.63% (mean value 74.44 ± 1.05%), while the sprayed rows no. 1 and 2 reached only 37.67% (mean value 29.99 ± 1.44%).

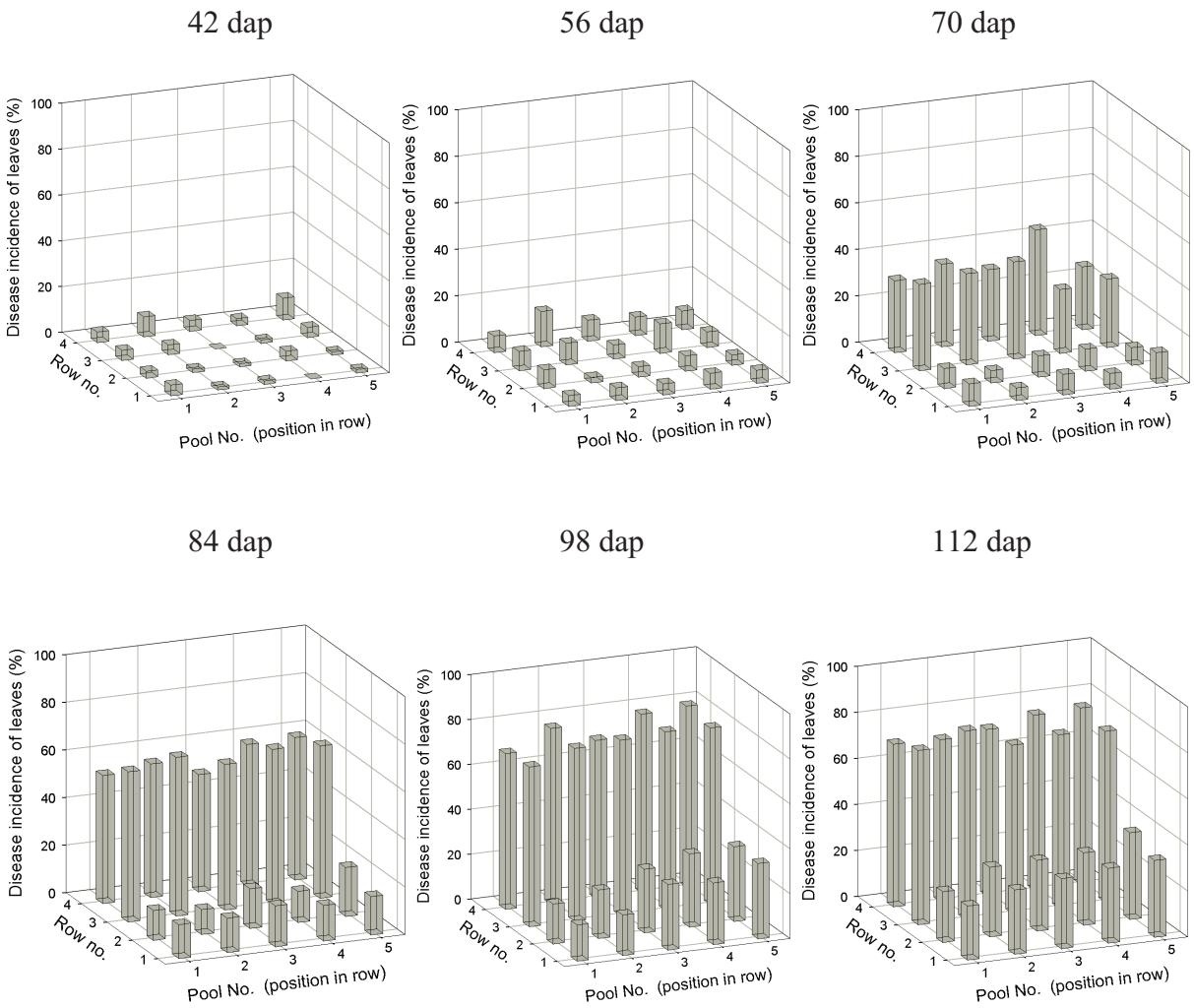


Figure 3.10: BLM incidence of leaves in the greenhouse at 6 assessment dates (42, 56, 70, 84, 98, and 112 dap), in experiment 4 conducted from November 2003 until March 2004. Incidence is calculated as the average of three plants.

3.4.2.2.3 Spatial analysis of disease severity

In Figure 3.11, the disease distribution over time is displayed for experiment 1. At 14 dap, the first disease symptoms appeared with a mean disease severity of less than 0.03 ± 0.01% (maximum value 0.58%). This first appearance of diseased leaves started around pool no. 1 to 5 in rows 5 and 6. At 28 dap, the disease severity increased to a maximum of 3.4% (mean value 0.61 ± 0.09%). Again, a slight gradient to the side-door was observable. At 42 dap, a clear difference between sprayed and non-sprayed treatments was noticeable. At 56 dap, there was a peak with 56.54% (mean value 6.06 ± 1.27%) at row no. 6, pool no. 10.

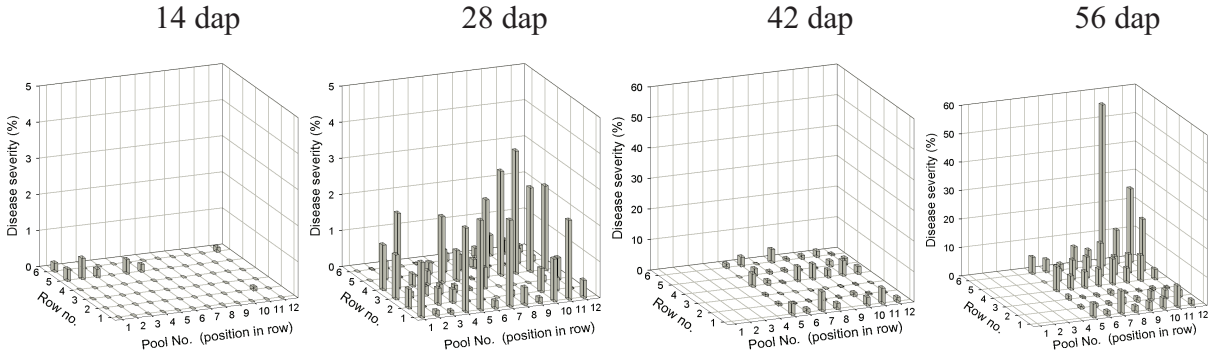


Figure 3.11: BLM severity of plants in the greenhouse at four assessment dates (14, 28, 42 and 56 dap), in experiment 1. Severity is calculated as the average of three plants. Notice the change of the disease scale at 42 dap from 5 to 60%.

For comparison, the disease severities at 28 dap are displayed for experiments 1, 2 and 3 in Figure 3.12. The first experiment had an average disease severity of 0.61 ± 0.09%, the second of 0.35 ± 0.06% and the third of 0.015 ± 0.003%.

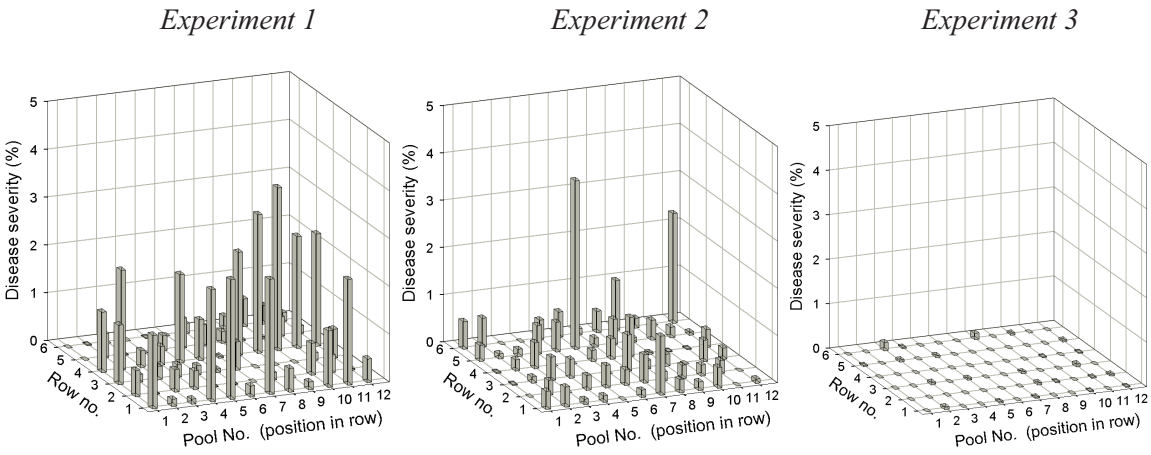


Figure 3.12: BLM severity of plants in the greenhouse at 28 dap, in experiments 1, 2 and 3. Severity is calculated as the average of three plants.

No differences between the sprayed and the non-sprayed parts were found at 28 dap and no disease gradient was observed. The spatial distribution of the first experiment showed a trend of a higher incidence of diseased plants towards the side door (area around row no. 6, pool no. 12). The second experiment had two peaks in the non-sprayed side of the greenhouse, one at row no.5, pool no. 6 and the other at row no. 6, pool no. 12. In the third experiment, BLM severity was extremely low at 28 dap. The spatial distribution of the BLM epidemic of experiment 4 is shown in Figure 3.13. Rows 1 and 2 were sprayed and rows 3 and 4 remained unsprayed. The epidemic started later than in the other experiments, therefore the first graph depicts the spatial distribution at 42 dap. The disease pattern is random.

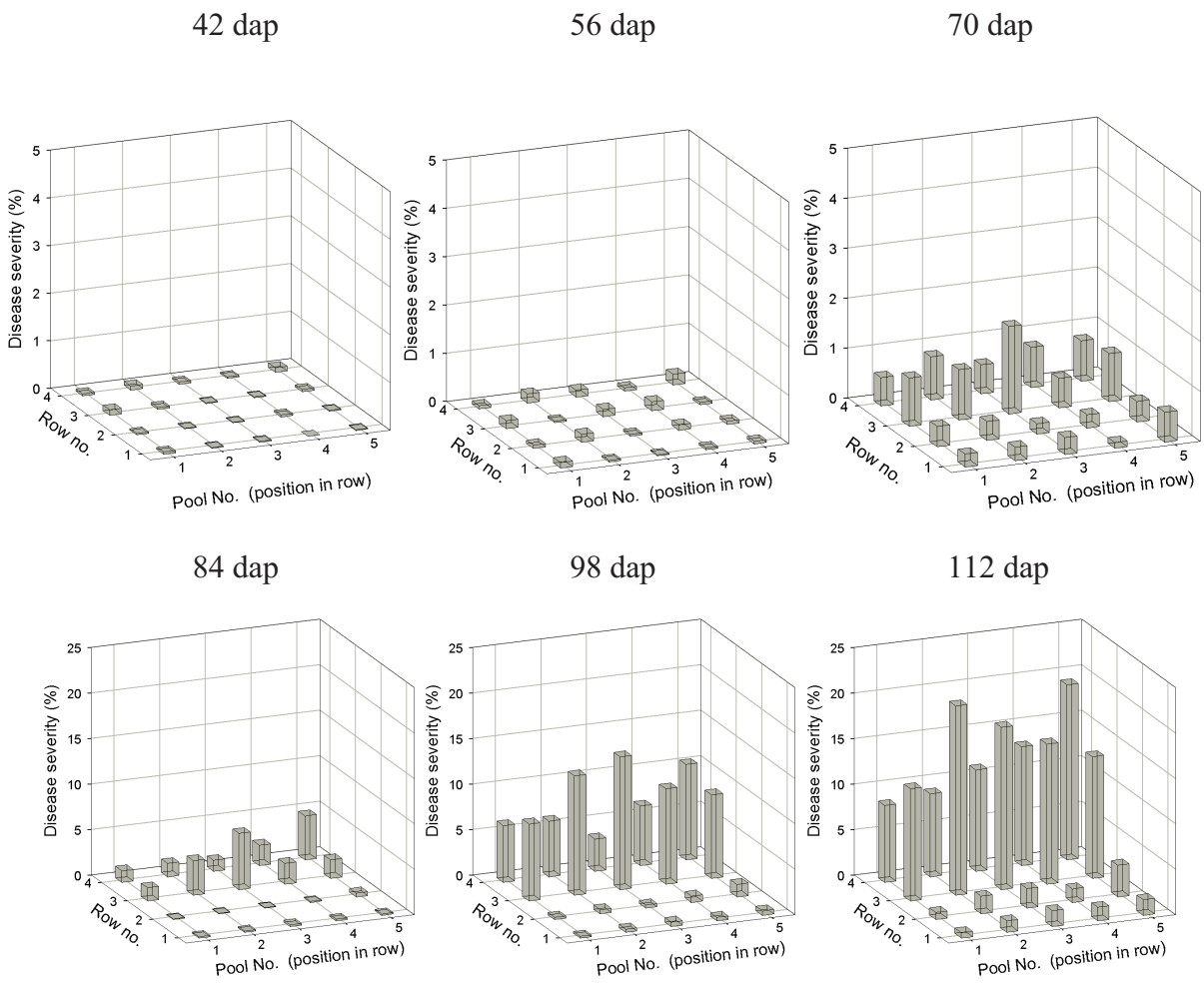


Figure 3.13: BLM severity of plants in the greenhouse at 6 assessment dates (42, 56, 70, 84, 98 and 112 dap), in experiment 4, conducted from November 2003 until March 2004. Incidence is calculated as the average of three plants. Notice the change of the disease scale at 84 dap from 5 to 25%.

A clear difference in disease severity was found between the rows no. 3 and 4 (no fungicide application) and rows 1 and 2 (fungicide application). The fungicide treatment reduced the maximum disease severity from 20.80% (mean value 14.07 ± 1.32%) to 3.47% (mean value 1.67 ± 0.25%).

3.4.2.3 *Vertical distribution of BLM severity*

Whereas the previous investigations of BLM dealt with the horizontal distribution from plant to plant, the following analyses focus on the vertical distribution within the plants as given by the disease severity of leaves.

As representatives of the four experiments, the results of experiments 1 and 4 are shown (Figures 3.14 and 3.15). Experiments 2 and 3 revealed the same tendency as experiment 4 and therefore their vertical disease distributions are displayed in the appendix (Figures 6.7 and 6.8).

In experiment 1 (Figure 3.14), we used a determinate variety with an average maximum of 26 (± 1.11) leaves. In all treatments, the height occurrence of BLM ended in a rapid decline around leaf no. 16. On the top leaves (the last third, no. 18 to 26), no visual symptoms of BLM were detectable. At the symptomatic part of the plants, the disease continuously raised over time with roughly 5% per week (Figure 3.14).

The maximum values of disease severity in the sprayed treatments were:

40.00 ± 4.39% at leaf no. 11 for P0-BLM0;

36.60 ± 6.04% at leaf no. 7 for P1-BLM0;

35.00 ± 6.88% at leaf no. 12 for P2-BLM0.

The maximum values in the non-sprayed treatments were:

90.62 ± 4.57% at leaf no. 13 for P0-BLM1;

85.00 ± 10.00% at leaf no. 15 for P1-BLM1;

75.90 ± 11.13% at leaf no. 15 for P2-BLM1.

An important observation of the experiments was that the disease severity was significantly lower on the older leaves (leaf no. 1 to 5) as compared to the middle leaves (no. 10 to 15). For example, in the treatment P1-BLM1, the disease severity was around 40% for the lower as compared to 60% for the upper leaves. Therefore, all single curves of experiment 1 showed a negative skewed trend, meaning that the upper leaves of the plant had a higher disease severity than the lower ones at the same time.

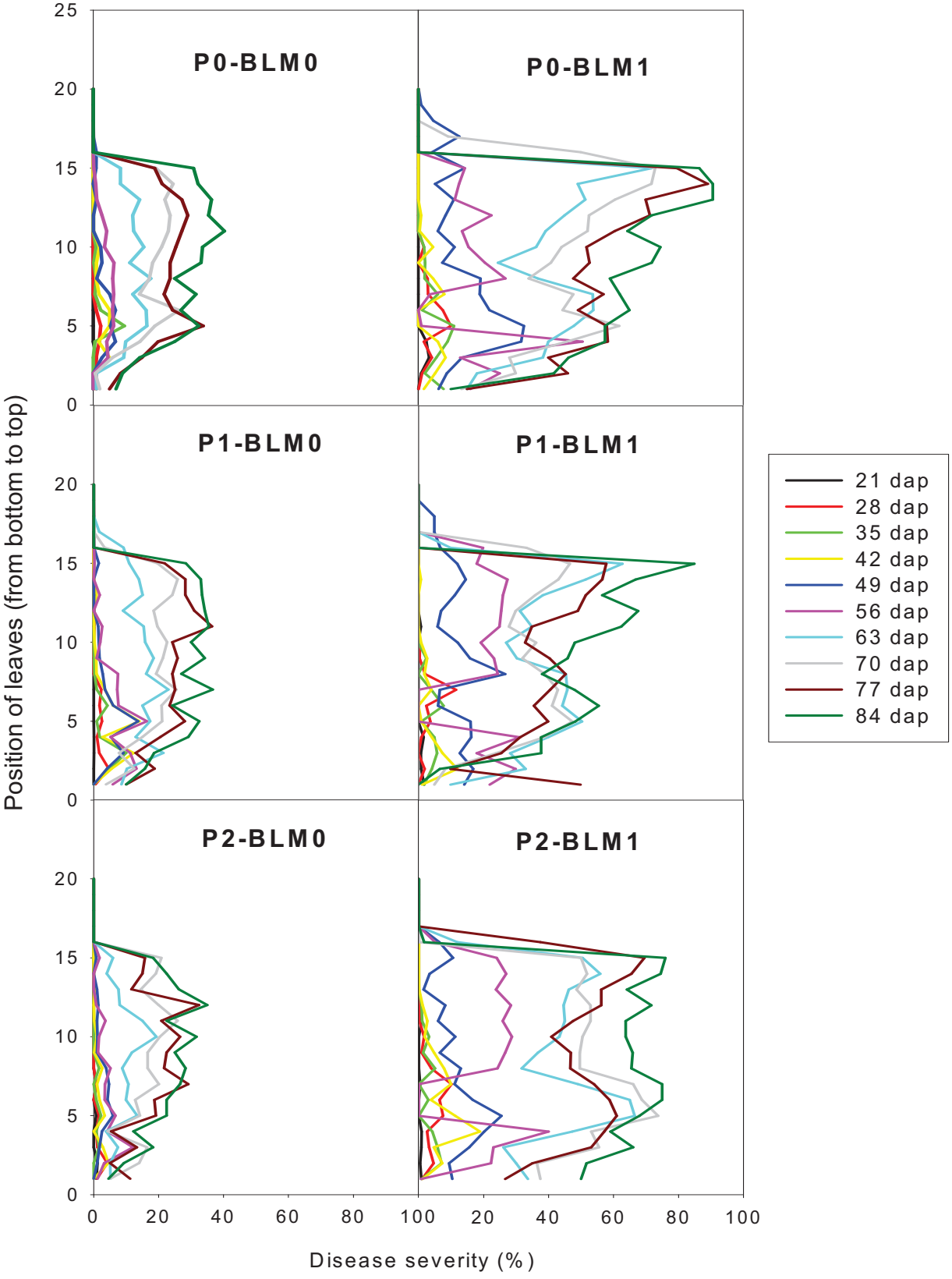


Figure 3.14: Vertical distribution of BLM severity (%) on leaves of tomato plants of the variety 'New King Kong' at different dap in experiment 1. Treatments: BLM0 – with control of BLM, and BLM1 – without control of BLM; P0 - non-inoculated, P1 - low level and P2 - high level of *P. aphanidermatum.*

While the vertical distribution for BLM in experiment 1 was more concentrated on leaves in the middle part of the plants, the distribution in experiment 4 showed a positive skewness (Figure 3.15). There was a higher disease level on the base than on the top of the plants, but because of the necessary pruning work of the indeterminate variety, the lower leaves were removed right before 84 dap. At 77 dap, the highest percentage was 11.4 ± 3.55% at leaf no. 5 for the P0-BLM1-treatment. For the $P0_T$-BLM1 the highest severity was by 5.55 ± 2.27% at leaf no. 4.

The vertical distribution was limited in height because young leaves on the top of the plants remained free of disease symptoms. Roughly one third of the leaves stayed disease free. On average, leaf no. 43 was the highest leaf insertion showing symptoms at 112 dap (Table 3.7). The total number of leaves per plant was 50.55 (± 0.7) in experiment 4. The maximum values of BLM severity assessed per leaf were less than 10% in the BLM0-treatments and less than 45% in the BLM1-treatments.

Taking a closer look on the leaf positions at a specific percentage of disease severity, for instance 5%, the vertical spread of the disease can be characterised. For example in experiment 4, the 5% mark was reached in the P0-BLM1-treatment at 70 dap at leaf no. 5. One week later, at 77 dap, the 5% level was at leaf no. 9. Another week later, at 84 dap, it climbed up to leaf no. 20. At 91 dap, 5% reached leaf no. 26; and again 7 days later (at 98 dap), leaf no. 30 showed 5% disease severity. Finally, at 112 dap, the 5% level reached leaf no. 34. For the $P0_T$-BLM1-treatment we got the following results: the 5% mark was observed for the first time at 77 dap at leaf no. 7. One week later, at 84 dap, the mark jumped to leaf no. 17. At 91 dap, the mark reached leaf no. 23, at 98 dap leaf no. 27. In the next week, at 105 dap, the mark arrived at leaf no. 31 and finally, at 112 dap, at leaf no. 35.

As mentioned before, the vertical trends in the BLM1-treatments of experiments 2 and 3 were similar to experiment 4. In experiment 2, the average total number of leaves was 42 (± 0.32) with a maximum disease severity of 24.5 (± 11.72%) at leaf no. 11 in the P2–BLM1-treatment. In experiment 3, the average total no. of leaves was 47 (± 0.6) with a disease severity maximum of 26.66 (± 4.4%) at leaf no. 11 in the P2–BLM1-treatment. Further details are given in the appendix (Tables 6.1 and 6.2).

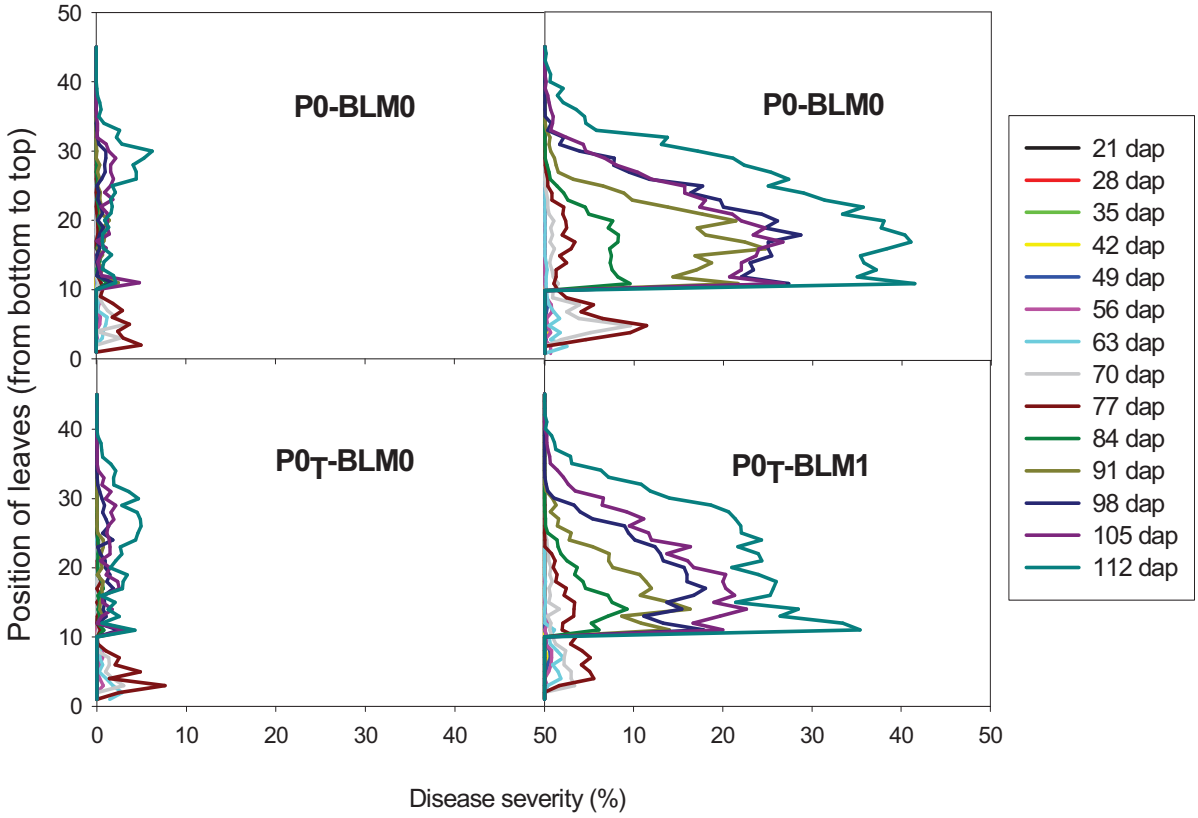


Figure 3.15: Vertical distribution of BLM severity (%) on leaves of tomato plants of the tomato variety 'King Kong 2' at different dap in experiment 4. Treatments: BLM0 – with control of BLM, BLM1 – without control of BLM, and different *Trichoderma* treatments (P0 - non-inoculated, $P0_T$ - inoculated with *T. harzianum*). Because of cultural practice, lower leaves (up to leaf no. 10) were removed after 77 dap.

