UNIVERSIDADE FEDERAL DO PARANÁ

ALEXANDRE DINNYS ROESE

PLANT DISEASES IN INTEGRATED CROP-LIVESTOCK SYSTEMS IN THE BRAZILIAN SUBTROPICS

CURITIBA

2017

ALEXANDRE DINNYS ROESE

PLANT DISEASES IN INTEGRATED CROP-LIVESTOCK SYSTEMS IN THE BRAZILIAN SUBTROPICS

Thesis presented to the Agronomy Post-Graduate Program, Crop Science Field Study, Department of Crop Protection, *Universidade Federal do Paraná*, as requirement to obtain the title of Doctor in Science.

Advisor: Prof. Dr. Louise Larissa May De Mio

CURITIBA
2017

R718 Roese, Alexandre Dinnys
Plant diseases in integrated crop-livestock systems in the brazilian subtropics / Alexandre Dinnys Roese. Curitiba: 2017.
123 f. il.

Orientadora: Louise Larissa May De Mio
Tese (Doutorado) – Universidade Federal do Paraná.
Setor de Ciências Agrárias. Programa de Pós-Graduação em Agronomia – Produção Vegetal.

1. Biologia do solo. 2. Microrganismos do solo. 3. Mofo. I. May De Mio, Louise Larissa. II. Universidade Federal do Paraná. Setor de Ciências Agrárias. Programa de Pós-Graduação em Agronomia – Produção Vegetal. III. Título.

CDU 631.46(816.2):632.4

UNIVERSIDADE FEDERAL DO PARANÁ
SETOR DE CIÊNCIAS AGRÁRIAS
PROGRAMA DE PÓS-GRADUAÇÃO EM
AGRONOMIA - PRODUÇÃO VEGETAL

PARECER

Os membros da Banca Examinadora designada pelo Colegiado do Programa de Pós-Graduação em Agronomia - Produção Vegetal, reuniram-se para realizar a argüição da Tese de DOUTORADO, apresentada pelo candidato **ALEXANDRE DINNYS ROESE**, sob o título **"PLANT DISEASES IN INTEGRATED CROP-LIVESTOCK SYSTEMS IN THE BRAZILIAN SUBTROPICS"**, para obtenção do grau de Doutor em Ciências do Programa de Pós-Graduação em Agronomia - Produção Vegetal do Setor de Ciências Agrárias da Universidade Federal do Paraná.

Após haver analisado o referido trabalho e argüido o candidato são de parecer pela "**APROVAÇÃO**" da Tese.

Curitiba, 02 de Fevereiro de 2017.

Professor Dr. Cícero Deschamps
Coordenador do Programa

Professora Dra. Tangriani Simioni Assmann
Primeira Examinadora

Dr. Alvaro Figueredo dos Santos
Segundo Examinador

Dr. Wagner Vicente Pereira
Terceiro Examinador

Professor Dr. Paulo Justiniano Ribeiro Junior
Quarto Examinador

Professora Dra. Louise Larissa May De Mio
Presidente da Banca e Orientadora

ACKNOWLEDGEMENTS

To my parents, who opted to have less food on the table for allow their children seek a better life.

To my wife, for all her encouragement and patience.

To Dr. Guilherme Lafourcade Asmus and all team of *Embrapa Agropecuária Oeste* at Dourados - MS, for allow and encourage me to this very important step in my career.

To Professor Louise Larissa May De Mio, for the opportunity, trust and guidance.

To the *Instituto Agronômico do Paraná*, for allow me to access their experimental field at Fazenda Modelo, in Ponta Grossa – PR.

To Dr. Vanderley Porfírio-da-Silva, Dr. Lucilia Maria Parron Vargas and to Namie Takii, of *Embrapa Florestas* at Colombo – PR, for the logistic support and for allow me to participate in their research projects.

To Professors Paulo Justiniano Ribeiro Junior and Walmes Marques Zeviani, for their teaching and support in statistical analysis.

To Dr. Tangriani Simioni Assmann of *Universidade Tecnológica Federal do Paraná* at Pato Branco – PR, Dr. Álvaro Figueredo dos Santos of *Embrapa Florestas* at Colombo – PR, and Dr. Wagner Vicente Pereira of *Universidade Federal do Paraná* at Curitiba – PR, for the relevant suggestions on the manuscript.

To all *LEMID* colleagues, in special to Gloria Soriano Vidal and Erica Camila Zielinski, who more directly contributed in this work.

To all brothers in faith of the Lutheran Church *Cristo Salvador* at Bairro Alto, for making our staying in Curitiba very pleasant.

ABSTRACT

Integrated crop-livestock systems (ICLSs) are efficient forms of agricultural diversification and have attracted research interest in recent years due to the benefits they bring to farmers and to the environment. These benefits are based on a more efficient use of the production resources and exploring benefic properties of the planed intensification in the systems. However, there have been few studies about the influence of different ICLS arrangements in plant diseases and soil microorganisms. Modifications in the microclimate and soil biology, for example, due to the system adopted may influence diseases. This thesis presents results of disease and microorganisms evaluations in a long-term field experiment of ICLSs installed in 2006 in the Region of Campos Gerais of Paraná, Brazil. The experiment has a total of 12 ha in area in an incomplete double factorial. Factor one is production system with three levels that are in order of increasing complexity: not integrated crop (CO), agropastoral (AP) and agrosilvopastoral (ASP) systems. Factor two is nitrogen rate applied on the winter pastures with 90 (in all production systems) and 180 kg/ha (in AP and ASP systems). Results revealed advantages of the ASP system concerned to the suppressiveness to the soil-borne pathogen *Rhizoctonia solani* and also lower density of native *Fusarium* spp. and higher density of native *Trichoderma* spp. in the soil. As the suppressiveness to *R. solani* was transferred by soil samples and eliminated by the soil sterilization, it is related to the soil microbiota. Contrary, powdery mildew (PM) on oats caused by *Blumeria graminis*, and on soybean caused by *Microsphaera diffusa*, was more severe in ASP system than in AP and CO. Severity of PM in ASP was about 20 times and four times, respectively for oats and soybean, more severe than in the others systems. These differences were influenced by the microclimate, mainly the shorter leaf wetness duration and higher daylight relative humidity promoted by trees. The survival period of *Sclerotinia sclerotiorum* sclerotia was lower on the soil surface than buried. Sclerotia survival was lower in the soil surface of AP system than on the CO and ASP. When buried to 8-10 cm depth, the survival period of sclerotia was lower in the ASP system compared to AP. In conclusion, agricultural diversification promoted by ICLSs revealed suppressiveness to soil-borne pathogens, but favored the biotrophic pathogens of the Erysiphaceae family.

Key-words: *Rhizoctonia solani*. *Sclerotinia sclerotiorum*. *Blumeria graminis*. *Microsphaera diffusa*. White mold. Soil suppressiveness.