Table 3.7: Characteristics of leaves in the four treatments of experiment 4

Treatment	Total no. of leaves	Highest leaf insertion with symptoms (position bottom to top)	max disease severity per leaf (%, at 112 dap)	Leaf position of max. severity
P0–BLM0	51.20 (± 0.48)	40	6.26 (± 1.40)	30
$P0_T$–BLM0	48.33 (± 2.37)	43	4.93 (± 1.74)	26
P0–BLM1	51.46 (± 0.88)	44	41.42 (± 4.64)	11
$P0_T$–BLM1	51.20 (± 1.11)	45	35.41 (± 4.82)	11

3.5 Discussion

3.5.1 Inoculum source of BLM

Black leaf mold of tomato, first reported in Asia more than 50 years ago, has become a serious threat for tomato production in recent years (Wang et al., 1994; Halfeld-Vieira et al., 2006), not only under field conditions but also in greenhouses.

In the newly established greenhouses at the AIT campus (in 2001), we observed a natural appearance of BLM in the experiments, starting in 2002. The spatial and temporal distributions of diseased plants were studied.

Our expectation was that spatial pattern analyses should give hints about possible ways used by the pathogen to enter the greenhouse, for instance expressed in a higher disease concentration close to the door or at the sidewalls. Join-count analyses, a more general method than the ordinary runs test (Madden et al., 2007), was applied to determine the pattern of diseased plants within the rows. However, under the conditions in the greenhouses of our study, the patterns for primary appearance of symptomatic plants differed among the experiments. In experiments 1 and 3, the spatial distribution of diseased plants within rows was aggregated, while in experiments 2 and 4 a random pattern was observed. In experiment 2, initially many experimental plants were removed and replaced because of infection by *P. aphanidermatum* due to the contaminated substrate. These replacements may have interfered with the natural primary occurrence of BLM. On the other hand, no intervention took place in experiment 4, but the analysis also showed a random disease occurrence. Concerning the disease distribution across rows, the analyses of all experiments did not show a gradient from the side rows towards the middle rows of the greenhouse.

Regarding the potential contamination of the substrate with *P. aphanidermatum*, it was observed that in experiment 2, a high amount of control plants (over 50% in P0*) died shortly after transplanting. The *P. aphanidermatum* inoculated treatments P1* and P2* were less affected. Compared to the other experiments, which were conducted under the same hygienic conditions, this was an exception. By investigating the possible way of contamination, we found out that a charge of the planting substrate was not sterilised in a proper way (Achilles, pers. com.). To avoid the risk with contaminated substrate, *Trichoderma harzianum* was mixed under the substrate in the following experiments 3 and 4. Consequently, only 2 control plants dropped out due to *P. aphanidermatum* in experiment 3.

After the spatial analysis, the source of the primary inoculum of *P. fuligena* was still not clear. Seeds as possible means for BLM transmission have not been mentioned in literature (AVRDC 2004; Hartman et al., 1991; Hartman and Wang, 1993). As possible sources of the

primary infection, spores drifted in the wind were feasible. From literature it is known that *P. fuligena* spores are disseminated over long distances by wind and over shorter distances by wind-driven rain or water (Hsieh and Goh, 1990; Wang et al., 1995; AVRDC, 2004). The research greenhouse had sidewalls made out of net (Econet M, pore size 0.18 mm, 40 x 37 mesh (40-mesh), Ludvig Swensson, Netherlands). This mesh size was big enough to allow conidia of size 15-60 x 3-5 µm (Hsieh and Goh, 1990) entering from outside to inside. The spatial pattern analyses of disease incidence of leaves or disease severity gave a hint that a possible entrance for the pathogen was the backside of the greenhouse, to which the fans of another greenhouse were directed. Another option for entrance might be the second entrance door at the back of the greenhouse. Compared to the main entrance door, which was a double sliding gate with a shoe disinfection device, the second door, at the opposite site of the greenhouse geared to the other greenhouses of the research site, was a single door but also equipped with a disinfection device. Therefore, it might be possible that the pathogen could penetrate easier at the second door, but as mentioned before, the spatial patterns in the experiments didn't show a gradient from the side rows towards the middle rows or from the back side to the front side and from the second door towards the middle.

As it is well known that wind transmission of BLM even over long distances is possible, the missing point in literature is a clear definition of long and short distances. If 1 km is already a long distance, then the Thalad Thai market could be a possible source for inoculum. This largest fruits and vegetables wholesale market in Thailand was one kilometre away from the greenhouse. At this place, fruits and vegetables were traded indoors as well as outdoors, so wind might be a possible carrier. A closer source might be a compost heap at the AIT campus around 200 m away from the research facilities. Plant debris was stored there from an experimental farm. Even if no visible symptoms of BLM were found, it is known that BLM is able to survive for a long time on debris (Wang et al., 1996).

Another explanation for the random distribution of primary appearance could be the introduction by people entering the greenhouse, e.g. workers, research assistants or visitors. Hsieh & Goh (1990), Wang et al. (1995) and AVRDC (2004) already confirmed this possibility. People visiting the market or their home gardens and afterwards joining the AIT, especially the greenhouse area, were possible carriers for BLM. Fruits and vegetables, which were purchased at the market, were possible carriers as well. Additionally, a short thought should be given to insects as possible carriers. It is known from literature that thrips are involved in the spread of fungal diseases (Fermaud et al., 1994; Ávila-Quezada et al., 2002; Dodd et al., 2004): Even if nothing like this has been described for BLM, it may be a possibility.

Another possible source of inoculum could be alternative host plants. Wang et al. (1995) identified the following alternative hosts: Black nightshade (*Solanum nigrum*), different species of eggplants (*Solanum indicum, S. melongena, S. macrocarpon*), different pepper species (*Capsicum annuum, C. chinense, C. baccatum, C. frutescens*) and different tomato species (for example: *L. chilense, L. hirsutum, L. pannellii, L. pimpinellifolium*). However, none of these plants occurred in the close (100 m) neighbourhood of the greenhouses.

3.5.2 Disease progression

In experiment 1, the determinate variety ´New King Kong´ was used, in experiments 2 to 4 the indeterminate variety ´King Kong 2´. Experiment 1, with a disease severity of nearly 30%, showed a higher level of BLM than the three following experiments. It is not yet figured out what kind of predisposition is necessary for a higher susceptibility of BLM. Wang et al. (1995) screened different accessions trying to find possible resistant cultivars. In their experiments, *L. hirsutum* was the most resistant one. A possible alternative to the current BLM management practice of intensive chemical treatment might be rearing resistant lines of breeding into commercial varieties (Wang et al., 1994).

The disease progress curves were typically S-shaped in all experiments. The only exception was experiment 2, in which we observed a plateau for a period of 23 days in the BLM0-treatment. In the BLM1-treatment, the plateau was also visible but not as much pronounced as in the BLM0-treatment. Investigating this delay it turned out that at the beginning of experiment 2, the greenhouse partitions were mixed up, so that the wrong side was treated with fungicide once a week over a period for 4 weeks.

The disease symptoms of BLM were observable latest 14 dap in our greenhouse experiments and increased steeply. Within 4 weeks, nearly 100% of the plants showed symptoms. In experiments 1 and 2, the first symptoms appeared 7 dap. In fact, this was quite too early for symptoms caused by naturally infection in the greenhouse. The incubation period observed in pre-experiments was longer and it was confirmed by literature that symptoms do not develop before 12 to 18 days after inoculation. Hartman and Wang (1992) mentioned 10 to 14 days after inoculation. Prior to them, Magda and Quebral (1970) also reported these results with experiments in moist chambers. The earliest time to observe visible lesions was six days after inoculation (Wang et al., 1996). Another exception was the 30°C pre-experiment, where it took 7 days to observe symptoms. The reason for the early appearance of BLM in the greenhouse after transplanting might be a hygienic problem in the nursery, where the seedlings already got pre-infected. The disease progressed slowly on young inoculated (or naturally infested) plants but increased rapidly as plants aged.

The technique of analysing plant diseases first by evaluation of simple disease progress curves is the first step for studying plant disease progression (Pennypacker et al., 1980). Our primary investigations led us to the following outcome: Even though a clear source for BLM was not detectable, the BLM incidence of plants increased very fast. After two months (> 56 dap) all plants inside the greenhouse were diseased. In contrast to disease incidence of plants, incidence of leaves never reached 100%. The vertical distribution showed that nearly one third of the leaves (from the top) stayed symptom free. This might be because new leaves continuously developed which stayed symptomless until they got old. In experiment 1, the disease incidence of leaves reached nearly 75%, in experiments 2 to 4 approximately 60%. By observation of the leaf position of the vertical distribution, it turned out, that single leaves were highly diseased. In experiment 4 in the BLM1-treatment, leaves reached approximately 40% disease severity, in experiment 2 almost 90%. The disease severities of single leaves were high, but the progress curves, given as disease severity of plants, stayed low. Disease severities were highest in experiment 1 with 30% in the BLM1-treatment and lowest in experiment 3. Hartman and Wang (1992) reported that diseased leaf area ranged from 10 up to 60% in natural field conditions.

In their first report, Hartman et al. (1991) observed that without fungicide control 54 to 86% of leaf area was diseased on several varieties and advanced breeding lines in replicated yield trials at AVRDC. Compared to our results with disease severities below 20% or even less than 10% of the variety ´King Kong´, the results of Hartman and Wang seem to be very high. This might be because Hartman and Wang used mycelium parts for inoculation (Hartman and Wang, 1993; Wang et al. 1995), whereas we used naturally infestation. Unfortunately, little information is available on the range of virulence among different isolates of *P. fuligena*. It is not known whether different races of the pathogen or pathotypes exist.

3.5.3 Laboratory experiments

The inoculum material needed was taken from tomato plants inside the greenhouses of the AIT. For lab experiments, we used naturally diseased leaves. Conidia can survive up to 6 months on infected tomato leaves stored in dry condition (Yamada, 1951). Studies showed that conidia can survive from one crop to the next without an intermediate host, and that crop debris can serve as an important source of primary inoculum (Wang et al., 1996). Unlike many other fungal spores, conidia of *P. fuligena* do not need free water for germination. *P. fuligena* conidia can germinate at 91% RH, and at 96.5 to 100% RH they germinate as well or even better than in free water (Hartman et al., 1991), but do not survive for 40 days on leaves maintained in moist conditions (Yamada, 1951). It might be free moisture that actually limits

black leaf mold. In earlier studies (Hartman and Wang, 1992), conidia survived and germinated well below 100% RH.

Our laboratory- and pre-experiments showed a similar trend compared to Hartman and Wang (1992) who indicated a high susceptibility of different tomato lines, an incubation time of approximately 14 days, a required high relative humidity, a temperature of approximately 30°C and the possibility of disease occurrence in the greenhouse. Wang et al. (1996) described that in in-vitro studies with the pathogen, 26 - 28°C was the optimum temperature for growth. No growth occurred at 34°C or above (Hartman et al., 1991). The optimum temperature for conidial germination was at 26°C and the maximum was 36°C (Yamada, 1951). In our laboratory experiments, we had chosen 25, 30 and 35°C, but at the highest temperature, no symptoms were expressed.

3.5.4 Disease management

Two different ways are possible to control BLM, either to choose a resistant variety or to apply fungicides. Hartman and Wang (1993) focussed their work on finding resistant cultivars to be used as commercial varieties, and noted that it may be feasible to incorporate resistance into commercial varieties. This way might be especially important to growers in developing countries, who cannot afford the cost of fungicide applications. Until now, the way of chemical treatment totally relies on protective fungicide applications (Hartman and Wang, 1993; Wanwilei, pers. com.). In our studies, we tried to keep one side of the greenhouse disease free by spraying. Although all greenhouses at the research site were treated with Maneb, the pressure of infection was high.

However, the disease incidence of treated and non-treated plants was very similar. For disease incidence of leaves, a significant difference between BLM0 as sprayed and BLM1 as non-sprayed treatment was detectable at the end of the experiments, in general between 10 and 20%. The difference between the treatments in disease severity was also significantly reduced.

Diseased severity, displayed in AUDPC values, in the sprayed compared to the non-sprayed treatments was recognizable, for example in experiment 1 in which the BLM0-treatments had an average of 300%-days and the BLM1-treatments showed an average of 700%-days.

Mersha (2008) found in his work an average disease severity range from 4 to 41% at the end of his experiments, after 106 days, with the variety FMTT260. In August to September, he found a disease severity up to 81%. However, plantings from November to January did not lead to severe epidemics.

3.5.5 Environment

Wang et al. (1996) mentioned a severe disease appearance during the hot, rainy season on tomato plants grown under rain shelter at AVRDC in Taiwan. They showed that BLM was most prevalent during the fall-winter dry season (September to March) in southern Taiwan when prolonged periods of high relative humidity were common that result in dew formation at night.

In our studies, we explored that the disease is prevalent throughout the year, not depending on rainfall or seasonal temperature; Hartman et al. (1991) and Mersha (2008) did the same observations. Periods of prolonged leaf wetness play a key role in BLM development. Long periods of high RH have been shown to be instrumental to the rapid disease build-up of two related diseases – *Cercospora* leaf spot of peanuts and *Cercospora* blight of celery (Hsieh and Goh, 1990; Berger, 1977; Wang et al., 1996). Except from the studies of Mersha (2008), no comparable research on *Pseudocercospora* spp. in the tropics has been carried out under similar environmental conditions. One exception is *Pseudocercospora musae,* which is a serious disease, able to cause 100% yield loss on susceptible banana varieties (Cordeiro and Matos, 2003).

4 Investigations of the joint effects of black leaf mold and Pythium root rot on tomato growth and yield parameters

4.1 Abstract

The complex of two tomato (*Solanum lycopersicon* L.) diseases composed of the aerial disease black leaf mold (BLM), caused by *Pseudocercospora fuligena,* and the soil-borne disease Pythium root rot (PRR), caused by *Pythium aphanidermatum*, was investigated under greenhouse conditions in Thailand in a closed net greenhouse (Econet M, pore size 0.18 mm, 40 x 37 mesh (40-mesh), Ludvig Swensson, Netherlands) with the base area of 10 x 20 m. Four experiments were conducted in different seasons and with two different tomato varieties (´New King Kong´ and ´King Kong 2´). In addition, *Trichoderma harzianum* was used as a biological antagonist in two experiments. *P. aphanidermatum* was inoculated in 2 different densities and the primary occurrence of PRR was monitored up to 14 days after positioning plants in the greenhouse. To keep the disease level in one part of the greenhouse low, Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}) was applied weekly.

Tests of substrate samples with the potato-baiting-method confirmed successful inoculations of in all experiments. No other pathogen than *P. aphanidermatum* was detected. Due to PRR, 30% to up to 64% of plants dropped out and were substituted in order to allow the progression of BLM that naturally occurred inside the greenhouse throughout the year without great differences among seasons. Neither the different *Pythium*-levels nor the treatment with *Trichoderma* changed the overall trend of the disease severity progress curves.

The results of tomato growth and yield parameters are in general very heterogeneous although there was an overall tendency of a negative interaction between the two diseases. In 3 of 4 plant growth parameters analysed, the joint losses due to the two diseases were smaller than the sum of losses of the individual diseases. For example in the first experiment, plants in treatment P0-BLM0 (i.e. without inoculation of *P. aphanidermatum*, but sprayed against BLM) had a fresh weight of 1030 ± 70 g, PRR in the P2-BLM0-treatment (i.e. inoculated with high level of *P. aphanidermatum*, but fungicide sprayed against BLM) reduced the weight by roughly 340 g compared to the control plants. The plants of the P0-BLM1-treatment (i.e. without inoculation of *P. aphanidermatum*, and without fungicide use) in which only BLM developed symptoms, had a value of 660 ± 73 g, thus a difference of 370 g compared to the control plants. Thus the plants of the P2-BLM1 treatment should have a value of approximately 320 g, but the measured value was 545 ± 73 g.

4.2 Introduction

The occurrence of two or more pathogens in simultaneous action on the same host is frequent, especially on tropical crops (Waller and Bridge, 1984; Savary and Zadoks, 1991). Most articles in literature, however, were focused on "one to one" host – pathogen interaction, but pathogen – pathogen interactions or multiple attacks were rarely investigated. Examples of disease complex studies include those of Powell (1971a), Latch and Potter (1977), Pieczarka and Zitter (1981), Johnson et al. (1987), Madden et al. (1987), Kranz and Jörg (1989), Weber et al. (1994), and Ngugi et al. (2001). In general, the interaction of pathogens complicates the control of diseases and the partitioning of the primary causes of losses. Usually the effects of a disease complex on yield are estimated by assuming that each disease acts independently. However, the simultaneous occurrence of diseases can lead to combined effects on crop yield and on the population dynamics of the pathogens. Depending on the simultaneous damage that pathogens cause to the host, the interaction between pathogens can be classified. If the damage caused by two concurrently infecting pathogens is similar to the sum of damages caused by the pathogens attacking the host separately, the effect is additive; if the damage is less, there is a negative interaction; if it is greater, a positive interaction (Waller and Bridge, 1984; Bassanezi et al., 1998).

The interaction may be a synergistic interaction in terms of combined effects of the pathogens, or an antagonistic interaction in terms of competitive exclusion (Zacheo, 1993). Synergistic interaction is important because the economic damage threshold for each disease can be significantly lowered by the presence of the interacting disease. Conversely, antagonistic interaction can increase the economic damage threshold of a disease in the presence of another (Johnson, 1990). Further on, the definitions of Odum (1953) can be helpful to interpret interaction concerning dynamics of pathogens. He suggested the following classifications for associations between organisms: neutralism, competition, mutualism, protocooperation, commensalism, amensalism, parasitism and predation. Powell (1971b) considered three theoretical mechanisms of bio-predisposition involving interacting pathogens: (1) the primary pathogen may make the host more susceptible to the secondary pathogen; (2) the primary pathogen may enhance the activity of the secondary pathogen; and (3) the secondary pathogen may even enhance the activity of the primary pathogen. Infection rates, maximum disease levels, and the shape of the progress curves may be changed by interacting diseases (Hau, 2001).

All these theories are important for both, the epidemiological perspective and for the standpoint of designing appropriate control strategies. Based on this, practicable, sustainable,

integrated disease and crop management strategies could be developed. Several scientists (Kranz, 2003; Strange, 2003; Cooke, 2006; Madden et al., 2007) pointed out that the measurement of plant disease and its effects on crop yield, quality and value are crucial for control priorities. Interactions between diseases caused by aerial and soil-borne pathogens may have significant implications for assessing crop losses and selecting appropriate control strategies (Paula Junior, 2002).

The subject of the following investigations was the complex of two tomato (*Solanum lycopersicon* L.) diseases composed of the aerial disease black leaf mold (BLM), caused by *Pseudocercospora fuligena,* and the soil-borne disease Pythium root rot (PRR), caused by *Pythium aphanidermatum.*

Tomato is after potato the most widely grown solanaceous vegetable (Rubatzky and Yamaguchi, 1997) and one of the most important crops in Thailand. For processed tomato, the major growing area is the north and northeast of Thailand. For table tomato, the planting area is distributed in various parts of the country (Intanoo, pers. com.; Pongam, pers. com.).

In 2001, first investigations on a sustainable tomato production under greenhouse conditions were done at the Asian Institute of Technology (AIT), Bangkok, Thailand. Several pests of tomatoes on fields outside the Bangkok area were observed, for example bacterial wilt, virus diseases, leaf mold. For soil-borne pathogens, wilts caused by bacterial wilt, Southern blight, Fusarium wilt and damping off including *Rhizoctonia* and *Pythium ssp.* were most significant. In the fields, foliar diseases such as early blight, late blight and powdery mildew were limiting factors of tomato production in Thailand (Pongam, pers. com.). As main leaf disease inside the greenhouse, black leaf mold (BLM), also formerly known as *Cercospora* leaf mold, was detected. BLM is caused by *Pseudocercospora fuligena* (Roldan) Deighton (= *Cercospora fuligena* (Roldan)) that belongs to the family of *Mycosphaerella* (Crous and Braun, 2003). The fungus is widespread in warmer regions or greenhouses around the world, especially in tropical and subtropical Asia (Hsieh and Goh, 1990; Crous and Braun, 2003). It was first reported on tomato in 1938 in the Philippines (Roldan, 1938), in 1951 in Japan, in 1955 in India, in 1974 in southern USA, in 1990 in Taiwan, in 1995 in Malaysia (Wang et al., 1995) and recently in Brazil (Halfeld-Vieira et al., 2006). In Thailand, it was first detected in 1979 in the Nongkham District, Amphoe Pasrijarern, Bangkok (Saranark and Chandrasrikul, 1980).

Pythium aphanidermatum was identified as the most important soil-borne pathogen causing damage in open fields in Thailand. *P. aphanidermatum* belongs to the species most frequently

associated with damping off. On matured plants, the pathogen is mainly affecting the roots, therefore the disease is named Pythium root rot (Moorman, 2001). As this species is a typical plant pathogen of warm regions (Van der Plaats-Niterink, 1981; Al-Sa'di et al., 2007), its occurrence in temperate climate is confined to greenhouses (Rafin and Tirilly, 1995). For long time survival, *P. aphanidermatum* forms thick walled oospores that remain slumbering in the soil until germination is triggered by external stimuli like moisture or root exudates (Hoppe, 1966; Kraft and Erwin, 1967). For short-term survival, asexually formed sporangia germinate, either directly or indirectly by formation of zoospores. The zoospores, which are initially wall-less and mobile in water are responsible for dispersion in moist environments (Jones et al., 1991). Grosch et al. (1999) reported yield reduction between 18 and 35% following heavy inoculation with *P. aphanidermatum*.

Possible control options for *P. aphanidermatum* are chemical treatment or genotypic plant resistance (Higginbotham et al., 2004). Widely used practices are soil sterilization by chemicals and fumigation (MacNab and Sherf, 1986). More environmentally friendly methods are treatments with antagonistic fungi and bacteria (Chen et al., 1998; Punja and Yip, 2003). One antagonistic fungus is *Trichoderma harzianum* that was explored as biological control agent in some of our experiments. *T. harzianum* has multiple mechanisms of actions, including mycoparasitism via production of chitinases, ß-1-3 glucanases and ß-1-4 glucanases (Lorito et al., 1996), antibiotics (Sivasithanparam and Ghisalberti, 1998), competition (Elad et al., 1999), solubilization of inorganic plant nutritions (Altomare et al., 1999), induced resistance (Bailey and Lumsden, 1998) and inactivation of the pathogen's enzymes involved in the infection process (Elad et al., 1999; Elad and Kapat, 1999). In Germany, it is listed as "plant strengthener" (www.bba.de, 2008). The bio-control agent can be directly applied to the substrate (Chamswarng and Intanoo, 2002). The control provided is equal to that by fungicides (Harman, 2000), with which it is mostly compatible, but it must be applied as a preventative before the disease occurs.

The purpose of this study was to determine possible effects of interaction between the aerial disease BLM and the soil-borne disease PRR. In the experiments, the plants were raised disease free in the nursery. After transplanting at an age of 4-6 weeks (depending on experiment), the plants were exposed to *P. aphanidermatum* in the infected substrate and to *P. fuligena* naturally occurring in the greenhouse. In our investigation, we studied plant growth parameters, yield and dynamics of both diseases occurring simultaneously under greenhouse conditions in Thailand.

4.3 Materials and Methods

The following experiments were carried out in laboratories and greenhouses of the Asian Institute of Technology (AIT) in Bangkok, Thailand during 2001 - 2004. They were part of a larger study, aiming to establish a sustainable and environmentally friendly vegetable production system under protected cultivation in the humid tropics.

4.3.1 Collection and selection of isolates of *Pseudocercospora fuligena*

Tomato plants in the greenhouses were naturally infected with *P. fuligena*. For maintenance, identification and storing as reference, isolates of *P. fuligena* were collected.

Naturally infected tomato plants with *P. fuligena* from the greenhouses on the campus of the AIT were used as source of inoculum. To obtain isolates from active BLM lesions, leaves were collected that had clearly delineated lesions. For each sampled greenhouse at least 10 leaves were randomly taken. In the laboratory, sections of 5 mm^2 were removed from the leading edge of lesions, washed in pure sodiumhyperchloride for 10 s, air dried for 4 s and then plated onto Petri dishes (100 x 15 mm) containing potato dextrose agar (PDA; Merck). The Petri dishes were incubated at 25°C under cool white fluorescent lights for 12 h and 12 h darkness. Putative *P. fuligena* colonies were randomly selected from these Petri dishes and sub-cultured onto dishes containing tomato oatmeal agar (TOA) until pure cultures were obtained. TOA was made by boiling 50 g of shredded tomato leaves and 15 g of oatmeal separately and then mixed. The tomato leaves suspensions were sieved through two layers of cheesecloth, mixed before adding 25 g of agar per litre of water, and autoclaved at 121°C for 15 min (Hartman and Wang, 1992). The pathogen was verified as *P. fuligena* by the Centraalbureau voor Schimmelcultures (reference det 321-2003), Utrecht, Netherlands and by Prof. Dr. Uwe Braun, Martin-Luther-University, Halle-Wittenberg, Germany. The most vigorous isolates were chosen for further experiments.

4.3.2 Isolate of *Pythium aphanidermatum*

For the inoculation of *P. aphanidermatum* in the greenhouse experiments, an isolate was used, which was kindly provided by Dr. Wanwilei Intanoo, Department of Plant Pathology, Kasetsart University, Thailand. This was confirmed as *P. aphanidermatum* by the Centraalbureau voor Schimmelcultures, Utrecht, Netherlands (reference det 273-2002).

4.3.3 Greenhouse experiments

4.3.3.1 *Experimental set-up*

The greenhouse (size 200 m^2), in which all experiments were conducted, was located at the AIT in Bangkok, Thailand. It was a closed net-house (Econet M, pore size 0.18 mm, Ludvig Swensson, Netherlands) equipped with two exhaust fans (550 m^3 min^{-1}, 1.5 HP, 960 rpm, Sriroz Company, India) at the front side of the net-house (Figure 4.1)

Figure 4.1: Closed net-house located at the campus of the Asian Institute of Technology (AIT) in Bangkok, Thailand.

The fans were operated by a computerized control system that automatically switched on one fan when temperature inside the net-house exceeded 25°C, and the second one at temperatures > 30°C. The climate in the greenhouse was monitored using a data logging system (ITG data logger, Leibniz Universität, Hannover, Germany). During the experiments, mean temperature and relative humidity was 28-30ºC and 70-80%. The total planting area of the greenhouse was 160 m^2. The greenhouse was lengthwise subdivided with a net (Econet M, pore size 0.18 mm, Ludvig Swensson, Netherlands) in two parts, each part with 3 rows and a separate entrance door.

Tomato seedlings were raised in the nursery under disease free conditions. At the required age for the experiments (4 to 6 weeks), they were planted in plastic pots (30 x 25 cm) filled with a commercial growing substrate. This was composed of clay, sand, and silt in proportions of 31, 30 and 39, respectively, and 29% of organic matter. The pots were placed on a black ground plastic cover (Chaisiri Nylon Canvas Factory Ltd., Bangkok, Thailand) and arranged in 6 rows with no inter-pot distance within a row, altogether 60 pots per row (Figure 4.2). The distance between rows was 160 cm and from sidewall and the middle partition to the row 55 cm.

Figure 4.2: Arrangement of pots in one part of a closed net-house at the campus of the AIT in Bangkok, Thailand.

Plants were irrigated and fertilized 7-9 times per day (2.5 L day^{-1}) with a drip irrigation system controlled by solar light integral. The fertilizers [Hakaphos® (N-P-K) (2.5 kg 100 L^{-1}), COMPO GmbH, Austria, and Bai-plus (calcium) (1.8 kg 100 L^{-1}), Bayer Ltd., Thailand] were injected into the irrigation system with mechanical injectors (DI 16, Dosatron®, France). Tomato plants were supported by ropes, which were fixed to the structure of the ceiling of the greenhouse. The plants were cultivated in a single-stem system. Necessary pruning work, for instance to remove side branches and to bind the plants, was done weekly.

In all experiments, *P. fuligena* occurred naturally in the greenhouse. To keep the disease level in one part of the greenhouse low, Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}) was applied weekly, beginning with the day after positioning (dap) plants in the greenhouse.

Between October 2002 and March 2004 altogether four experiments were conducted (Table 4.1).

Table 4.1: Characteristics of the experiments in the greenhouse

Exp No.	Start	End	Duration (dap)	Mean Temp (°C)	Min Temp (°C)	Max Temp (°C)	Mean RH (%)
1	21 Oct´02	13 Jan´03	84	28.3 ± 0.3*	20.9 ± 0.9 *	35.2 ± 0.1 *	**
2	19 May´03	11 Aug´03	84	29.1 ± 0.1	25.4 ± 0.1	35.2 ± 0.3	77.28 ± 0.78
3	21 Oct´03	10 Feb´04	112	26.6 ± 0.2	21.9 ± 0.2	33.4 ± 0.2	73.54 ± 0.53
4	26 Nov´03	17 March´04	112	25.9 ± 0.2	21.0 ± 0.2	33.0 ± 0.2	72.98 ± 0.65

* values from the Deutsche Wetterdienst databank measuring point Bangkok, Data logger were not yet already in function at that time; ** no values available.

4.3.3.2 *First Experiment*

This experiment was carried out with the determinate tomato variety ´New King Kong´. The seeds were sown on 26 August 2002 and transplanted eight weeks later. At 21 October 2002 with the day of transplanting and positioning plants in the greenhouse (plants had already 10 to 12 leaves), the substrate was inoculated with *P. aphanidermatum* in 3 different densities (P0, P1 and P2). The previously mentioned isolate was inoculated using roughly 1-cm^2 pieces of mycelium, grown for 4 days in darkness at 25°C on agar in Petri dishes. For the different levels of inoculum, different numbers of Petri dishes containing *P. aphanidermatum* mycelium were mixed in the substrate before transplanting the tomato plants, e.g. for P1 one Petri dish per pot, for the high level, P2, three Petri dishes were used. In the pots of control plants P0, plain PDA of 1 Petri dish was mixed.

Plants of both halves of the greenhouse were naturally infected with *P. fuligena.*

Additionally, plants were inoculated with *A. solani* in one-half of the greenhouse. Three leaves (number 5, 6 and 7 counted from bottom to top) were scratched, the agar plates with mycelium were directly pressed for 5 seconds smoothly onto the leaves. Thereafter, the leaves were moistened by spraying tap water and covered separately with plastic bags for 24 h in order to increase the relative humidity. Control plants were treated with sterile agar plates.

To avoid the spread of black leaf mold and early blight, the side without *A. solani* inoculation was weekly treated with Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}), beginning with the day after positioning (dap).

Due to the fact that the inoculation with early blight was not successful (see chapter 2), the main focus was on the observation of the interaction of PRR and BLM.

The plants were arranged in a split-block design (with 4 replications). Every split-block was subdivided by 3 levels of *P. aphanidermatum* (P0/P1/P2) as sub-plot factor. The sub-plots were repeated 4 times; each sub-plot contained 5 plants in a row. Border plants of each sub-plot were not used as data plants. The total number of plants in the greenhouse was 360 (270 were used for data collection), arranged in 6 rows (Figure 4.3).

The treatments were:

P0–BLM0: 1 plain Petri dish per pot, with control of *P. fuligena*

P1–BLM0: 1 Petri dish with *P. aphanidermatum* per pot, with control of *P. fuligena*

P2–BLM0: 3 Petri dishes with *P. aphanidermatum* per pot, with control of *P. fuligena*

P0–BLM1: 1 plain Petri dish per pot, without control of *P. fuligena*

P1–BLM1: 1 Petri dish with *P. aphanidermatum* per pot, without control of *P. fuligena*

P2–BLM1: 3 Petri dishes with *P. aphanidermatum* per pot, without control of *P. fuligena*

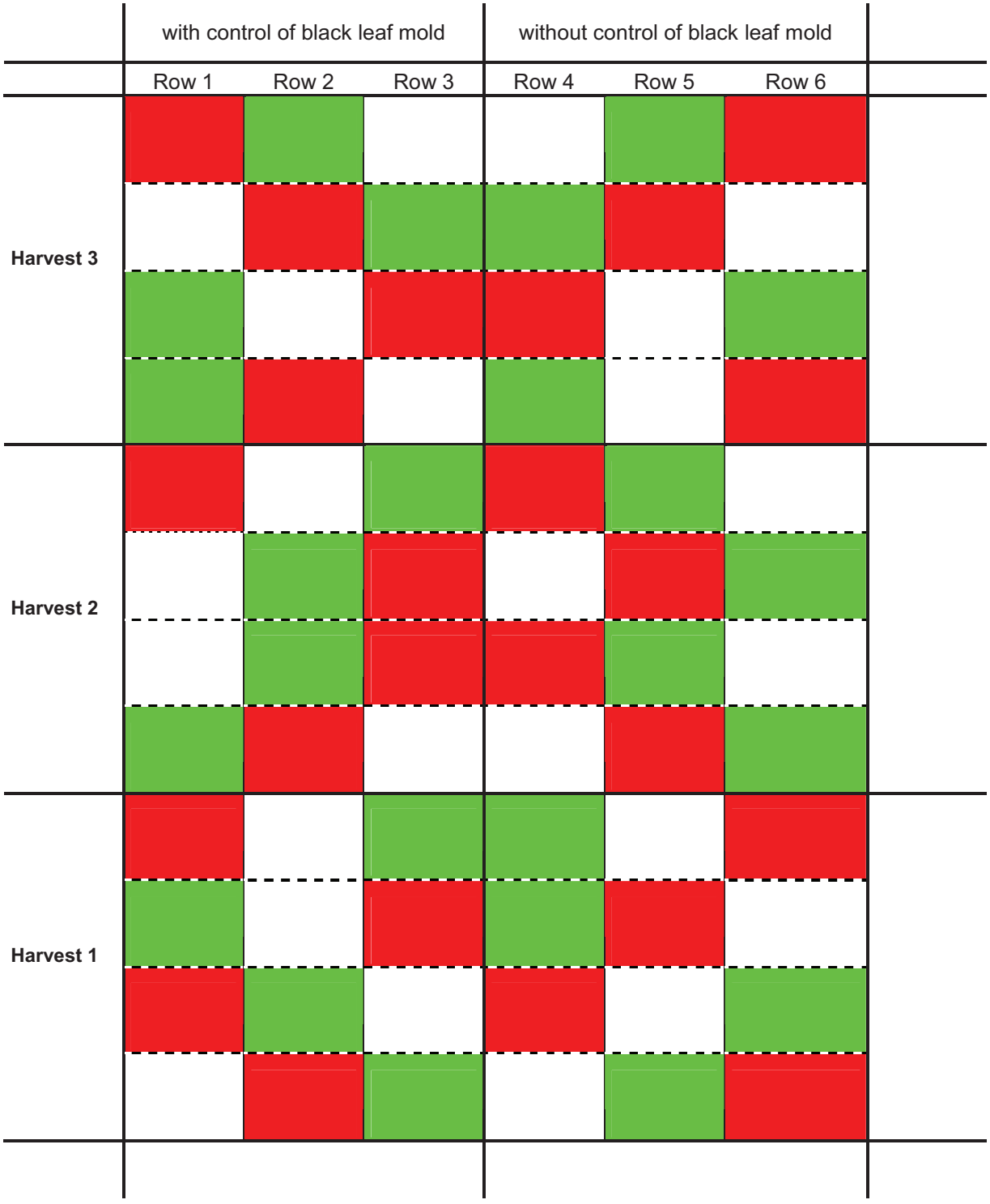


Figure 4.3: Greenhouse plan for experiment 1 laid out in a split-block design (with 3 replications). Control of BLM as main factor and 3 levels of *P. aphanidermatum* (P0/P1/P2) as sub-plot factor. Each rectangle represents 5 plants.
Colour Code: White: P0 = 1 plain Petri dish per pot
Red : P1 = 1 Petri dish with *P. aphanidermatum* per pot
Green: P2 = 3 Petri dishes with *P. aphanidermatum* per pot

A destructive sampling of 20 plants per treatment was done every month. Thus one third of the greenhouse plants, starting on the opposite side of the ventilation, was removed at each sampling. The leaf area was measured, roots were washed, air dried and weighed, fruits were harvested (for a detailed method description see 4.3.4.3). Additionally, disease incidences of plants and leaves were determined and disease severity was visually estimated leaf by leaf once a week (see 4.3.4). These observations were done for 3 months after inoculation.

4.3.3.3 *Second Experiment*

The second experiment was carried out with the variety ´King Kong 2´, an indeterminate variety. The experiment was conducted in the same way as the first one (see above). Seeds were sown on 21 April 2002. One month later (on 19 May 2002) the inoculation of *P. aphanidermatum* was carried out using a modified technique of Chaengchaiyasakulthai and Chamswarng (1986). As stock culture, a mixture of sand, soil, and maize flour (3/1/1) was infested with mycelium plugs from four days old cultures of *P. aphanidermatum* (grown on PDA, and incubated in darkness). After two weeks at 35°C, the commercial substrate was mixed with 5% of the stock culture and 1.5% maize flower. This mixture was incubated overnight at 25°C and then used as inoculum (P0* = 0.2 g sterilized inoculum per pot, P1* = 0.2 g and P2* = 1 g of inoculum per pot). The experimental control plants received 0.2 g sterilized inoculum material per pot.

Immediately after inoculation of the substrate, the plastic pots, as previously described, were placed in the greenhouse and plants, which had already 10 to 12 leaves, were planted.

The treatments in this experiment were:

P0*–BLM0: 0.2 g sterile inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*

P1*–BLM0: 0.2 g inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*

P2*–BLM0: 1 g inoculum of *P. aphanidermatum* per pot, with control of *P. fuligena*

P0*–BLM1: 0.2 g sterile inoculum of *P. aphanidermatum* per pot, without control of *P. fuligena*

P1*–BLM1: 0.2 g inoculum of *P. aphanidermatum* per pot, without control of *P. fuligena*

P2*–BLM1: 1 g inoculum of *P. aphanidermatum* per pot, without control of *P. fuligena*

The experiment was planned for three months including three destructive samplings, as described above. The data collection was done weekly (see first experiment, 4.3.3.2).

4.3.3.4 *Third Experiment*

The third experiment was carried out similar to the previous one. Inoculation densities of *P. aphanidermatum* were chosen as in the first experiment (see 4.3.3.2). As a special treatment, *T. harzianum,* a biological antagonist of *P. aphanidermatum,* was added to the substrate of the P0-treatments. According to the method of Chamswarng and Intanoo (2002), the antagonist was mixed into the substrate in 0.2% proportion prior to the transplanting of tomato plants. The *T. harzianum* isolates were kindly provided by Dr. Wanwilei Intanoo, Department of Plant Pathology, Kasetsart University, Thailand.

Seeds of the variety ´King Kong 2´ were sown on 20 September 2003. One month later (on 21 October 2003), the plants were transplanted in pots filled with substrate and inoculated with *P. aphanidermatum*, placed in the greenhouse and were naturally infected by *P. fuligena.*

The treatments were:

$P0_T$–BLM0: 1 plain Petri dish per pot, with control of *P. fuligena,* with *T. harzianum*

P1–BLM0: 1 Petri dish with *P. aphanidermatum* per pot, with control of *P. fuligena*

P2–BLM0: 3 Petri dishes with *P. aphanidermatum* per pot, with control of *P. fuligena*

$P0_T$–BLM1: 1 plan Petri dish per pot, without control of *P. fuligena,* with *T. harzianum*

P1–BLM1: 1 Petri dish with *P. aphanidermatum* per pot, without control of *P. fuligena*

P2–BLM1: 3 Petri dishes with *P. aphanidermatum* per pot, without control of *P. fuligena*

All experimental plants were placed at the determined position in the greenhouse (Figure 4.3), the surplus potted plants were placed at the edge of the greenhouse. At 2, 3, 5, 10, 13 and 15 dap, all plants were monitored for disease symptoms mainly caused by *P. aphanidermatum,* e.g. wilting. Our research focused on the interaction within the disease complex PRR and BLM. As interaction is relevant only on living plants, the plants dropped out until 14 dap were replaced,. At 5 dap, pots with dead plants were replaced by spare pots with healthy plants of the same inoculation density which were stored at the edge of the greenhouse. If more plants dropped out than were replaceable, the positions stayed empty within the row. A detailed sketch of the replaced plants can be found in the appendix (Figure 6.1).

The experiment was designed for 4 months with 4 destructive samplings. Disease observations were done weekly as previously described. A destructive sampling of 15 plants

per treatment was done every month, 9 plants were used for data collection. At each sampling, one fourth of the greenhouse plants was removed.

4.3.3.5 *Fourth Experiment*

In this experiment the variety ´King Kong 2´ was used. The design of this experiment differed from the previous ones. In addition, *T. harzianum* was inoculated but not *P. aphanidermatum*. The inoculation method of *T. harzianum* was identical to the third experiment (see 4.3.3.4).

The seeds were sown on 25 October 2003. The tomato seedlings were planted in trays and stored in the nursery. One month later (on 26 November 2003) they were transplanted in pots pre-inoculated with *T. harzianum,* placed in the greenhouse and naturally infected with *P. fuligena.*

The total number of plants in the greenhouse was 60, so that 15 plants for each treatment were available. Plants were arranged in four rows, two rows on each side of the subdivided greenhouse. Plants of both halves of the greenhouse were naturally infected with *P. fuligena*, but one side was weekly treated with Maneb (Dithane M-45, 1.6 kg a. i. ha^{-1}), beginning with the day after positioning.

The treatments were:

P0–BLM0:	with control of *P. fuligena*
$P0_T$–BLM0:	with control of *P. fuligena*, inoculated with *T. harzianum*
P0-BLM1:	without control of *P. fuligena*
$P0_T$–BLM1:	without control of *P. fuligena,* inoculated with *T. harzianum*

Disease incidences of plants and leaves were determined by counting and disease severity was visually estimated leaf by leaf once a week. After 4 months of observation, the experiment was completed with a final destructive sampling of the plants.

4.3.4 Disease assessment

4.3.4.1 *Assessment of BLM*

For disease assessment of BLM, the leaves of all plants were visually rated for percent diseased foliage using a modified Beaumont rating scheme (Beaumont, 1954) with 0% indicating no visible symptoms of *P. fuligena* infection and 100% indicating completely diseased foliage. The rating scale was modified as followed:

no recognizable lesions : 0%
0.5 – 2.5% diseased foliage : 1%
2.5 – 7.5% diseased foliage : 5%
7.5 – 15% diseased foliage : 10%
15 – 25% diseased foliage : 20%
25 – 35% diseased foliage : 30%
35 – 45% diseased foliage : 40%
45 – 62% diseased foliage : 50%
63 – 82% diseased foliage : 75%
83 – 100% diseased foliage : 100%

The disease assessment started in experiment 1 at the day after positioning the potted tomatoes in the greenhouse. In experiments 2, 3 and 4 it began at the day of transplanting, which was similar to the day after positioning potted tomatoes in the greenhouse.

4.3.4.2 *Assessment of Pythium aphanidermatum*

Besides the visual monitoring of BLM, additional attention was given to any symptoms related to the soil-borne fungus, e.g. wilting of leaves, indefinable leaf spots, dwarfing or dying off which could give information about infection.

As the root pathogen *P. aphanidermatum* rarely shows lesions on the upper part of the plant, a potato baiting method was used for detection and re-isolation (Stanghellini and Kronland, 1985). Soil samples were taken weekly from 10 randomly selected pots (about 50 mL), placed in a Petri dish and saturated with distilled water. A slice of potato (0.25 cm^2 and 3 mm thick) with a piece of water agar on top (same size as potato slice) was used as bait and placed on the saturated soil surface (Figure 4.4). After incubating for 48 hours in darkness at 30°C, the water agar slice was removed and placed onto a special detection medium for *P. aphanidermatum*, containing PDA with 100 ppm Pimaridin + 100 ppm Streptomycin (Hine and Luna, 1963). After 24 hours at 32°C in darkness, the Petri dishes were evaluated under the binocular (Leica MZ75, Fa. Leica, Germany) with a cold light source (KL 1500 LCD, Fa. Schott, Germany). If the soil sample was infested, cotton wool pad-like mycelium could be identified using the book of Domsch et al. (1993).

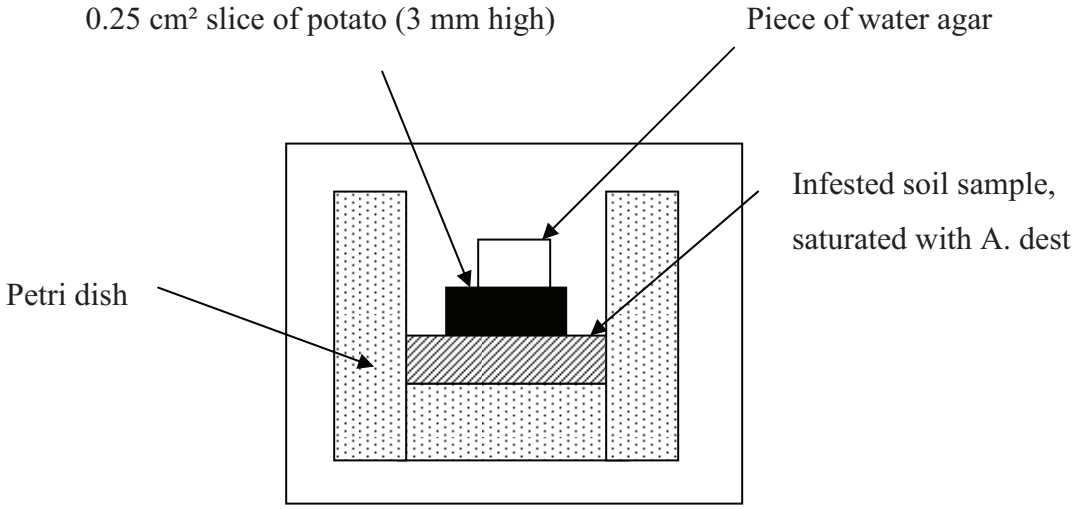


Figure 4.4: Schematic diagram (lateral cut) of the potato baiting method (after Stanghellini and Kronland, 1985).

4.3.4.3 *Assessment of plant growth parameters*

The shoot and root fresh weight was determined at each destructive sampling date (once in a month) until the end of the experiment. The roots were cut of the shoot at substrate level, washed under running water to remove the substrate, dried with household paper and weighed.

The shoot fresh weight including the leaves was taken by using an overhead panel balance (Fa. Scaltec Instruments, Heiligenstadt, Germany, max. 3000 g, d = 0.1 g).

The plant height was measured and the leaves of each experimental plant were counted on a weekly basis until the end of the experiment.

To determine the leaf area in cm^2, each leaf was removed from the shoot and individually measured with a leaf area meter (LI-COR; Model Li-3100 AREA meter, left-Cor. Inc. Lincoln, Nebraska, USA).

Fruits were harvested on a regular basis (in 7 days interval) after the first ripe fruits were found. In addition, the unripe tomatoes were harvested at the final destructive sampling.

To determine dry weight, fresh shoots were singly packed in paper bags, stored in the drying oven (ventilated oven; 80°C) and weighed after 1 week.

4.3.5 Statistical analyses

The quantitative data of the plants, i.e. total leaf number, plant height, yield, root weight, dry weight, leaf area etc. were subjected to two-way analyses of variance (ANOVA) using the PROC GLM procedure of the SAS software package (SAS 9.1, Users Guide, SAS Institute, Cary, NC) and means separated by last significant difference (LSD $p < 0.05$). The two factors investigated were the influence of BLM (marked with capital letters A and B) and PRR (characterized with small letters a and b) and their interaction (marked with ***). Mean comparisons were conducted using Tukey's t-test ($p \leq 0.05$).

The computed standard errors (SE) are displayed in the figures while within the text, the standard error of the statistical analysis of the PROC GLM are mentioned.

4.4 Results

After inoculation of *P. aphanidermatum* and natural infection by *P. fuligena*, all tomato plants showed symptoms of both diseases, PRR more in the early, BLM in the later stage of the experiments. It should be noted that the experiments were carried out at different times of the year so that plant growth parameters cannot be compared easily among the experiments.

4.4.1 Observation of Pythium root rot

Tests of substrate samples from experimental pots with the potato-baiting-method confirmed a successful inoculation of *P. aphanidermatum* in all experiments. In addition, in experiment 2, sample tests also detected a contamination of the P0*-treatments which were not inoculated. Therefore, samples of the general planting substrate were taken showing that the whole lot of substrate was contaminated. No other pathogens than *P. aphanidermatum* could be re-isolated.

In experiment 1, tomato plants didn't show any specific symptoms related to PRR, e.g. wilting.

In experiment 2, at 6 dap, 64% of the plants in the P0*-treatments (BLM0 and BLM1), and 34% of the P1*- and P2*-treatments were replaced (Table 4.2). For further details of replaced plants see appendix (Figure 6.1). One week later (at 13 dap), 44% of the P0*-treatments and 31% of P1* and 29% of P2* were replaced again with surplus seedlings. Without inoculation, the P0*-treatment was heavily attacked by PRR.