RESUMO

Sistemas integrados de produção agropecuária (SIPAs) são formas eficientes de diversificação agrícola e tem atraído interesse da pesquisa nos últimos anos devido aos benefícios que trazem para os agricultores e para o ambiente. Estes benefícios estão baseados em um uso mais eficiente dos recursos de produção e exploração de propriedades benéficas da intensificação planejada nos sistemas de produção. No entanto, existem poucos estudos sobre a influência de diferentes arranjos de SIPA em doenças de plantas e microrganismos de solo. Modificações no microclima e na biologia do solo, por exemplo, devido ao sistema adotado podem influenciar as doenças. Esta tese apresenta resultados de avaliações de doenças e patógenos em um experimento de campo de longa duração de SIPAs instalado em 2006 na região dos Campos Gerais do Paraná, Brasil. O experimento tem um total de 12 ha em área em um fatorial duplo incompleto. O fator um é sistema de produção com três níveis que são em ordem de aumento de complexidade: lavoura não integrada (CO), sistema agropastoril (AP) e agrosilvipastoril (ASP). O fator dois é dose de nitrogênio aplicada sobre as pastagens de inverno com 90 (em todos os sistemas de produção) e 180 kg/ha (nos sistemas AP e ASP). Os resultados revelam vantagens do sistema ASP relacionados à supressividade ao patógeno de solo *Rhizoctonia solani* e também menor densidade de *Fusarium* spp. nativo e maior densidade de *Trichoderma* spp. nativo no solo. Como essa supressividde foi transferida por amostras de solo e eliminada com a esterilização do solo, ela está relacionada com a microbiota do solo. Ao contrário, oídio em aveia causado por *Blumeria graminis,* e em soja causado por *Microsphaera diffusa,* foi mais severo no sistema ASP do que no AP e no CO. A severidade de oídio no ASP foi cerca de 20 vezes e quatro vezes maior, respectivamente pra aveia e soja, do que nos outros sistemas. Essas diferenças foram influenciadas pelo microclima, principalmente a menor duração do molhamento foliar e a maior umidade relativa do ar durante o dia promovido pelas árvores. O período de sobrevivência de escleródios de *Sclerotinia sclerotiorum* foi menor na superfície do solo do que enterrados. A sobrevivência de escleródios foi menor na superfície do solo do sistema AP do que no CO e no ASP. Quando enterrados a 8-10 cm de profundidade, o período de sobrevivência dos escleródios foi menor no sistema ASP comparado com o AP. Em conclusão, a diversificação agrícola promovida por SIPAs revelou supressividade a patógenos de solo, mas favoreceu os patógenos biotróficos da família Erysiphaceae.

Palavras-chave: *Rhizoctonia solani*. *Sclerotinia sclerotiorum*. *Blumeria graminis*. *Microsphaera diffusa*. Mofo branco. Supressividade do solo.

SUMMARY

LIST OF FIGURES

LIST OF TABLES

1 GENERAL INTRODUCTION

Integrated crop-livestock systems (ICLSs) are longstanding production systems that include diversified agricultural practices, like crops and animals grazing in the same area (FAO, 2010; MORAES et al., 2014a). These systems are considered the main form of agricultural land use in the world (BELL; MOORE, 2012; HAAN; STEINFELD; BLACKBURN, 1997). They are responsible for more than a half of food production in the world (HERRERO et al., 2010), and considered as a sustainable intensification way to approach the increasing demand for food production with reduced land availability (GODFRAY et al., 2010; THORNTON; HERRERO, 2001).

Studies in ICLSs have been intensified in recent years (CARVALHO et al., 2014) due to its benefits when compared to the monoculture or not integrated crops. The benefits of ICLSs include the increase in the efficiency of land and machinery use, reductions in plant diseases and weed incidence, increased profitability and incomes and greenhouse gas mitigation (ALTIERI, 1999; BELL; MOORE, 2012; CARVALHO et al., 2010; RYSCHAWY et al., 2012).

In the subtropical region of Brazil, ICLSs are characterized by annual rotation of pastures and crops in the same area (MORAES et al., 2014b). In Brazil's tropical region, ICLSs include permanent pastures for two years, rotated with one or two years of soybean or corn in the summer and annual winter pastures (SALTON et al., 2014). Even that trees are encompassed in the definition of ICLS, their inclusion in such production systems is still not common (MORAES et al., 2014a).

Agricultural diversification promoted by ICLS influences the ecosystem properties and pathogen growth can either be facilitated or inhibited, depending on the particular requirements of the organism, so that generalizations about reduction in plant disease are difficult (MATSON, 1997). Little is known about the influence of different arrangements of ICLSs in plant disease occurrence or progress, particularly when the forestry component is added (GAMBLE et al., 2014; SCHROTH et al., 2000).

Disease suppression is frequently associated to soil microbiota (BONILLA et al., 2012). Microorganisms present in a particular soil are not well known and have been underexplored in agriculture (STOCKDALE; BROOKES, 2006; VAN DER HEIJDEN; WAGG, 2013). An exception is the genus *Trichoderma*, reported as an antagonist of several plant pathogens by different mechanisms of action (LORITO et al., 2010).

Soils suppressive to plant pathogens are found throughout the world and can be defined as an inhospitable soil, that limits either the survival or the growth of the pathogens in a general or specific way (GARBEVA; VAN VEEN; VAN ELSAS, 2004). Although the mechanisms by which soil are suppressive to different pathogens can involve biotic and abiotic factors (GHINI; MORANDI, 2006), microorganisms play key roles in suppressing soil-borne pathogens (GARBEVA; VAN VEEN; VAN ELSAS, 2004). Soil suppressiveness to pathogens is a relevant attribute of healthy soils (GARBISU; ALKORTA; EPELDE, 2011; LARKIN, 2015). Research on soil suppressiveness to plant pathogens in production systems that integrate grain production, livestock, and forestry is still limited.

The presence of trees in a production system can also implies in a drastic modification of the microclimate, including light intensity, temperature and relative humidity (RH) and its consequences on dew formation and leaf wetness duration (DIETZ et al., 2007; LUO; GOUDRIAAN, 2000). Leaf wetness promoted by dew is favored by low nocturnal temperature, more common during clear-sky nights (ROWLANDSON et al., 2015). And the RH is higher below the tree canopy than outside then (DIETZ et al., 2007; KOECH; WHITBREAD, 2006).

The microclimate is relevant for powdery mildew (PM) and several microclimatic characteristics have been studied. Particularly, leaf wetness and RH are important components for spore germination and infection. PM is one of the most frequent and important disease for a variety of summer and winter cereals, fruit and vegetable crops (GLAWE, 2008).

White mold is an ancient and important plant disease, caused by *Sclerotinia sclerotiorum*, a cosmopolitan plant pathogen (BOLTON; THOMMA; NELSON, 2006), hosted by more than 500 species of 75 botanical families (SAHARAN; MEHTA, 2008). Sclerotia produced by the pathogen are the central component of epidemics as they are the primary long-term survival structures and survive in the soil for one to eight years, depending on several factors as the soil biology, chemical and physical characteristics, the presence of susceptible plants, depth of sclerotial burial in the soil and soil atmosphere (ADAMS; AYERS, 1979; COLEY-SMITH; COOKE, 1971; REIS; TOMAZINI, 2005; SAHARAN; MEHTA, 2008; WU; SUBBARAO, 2008).