Table 4.2: Replaced plants in the different *Pythium* – treatments of experiment 2, at 6 dap and 13 dap

Treatments	Total number of plants	Number of plants replaced at 6 dap	Number of plants replaced at 13 dap
P0*-BLM0	60	40	29
P1*-BLM0	60	13	14
P2*-BLM0	60	13	17
P0*-BLM1	60	37	24
P1*-BLM1	60	28	23
P2*-BLM1	60	28	18

In experiment 3, the plants were checked for PRR beginning at 2 dap. Altogether 150 plants were stored in the greenhouse (120 experimental plants and 30 additional ones). Plants which dropped out at 5 dap were replaced with the 30 surplus plants. No further exchange was done

later. In the $P0_T$-treatments (combined BLM0 and BLM1) with *T. harzianum,* only 2 plants wilted after 15 dap. In the P1-treatments 33% and in the P2-treatments 27% dropped out (Figure 4.5).

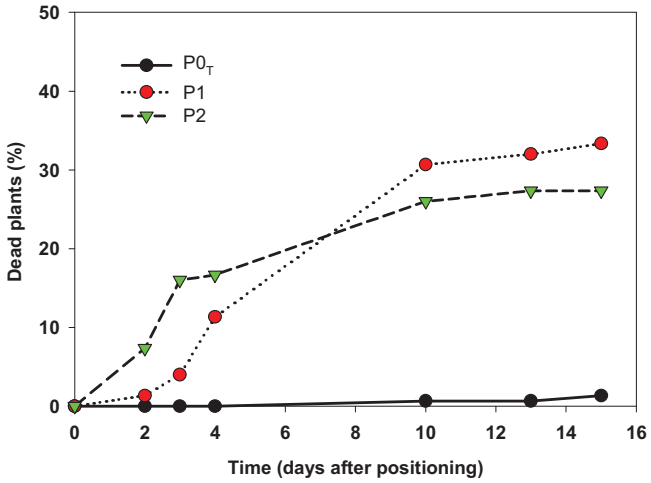


Figure 4.5: Dead plants (%) in experiment 3 due to *P. aphanidermatum* in 3 treatments: $P0_T$ - no *Pythium* with *T. harzianum*, P1- low level and P2- high level of *Pythium*.

BLM symptoms appeared at 14 dap. Later, lower leaves started wilting and dropped off. P1- and P2-treatments showed significantly less mortality than the $P0_T$–treatment (Figure 4.6). These unexpected results are probably caused by replacing dead plants in the P1 and P2 treatments shortly after transplanting.

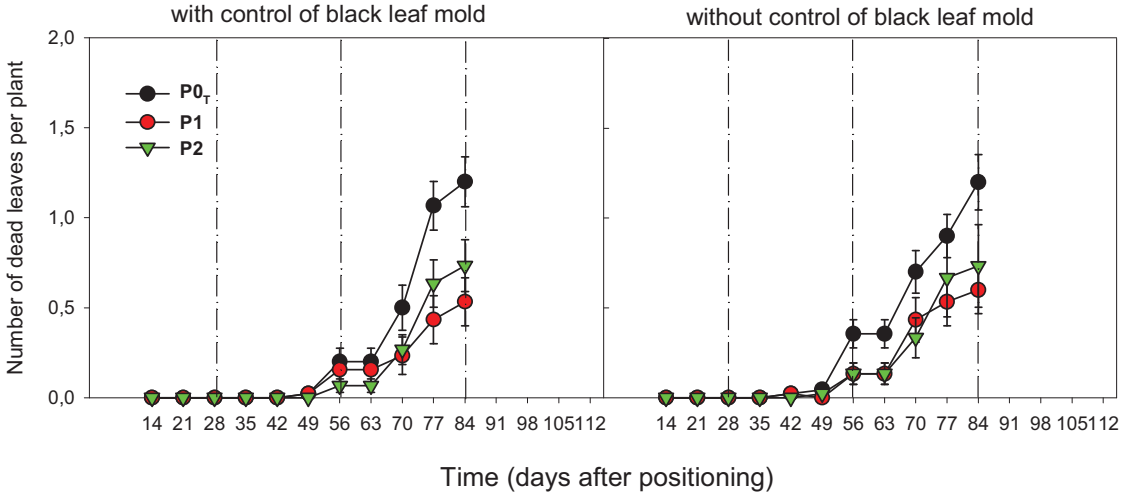


Figure 4.6: Number of dead leaves per plant in experiment 3 with different *Pythium*-treatments: $P0_T$ - no *Pythium* inoculation with *T. harzianum*, P1 - low level and P2 - high level of *Pythium*. Vertical lines show the times when part of the plants were removed, resulting in a reduced sample size. After 84 dap no further dead leaves were observed because the 10 lowest leaves were removed on that day, following the normal cultural practice.

In experiment 4, no plants were replaced. Here wilting symptoms of the lower leaves appeared at 56 dap. The differences between the P0- and $P0_T$–treatments were small. The BLM0-treatment showed significant less leaf mortality compared to the BLM1-treatment (Figure 4.7).

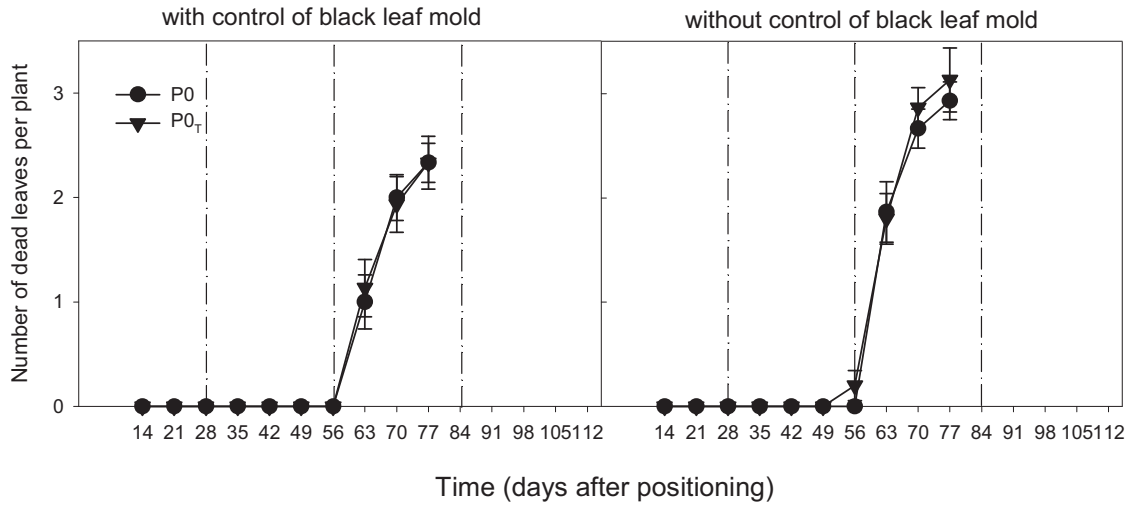


Figure 4.7: Number of dead leaves accumulated per plant in experiment 4 with: P0 – non-inoculated, $P0_T$ - with *T. harzianum.* Vertical lines show the times when part of the plants were removed resulting in a reduced sample size. After 77 dap no further dead leaves were observed because the 10 lowest leaves were removed on that day, following the normal cultural practice.

4.4.2 Observation of BLM

BLM naturally occurred on tomato plants inside the greenhouse throughout the year without great differences among seasons. Both tested cultivars, ´New King Kong´ and ´King Kong 2´, were susceptible.

The highest disease severity was reached in experiment 1. Plants in the non-controlled part of the greenhouse reached approximately 30% disease severity. Plants in experiments 2 and 3 remained below a disease severity level of 10% in the non-sprayed parts of the greenhouse, while in the P0-treatment in experiment 4, BLM severity reached 15%.

Neither the different *Pythium*-levels nor the treatment with *Trichoderma* changed the overall trend of the disease severity progress curves.

Results on BLM epidemics were displayed and discussed in detail in chapter 2. Here the effects of the epidemics of the plant growth parameters will be presented.

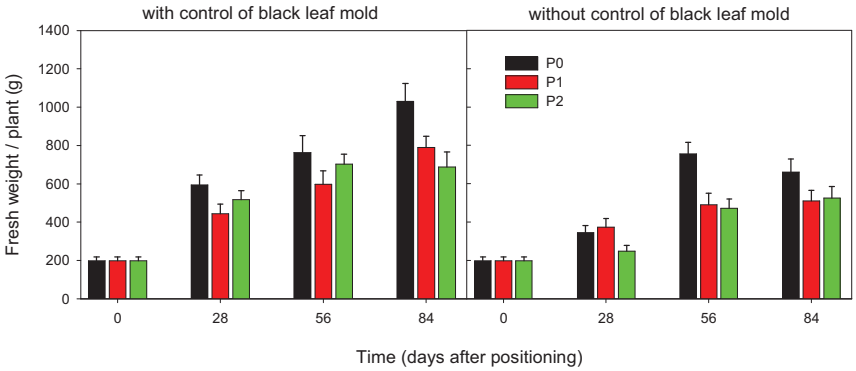
4.4.3 Plant growth parameters

The following results were based on monthly destructive samplings. In experiments 1 and 2, conducted over 3 months, 12 plants were harvested and analysed at every sampling day. In experiments 3 and 4, conducted for 4 months, 9 plants were monthly harvested.
Generally, the results were very heterogeneous without showing overall tendencies. Data are displayed in figures and statistical analyses are given in the appendix. Results of experiment 2 should be interpreted with care, because the plants showed an unplanned infestation with *P. aphanidermatum* even in the P0*-treatments, in which no inoculation was carried out.

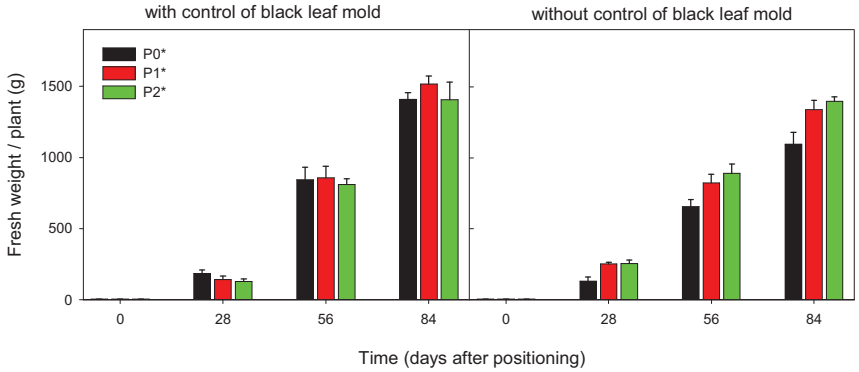
4.4.3.1 *Fresh biomass*

After abscising the tomato plants and removing all fruits, the plants were balanced (Figure 4.8). Details can be found in the appendix (Table 6.3-6.6).
At the beginning of experiment 1, the plants started with an initial plant fresh weight of 200 g per plant. At 28, 56 and 84 dap, the plant fresh weight in the BLM1-treatments were significantly reduced compared to BLM0. PRR lessened the weight at 56 and 84 dap. The plants of the P0–BLM0 treatment had a final weight of 1029.71 ± 69.56 g at 84 dap, while plants in the P2–BLM0-treatments had only 686.94 ± 69.56 g.
In experiment 2, a significant interaction appeared at 28 dap, because plant weights of the P1*-and P2*-treatments in BLM0 and BLM1 showed an opposed tendency. At 28 dap, mean plant weight of the BLM0-treatments was lower than of the BLM1-treatments, but at 84 dap higher. At 84 dap, the weight of plants in the P0*-treatments was lighter compared to the plants in the P1*- and P2*-treatments (P0*-BLM1: 1097.18 ± 76.67 g and P2*-BLM1: 1398.25 ± 73.41 g).
In experiment 3, *T. harzianum* was inoculated as an antagonist for *P. aphanidermatum* in the $P0_T$-treatments. The plants in the *P. aphanidermatum* inoculated treatments (P1 and P2) were reduced in fresh weight at 28, 56 and 84 dap. At these days also the fresh weights in the BLM0-treatments were lower than in the BLM1-treatments. A significant interaction was found at the beginning of the experiment (28 dap) with a fresh weight of around 72 g per plant in P1-BLM0 and P2-BLM0, nearly three times smaller than in the $P0_T$-treatments with and without spraying. At 112 dap, no significant influences of BLM and PRR were detectable.
In experiment 4, only one sample was taken at the end of the experiment (112 dap). The fresh weight of plants was higher in the BLM1- than in the BLM0-treatments. *T. harzianum* did not show a significant influence (P0-BLM0: 987.89 ± 48.43 g; $P0_T$-BLM0: 1010.11 ± 48.43 g).

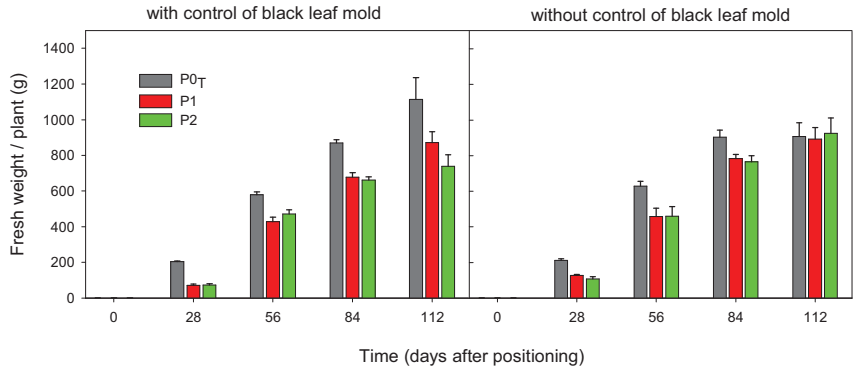
Experiment 1: *October 2002 – January 2003*



Experiment 2: *May – August 2003*



Experiment 3: *October 2003 – February 2004*



Experiment 4: *November 2003 – March 2004*

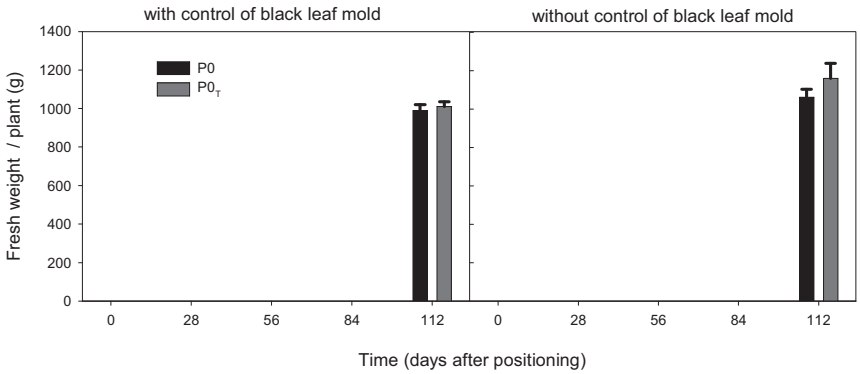


Figure 4.8: Fresh weight of plants ± SE (g) in four experiments with different *Pythium*-treatments: P0– non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1- low level and P2- high level of *Pythium*. P0*-, P1*- and P2*-treatments refer to another inoculation method (see 4.3.3.3). Sample sizes: experiments 1 and 2 n=12, experiment 3 n=9 and experiment 4 n=15. Because of cultural practice, the 10 lowest leaves were removed in experiments 3 (at 84 dap) and 4 (after 77 dap).

4.4.3.2 *Dry weight*

After taking the fresh weight, the plants were dried in a drying oven and then again weighed (Figure 4.9). Details of the measurements can be found in the appendix (Table 6.7 – 6.9). The results showed a strong correlation between dry and fresh weight with high coefficients of determination, e.g. in experiment 1 $r^2 > 0.91$, in experiment 2 and 3 $r^2 > 0.98$.

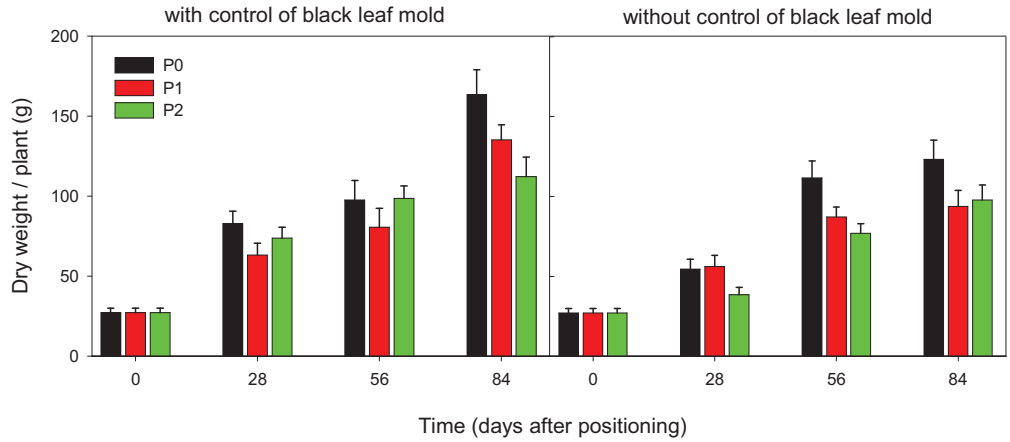
In experiment 1, at 28 dap and 84 dap the dry weight was significantly reduced in the BLM1-treatments, e.g. at 84 dap P0-BLM0 had a dry weight of 163.33 ± 11.66 g and P0–BLM1 of 123.3 ± 11.66 g. Also at 84 dap, plants inoculated with *P. aphanidermatum* (P1 and P2) were reduced in weight compared to plants in the P0-treatment.

The second experiment did not show significant effects at 28 and 56 dap, neither for BLM nor for PRR. At 84 dap, the dry weight of plants in the control-treatments (BLM0) was higher compared to the plants of the BLM1-treatment. The dry weight was significantly reduced in the P0*-treatments compared to the P1*- and P2*-treatments.

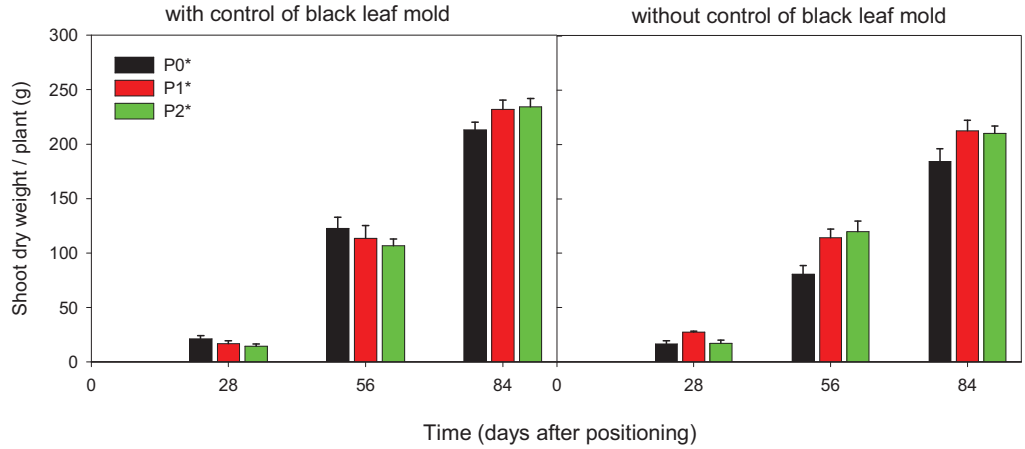
Experiment 3 showed a different trend. Only at 28 dap, the non-sprayed plants (BLM1) had a noticeable higher weight than the sprayed ones. Within the *Pythium*-treatments, $P0_T$ had the highest values at 28, 56 and 84 dap. At 112 dap, no significant effects of BLM and PRR were detectable.

Dry weight was not measured in experiment 4.

Experiment 1: *October 2002 – January 2003*



Experiment 2: *May – August 2003*



Experiment 3: *October 2003 – February 2004*

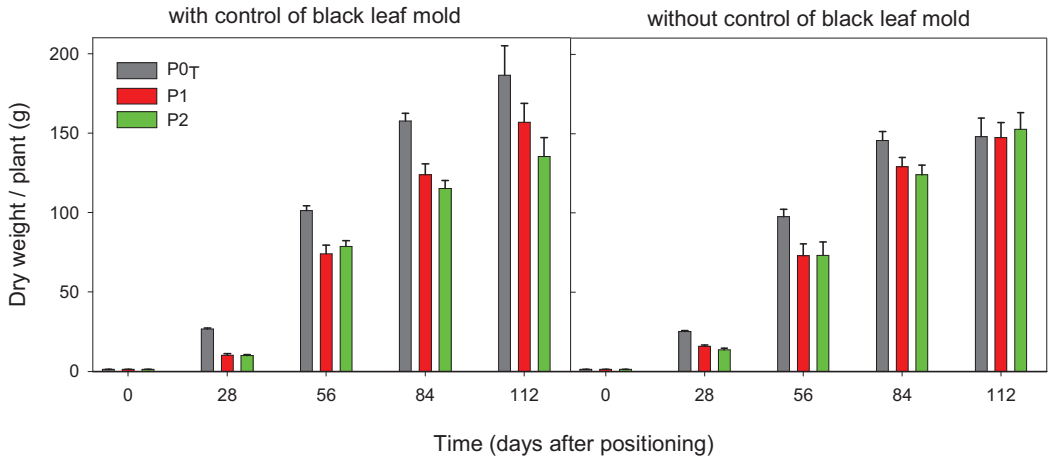


Figure 4.9: Dry weight of plants ± SE (g) in three experiments with different *Pythium*-treatments: P0 – non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1 - low level and P2 - high level of *Pythium*. P0*-, P1*- and P2*-treatments refer to another inoculation method (see 4.3.3.3). Sample sizes: experiments 1 and 2 n=12, experiment 3 n=9. Because of cultural practice, the 10 lowest leaves were removed in experiment 3 (at 84 dap) .

4.4.3.3 *Leaf area*

From the abscised tomato plants the leaves were chopped off and measured with a leaf area meter. The results were displayed in Figure 4.10, further details can be found in the appendix (Tables 6.10 – 6.13).

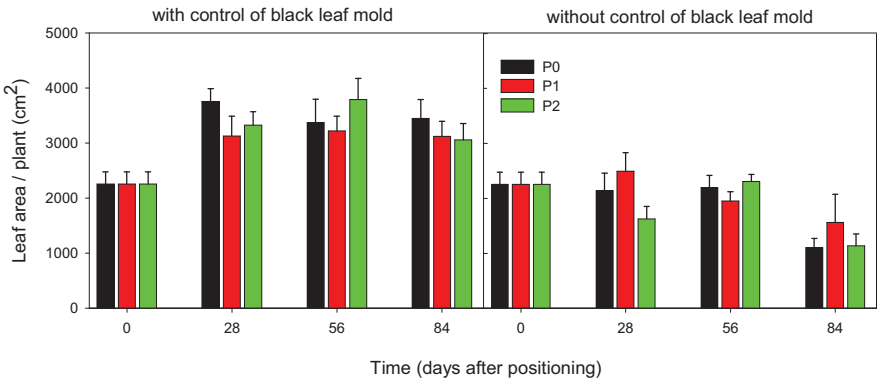
In experiment 1, the plants of the determinate variety ´New King Kong´ already started with an average of 2200 cm^2 leaf area. The leaf area of the non-sprayed treatments (BLM1) were significantly reduced in all samplings, e.g. at 84 dap from 3447.64 ± 323.17 cm^2 for P0-BLM0 to 1101.56 ± 323.17 cm^2 for P0-BLM1. Here, PRR did not have a significant influence at all sampling dates.

In experiment 2, conducted with an indeterminate variety, leaf area had a different range. At 28 and 56 dap, a significant interaction between the different *Pythium*-levels and the BLM-treatments was detectable. At 28 dap, the plants of the P0*-BLM0-treatments had the biggest size and the P1*- and P2*-BLM0-treatments were smaller. Within the BLM1-treatments, P1*-BLM1 had the largest leaf area (2791.58 ± 22.14 cm^2). At 56 dap, again the plants of the P0*-BLM0-treatments had the biggest area, while the areas of P1*- and P2*-BLM0-treatments were lower. At this time, the trend in the BLM1-treatments was vice versa. At 84 dap, no significant differences were observable.