The decline in population of sclerotia in the soil can be predominantly attributed to their germination or decomposition by microorganism, both influenced by physical and chemical characteristics of the soil and atmosphere (MERRIMAN, 1976;

WILLIAMS; WESTERN, 1965). The carpogenic germination of sclerotia is favored after passing through a cold period, near to 10 to 15 °C (COLEY-SMITH; COOKE, 1971) and is stimulated by the light incidence (SUN; YANG, 2000; VENTUROSO et al., 2013).One or more apothecia arise from the sclerotia (GRAU, 1989; LIU; PAUL, 2007). Ascospores germination requires an external nutrient source, typically provided by plant tissue under decomposition, hence the relation between the bloom and the beginning of the epidemic (ABAWI; GROGAN, 1979; DANIELSON; NELSON; HELMS, 2004).

In summary, ICLSs modifies the soil biology and the microclimate, and these modifications can influence plant diseases. For this reason, the objective of this study was to assess the influence of agricultural diversification promoted by ICLSs on plant diseases, using some important plant pathogens and microorganisms as models. In the chapter 3 it was shown the soil suppressiveness to soybean damping-off caused by *R. solani,* the biotic influence on this suppressiveness, and also how ICLSs influence the native *Fusarium* and *Trichoderma* densities in the soil. Chapter 4 explores the PM severity on oats and soybean, related to the microclimatic characteristics of the production systems. And the chapter 5 presents a low survival period of sclerotia of *S. sclerotiorum*, buried and in the soil surface, in ICLSs. Agropastoral (AP) and agrosilvopastoral (ASP) systems were compared to a not integrated crop (CO) in a long term ICLS experiment in the Ponta Grossa municipality of the Campos Gerais region of Paraná State, in the Brazilian subtropics.

2 LITERATURE REVIEW

2.1 AGRICULTURAL DIVERSIFICATION PROMOTED BY INTEGRATED CROP-LIVESTOCK SYSTEMS

Over the decades agriculture have become more specialized and focused on each production component separately, which has caused larger economic scale and resulted in the uncoupling of many crop and livestock production enterprises (TANAKA; KARN; SCHOLLJEGERDES, 2008). Diversity provides the key to overcoming many problems associated with monoculture in cropping systems (ALTIERI, 1999), and one way of increasing diversity in agricultural systems is through integration of crops and livestock - a sustainable intensification way to approach the increasing demand for food production with reduced land availability (GODFRAY et al., 2010; THORNTON; HERRERO, 2001).

The integration of agricultural practices like crops and animals grazing in the same area are longstanding production systems and considered the main form of agricultural land use in the world, occupying a total of about 25 M Km^2 of land (BELL; MOORE, 2012; HAAN; STEINFELD; BLACKBURN, 1997; THORNTON; HERRERO, 2001), and responsible for more than a half of food production in the world, mainly in developing countries (HERRERO et al., 2010). These production systems are included in the definition of Integrated Crop-Livestock System (ICLS), as proposed by FAO (2010) and Moraes et al. (2014a). Studies in this subject have been intensified in recent years (CARVALHO et al., 2014) due to its benefits when compared to the monoculture.

Examples of the benefits that ICLSs can bring to farmers and also to the environment include reductions in costs and risks, increase in the efficiency of land and machinery use, reductions in plant diseases and weed incidence, increased profitability and incomes, increased diversity and greenhouse gas mitigation (ALTIERI, 1999; BELL; MOORE, 2012; CARVALHO et al., 2010; RYSCHAWY et al., 2012). These benefits are referred as emergent properties resulting from the synergy among the soil, plants, animals, and atmosphere (MORAES et al., 2014b) and the soil is the compartment in the system that best expresses these results (CARVALHO et al., 2010). Natural resources are better used in ICLS, where crop residues contribute to the diet for animals, and animal manure provide nutrient inputs to crops, freshwater is more efficiently used and risks are spread across several enterprises, what is

especially important in smallholder systems under certain climate change scenarios (WRIGHT et al., 2012).

ICLSs can assume different arranges depending on the region and on the objectives of the land exploration. In the subtropical region of Brazil, ICLSs are characterized by annual rotation of pastures and crops in the same area, under a direct seeding system. Most common systems comprise rotation or succession of summer crops, such as soybean (*Glycine max*), corn (*Zea mays*), bean (*Phaseolus vulgaris*), or rice (*Oryza sativa*), with annual winter pastures, such as black oat (*Avena strigosa*) and annual ryegrass (*Lolium multiflorum*), alone or mixed (MORAES et al., 2014b). In Brazil's tropical region, the proposed arrangements of ICLSs include permanent pastures (*Brachiaria and Urochloa* genres) for two years, rotated with one or two years of soybean or corn in the summer and *Brachiaria* or black oat in winter (SALTON et al., 2014).

Even that trees are encompassed in the definition of ICLS (CARVALHO et al., 2014; FAO, 2010), their inclusion in such production systems is still not common (MORAES et al., 2014a), probably due to difficulties in the implementation and maintenance of trees. For this reason, little is known about the influence of this component in the production system (GAMBLE et al., 2014; SCHROTH et al., 2000). Competition for resources across the crop-tree interface was observed by Miller and Pallardy (2001). The authors discuss the use of root barriers, especially in regions subject to depletion of soil water. Aboveground, the presence of trees in a production system can implies in microclimatic modifications, including light intensity, temperature and relative humidity and its consequences on dew formation and leaf wetness duration (DIETZ et al., 2007; LUO; GOUDRIAAN, 2000), what can influence plant diseases. Agricultural intensification promoted by ICLS influences the ecosystem properties and, especially about plant diseases, pathogen growth can either be facilitated or inhibited, depending on the particular requirements of the organism, so that generalizations are difficult (MATSON, 1997).

FIGURE 2.1 – AGROSSILVOPASTORAL SYSTEM DURING THE AGROFORESTRY PHASE (UPPER) AND SILVIPASTORAL PHASE (LOWER)

SOURCE: the author (2016).

2.2 SOIL SUPPRESSIVENESS TO PATHOGENS

Alternative methods to chemical means of disease control can decrease dependence on fungicides, reduce costs, and mitigate damage to the environment, especially in the absence of resistant cultivars. One of such alternative methods of disease control is the induced or natural soil suppressiveness to pathogens. Although agricultural diversification promotes changes in the environment, little is known about the influence of different arrangements of ICLSs in soil-borne pathogens and benefic microorganisms, particularly when the forestry component is added.

Soils suppressive to plant pathogens are found throughout the world and can be defined as an inhospitable soil, that limits either the survival or the growth of the pathogens in a general (broad range of pathogens) or specific (only one or a few pathogens) way (GARBEVA; VAN VEEN; VAN ELSAS, 2004). Although the mechanisms by which soil are suppressive to different pathogens can involve biotic and abiotic factors (GHINI; MORANDI, 2006), microorganisms play key roles in suppressing soil-borne pathogens (GARBEVA; VAN VEEN; VAN ELSAS, 2004)

ICLS can increase crop health and productivity (FRANZLUEBBERS, 2007). The biological components of soil are critical to soil health (LARKIN, 2015). Disease suppression is frequently associated to soil microbiota, such as microbial biomass and the abundance of specific microbial groups (BONILLA et al., 2012). Microorganisms present in a particular soil are not well known and have been underexplored in agriculture, even though they are responsible for the function of agricultural and natural ecosystems (STOCKDALE; BROOKES, 2006; VAN DER HEIJDEN; WAGG, 2013). An exception is the genus *Trichoderma*, reported as an antagonist of several plant pathogens (LORITO et al., 2010; VERMA et al., 2007) by different mechanisms of action (DENNIS; WEBSTER, 1971; SIVAN; CHET, 1989) and also involved in promoting plant growth (MARÍN-GUIRAO et al., 2016; PEREIRA et al., 2014) and stress resistance (MASTOURI; BJÖRKMAN; HARMAN, 2010).

As a benefic microorganism, *Trichoderma* is frequently associated with quality or healthy soils (GARBISU; ALKORTA; EPELDE, 2011; LARKIN, 2015; LIU; GLENN; BUCKLEY, 2008) and responsible for natural or induced soil suppressiveness to several pathogens (JANVIER et al., 2007; WELLER et al., 2002), including the well documented suppressiveness to *Rhizoctonia solani* (ANEES et al., 2010; GROSCH et al., 2006; LEWIS; LUMSDEN, 2001; LORITO et al., 2010; PEREIRA et al., 2014).

Soil suppressiveness to pathogens is a relevant attribute of healthy soils (GARBISU; ALKORTA; EPELDE, 2011; VAN BRUGGEN et al., 2015). The terms soil health and soil sustainability indicate the capacity of a soil to function as a vital living system, to sustain biological productivity, promote environmental quality and maintain plant and animal health (DORAN; ZEISS, 2000). And soil organisms are frequently used as indicators of soil quality and health (DORAN; ZEISS, 2000).

2.3 MICROCLIMATE IN AGRICULTURAL DIVERSIFICATION SYSTEMS AND ITS INFLUENCE ON POWDERY MILDEW

Agricultural diversification can lead to environment modifications, which can increase or decrease plant diseases, depending on the particular requirement of the organisms (MATSON, 1997). But there is a lack of studies on the mechanisms governing these effects (MALÉZIEUX et al., 2009), and little is known about the influence of different arrangements of ICLSs in plant disease occurrence or progress, particularly when the forestry component is added (GAMBLE et al., 2014; SCHROTH et al., 2000). The presence of trees in a production system can implies in a drastic modification of the microclimate, including light intensity, temperature and relative humidity (RH) and its consequences on dew formation and leaf wetness duration (DIETZ et al., 2007; LUO; GOUDRIAAN, 2000).

In a meta-analysis to evaluate effects of agroforestry on pest, disease and weed control (PUMARIÑO et al., 2015), only one paper (KOECH; WHITBREAD, 2006) was about disease. This work showed lower bean rust (*Uromyces appendiculatus*) inside an alley cropping than outside them and results were related with microclimate, especially diurnal RH and leaf wetness duration, which were less favorable to the disease inside low spacing alleys.

Powdery mildew (PM) is one of the most frequent and important disease for a variety of summer and winter cereals, fruit and vegetable crops (DEAN et al., 2012; GLAWE, 2008; KANG; MIAN, 2010; TWOMEY et al., 2015). Although the diversity of PM is underestimated in many parts of the world, the geographical distribution of some species appear to be expanding (GLAWE, 2008). This disease is caused by obligate biotroph ascomycetes. This biotrophic lifestyle is also present in very distantly related phytopathogens such as the rust fungi (basidiomycetes) and downy mildews (oomycetes) (KEMEN; JONES, 2012; PANSTRUGA; SPANU, 2014).

The microclimate is relevant for PM and several microclimatic characteristics have been studied. Particularly, leaf wetness and RH are important components for spore germination and infection. Leaf wetness promoted by dew is favored by low nocturnal temperature, more common during clear-sky nights (ROWLANDSON et al., 2015). And the RH is higher below the tree canopy than outside then (DIETZ et al., 2007; KOECH; WHITBREAD, 2006). Leaf wetness occurs predominantly due precipitation/irrigation or dew formation (ROWLANDSON et al., 2015). Since

precipitation does not occur every day but dew is frequent in humid tropical regions (DIETZ et al., 2007), dew plays an important role in the leaf wetness. Experimental and observational results shown that duration and amount of dew is higher on the exposed than on covered surfaces, either in forest (DIETZ et al., 2007) or in cereals (LUO; GOUDRIAAN, 2000).

PMs can be divided in two groups according their ability to germinate on free water: one group with germination in water comparable to that on the leaf surface and the other showing poor germination (SIVAPALAN, 1993). The author also observed that growth and conidia production of *Blumeria graminis* was lower after conidia that originated the pustules were in contact with free water. Earlier results (GRAINGER, 1947; MANNERS; HOSSAIN, 1963) suggest that a saturated atmosphere provides optimum conditions for spore germination in *B. graminis* if care is taken to exclude liquid water. The production of conidia by pustules of *B. graminis* which had been wetted with a few drops of water was reduced less than a half (WARD; MANNERS, 1974). If pustules were kept for a second day after wetting, the sporulation returned to normal, so there seemed to be no permanent damage on germination for wetting. In soybean eight hours of leaf wetness was the ideal period to the progress of PM (*Microsphaera diffusa*) in Brazil (ALVES et al., 2009).

Radiation also promotes great influence on PM. Shaded environment increased severity of *Erysiphe necator* on grapevine and this result was related to the reduction of ultraviolet (UV) radiation and temperature provided by the shadow (AUSTIN; WILCOX, 2012). PM on *Poa* and *Festuca* spp. have been reported with greater severity in shaded environment (SMILEY; DERNOEDEN; CLARKE, 1992). High light intensity reduced germination and hyphal growth of *Sphaerotheca macularis* f. sp. *fragariae* on strawberry (AMSALEM et al., 2006) and shaded environment was more favorable for spore germination and mycelial growth of the *Uncinula necator* (WILLOCQUET et al., 1996). The light spectra has also influence on PM: cucumber plants grown under red light were more resistant to *Sphaerotheca fuliginea* while plants grown under other monochromatic spectra were less resistant than plants grown under white light (WANG et al., 2010).

Although *B. graminis* develops in a wide range of temperature, the optimum temperature for spore germination is near 20 ºC (CHEREWICK, 1944; MANNERS; HOSSAIN, 1963). Better development of *M. diffusa* on soybean plants was observed at temperatures of 23 and 24 ºC in Brazil, depending on the cultivar (ALVES et al.,

2009), in accordance with previous reported (MIGNUCCI, 1989).

FIGURE 2.2 - POWDERY MILDEW CAUSED BY *BLUMERIA GRAMINIS* ON OAT

SOURCE: the author (2016).

FIGURE 2.3 – POWDERY MILDEW CAUSED BY *MICRHOSPHAERA DIFFUSA* ON SOYBEAN

SOURCE: the author (2016).

2.4 SURVIVAL AND GERMINATION OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM*

White mold (WM) is an ancient and important plant disease, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, one of the most devastating and cosmopolitan plant pathogen (BOLTON; THOMMA; NELSON, 2006), hosted by more than 500 species of 75 botanical families, mainly Asteraceae, Fabaceae, Brassiccease and Solanaceae (BOLAND; HALL, 1994; SAHARAN; MEHTA, 2008). The disease results in variable productivity damages that may be as high as 100% (GRAU, 1989; KAMAL et al., 2016; MEYER et al., 2014; WRATHER et al., 2001). WM incidence in soybean is increasing in Brazil since 2008, with an estimation of 23.7% of the cultivated area infested in the 2012/2013 crop season (MEYER et al., 2014).