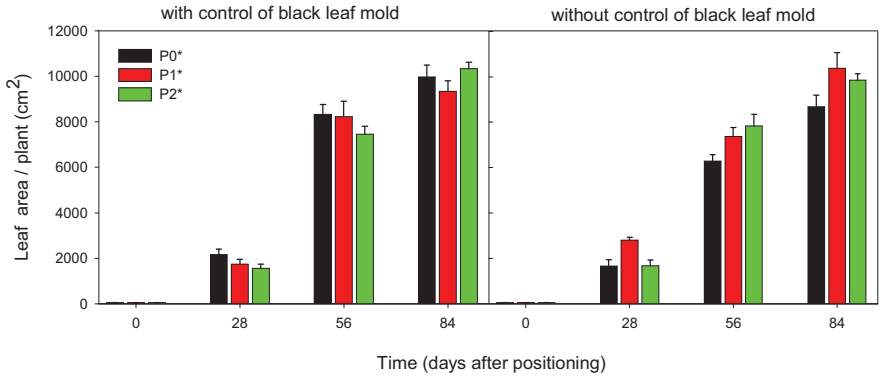
In experiment 3, at 28 dap, a significant interaction occurred. Within the different Pythium-treatments, plants of the $P0_T$-treatments had the biggest area while plants of the P1- and P2-BLM1-treatments were smaller but with reverse trends within the P1- and P2-treatments. At 28, 56 and 84 dap, the leaf area of plants in the sprayed treatments (BLM0) was significantly reduced compared to the BLM1-treatments. Plants in the inoculated *P. aphanidermatum*-treatments (P1 and P2) were as well reduced in size, at 56 dap. At 112 the treatments didn't show significant differences.

In experiment 4, the leaf area of the plants was higher in the non-sprayed treatments compared to the sprayed ones. Plants inoculated with *T. harzianum* had a significantly increased area (P0-BLM0: 4940.88 ± 363.67 cm^2 versus $P0_T$-BLM0: 5186.59 ± 363.67 cm^2).

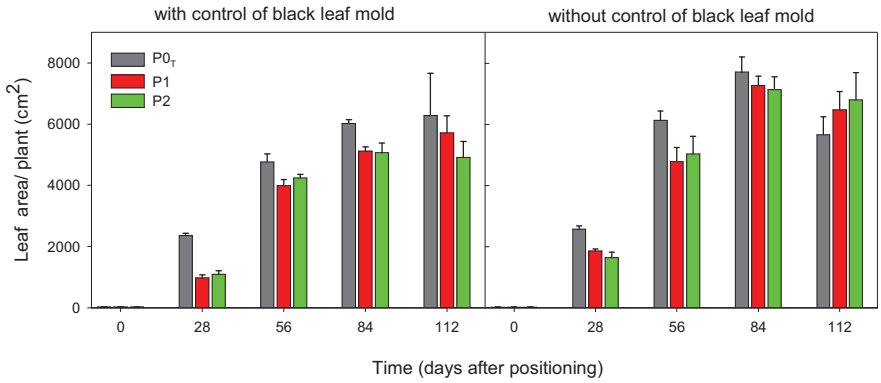
Experiment 1: *October 2002 – January 2003*



Experiment 2: *May – August 2003*



Experiment 3: *October 2003 – February 2004*



Experiment 4: *November 2003 – March 2004*

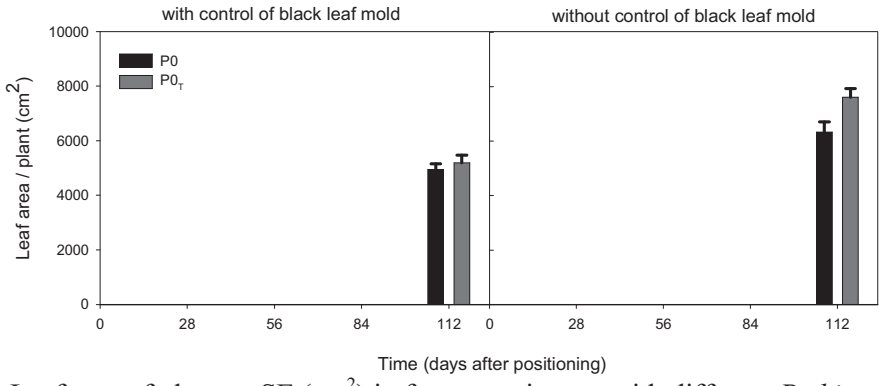


Figure 4.10: Leaf area of plants ± SE (cm^2) in four experiments with different *Pythium*-treatments: P0– non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1- low level and P2- high level of *Pythium*. P0*-, P1*- and P2*-treatments refer to another inoculation method (see 4.3.3.3). Sample sizes: experiments 1 and 2 n=12, experiment 3 n=9 and experiment 4 n=15. Because of cultural practice, the 10 lowest leaves were removed in experiments 3 (at 84 dap) and 4 (after 77 dap).

4.4.3.4 *Root weight*

After washing, the roots showed a light creamy colour (Figure 4.11) without discoloration. The roots in the treatments inoculated with *P. aphanidermatum* (P1 and P2) were in general shorter and reduced in size compared to the non-inoculated (P0) ones, no matter if BLM was controlled or not.

a) 

b) 

c) 

Figure 4.11: Washed out roots of experiment 1 in three treatments:
a) P0–BLM0: 1 plain Petri dish per pot, with control of *P. fuligena*
b) P1–BLM1: 1 Petri dish with *P. aphanidermatum* per pot, without control of *P. fuligena*
c) P2–BLM1: 3 Petri dishes with *P. aphanidermatum* per pot, naturally infected with *P. fuligena*

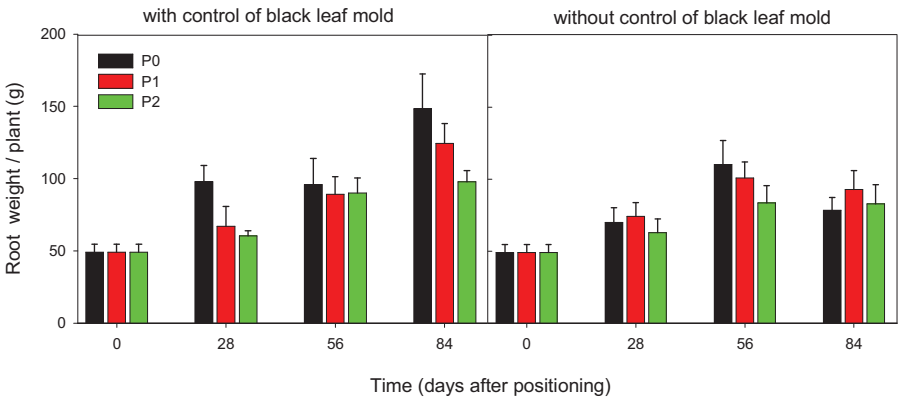
After the visual rating, the clammy roots were weighed (Figure 4.12), the details can be found in the appendix (Tables 6.14 – 6.17).

In experiment 1, at 28 and 56 dap no significant differences occurred. At the end of the experiment (at 84 dap), the roots of the plants in the treatments without control (BLM1) showed a significant reduction of weight, e.g. 148.42 ± 14.47 g in P0-BLM0 compared to 78.47 ± 14.47 g in P0-BLM1.

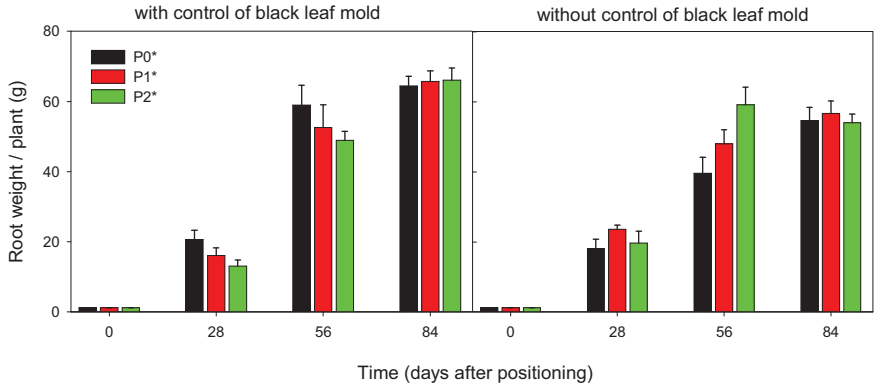
In experiment 2, at 56 dap a significant interaction appeared. In the fungicide treatments, the P0*-treatments had the highest weight with 57.23 ± 4.69 g, while in the non-sprayed treatments, the highest root weight was measured in the P2*-treatments with 59.30 ± 5.35 g. Later (at 84 dap), the root weight was significantly reduced in the non-sprayed treatments (BLM1). The *P. aphanidermatum* inoculated treatments did not show any significant influence on the root weight.

In experiment 3, the root weight was reduced in the BLM non-sprayed treatments at 56 dap. At 84 dap, a significant interaction appeared. The plants of the $P0_T$-treatments had always significantly higher weights than the ones in the P1- and P2-treatments.

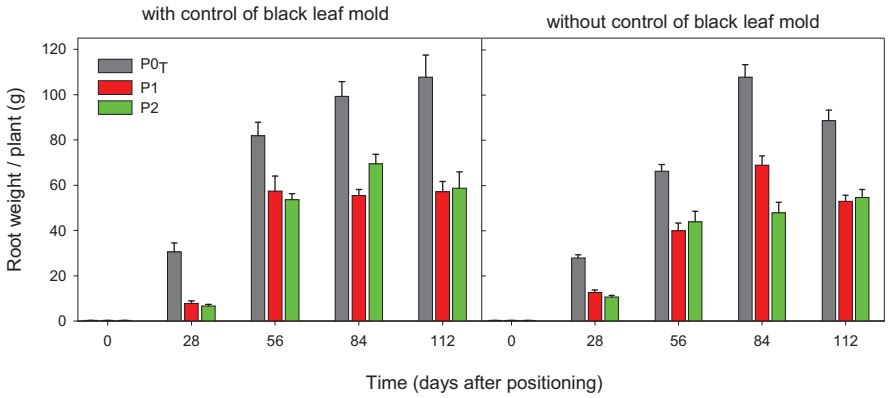
Experiment 1: *October 2002 – January 2003*



Experiment 2: *May – August 2003*



Experiment 3: *October 2003 – February 2004*



Experiment 4: *November 2003 – March 2004*

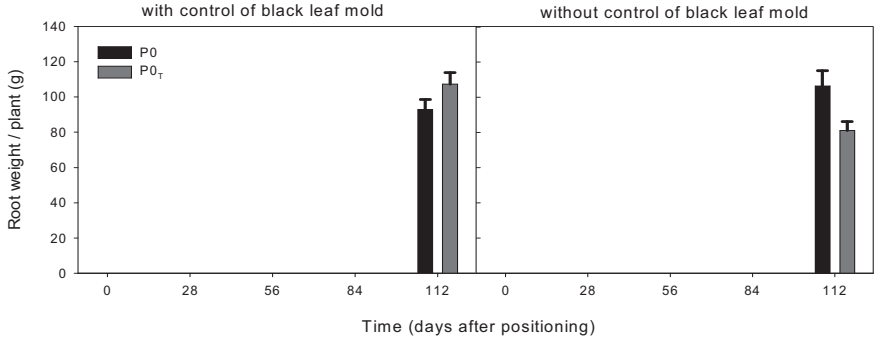


Figure 4.12: Root fresh weight of plants (± SE) (g) in four experiments with different *Pythium*-treatments: P0– non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1 - low level and P2 - high level of *Pythium*. P0*-, P1*- and P2*-treatments refer to another inoculation method (see 4.3.3.3). Sample sizes: experiments 1 and 2 n=12, experiment 3 n=9 and experiment 4 n=15. Because of cultural practice, the 10 lowest leaves were removed in experiments 3 (at 84 dap) and 4 (after 77 dap).

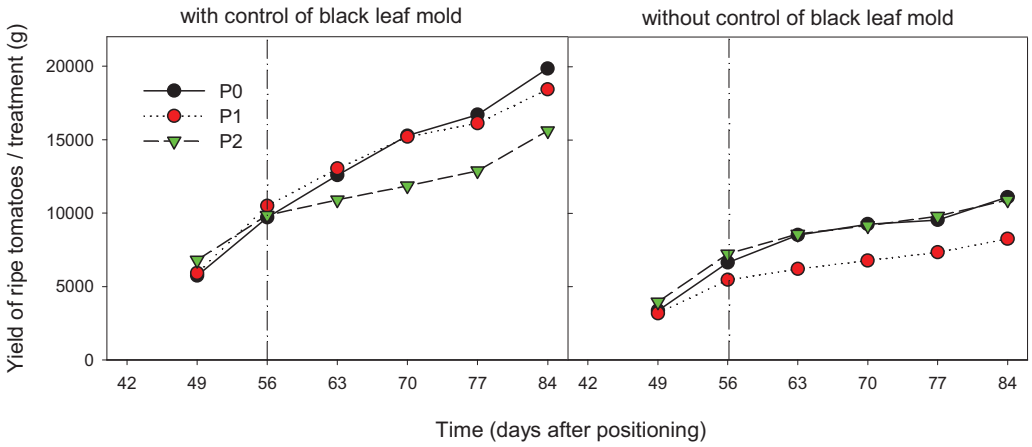
At the end of experiment 4 (112 dap), no significant difference was detectable, but again a significant interaction appeared. The maximum root weight reached up to 107.31 ± 6.95 g in the $P0_T$-BLM0-treatment. In P0-BLM0, root weight was 92.79 ± 6.95 g. In the P0-BLM1-treatment, the weight was 102.19 ± 6.95 g and the $P0_T$-BLM1-treatment 77.80 ± 6.95 g.

4.4.3.5 Fruit weight

For the results of yield, it should be mentioned that expected marketable yield of 100 g per fruit was rare.

The fruit weights were taken continuously while the fruits were yielding on the tomato plants. In experiment 1, the ripening of fruits started at 49 dap (Figure 4.13).

a)



b)

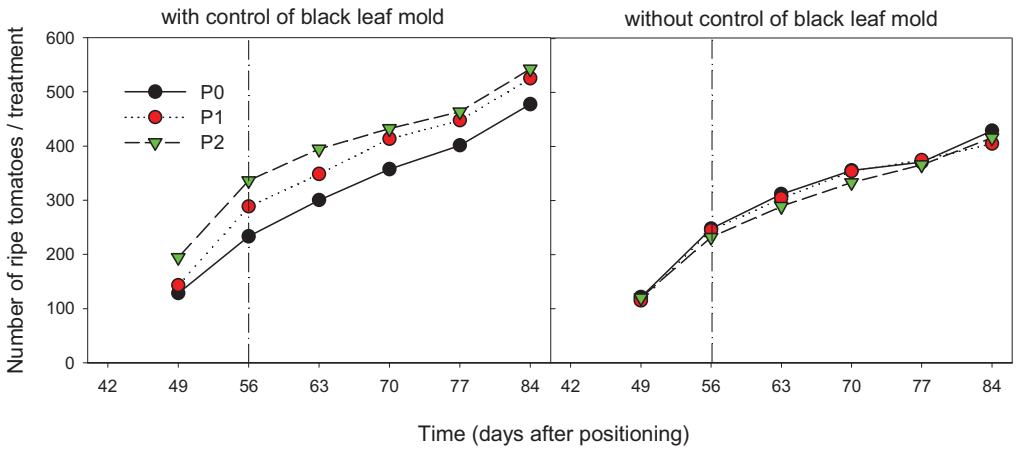


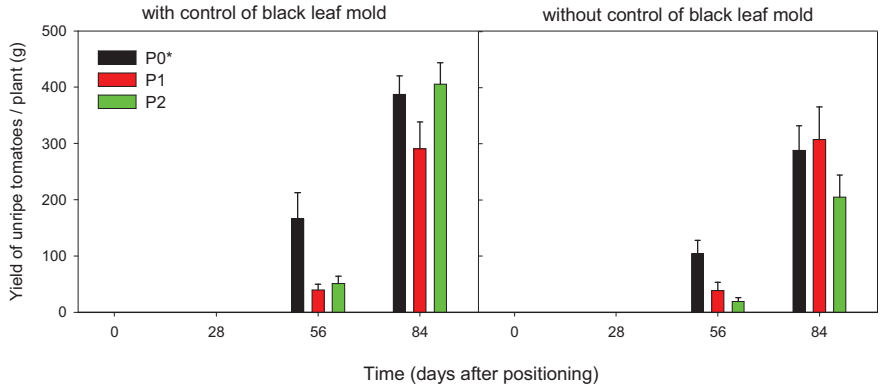
Figure 4.13: a) Accumulated yield of ripe tomatoes per treatment (g) and b) accumulated number of ripe fruits per plant in experiment 1 with different *Pythium*-treatments: P0– non-inoculated, P1 - low level and P2 - high level of *Pythium.* Variety: ´New King Kong´. Vertical lines show the times when part of the plants were removed, resulting in a reduced sample size.

The plants of the BLM0-treatment had a significant higher amount of ripe tomatoes compared to the BLM1-treamtent at 84 dap.

The P0-BLM0-treatment had the highest accumulated yield of ripe tomatoes (altogether 19834 g) as well as the highest average total fruit weight per plant (262.58 ± 48.62 g), with an average fruit weight of 41.48 g, at 84 dap. The lowest weight per plant was found in the P2-BLM1-treatment (101.04 ± 26.27 g) with an average fruit weight of 22.21 g. The number of fruits harvested from plants in the BLM0-treatments was significantly higher (around 500) compared to the number of ripe tomatoes in the BLM1-treatments (around 400).

In experiment 2, conducted with the tomato variety ´King Kong 2´, the plants started yielding at 56 dap. The harvested unripe fruits at 56 dap and 84 dap are shown in Figure 4.14a. At 56 dap, the yields in the P1*- and P2*-treatments were significantly reduced compared to the P0*-treatments. At 84 dap a significant interaction appeared.

a)



b)

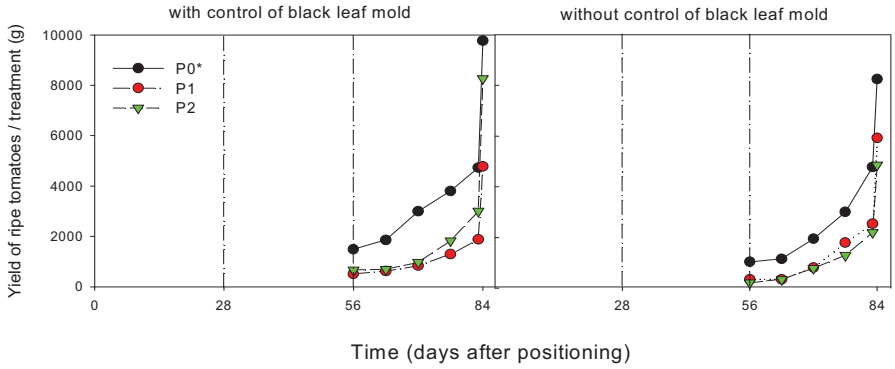
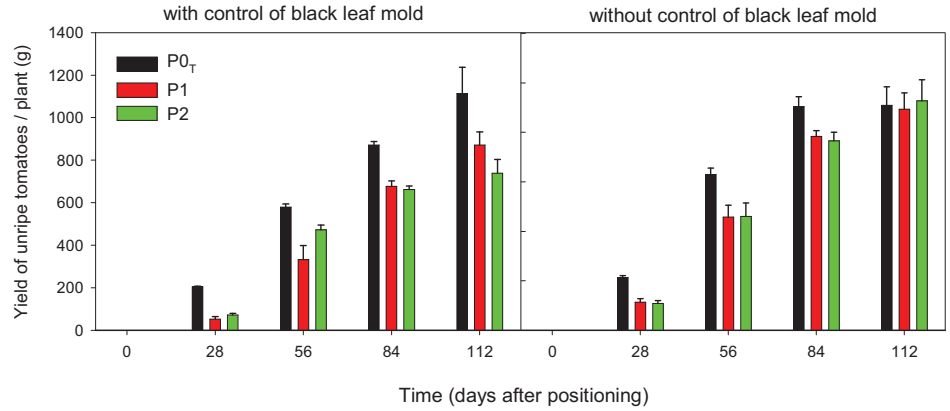


Figure 4.14: a) Average yield of unripe tomatoes per plant (± SE) (g) and b) accumulated yield of ripe tomatoes per treatment in experiment 2 with different *Pythium* treatments: P0- non-inoculated, $P0_T$ – no *Pythium* with *T. harzianum*, P1 - low level and P2 - high level of *Pythium*. P0*-, P1*- and P2*-treatments refer to another inoculation method (see 4.3.3.3), n=12.

The accumulated values are given in Figure 4.14b. The plants in the P0*-treatments had the highest yield, e.g. in P0*-BLM0 altogether 9763.85 g were harvested, leading to an average of 813.65 g yield per plant. Yields in the P1*- and P2*-treatments were lower and did not show a homogenous trend.

In experiment 3, the yield of green tomatoes started at 28 dap (Figure 4.15a). At 28, 56 and 84 dap the plants inoculated with *P. aphanidermatum* produced less yield than the plants of the $P0_T$-treatment. The accumulated yield of ripe tomatoes started again at 56 dap (Figure 4.15b). The yield of the plants in the $P0_T$-BLM0-treatment reached 25 kg, in P2-BLM0 only 19.6 kg. No significant differences were observable

a)



b)

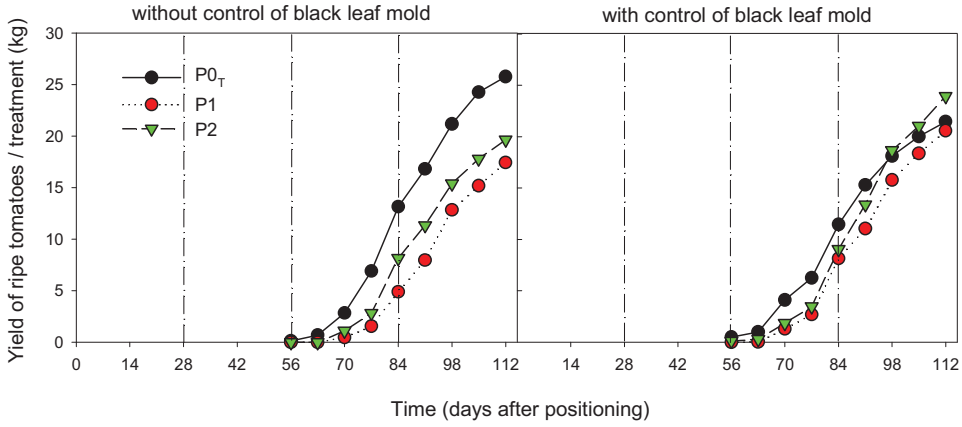
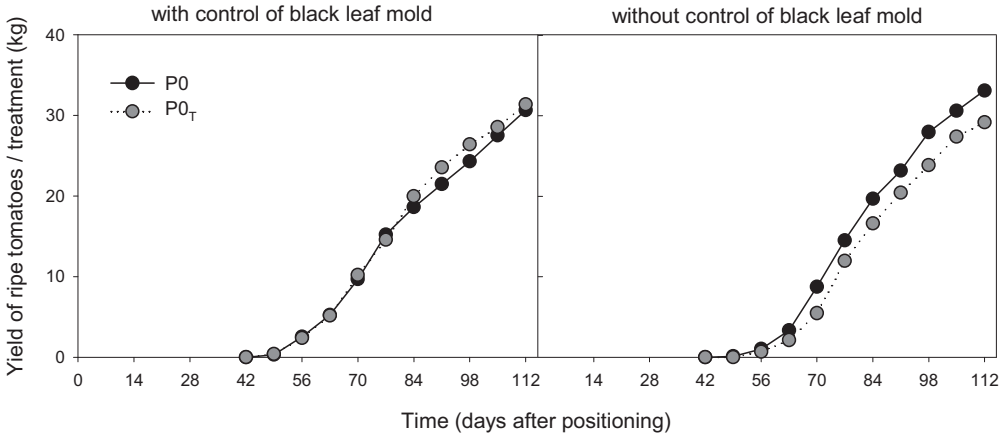


Figure 4.15: a) Yield of tomato plants, average yield of unripe tomatoes per plant (± SE) (g) and b) accumulated yield of ripe tomatoes per treatment (kg) of experiment 3, with different *Pythium*-treatments: P0- non-inoculated, $P0_T$ - no *Pythium* with *T. harzianum*, P1- low level and P2- high level of *Pythium*. Vertical line shows the times, when the sample size was reduced from 27 to 18 and to 9 plants.

In experiment 4, no data of unripe tomatoes were taken. The average fruit weight of ripe tomatoes per treatment increased up to 30 kg per treatment (Figure 4.16a), that corresponded

to approximately 2 kg per plant. The average fruit weight per plant (Figure 4.16b) was up to 400 g in the treatment without *T. harzianum*.

a)



b)

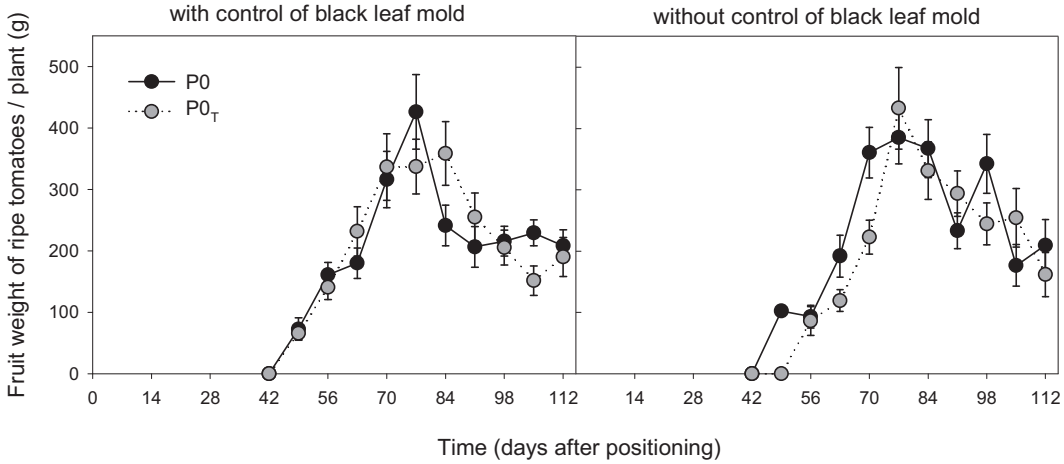


Figure 4.16: a) Accumulated yield of ripe tomatoes per treatment and b) Yield (± SE) of ripe tomatoes per plant (g) of experiment 4, with different *Pythium* treatments: P0- non-inoculated, $P0_T$- no *Pythium* with *T. harzianum* (n=15).

4.5 Discussion

In our investigations, we studied plant growth parameters and yield affected by the two diseases, BLM and PRR, occurring simultaneously on tomato under greenhouse conditions in Thailand. As the experiments were carried out at different times of the year, the plant growth parameters cannot be compared easily among the experiments.

4.5.1 Observation of Pythium root rot

The aim of our study was to observe interactions of both pathogens infecting the same tomato plant. However, when the plants were already heavily damaged or even killed by PRR, it was not possible to look at the additional effects caused by BLM. The most critical point in the experiments was therefore to choose an inoculum level of *P. aphanidermatum* that affected the plants but also allowed to observe symptoms of BLM. In an additional experiment not reported here, all plants inoculated with *P. aphanidermatum* died within 14 dap, because the inoculum levels chosen were too high. To avoid this problem of dropping out of plants due to PRR, we inoculated much more plants than necessary and used them to replace heavily affected plants by those plants treated in the same way but expressing the disease only weakly. In experiment 2, these plants remained in the disease free nursery, in experiment 3 the plants were stored inside the experimental greenhouse close to the sidewalls. From there, the additionally stored plants were placed in the positions of the plants to be substituted. As *P. aphanidermatum* was already inoculated in the substrate in the quantity needed, the progress of PRR was not disturbed. Regarding BLM, it was possible that tomato plants which were replaced had already been infected and that due to the replacement or new positioning in experiment 3, the spatial distribution of the primary occurrence of BLM may be affected. On the other hand, the substitution of plants was restricted to a narrow time frame of 14 days only. Later the epidemics could progress without further disturbance.

An additional problem occurred in experiment 2, when a high number of plants of the P0-treatments dropped out. As the plants of these control treatments, such as P0-BLM0, were raised under special hygienic provisions, any kind of contamination during the experiment could be excluded. However, in the pots with dead plants *P. aphanidermatum* could be re-isolated although no inoculation of this pathogen had taken place. In other samples of the substrate lot, a contamination with *P. aphanidermatum* was observed, too. Although this substrate was ordered from a company that disinfected soil by steaming, the substrate was contaminated with *P. aphanidermatum*.

As other subprojects within the main project were also affected by this contamination and because of the fact that there was no guarantee for a pathogen-free substrate in the future, it was decided to apply the biological antagonist *Trichoderma harzianum* to the substrate of the control treatments. The results in experiments 3 and 4 clearly showed a reduction of dropped out plants.

During the course of the experiments, leaves shed, especially in experiment 3, but also in experiment 2. However, it is difficult to relate the loss of lower leaves to PRR or BLM, because plants of the control treatments also lost leaves. Therefore, the loss could be due to the normal process of aging, i.e. the senescence of the plants.

4.5.2 Plant growth parameters

The parameters investigated were fresh weight, dry weight, leaf area and root weight. Additionally, ripe tomatoes were harvested on a weekly basis. The results of the plant growth parameters were very heterogeneous. If two diseases were affecting the same host plant, it was expected that the host parameters in general were reduced and the sum of losses was higher than the single loss. However, a clear trend was not detectable, for instance for the fresh weight: In the first experiment, BLM and PRR had a significant negative influence at all three sampling dates, resulting in lower weights in the inoculated treatments. In the second experiment at the first sampling date, the occurrence of BLM increased the fresh weight, PRR had no influence, and additionally an interaction appeared. At the second sampling date, no significant differences were detectable, and at the third sampling date, plants of BLM0 had the highest fresh weight, those of P0-treatments the smallest. In the third experiment with four sampling dates, the unsprayed plants, allowing the progress of BLM, had the highest weight, while the inoculation of *Pythium* reduced the fresh weight of the plants in the P1- and P2-treatments. At the first sampling date, a significant interaction appeared and at the end of the experiment no significant differences were detectable.

The dry weight, which was highly correlated with fresh weight, was expected to show the same statistical tendencies found for the fresh weight, but less differences appeared.

The leaf area was mainly influenced by the foliar disease BLM. The indeterminate variety ´New King Kong´ seemed to react to BLM with a reduction of leaf area, the indeterminate variety ´King Kong 2´, on the other hand, reacted with an increasing area. The influence of the *Pythium*-treatments was marginal and only in the beginning of the experiments. The same tendencies were recognisable for the plants of the indeterminate variety which revealed significant interactions in the beginning of the experiment.

The analyses of root weight showed significant reductions only in experiment 3 in the P1-and P2-treatments. The influence of BLM was significant at the end of the experiments. An analysis of plants based on visual monitoring would lead to another impression, because roots from plants of the P2-treatments, for example, had no light cream colour and less fine roots than those of the P0-treatments.

Fruit weight was taken continuously during the yielding of the tomato plants or discretely at the sampling days. The yields in our experiments were lower than the expected values known from literature, maximum yield losses of 25% were measured in the non-sprayed-BLM1-treatments compared to the BLM0-treatments. Our results were in accordance with those of Kleinhenz et al. (2006), who confirmed that most fruits produced were non-marketable with a fruit fresh weight averaging 32 g $fruit^{-1}$.

The experiments showed mainly negative interactions (in 65 %) in relation to yield, less positive interactions (in 7.5 %), and additive (in 10%). To re-capitulate, if the damage caused by two concurrently occurring diseases is similar to the sum of damages caused by the diseases attacking the host separately, the effect is additive; if the damage is less, there is a negative interaction; if it is greater, a positive interaction exists (Waller and Bridge, 1984; Bassanezi et al., 1998).

BLM and PRR showed a negative interaction with respect to the fresh weight, besides the normal significant influences. For example, in the first experiment at 84 dap, plants in treatment P0-BLM0 had a fresh weight of 1030 ± 70 g, in the P2-BLM0 treatment (i.e. inoculated with high level of *P. aphanidermatum*, but fungicide sprayed against BLM) the weight was reduced by roughly 340 g compared to the control plants. The plants of the P0-BLM1 treatment in which only BLM developed symptoms, had a value of 660 ± 73 g, thus a difference of 370 g compared to the control plants. If the interaction would be additive, the plants of P2-BLM1 treatment should have a value of approximately 320 g. As the actual value was 545 ± 73 g, the outcome showed a negative interaction of the two diseases with respect to the combined loss.

Additionally we found in our results some unexpected interactions. In 17.5 % of all the analyzed results across the experiments, the effects caused by two concurrently occurring diseases canceled out each other and the interaction was conducive to the host growth, a kind of supplementary interaction. For example, at 112 dap in the third experiment, plants in the $P0_T$-BLM0-treatment had a leaf area of 6282 ± 810 cm^2.while the leaf area was reduced by roughly 1370 cm^2 in the P2-BLM0 treatment. The plants of the $P0_T$-BLM1-treatments had a

value of 5671 ± 810 cm^2, thus a difference of 610 cm^2 compared to the control plants. The plants in the P2-BLM1-treatments, however, had a final leaf area of 6810 ± 810 cm^2, a plus of roughly 530 cm^2.

As mentioned before, many plants dropped out in the beginning of the second experiment, so that in the third experiment *T. harzianum* was inoculated as prevention. Due to the lack of comparable results for *T. harzianum* under greenhouse conditions in Thailand, the fourth experiment was conducted, to compare plants without any treatment with those plants inoculated with *T. harzianum*. The trials on tomato plants in greenhouses at the AIT were the first in the central region in Thailand. *T. harzianum* had a very positive influence on the plants in the third and fourth experiment leading to only 1 and none dead plant, respectively. However, no conclusion could be drawn if there was an effect of *Trichoderma* on the plant even if no *Pythium* was inoculated.
In the fourth experiment, the inoculated $P0_T$-BLM1-treatment had the highest fresh weight, the biggest leaf area, and the smallest root weight. As biological control agent against PRR, *T. harzianum* showed positive effects.

Heine (2005) also conducted experiments under greenhouse conditions in Thailand on tomatoes with the pathogen *P. aphanidermatum*. He showed that the root fresh weights of plants, inoculated with *P. aphanidermatum* and non-inoculated, did not differ. In our results, we found significant differences between the root weights of the P0- and P1/P2-treatments; BLM had a significant negative influence on root growth.

In general, the reduction of plant vitality in our four experiments could be explained by a decreased ability of the plants to absorb nutrients and water due to partial destruction of the root system by PRR and decreased root activity in the early stage of plant growth (van Noordwijk and de Willingen, 1997). As a general rule Calvert (1957) mentioned that stress during the early stage of tomato development was correlated with the subsequent growth of the plant.

Even though Mersha (2008) showed that the occurrence of BLM could be reduced by changing the greenhouse net or by using special cooling systems, the disease always occurred. In the future, BLM will become a more and more relevant disease in- and outside the greenhouse. The favourable climatic conditions will be widespread, not only inside

greenhouses but also outside, due to climatic change. Recently, Halfeld-Vieira et al. (2006) reported already that BLM, usually uncommon in Brazil, caused a severe foliar blight epidemic under protected cultivation. Thus it can be expected that BLM will become more important and that it will interact with other diseases. Therefore these investigations on disease interactions were only the onset demanding for further research on this aspect.

5 General Discussion

As many details of this study are already discussed in the individual chapters, we will give only a final short and comprehensive discussion and valuation of the achieved results and of their further application.

It should be taken into account that this study included the first investigations of disease interactions of tomatoes under greenhouse conditions in Thailand. In the beginning of our investigations, we tried to answer the following four main questions:

- What are the most important diseases for a tomato crop under greenhouse conditions in the humid-tropics?
- What kind of disease complex will appear?
- Are there seasonal variations throughout the year?
- What type of interactions between diseases will occur?

A literature review identified two tomato diseases of probably high importance in Thailand: early blight (*Alternaria solani*), a disease favoured by warm, humid conditions, as the main foliar disease, and *P. aphanidermatum* as pathogen for the major soil-borne disease. In subsequent field surveys conducted throughout the country, early blight (EB) was confirmed as one of the main diseases on tomatoes in the field. The surveys also showed that the winter (cool / dry) season is the favoured period for tomato production in Thailand. Therefore the first experiments in the greenhouse were conducted in the time when the local farmers were growing tomatoes in the field, November to March. Later the experiments were expanded throughout the year.

Subsequently, experiments in the greenhouse and under laboratory conditions were started, but after artificial inoculation no further disease cycle of EB was observable, because the temperature was too high (above 35°C) in the greenhouse. Even by scratching the leaf surface to facilitate the penetration of the pathogen, no further symptoms appeared. On the other hand, Mersha (2008) reported in his work that EB occurred in the greenhouse at the same site during the cool season plantings in October and November, but that it was only sporadic for the first 3 to 6 weeks after transplanting.

In the second experiment, a leaf disease not expected occurred naturally, namely black leaf mold (BLM), caused by *Pseudocercospora fuligena*. As this disease was frequently observed in all other greenhouses of the AIT research site, BLM was considered as the most important foliar disease under the greenhouse conditions in this part of Thailand.

Soil-borne diseases should be of minor importance in production systems using soil substrates because of the usually practised disinfection or solarisation. In the fields, *P. aphanidermatum*

was identified as a pathogen frequently affecting tomato production. Our tests under controlled laboratory conditions showed that this pathogen could be successfully inoculated and re-isolated under the conditions in our greenhouses. In fact, the conditions for *P. aphanidermatum* were so favourable that it was a problem to choose the right inoculum level, needed to affect the plants but not to kill them quickly. For instance, the plants of the first experiment started wilting so early, that this experiment was aborted already after 56 dai, although it was planned for 84 dai. The choice of *P. aphanidermatum* as relevant pathogen was confirmed in experiment 2 which by chance was carried out with a commercial substrate heavily contaminated by this pathogen.

Thus the first conclusion was that BLM but not EB was the most relevant foliar disease under the given conditions and that *P. aphanidermatum*, causing Pythium root rot (PRR) on transplanted plants, could be an important soil-borne pathogen, especially when plants are grown in contaminated substrate not completely disinfected. Thus also an answer to the second question can be given: BLM and PRR can form a relevant disease complex to be investigated in more details.

Prior to the study of the disease complex, intensive investigations of the temporal progress and the spatial distribution of BLM were accomplished at the AIT research site. BLM occurred for the first time in 2001 in the greenhouses of the AIT campus, but the origin of the inoculum could not be identified. Later, BLM was prevalent in the greenhouses throughout the year without any artificial inoculation. This was in contrast to the results of Mersha (2008), who figured out that there was a seasonal variation of BLM. The natural infection pressure of *P. fuligena* was so high that one side of the greenhouse has to be sprayed on a regular basis to keep BLM under a certain limit. It was not possible to eliminate BLM totally, but with fungicide sprays the disease severity could be held below 15% in the first experiment and below 3% in the three other experiments. Plants in the non-controlled part of the greenhouse reached approximately 30% disease severity in experiment 1, 10% in experiments 2 and 3 and 15% in experiment 4. Mersha (2008) reported a maximum disease severity of individual plants in a range from 4% to 41% during periods with lower infection pressure, but from 68% to 81% during the peak seasons. These results support those of Wang et al. (1995) who showed that BLM severity ranged from 10 to 40% for individual plants of resistant parents and 50 to 90% for plants of susceptible parents in their screening trials. Hartman et al. (1991) similarly reported a disease severity of 87% on a plant basis after artificial inoculations and exposing the plants for two days to high RH.

To compare disease progress curves, the area under disease progress curves was chosen (AUDPC) as an alternative method to fitting growth models. This procedure usually applied when observed disease progression cannot be described by simple growth functions (Shaner and Finney, 1977; Campbell and Madden 1990, Xu, 2006).

The AUDPC values of disease severity in our experiments showed a significant reduction in the sprayed compared to the non-sprayed treatments. For example in experiment 1 in which the BLM0-treatments had an average AUDPC of 300%-days and the BLM1-treatments showed an average of 700%-days.

With respect to the spatial distribution, joint-count analyses, a more general method than the ordinary runs test (Madden et al., 2007), were applied to determine the pattern of diseased plants within the rows. In experiments 1 and 3, the spatial distribution of diseased plants within rows was aggregated, while in experiments 2 and 4 a random pattern was observed. In experiment 2, initially many experimental plants were removed and replaced because they were infected by *P. aphanidermatum* from the contaminated substrate. This may have influenced the disease pattern of BLM. On the other hand, at some dates the number of diseased plants was rather small compared to the total number, a fact that should be taken into account when the results showing aggregation were interpreted. In some of our cases, for instance, aggregation was detected, because of the relatively unlike situation that out of a few plants, two neighbouring plants were diseased. The further analyses showed that no gradients appeared which would allow concluding about the way used by the pathogen to invade the greenhouses. So far the inoculum source of *P. fuligena* in- and outside the greenhouses could not be identified. It could be plant debris from the campus, infected tomatoes sold at the nearby vegetable market or alternative host plants.

In addition to the horizontal BLM distribution within rows, the vertical distribution of BLM on tomato plants was analysed showing that nearly one third of the leaves (from the top) stayed symptom free. This might be due to the fact that new leaves continuously develop, which stayed symptomless. Mersha (2008) showed the same tendencies in his work. *P. fuligena* preferred fully expanded younger leaves for infection as compared to the older leaves, considering the real age of leaves across plant strata. On plants artificially inoculated, the lower leaves close to the pot and the leaves on the top stayed relatively disease free. In spite of the cumulative trend towards the middle part of the canopy, spore landing and infection started randomly from about the 5th to 10th leaf (counted from bottom to top).

The other disease of the complex, Pythium root rot, could be a severe soil-borne disease under the given conditions. PRR, in literature also named damping off, was more relevant in the beginning of the experiments (up to 14 dap), when a huge amount (up to 60%) of plants dropped out. These plants were replaced because interactions between both diseases could be monitored only on living plants. After replacing the highly affected plants, the drop out became less. Thus with this kind of manipulation, the hazardous disease is getting moderate allowing the progress of the foliar disease. Heine (2005) pointed out that the damage remained at a low level when the host plant had survived the initial stage, and that the further impact on plant growth parameters is low.

P. aphanidermatum was inoculated in two different levels. In addition, two different inoculum methods were applied, one using directly the mycelium and the other based on a maize mix stock culture, but the overall trend did not significantly differ. To determine the concentration needed was difficult in both methods. The inoculation density should give us a hint about the existing inoculum amount in the substrate and should reveal mistakes of too strong inoculation before the experiment started.

Possible ways to determine the inoculum concentration could be to weigh the mycelium used or to enumerate the oospores of the inoculum solution under the microscope. The problem of the first method is that mycelium includes not only hyphae and spores but also propagation material which then is also weighed. With the second method, the inoculum density can be determined more accurately than with the mycelium weighing. However, the equation “1 oospore = 1 colony” is not valid because the oospores are only the “collecting box” for the spores (Grosch and Schwarz, 1998). Nevertheless, it is possible to count the single spores after dissolving them from the oospores with chemotactical impulses and isolation from other particles of the sample (Martin, 1992). For future research we recommend an additional counting of the oospores (Grosch and Schwarz, 1998) and re-isolation and enumeration of the pure spores (Moorman, 1996).

To detect the type of interaction between both diseases, plant growth parameters and yield were analysed, but the experimental results were very heterogeneous, showing no clear trend. Both diseases together did not reduce the parameters, e.g. shoot dry weight, root weight, fresh weight, as much as the sum of both diseases acting individually. In 65% of the experiments, the combined loss was smaller than the sum of the single losses. Thus, in most cases the diseases were negatively interacting, but also exceptions occurred.

Fruit weight was taken continuously during the yielding of the tomato plants or discretely at the sampling days. The yields in our experiments were lower than the expected values from

the seed supplier. Maximum yield losses of 25% were measured. Mersha (2008) showed that there was a clear tendency of higher yield during the cool-dry seasons as compared to the hot-wet seasons. He reported yield losses of 31.10% due to BLM, but pointed out that yield loss attributed to BLM might not be as high as those due to other tomato foliar diseases, such as bacterial spot, early and late blight or Septoria leaf spot (Hartman and Wang, 1992). This might be mainly because of the late beginning of epidemics as well as the restriction of BLM to the lower stratum of the canopy.
Our results showed the same tendencies as those of Kleinhenz et al. (2006), who confirmed that most of the fruits produced were non-marketable, with a fruit fresh weight averaging of 32 g fruit^{-1}.

In the second experiment, heavy losses occurred caused by substrate contamination. As the plants of the control treatments were raised under special hygienic provisions, any kind of contamination during the experiment could be excluded. Nevertheless, in the pots with dead plants *P. aphanidermatum* could be re-isolated although no inoculation of this pathogen has taken place. In other samples of the substrate lot, a contamination with *P. aphanidermatum* was observed, too. Although this substrate was ordered from a company that disinfected soil by steaming, the substrate was contaminated with *P. aphanidermatum*. Therefore, it was decided to use the antagonist *T. harzianum* that had positive side effects by increasing several plant growth parameters, like fresh weight and leaf area. Therefore it could be considered as plant strengtheners. In addition, it could be investigated if *T. harzianum* had as well antagonistic effects against BLM and if a disease reduction would be possible by foliar applications.

As there are still a lot of questions open, we strongly emphasize the need for more studies dealing with disease complexes of tomatoes and interactions in- and outside the greenhouses, in order to develop adequate management strategies to control simultaneously occurring diseases.

6 Appendix

	With control of black leaf mold						Without control of black leaf mold					
	Row 1		Row 2		Row 3		Row 4		Row 5		Row 6	
	6 dap	13 dap	6 dap	13 dap	6 dap	13 dap	6 dap	13 dap	6 dap	13 dap	6 dap	13 dap
Harvest 3	1	1	1	0	4	3	3	3	2	2	1	1
	2	2	2	0	2	0	1	0	4	2	3	1
	2	0	2	3	2	0	0	1	4	3	1	0
	1	1	0	0	2	3	0	1	4	3	0	1
Harvest 2	2	2	4	4	0	0	4	2	2	1	0	1
	0	2	2	0	2	1	2	4	1	1	1	1
	5	3	0	0	3	3	0	0	2	1	1	2
	2	0	0	0	3	2	2	1	1	2	1	1
Harvest 1	0	0	4	3	5	1	2	3	0	3	2	0
	0	0	2	3	2	2	1	1	2	3	2	0
	0	0	0	1	2	2	0	1	1	4	3	4
	0	0	0	0	2	2	0	4	1	3	4	0

Figure 6.1: Detailed map of replaced plants in the different levels of PRR (P0/P1/P2) of experiment 2 at 6 dap and 13 dap. Each rectangular represents 5 plants. Colour Code: White:P0 = 1 plain Petri dish per pot; Red:P1 = 1 Petri dish with *P. aphanidermatum* per pot, Green:P2 = 3 Petri dishes with *P. aphanidermatum* per pot.

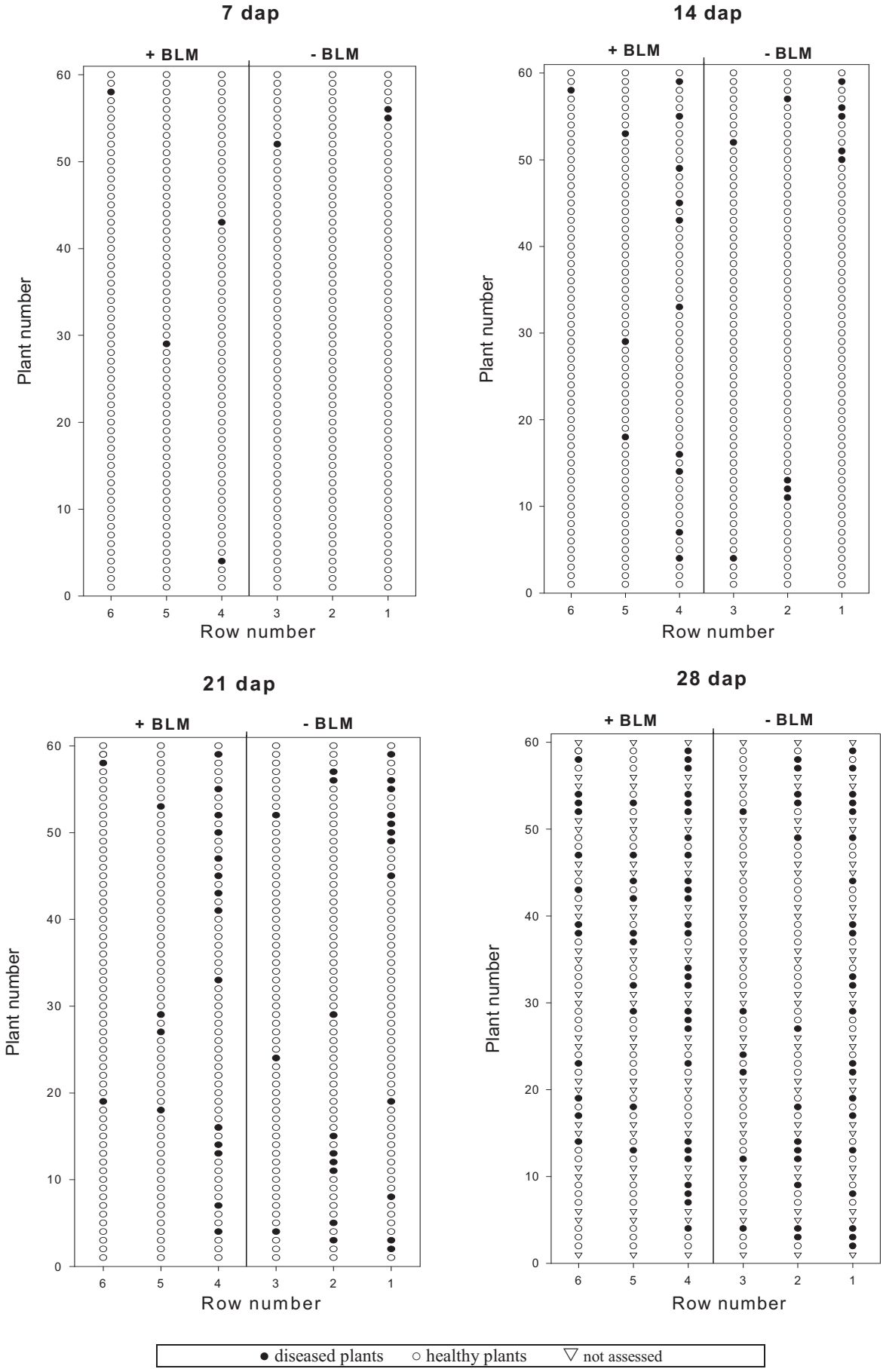


Figure 6.2: Spatial patterns of *Pseudocercospora fuligena* diseased plants in the greenhouse at four disease assessment dates (7, 14, 21 and 28 dap) of the 1st experiment, n=360, variety: ´New King Kong´.