Sclerotia produced by the pathogen are the central component of epidemics as they are the primary long-term survival structures and survive in the soil for at least one, with references of more than eight years, depending on several factors as the soil biology, chemical and physical characteristics, the presence of susceptible plants, depth of sclerotial burial in the soil and soil atmosphere (ADAMS; AYERS, 1979; BRUSTOLIN et al., 2016; COLEY-SMITH; COOKE, 1971; REIS; TOMAZINI, 2005; SAHARAN; MEHTA, 2008; WU; SUBBARAO, 2008). The production of secondary sclerotia is reported for several authors, but with no significant importance in epidemics (WILLETTS; WONG, 1980; WILLIAMS; WESTERN, 1965). Although better humidity and temperature for sclerotia germination vary depending on the geographical origin of sclerotia, moisture and soil temperature are critical factors in germination and production of apothecia, and this usually occurs after the closing lines of the host culture, when shading contributes to low temperature and high soil moisture (BOLTON; THOMMA; NELSON, 2006; MILA; YANG, 2008; SUN; YANG, 2000).

A sclerotium is a hyphal aggregate with an outer rind of cells containing melanin, a compound that is believed to play an important role in protection from adverse conditions and microbial degradation in many fungi (HENSON; BUTLER; DAY, 1999; SAHARAN; MEHTA, 2008; SHARMA et al., 2015). Infection can occur either by miceliogenic or carpogenic germination of sclerotia, although for *S. sclerotiorum* ascospores released in the air after carpogenic germination is the more important inoculum (ABAWI; GROGAN, 1979). The carpogenic germination is favored

after passing through a cold period, near to 10 to 15 °C (COLEY-SMITH; COOKE, 1971).

Carpogenic germination of *S. sclerotiorum* was not observed in soil depths exceeding 4 cm (WU; SUBBARAO, 2008) and sclerotia survival period was higher when buried to 10 cm depth (36 months) than when left on the soil surface (14 months) in the subtropical region of Brazil (REIS; TOMAZINI, 2005). No differences were observed in sclerotia survival at depths of 5 and 15 cm (ALEXANDER; STEWART, 1994). Sclerotia kept on the soil surface after soybean harvest lost their viability after 12 months in the subtropical region of Brazil, with black oats (*Avena strigosa*) cultivated in the winter (BRUSTOLIN et al., 2016). Twenty-seven percent of sclerotia buried at 15 cm depth were recovered after 11 month, and only 50% of they were viable to germinate miceliogenicaly in a New Zealand horticultural soil (ALEXANDER; STEWART, 1994). With information provided by grower's experience (ADAMS; AYERS, 1979), sclerotia of *S. sclerotiorum* survive in nature for about 4 to 5 years.

The above examples indicates that extrapolations on longevity between different types of sclerotia and soils cannot be made (MERRIMAN, 1976; MERRIMAN et al., 1979). Nevertheless, the rates of sclerotia of *S. sclerotiorum* decline were not different in an survival evaluation carried out in four fields located in two different regions with different annual temperature, rainfall, soil type and crop sequence (BEN-YEPHET; GENIZI; SITI, 1993). In this experiment, smallest sclerotia (near 0.2 mm diameter) were predominant at the end of evaluation time (five or six years, depending on the field), with rates over than 70 %.

The decline in population of sclerotia in the soil can be predominantly attributed to their germination or decomposition by microorganism, both influenced by physical and chemical characteristics of the soil and atmosphere (MERRIMAN, 1976; SHARMA et al., 2015; WILLIAMS; WESTERN, 1965). Germinability of recovered sclerotia was not affected during a seven year evaluation, while the number and size of recovered sclerotia declined (BEN-YEPHET; GENIZI; SITI, 1993). Despite the recovering rates of sclerotia after 11 month varied between 21 to 50 % depending on the depth buried (5 to 15 cm), 98 to 100 % of control sclerotia kept in plastic bags in the dark at room temperature were still viable, showing that degradation of sclerotia in the soil is the main reason for the inoculum reduction (ALEXANDER; STEWART, 1994).

A rapid decline in sclerotinia wilt incidence was noted during a six-year monocroping of sunflower even with the re-infestation of the soil with additional

sclerotia at each seeding period (HUANG; KOZUB, 1991). This result was attributed to the hyperparasitism of biological control agents. Similar results were observed for other plant pathogens like *Gaeumannomyces graminis* (LEBRETON et al., 2004) and *Rhizoctonia solani* (ANEES et al., 2010).

One or more apothecia arise from the sclerotia (GRAU, 1989; HAO; SUBBARAO; DUNIWAY, 2003; KOHN, 1979; LIU; PAUL, 2007). The ascospores are continuously discharged from the asci for about 10 to 15 days in optimal conditions, and one apothecium can produce more than one million ascospores, which can fall in the same crop or be transported by air for several kilometers (CLARKSON et al., 2003; LIU; PAUL, 2007).

Ascospores germination requires an external nutrient source, typically provided by plant tissue under decomposition, hence the relation between the bloom and the beginning of the epidemic, due to senescence parts of flowers that fall on the leaves, petioles and stems of the plants (ABAWI; GROGAN, 1979; DANIELSON; NELSON; HELMS, 2004; GRAU, 1989; MC LEAN, 1958; TURKINGTON; MORRAL, 1993). As the flowering in soybean plants is close to the complete closure of lines and soil shading, the source of nutrient for germination of ascospores is available when conditions are more favorable for carpogenic germination of sclerotia, thus facilitating the occurrence of epidemics (BOLTON; THOMMA; NELSON, 2006).