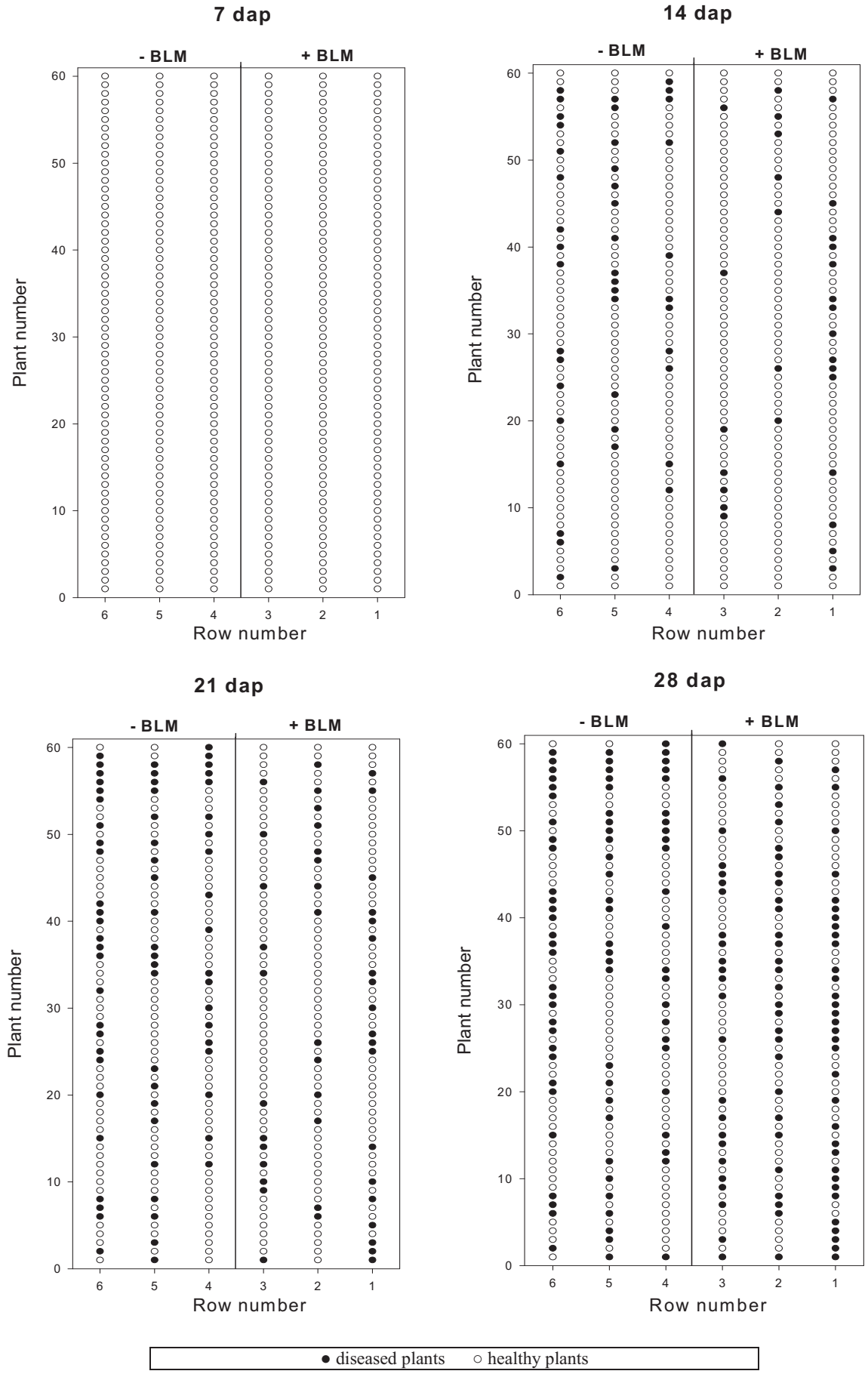


Figure 6.3: Spatial patterns of *Pseudocercospora fuligena* diseased plants in the greenhouse at four disease assessment dates (7, 14, 21 and 28 dap) of the 2nd experiment, n=360, variety: ´King Kong 2´.

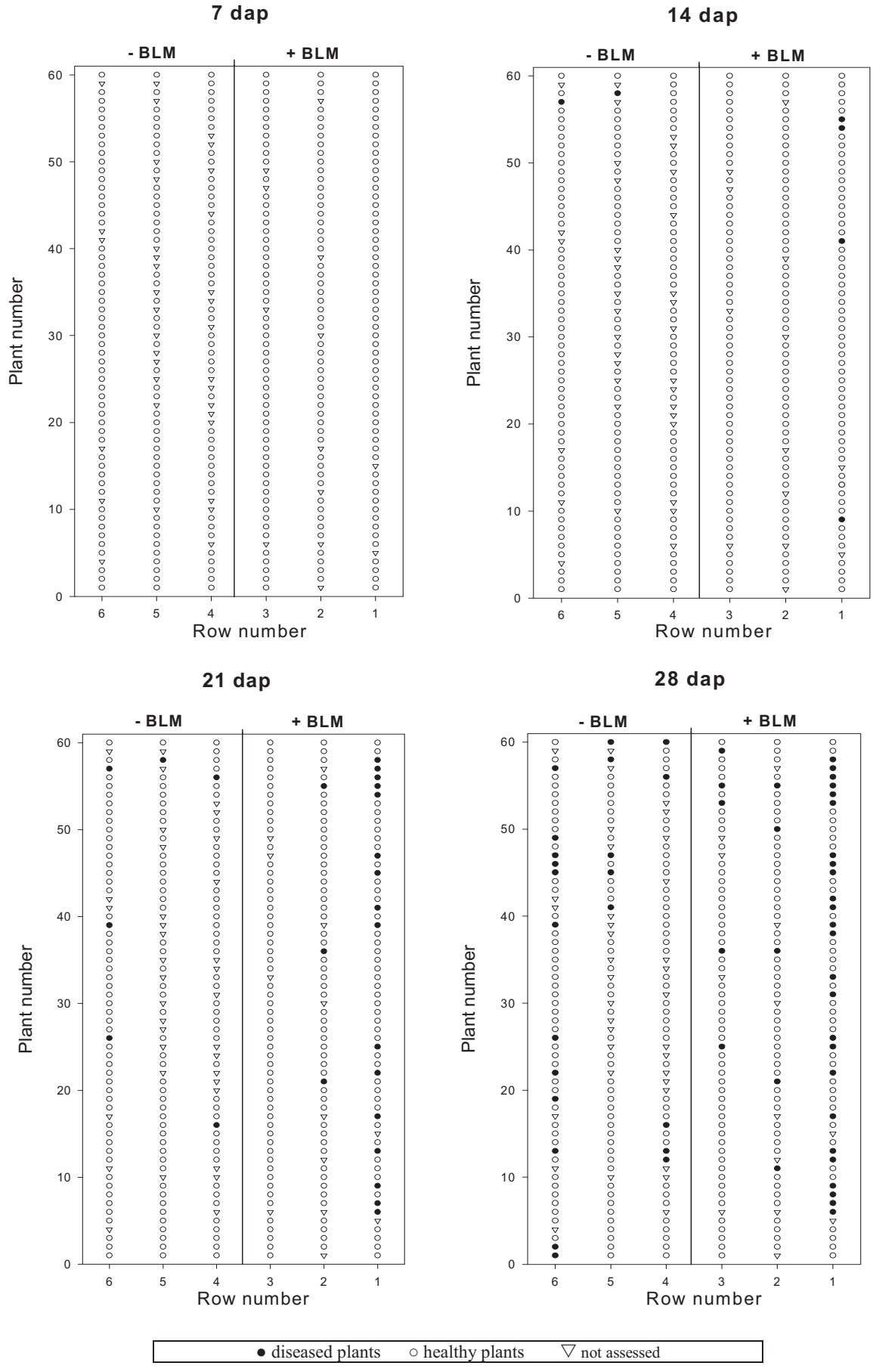
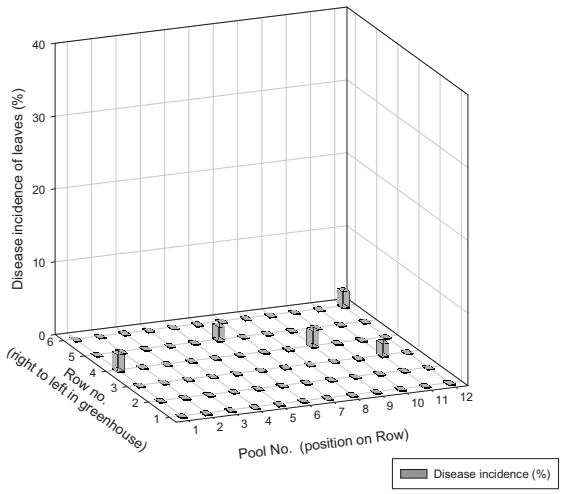
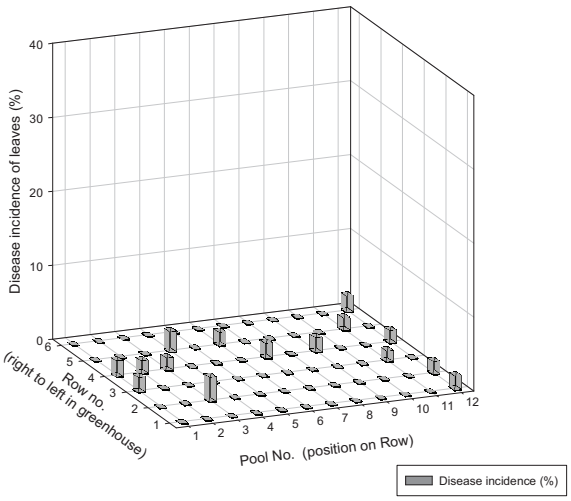


Figure 6.4: Spatial patterns of *Pseudocercospora fuligena* diseased plants in the greenhouse at four disease assessment dates (7, 14, 21 and 28 dap) of the 3rd experiment, n=360, variety: ´King Kong 2´.

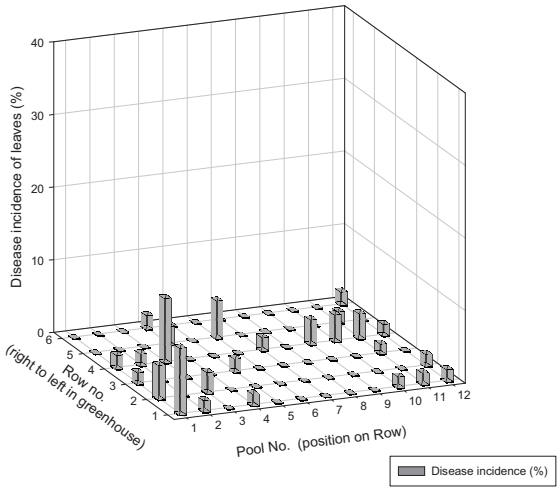
7 dap



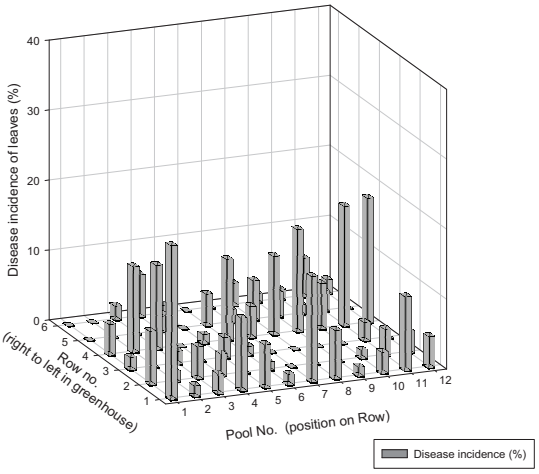
14 dap



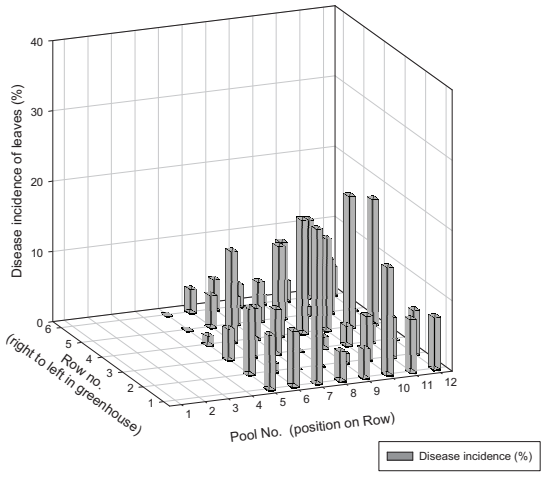
21 dap



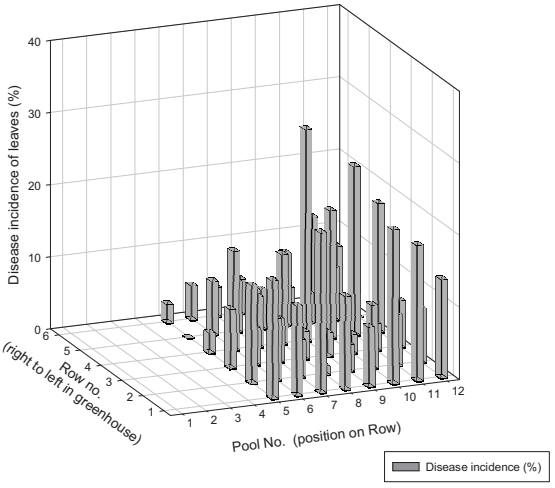
28 dap



35 dap



42 dap



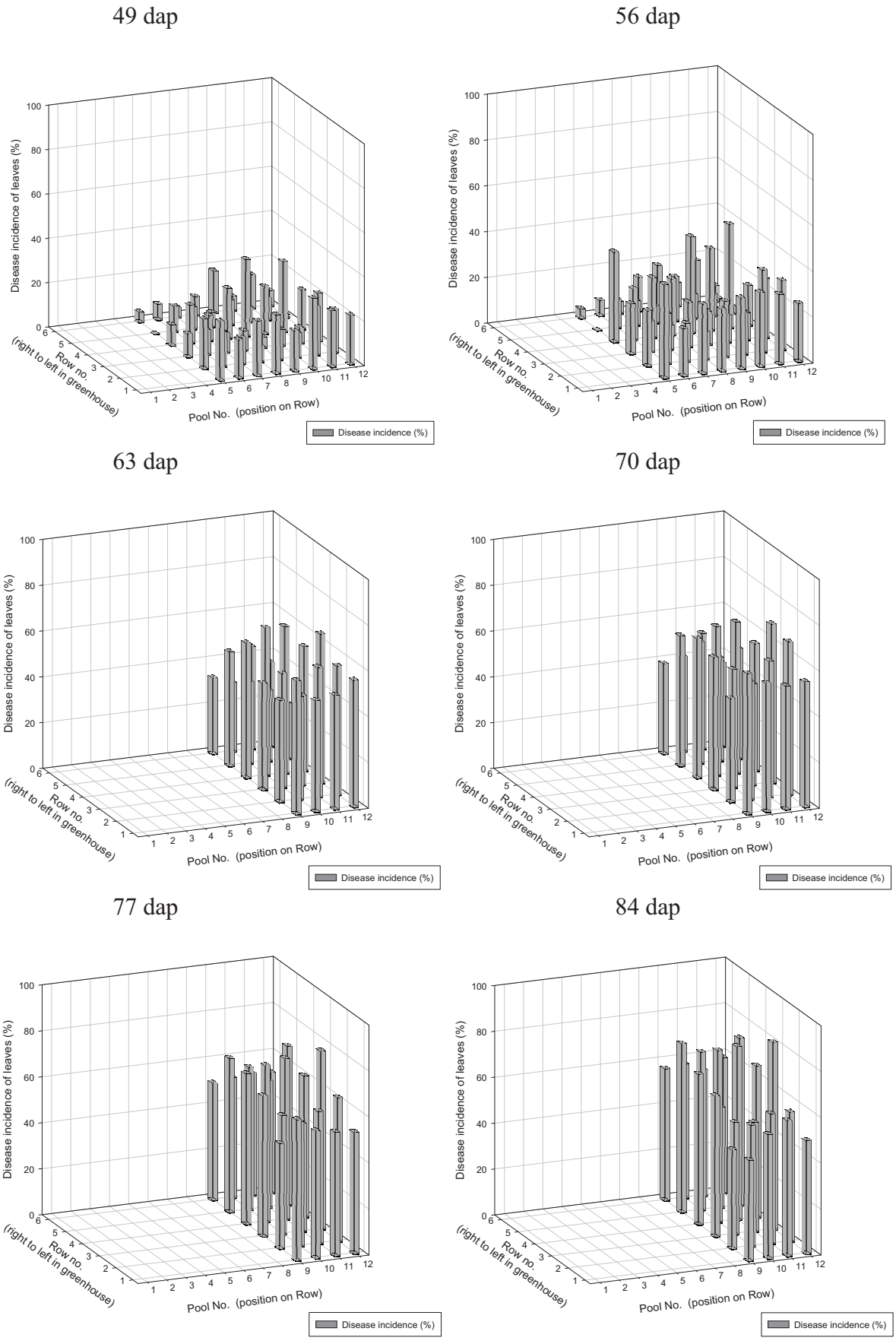
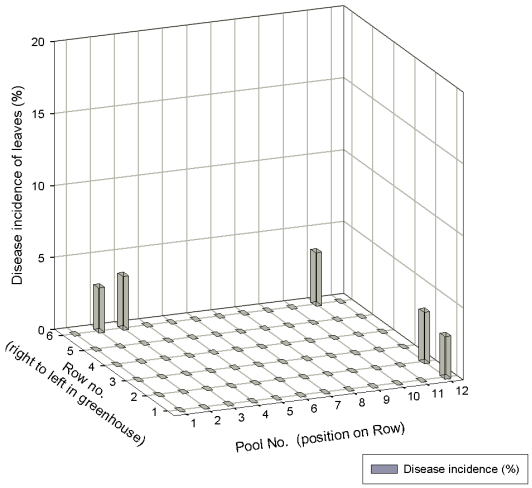
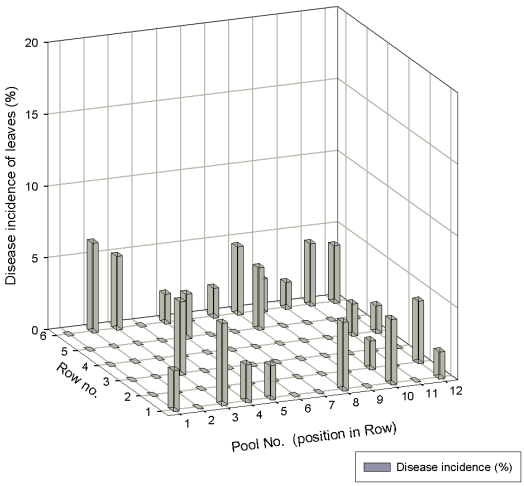


Figure 6.5: BLM incidence of leaves in the greenhouse at 12 assessment days (7 till 84 dap) in experiment 1. Incidence is calculated as the average of three plants.
Notice the change of the disease scale at 49 dap (from 40 to 100%).

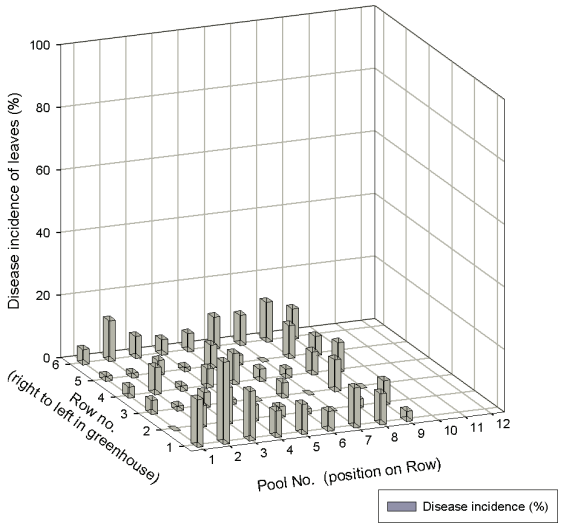
14 dap



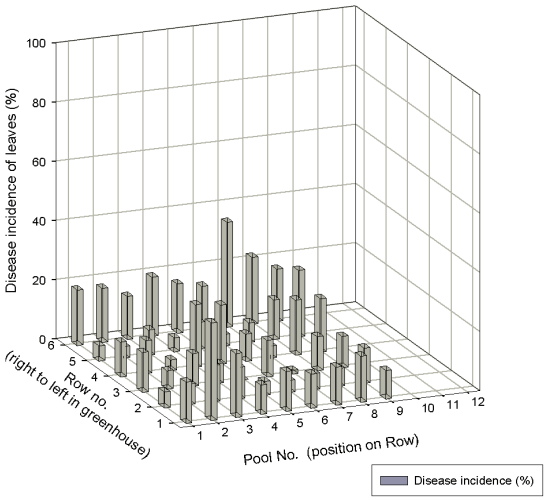
28 dap



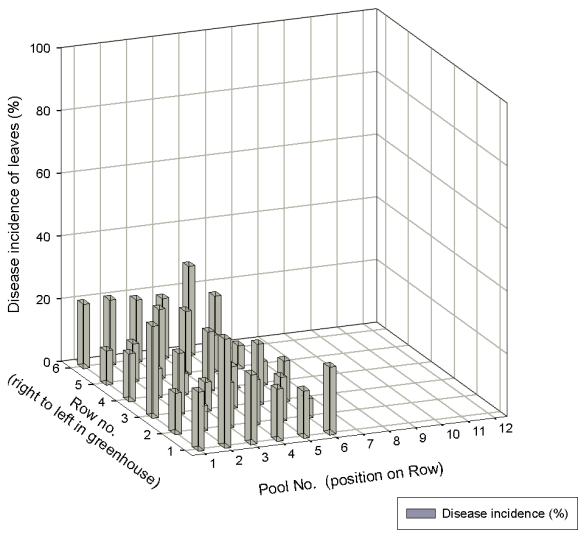
42 dap



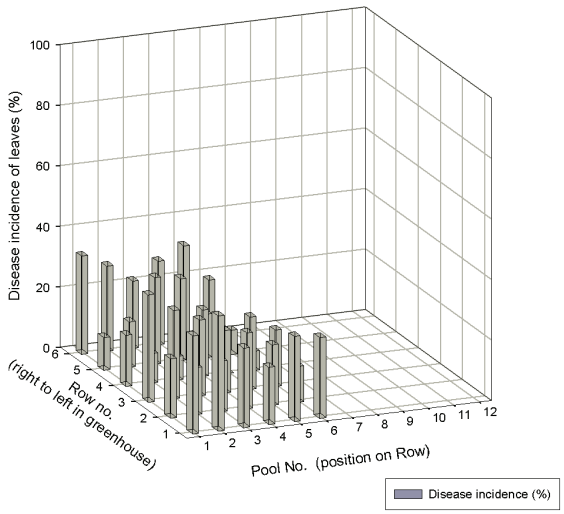
56 dap



70 dap



84 dap



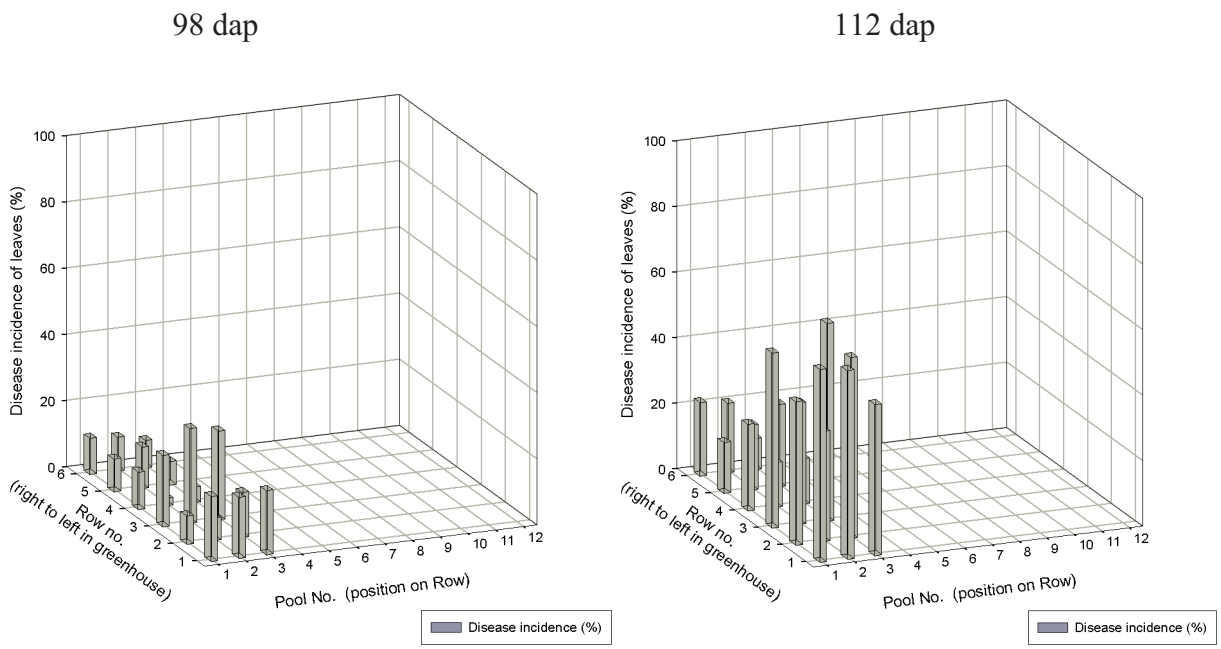


Figure 6.6: BLM incidence of leaves in the greenhouse at 12 assessment days (7 till 84 dap) in experiment 3.
Incidence is calculated as the average of three plants.
Notice the change of the disease scale at 42 dap (from 40 to 100%).

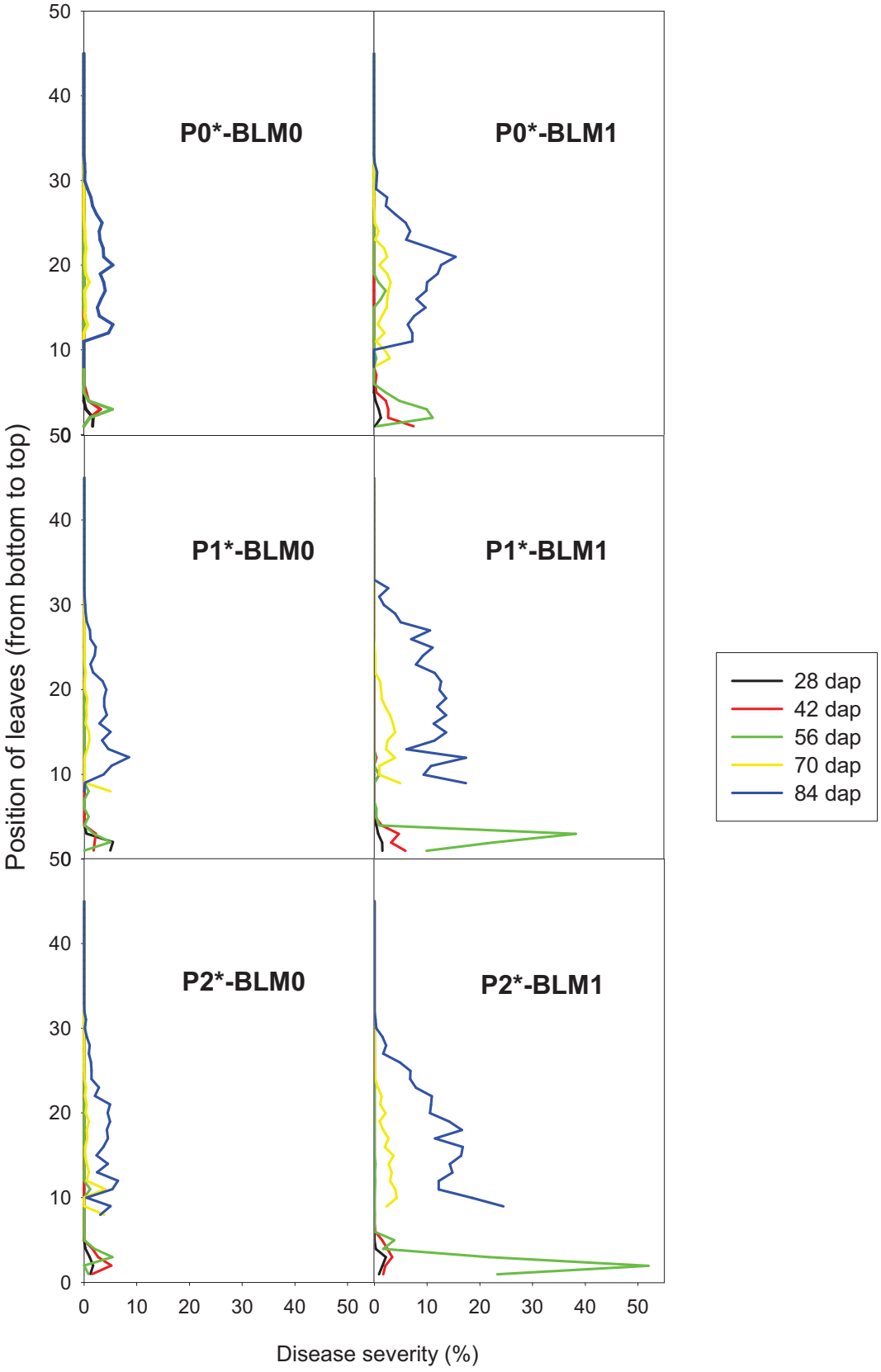


Figure 6.7: Vertical distribution of BLM severity (%) on leaves of tomato plants of the tomato variety 'King Kong 2' at different dap in experiment 2. Treatments: BLM0 – with control of BLM, and BLM1 – without control of BLM; P0- non-inoculated, P1- low level and P2- high level of *P. aphanidermatum*.

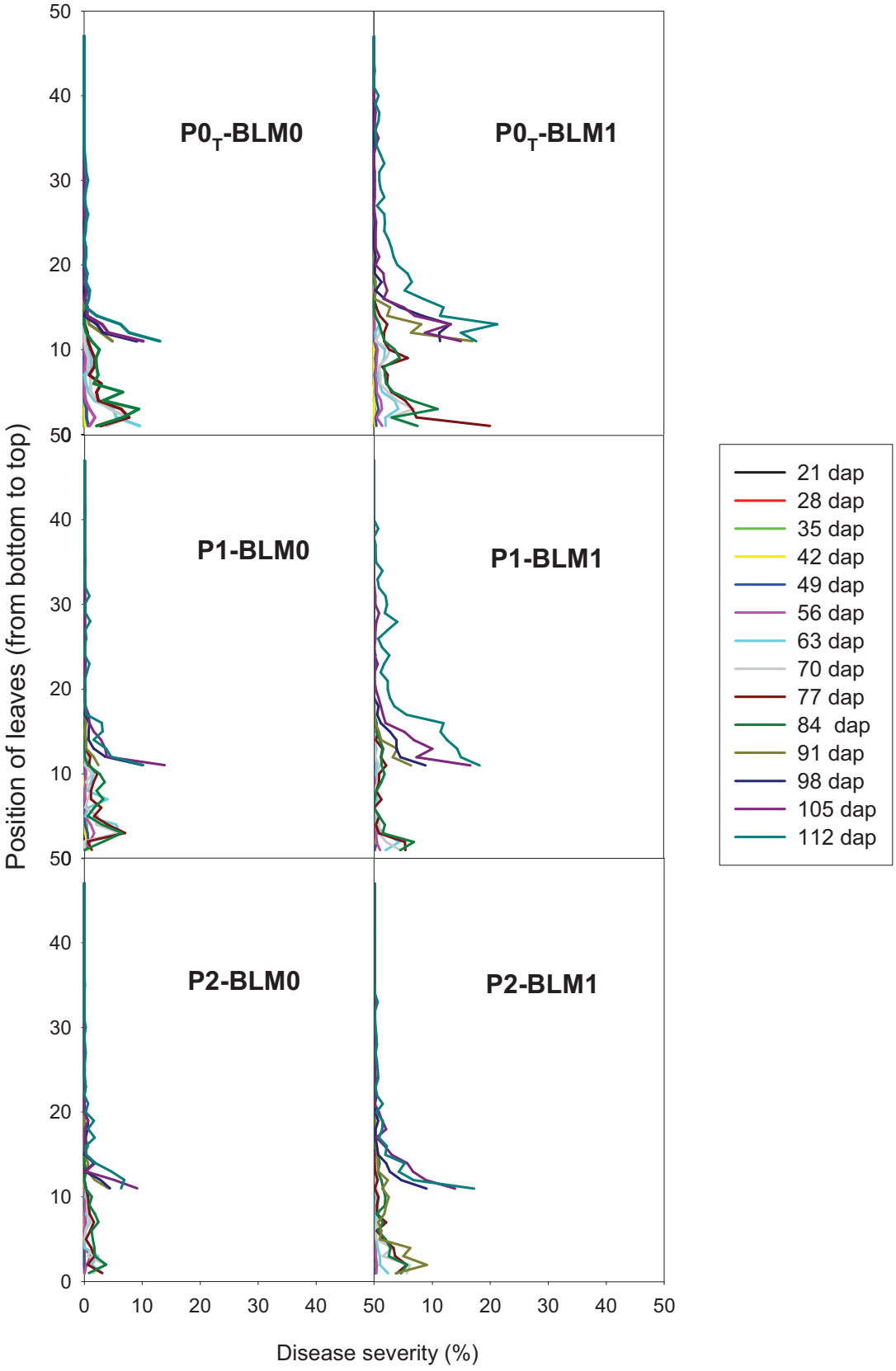


Figure 6.8: Vertical distribution of BLM severity (%) on leaves of tomato plants of the tomato variety 'King Kong 2'at different dap in experiment 3. Treatments: BLM0 – with control of BLM, and BLM1 – without control of BLM; P0- non-inoculated, P1- low level and P2- high level of *P. aphanidermatum*.

Table 6.1: Maximum %-disease severity, per plant, leaf position with the maximum disease severity, highest leaf position insertion with disease symptoms and total number of leaves, for experiment 2

Treatment	Total no. of leaves	Highest leaf insertion with symptoms (position bottom to top)	max disease severity (%, at 112 dap)	Leaf position of max. severity
P0*–BLM0	42.50 (± 0.65)	32	5.53 (± 1.96)	13
P1*–BLM0	42.50 (± 0.77)	31	8.50 (± 3.16)	12
P2*–BLM0	42.91 (± 0.48)	32	5.00 (± 2.65)	9
P0*–BLM1	39.36 (± 0.88)	32	15.50 (± 2.94)	21
P1*–BLM1	40.25 (± 0.53)	32	17.50 (± 8.29)	11
P2*–BLM1	42.66 (± 0.55)	31	24.50 (± 11.72)	11

Table 6.2: Maximum %-disease severity, per plant, leaf position with the maximum disease severity, highest leaf position insertion with disease symptoms and total number of leaves, for experiment 3

Treatment	Total no. of leaves	Highest leaf insertion with symptoms (position bottom to top)	max disease severity (%, at 112 dap)	Leaf position of max. severity
$P0_T$–BLM0	45.77 (± 2.39)	33	13.11 (± 4.04)	11
P1–BLM0	47.88 (± 1.48)	41	7.88 (± 3.20)	11
P2–BLM0	47.33 (± 1.33)	35	6.88 (± 2.12)	12
$P0_T$–BLM1	47.55 (± 1.09)	43	18.88 (± 6.27)	13
P1–BLM1	46.88 (± 0.85)	39	16.22 (± 5.07)	11
P2–BLM1	48.11 (± 0.35)	38	26.66 (± 4.40)	11

Table 6.3 – 6.17

The Data of each experiment were subjected of a two-way analysis of variance (ANOVA) and means separated by LSD ($p < 0.05$). The two factors, investigated, are the influence of PRR within column (characterized with small letters), BLM within rows (marked with capital letters), and Interactions (characterized with *** at the value). Means followed by the same letter are not significantly different ($P = 0.05$).

Table 6.3 Fresh weight, Experiment 1

Experiment 1; 28 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	594.67 ± 43.63	344.88 ± 43.63	a
P1	443.27 ± 43.63	372.51 ± 43.63	a
P2	518.09 ± 43.63	267.68 ± 45.57	a
Statistic for BLM	A	B	

Experiment 1; 56 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	762.24 ± 64.33	755.98 ± 67.19	a
P1	596.95 ± 64.33	490.37 ± 67.19	b
P2	703.18 ± 64.33	470.94 ± 64.33	b
Statistic for BLM	A	B	

Experiment 1; 84 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	1029.71 ± 69.56	660.37 ± 72.66	a
P1	790.30 ± 69.55	509.71 ± 69.56	b
P2	686.94 ± 69.56	545.16 ± 72.66	b
Statistic for BLM	A	B	

Table 6.4 Fresh weight, Experiment 2

Experiment 2; 28 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	185.25 ± 22.87	130.83 ± 22.87	a
P1*	140.33 ± 22.87***	252.00 ± 22.87***	a
P2*	127.25 ± 22.87***	254.67 ± 22.87***	a
Statistic for BLM	B	A	

Experiment 2; 56 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	843.08 ± 65.65	655.92 ± 65.64	a
P1*	856.83 ± 65.64	823.50 ± 65.65	a
P2*	809.09 ± 68.57	890.30 ± 71.93	a
Statistic for BLM	A	A	

Experiment 2; 84 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	1409.50 ± 73.41	1097.18 ± 76.67	b
P1*	1515.83 ± 73.41	1339.83 ± 73.41	a
P2*	1406.25 ± 73.41	1398.25 ± 73.41	a
Statistic for BLM	A	B	

Table 6.5 Fresh weight, Experiment 3

Experiment 3; 28 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	204.01 ± 7.85***	212.99 ± 7.85***	a
P1	71.20 ± 7.85***	127.01 ± 7.85***	b
P2	72.69 ± 7.85***	108.10 ± 7.85***	b
Statistic for BLM	B	A	

Experiment 3; 56 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	579.46 ± 34.79	629.17 ± 34.79	a
P1	427.72 ± 39.45	458.47 ± 34.79	b
P2	471.80 ± 34.79	460.08 ± 34.79	b
Statistic for BLM	B	A	

Experiment 3; 84 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	870.37 ± 27.31	903.15 ± 27.31	a
P1	677.40 ± 27.31	783.73 ± 27.31	b
P2	662.18 ± 27.31	766.07 ± 27.31	b
Statistic for BLM	B	A	

Experiment 3; 112 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	1112.95 ± 62.18	907.99 ± 82.18	a
P1	871.18 ± 82.18	893.66 ± 82.18	a
P2	739.23 ± 82.18	926.53 ± 82.18	a
Statistic for BLM	A	A	

Table 6.6 Fresh weight, Experiment 4

Experiment 4; 112 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for *T. harzianum*
P0	987.89 ± 48.43	1061.38 ± 48.43	a
$P0_T$	1010.11 ± 48.43	1159.88 ± 48.43	a
Statistic for BLM	B	A	

Table 6.7 Dry weight, Experiment 1

Experiment 1; 28 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	82.84 ± 6.63	54.51 ± 6.63	a
P1	63.31 ± 6.63	56.15 ± 6.63	a
P2	73.68 ± 6.63	40.99 ± 6.92	a
Statistic for BLM	A	B	

Experiment 1; 56 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	97.52 ± 9.39	111.65 ± 9.39	a
P1	80.59 ± 9.39	87.20 ± 9.39	a
P2	98.60 ± 9.39	77.07 ± 9.39	a
Statistic for BLM	A	A	

Experiment 1; 84 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	163.33 ± 11.66	123.20 ± 11.66	a
P1	135.17 ± 11.66	93.76 ± 11.66	b
P2	112.13 ± 11.66	101.20 ± 12.18	b
Statistic for BLM	A	B	

Table 6.8 Dry weight, Experiment 2

Experiment 2; 28 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	21.16 ± 2.54	16.33 ± 2.54	a
P1*	16.91 ± 2.54	27.33 ± 2.54	a
P2*	14.33 ± 2.54	17.25 ± 2.54	a
Statistic for BLM	A	A	

Experiment 2; 56 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	122.75 ± 9.00	80.75 ± 9.00***	a
P1*	113.25 ± 9.00	114.09 ± 9.40	a
P2*	106.64 ± 9.04	119.60 ± 9.80	a
Statistic for BLM	A	A	

Experiment 2; 84 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	213.17 ± 8.67	184.45 ± 9.00	b
P1*	231.83 ± 8.67	212.75 ± 8.67	a
P2*	234.17 ± 8.67	210.25 ± 8.67	a
Statistic for BLM	A	B	

Table 6.9 Dry weight, Experiment 3

Experiment 3; 28 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	26.57 ± 0.85	25.01 ± 0.85	a
P1	10.21 ± 0.85	15.88 ± 0.85	b
P2	9.86 ± 0.85	13.53 ± 0.85	b
Statistic for BLM	B	A	

Experiment 3; 56 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	101.11 ± 5.75	97.50 ± 5.75	a
P1	73.95 ± 6.52	72.98 ± 5.75	b
P2	78.73 ± 5.75	73.20 ± 5.75	b
Statistic for BLM	A	A	

Experiment 3; 84 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	157.65 ± 5.77	145.70 ± 5.77	a
P1	123.75 ± 5.77	129.22 ± 5.77	ab
P2	115.09 ± 5.77	124.19 ± 5.77	b
Statistic for BLM	A	A	

Experiment 3; 112 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	186.52 ± 12.64	148.02 ± 12.64	a
P1	156.88 ± 12.64	147.51 ± 12.64	a
P2	135.28 ± 12.64	152.64 ± 12.64	a
Statistic for BLM	A	A	

Table 6.10 Leaf area, Experiment 1

Experiment 1; 28 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
P0	3751.53 ± 288.77	2137.75 ± 288.77	a
P1	3126.84 ± 288.77	2493.08 ± 288.77	a
P2	3325.27 ± 288.77	1771.96 ± 301.61	a
Statistic for BLM	A	B	

Experiment 1; 56 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
P0	3370.73 ± 287.73	2195.65 ± 287.73	a
P1	3217.21 ± 287.73	1951.42 ± 287.73	a
P2	3790.09 ± 287.73	2307.59 ± 300.53	a
Statistic for BLM	A	B	

Experiment 1; 84 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
P0	3447.64 ± 323.17	1101.56 ± 323.17	a
P1	3121.81 ± 323.17	1562.05 ± 323.17	a
P2	3053.62 ± 323.17	1187.25 ± 337.54	a
Statistic for BLM	A	B	

Table 6.11 Leaf area, Experiment 2

Experiment 2; 28 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
P0*	2163.00 ± 22.14	1655.50 ± 22.14***	ab
P1*	1738.50 ± 22.14***	2791.58 ± 22.14***	a
P2*	1561.58 ± 22.14***	1680.25 ± 22.14	b
Statistic for BLM	A	A	

Experiment 2; 56 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
P0*	8330.92 ± 449.99	6281.75 ± 449.99***	a
P1*	8234.00 ± 449.99***	7372.58 ± 449.99	a
P2*	7443.27 ± 470.00	7831.80 ± 492.94	a
Statistic for BLM	A	B	

Experiment 2; 84 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
P0*	9974.58 ± 676.36	8679.18 ± 706.44	a
P1*	9339.58 ± 676.36	10380.00 ± 676.36	a
P2*	10335.00 ± 676.36	9855.00 ± 676.36	a
Statistic for BLM	A	A	

Table 6.12 Leaf area, Experiment 3

Experiment 3; 28 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
$P0_T$	2357.66 ± 112.97	2567.26 ± 112.97	a
P1	982.36 ± 112.97***	1860.12 ± 112.97	b
P2	1095.20 ± 112.97***	1640.02 ± 112.97	b
Statistic for BLM	B	A	

Experiment 3; 56 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
$P0_T$	4763.96 ± 361.47	6138.89 ± 361.47	a
P1	3992.84 ± 409.87	4781.29 ± 361.47	b
P2	4243.74 ± 361.47	5038.82 ± 361.47	b
Statistic for BLM	B	A	

Experiment 3; 84 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
$P0_T$	6021.38 ± 331.29	7718.90 ± 331.29	a
P1	5113.38 ± 331.29	7276.25 ± 331.29	a
P2	5072.88 ± 331.29	7139.61 ± 331.29	a
Statistic for BLM	B	A	

Experiment 3; 112 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for PRR
$P0_T$	6281.78 ± 810.48	5671.40 ± 810.48	a
P1	5714.58 ± 810.48	6488.94 ± 810.48	a
P2	4913.52 ± 810.48	6809.98 ± 810.48	a
Statistic for BLM	A	A	

Table 6.13 Leaf area, Experiment 4

Experiment 4; 112 dap

	BLM0 Mean value ± SE, (cm^2)	BLM1 Mean value ± SE, (cm^2)	Statistic for *T. harzianum*
P0	4940.88 ± 363.67	6332.72 ± 363.67	b
$P0_T$	5186.59 ± 363.67	7607.08 ± 363.67	a
Statistic for BLM	B	A	

Table 6.14 Root weight, Experiment 1

Experiment 1; 28 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	97.67 ± 9.97	69.99 ± 9.97	a
P1	67.06 ± 9.97	74.10 ± 9.97	a
P2	60.40 ± 9.97	67.95 ± 10.42	a
Statistic for BLM	A	A	

Experiment 1; 56 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	95.77 ± 13.75	110.18 ± 13.75	a
P1	89.07 ± 13.75	100.72 ± 13.75	a
P2	90.03 ± 13.75	83.49 ± 13.75	a
Statistic for BLM	A	A	

Experiment 1; 84 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0	148.42 ± 14.47	78.47 ± 14.47	a
P1	124.30 ± 14.47	92.90 ± 14.47	a
P2	97.68 ± 14.47	87.18 ± 15.12	a
Statistic for BLM	A	B	

Table 6.15 Root weight, Experiment 2

Experiment 2; 28 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	20.50 ± 2.39	18.00 ± 2.39	a
P1*	16.00 ± 2.39	23.50 ± 2.39	a
P2*	13.00 ± 2.39	19.66 ± 2.39	a
Statistic for BLM	A	A	

Experiment 2; 56 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	57.23 ± 4.69***	40.18 ± 5.10***	a
P1*	52.75 ± 4.88***	48.00 ± 4.88***	a
P2*	49.00 ± 5.10***	59.30 ± 5.35***	a
Statistic for BLM	A	A	

Experiment 2; 84 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
P0*	64.41 ± 3.16	54.66 ± 3.16	a
P1*	65.58 ± 3.16	56.75 ± 3.16	a
P2*	66.16 ± 3.16	54.08 ± 3.16	a
Statistic for BLM	A	B	

Table 6.16 Root weight, Experiment 3

Experiment 3; 28 dap

	BLM 0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	30.61 ± 1.83	27.90 ± 1.83	a
P1	7.85 ± 1.83	12.70 ± 1.83	b
P2	6.70 ± 1.83	10.62 ± 1.83	b
Statistic for BLM	A	A	

Experiment 3; 56 dap

	BLM 0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	81.83 ± 4.43	66.29 ± 4.43	a
P1	57.37 ± 5.03	39.98 ± 4.43	b
P2	53.55 ± 4.43	43.87 ± 4.43	b
Statistic for BLM	A	B	

Experiment 3; 84 dap

	BLM 0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	99.23 ± 5.42	107.94 ± 5.42	a
P1	55.32 ± 5.43***	69.06 ± 5.42***	b
P2	69.43 ± 5.42***	47.85 ± 5.42***	b
Statistic for BLM	A	A	

Experiment 3; 112 dap

	BLM 0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for PRR
$P0_T$	107.78 ± 6.00	88.67 ± 6.00	a
P1	57.21 ± 6.00	53.05 ± 6.00	b
P2	58.69 ± 6.00	54.78 ± 6.00	b
Statistic for BLM	A	A	

Table 6.17 Root weight, Experiment 4

Experiment 4; 112 dap

	BLM0 Mean value ± SE, (g)	BLM1 Mean value ± SE, (g)	Statistic for *T. harzianum*
P0	92.79 ± 6.95	102.19 ± 6.95	a
$P0_T$	107.31 ± 6.95***	77.80 ± 6.95***	a
Statistic for BLM	A	A	

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