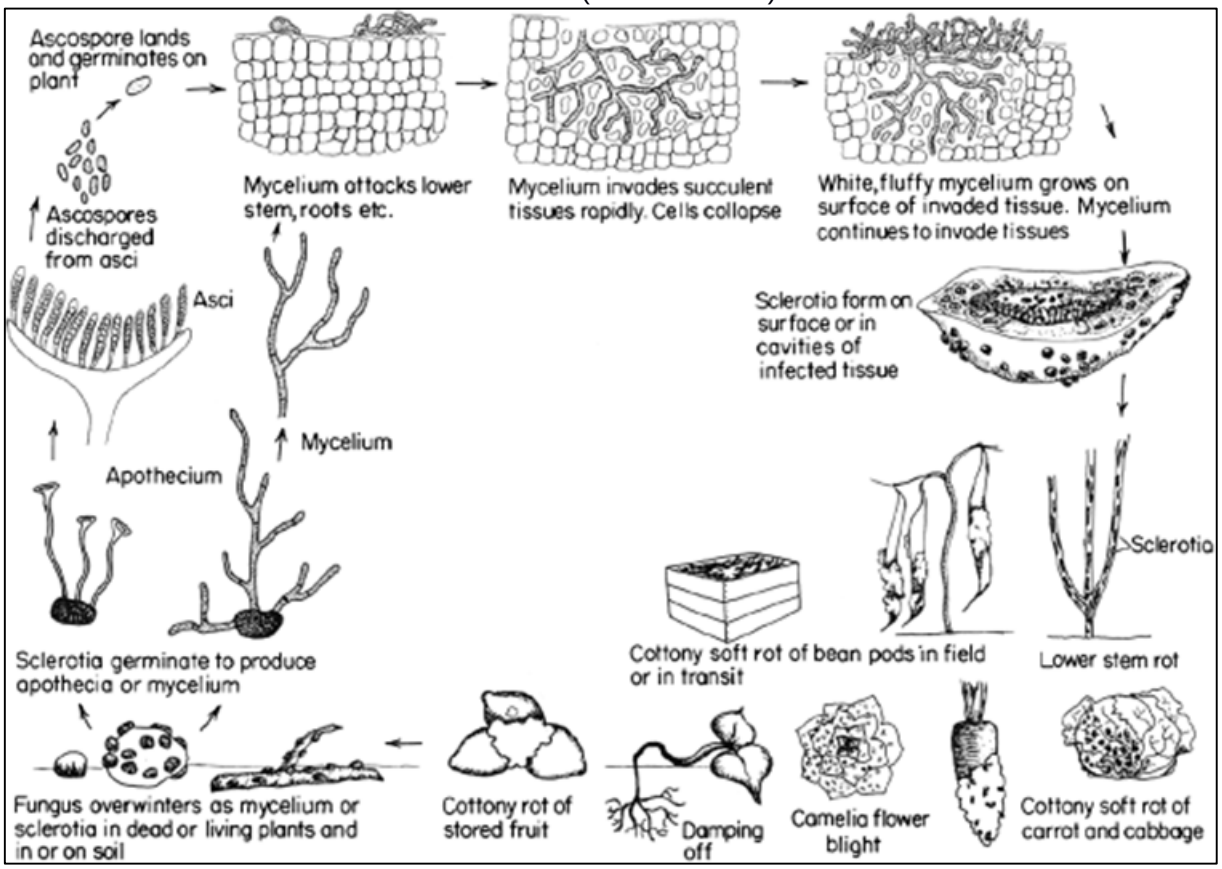
FIGURE 2.4 – SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* SHOWING APOTHECIA

SOURCE: the author (2016).

FIGURE 2.5 – WHITE MOLD (*SCLEROTINIA SCLEROTIORUM*) IN SOYBEAN

SOURCE: the author (2016).

FIGURE 2.6 – WHITE MOLD (*SCLEROTINIA*) DISEASE CICLE



SOURCE: AGRIOS (1997).

3 AGROSILVOPASTORAL SYSTEM IN THE BRAZILIAN SUBTROPICS ENHANCES SUPPRESSIVENESS TO SOYBEAN DAMPING-OFF CAUSED BY *RHIZOCTONIA SOLANI* AND ALTERS *FUSARIUM* AND *TRICHODERMA* DENSITY

ABSTRACT

Integrated Crop-Livestock Systems (ICLSs) are considered efficient forms of agricultural diversification and have attracted research interest in the past few years, but there have been few studies on the influence of different ICLS arrangements, especially considering afforestation, on plant diseases and density of soil microorganisms. This work investigated the influence of two ICLS arrangements in a long-term field experiment: agropastoral (AP) and agrosilvopastoral (ASP), compared to a not integrated crop (CO) on the suppression of post-emergence damping-off caused by *Rhizoctonia solani* and on the density of *Fusarium* and *Trichoderma* propagules in the soil. In the first assay, soil samples were evaluated for damping-off incidence in: a) the fresh soil infested with *R. solani*; b) the sterilized and re-infested soil and c) after substituting 10% of the volume of the sterilized and re-infested soil by fresh soil collected from the same plots in the field. In the second assay, native *Fusarium* and *Trichoderma* propagules were quantified in selective medium. Damping-off incidence in ASP was 0.7X compared to AP and CO. Evaluations with sterilized soil and with transferred soil samples attributed this suppression to biotic factors. *Fusarium* propagules were retrieved in the ASP soil at 0.7X and 0.6X the amounts observed in AP and CO soils, respectively. *Trichoderma* propagules were retrieved in the ASP soil at 1.5X the amounts compared to AP, but at similar amounts compared to CO. The diversification promoted by agrosilvopastoral system in the region of Campos Gerais of Paraná State, Brazil, has the potential to reduce pathogens and enhance beneficial microorganisms in the soil.

key words: Agricultural diversification. Integrated crop-livestock system. Agropastoral system. Agroforestry. Soil health.

RESUMO

Sistemas integrados de produção agropecuária (SIPAs) são considerados formas eficientes de diversificação agrícola e tem atraído interesse da pesquisa nos últimos anos, mas existem poucos estudos sobre a influência de diferentes arranjos de SIPA, especialmente considerando florestamento, em doenças de plantas e densidade de microrganismos do solo. Este trabalho investigou a influência de dois arranjos de SIPA em um experimento de campo de longa duração: agropastoril (AP) e agrosilvipastoril (ASP), comparados com uma lavoura não integrada (CO) na supressão do tombamento de pós-emergência causado por *Rhizoctonia solani* e na densidade de propágulos de *Fusarium* e *Trichoderma* no solo. No primeiro experimento, amostras de solo foram avaliadas quanto à incidência de tombamento em: a) solo fresco infestado com *R. solani*; b) solo esterilizado e reinfestado e c) após substituir 10% do volume do solo esterilizado e reinfestado por solo fresco coletado das mesmas parcelas no campo. No segundo experimento, propágulos de *Fusarium* e *Trichoderma* nativos foram quantificados em meios seletivos. A incidência de tombamento no ASP foi 0,7X comparado com o AP e o CO. Avaliações com solo esterilizado e após a transferência de amostras de solo atribuem essa supressão a fatores bióticos. Propágulos de *Fusarium* foram recuperados do solo do ASP em 0,7X e 0,6X em relação às quantidades recuperadas dos solos do AP e do CO, respectivamente. Propágulos de *Trichoderma* foram recuperados do solo do ASP em 1,5X comprado com o AP, porém em quantidades similares com o CO. A diversificação promovida por sistema agrosilvipastoril na região dos Campos Gerais do Paraná tem potencial para reduzir patógenos e aumentar microrganismos benéficos no solo.

Palavras-chave: Diversificação agrícola. Integração lavoura-pecuária. Sistema agropastoril. Agrofloresta. Saúde do solo.

3.1 INTRODUCTION

Integrated systems of agricultural production, including crops and animals grazing in the same area, are longstanding production systems and considered the main form of agricultural land use in the world (BELL; MOORE, 2012; HAAN; STEINFELD; BLACKBURN, 1997; THORNTON; HERRERO, 2001). These production systems are included in the definition of Integrated Crop-Livestock System (ICLS) by FAO (2010) and Moraes et al. (2014b). Studies in this area have been intensified in recent years due to the benefits that these systems bring to the farmer and to the environment when compared to the monoculture. Examples of the benefits of ICLS include reductions in costs and risks, increase in the efficiency of land and machinery use, increased diversity, greenhouse gas mitigation, reductions in plant diseases and weed incidence, and increased profitability and incomes (ALTIERI, 1999; BELL; MOORE, 2012; CARVALHO et al., 2010; RYSCHAWY et al., 2012). The soil is the compartment in the system that best expresses these results (CARVALHO et al., 2010). These benefits are considered as emergent properties resulting from the synergy among the soil, plants, animals, and atmosphere (MORAES et al., 2014b).

In the subtropical region of Brazil, ICLSs are characterized by annual rotation of pastures and crops in the same area, under a direct seeding system. Most common systems comprise rotation or succession of summer crops, such as soybean (*Glycine max*), corn (*Zea mays*), bean (*Phaseolus vulgaris*), or rice (*Oryza sativa*), with annual winter pastures, such as black oat (*Avena strigosa*) and annual ryegrass (*Lolium multiflorum*), alone or mixed (MORAES et al., 2014b). In Brazil's tropical region, the proposed ICLSs arrangements include permanent pastures (*Brachiaria and Urochloa* genres) for two years, rotated with one or two years of soybean or corn in the summer and *Brachiaria* or black oat in the winter (SALTON et al., 2014). Planned inclusion of tree species in such production systems is still not common (MORAES et al., 2014a), probably due to difficulties in the implementation and maintenance of trees, even though this component is clearly encompassed in the definition of ICLS (CARVALHO et al., 2014; FAO, 2010).

Soybean is a major crop in tropical and subtropical regions, and Brazil is the second largest soybean producer worldwide (FAO, 2014). Diseases, such as damping-off caused by *Rhizoctonia solani* Kühn [teleomorph: *Thanatephorus cucumeris* (A.B.Frank) Donk], cause damage to soybean and several others crops, and the

availability of resistant cultivars is limited. This fungus is found in soils worldwide and is considered a destructive pathogen with a broad host range. It causes diseases on several agronomic crops, ornamentals, and forestry species (GONZÁLEZ GARCÍA; PORTAL ONCO; RUBIO SUSAN, 2006).

Saprophytic and pathogenic populations of the genus *Fusarium* are widespread in the world. Pathogens in this genus are responsible for vascular wilt diseases either in temperate and tropical regions (FINLAY, 2007). *Fusarium* is frequently reported causing soybean diseases and yield reduction in the major soybean producers countries (WRATHER et al., 2010), with emphasis for sudden death (AOKI et al., 2003; FARIAS NETO et al., 2013; WESTPHAL et al., 2014). The genus *Fusarium* is also one of the most important associated with wheat (ZHANG et al., 2012), corn and rice (KIM et al., 2012).

Alternative methods to chemical means of disease control can decrease dependence on fungicides, reduce costs, and mitigate damage to the environment, especially in the absence of resistant cultivars. Such methods usually focus on the host, the environment, or both. Although ICLS promote changes in the environment, little is known about the influence of different arrangements of these production systems in plant disease.

Microclimate also influences naturally occurring microorganisms. Microorganisms present in a particular soil are not well known and have been underexplored in agriculture, even though they are responsible for the function of agricultural and natural ecosystems (STOCKDALE; BROOKES, 2006; VAN DER HEIJDEN; WAGG, 2013). An exception is the genus *Trichoderma*, reported as an antagonist of several pathogens by different mechanisms of action and also involved in promoting plant growth and stress resistance (LORITO et al., 2010; VERMA et al., 2007).

Current research on soil suppressiveness to plant pathogens and microbial density in the soil does not include environments that integrate grain production, livestock and forestry. The current situation indicates a lack of knowledge on how different arrangements of ICLSs influence the soil microbiota. Studies on this subject can guide better management practices in diversified environments.

The objectives of this work were to assess the influence of two ICLS arrangements on: a) the soil suppressiveness to plant pathogens, using the soybean damping-off caused by *R. solani* as an indicator, and b) the microorganisms density in

the soil using the native *Fusarium* and *Trichoderma* propagules as indicators. The integration between crop and livestock (agropastoral system - AP) and integration of crop, livestock, and forestry (agrosilvopastoral system - ASP), with a control system (not integrated crop - CO) were compared in a long term ICLS experiment installed in the Ponta Grossa municipality of the Campos Gerais region of Paraná, Brazil.

3.2 MATERIALS AND METHODS

3.2.1 *Rhizoctonia solani* inoculum

An isolate of *R. solani* anastomosis group 4 was obtained from the Plant Pathology Laboratory of *Embrapa Agropecuária Oeste*. The pathogenicity of the isolate was determined by placing mycelium discs grown for 3 days in contact with the hypocotyls of soybean seedlings grown for 7 days in sterilized soil. After 7 more days, seedling tissues presenting symptoms were transferred to PDA (Potato Dextrose Agar) and the mycelia growth examined by light microscopy, confirming the growth of *R. solani* by the morphological characteristics of the hyphae.

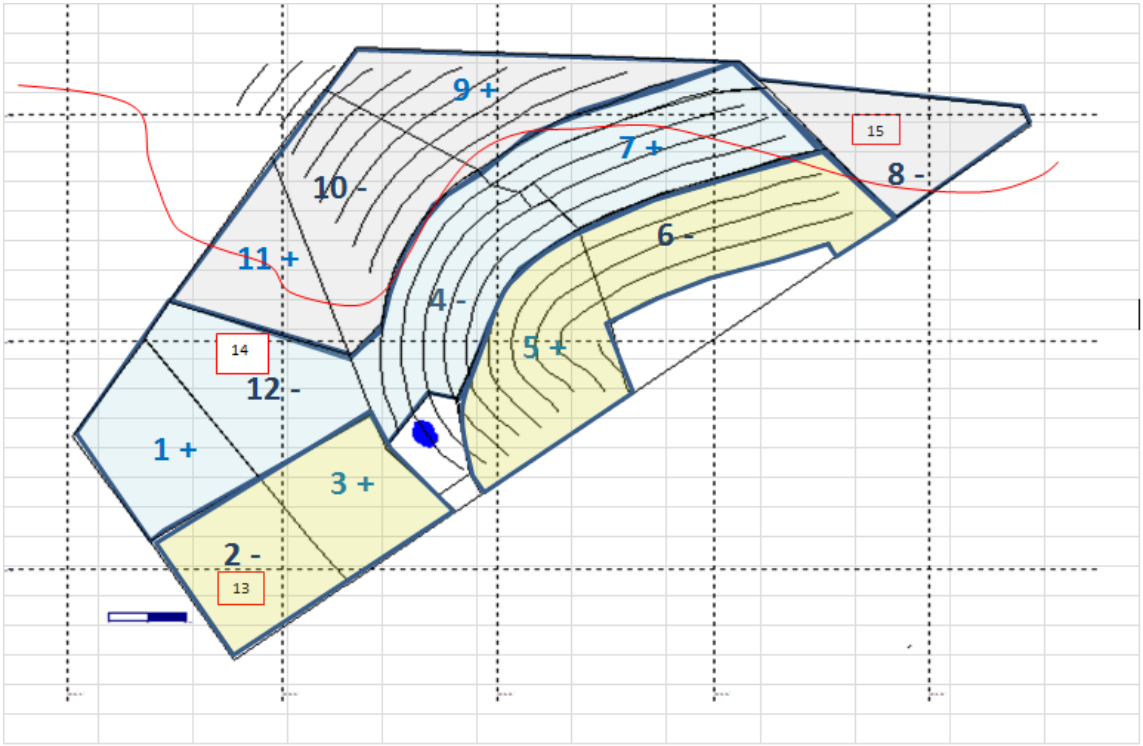
Inoculum was produced as described in Kinsbursky and Weinhold (1988). The isolate was grown for 7 days on PDA and cut into pieces of approximately 1 cm², and the content of each Petri dish was distributed in one 1 l Erlenmeyer flask. The flasks contained 100 g of black oat, previously moistened and autoclaved for one hour at 120 °C in three consecutive days. After 35 days of incubation, during which the flasks were manually shaken every 3-4 days, the colonized oat grains were transferred to a tray and dried for 3 days in a glasshouse. This inoculum was then ground to 1 mm in a Wiley-type knife mill. The inoculum was placed in plastic bags and kept in a 4 ºC refrigerator until use.

To determine the quantity of inoculum to be used in the experiments, 5 soil samples from zero to 15 cm depth of the field experiment were collected, and 3 different amounts of inoculum were applied to each sample, followed by soybean sowing. Based on this preliminary test, a dose of 0.6 g inoculum for each pot with 1.5 liter of soil was chosen, as this was the amount at which approximately 80% of the plants showed symptoms 35 days after sowing.

3.2.2 Description of the evaluated agricultural systems and soil sampling

The field experiment evaluated (Figure 3.1) consisted of an ICLS long-term experiment installed in 2006 within an experimental station belonging to the *Instituto Agronômico do Paraná* (IAPAR) in Ponta Grossa municipality, Paraná State, Brazil (25°07´S; 50°03´W; and 953m asl). This municipality is located in Campos Gerais of Paraná region. The experiment has two distinct soil classes that were separated by blocks: Dystric Cambisol (one block) and Dystric Ferralsol (two blocks) originated on a geological substrate based mainly on quartz sandstones with basalt intrusions (GUIMARÃES et al., 2007). The climate according to Köppen classification is Cfb, mesothermal humid subtropical.

FIGURE 3.1 – CROQUI OF THE FIELD EXPERIMENT



Long-term field experiment of ICLSs in the *Estação Experimental Fazenda Modelo*, owned to *Instituto Agronômico do Paraná*, at Ponta Grossa, PR. Agropastoral system is in the plots 1, 2, 3, 8, 11 and 12. Agrosilvopastoral system is in the plots 4, 5, 6, 7, 9 and 10. Not integrated crop is in the plots 13, 14 and 15. Blocks are separated by colors. Signs after the plot numbers indicates de lower (90 kh/ha) or higher (180 kg/ha) nitrogen rates applied on the winter pastures. Total experimental area is about 12 ha.

The experiment has treatments arranged in an incomplete factorial with 3 replications considered in randomized blocks. Experimental factor one is production

system with three levels: integration between crop and livestock (agropastoral system - AP), integration of crop, livestock, and trees (agrosilvopastoral system - ASP), and a control with not integrated crop (CO). Experimental factor 2 is amount of nitrogen (N) applied as urea on cover in total area about 40 days after sowing of the winter pasture, with two levels: 90 kg/ha (in all production systems) and 180 kg/ha (in AP and ASP systems). The lower (90 kg/ha) dose of N is the normally applied on winter pastures in the Region, and the upper (180 kg/ha) dose was included in the original experiment expecting to partially compensate the reduction in the forage growth due the light interception by trees.

The winter grazing in all experimental area consists of black oat and annual ryegrass intercropped. Cattle grazing occur for an uninterrupted period of 90 to 120 days every winter except on CO plots, and stocking rate in each plot is managed to maintain the pasture with about 20 cm height. The summer crop in all experimental area consists of soybean and corn on alternated crop seasons. Summer crops and winter pastures are established by direct seeding (no tillage). Chemical fertilization and pesticides used are listed in Tables 3.1 and 3.2.

The tree component of the ASP is composed of eucalyptus (*Eucalyptus dunnii*) and silver oak (*Grevillea robusta*) alternated in single rows, with an average of 4.5 m between trees and 14 m between rows. Pink pepper (*Schinus terebinthifolius*) was included in the original experiment but was cut during the summer of 2013 in order to decrease shading level by trees. The decrease in light availability for the understory (crops and grazing) vegetation calculated as the difference between two ceptometers, compared to the treeless systems was 53 ± 1.5% in 2014 and 56 ± 0.9% in 2015 winters (PONTES et al., 2017). Each plot has an area of about one hectare, except CO plots, which were established within the AP plots in 2010 and sized at 100 m^2.

TABLE 3.1 – CHEMICAL FERTILIZATION APPLIED IN THE SUMMER AND WINTER CROPS IN THE EXPERIMENTAL AREA

Crop season	Fertilizing	Quantity (kg/hectare)
2013/2014 corn	Dolomitic limestone (37 days before sowing)	500
	10-30-101 (sowing day)	400
	Urea (40 days after sowing)	250
2014 oat + ryegrass	Dolomitic limestone (11 days before sowing)	500
	4-30-101 (sowing day)	400
	Urea (40 days after sowing)	200 or 400 (depending on the N treatment)
2014/2015 soybean	0-20-20 (sowing day)	400
2015 oat + ryegrass	4-30-101 (sowing day)	400
	Urea (40 days after sowing)	200 or 400 (depending on the N treatment)
2015/2016 corn	4-30-101 (sowing day)	400
	Urea (30 days after sowing)	300
2016 oat + ryegrass	4-30-101 (sowing day)	400

[1]Proportional quantities of N, P_2O_5 and K_2O.

TABLE 3.2 - PESTICIDES (kg/hectare) USED IN SUMMER CROPS IN THE EXPERIMENTAL AREA

	Crop season		
Type of pesticide	2013/2014 corn	2014/2015 soybean	2015/2016 corn
Herbicide	Glyphosate (1.44) Atrazine (2.5) Mesotrione (0.14)	Glyphosate (1.44) Glyphosate (1.44)	Glyphosate (1.44) Atrazine (0.4) Tembotrione (0.1)
Insecticide	Parathion methyl (0.6) Thiamethoxam (0.04) Triflumuron (0.04) Deltamethrin (0.008)	Fipronil (0.03) Novaluron (0.008) Imidacloprid (0.08) Beta-Cyfluthrin (0.01) Imidacloprid (0.1) Bifenthrin (0.02)	Imidacloprid (0.04) Thiodicarb (0.14)
Fungicide	Captan (0.04)	Pyraclostrobin (0.03) Thiophanate-methyl (0.02) Azoxystrobin (0.06) Cyproconazole (0.02) Pyraclostrobin (0.07) Epoxiconazole (0.03)	Captan (0.04)

Four representative 1.5 l soil samples from zero to 15 cm depth were collected erratically from each plot, with about 40 m between each soil sampling inside each plot, avoiding the sites with recent (visible) manure deposition. In total, 60 samples were collected corresponding to five combinations of production system and amount of N times 3 blocks times 4 samples per plot. Samples were analysed separately. In the ASP treatment, soil samples were collected 3.5 m from the tree rows (2.5 m from the edge of the crops), since this is the middle point between tree line and the center

of alleys. Samples were taken with the aid of a shovel, taking care to collect a uniform width block of soil from the surface until 15 cm deep. The soil was packed in plastic bags to retain moisture and transferred to aluminium pots of 1.5 L capacity on the following day. For further evaluation of *Fusarium* and *Trichoderma* density, an aliquot of approximately 100 g was taken from each soil sample, sieved through a 1 mm opening sieve, and stored at 4 ºC inside acrylic gerboxes for three weeks.

Soil samples were collected twice: during the grazing phase on August (winter) and after the desiccation of the forages for the soybean seeding on November (spring) 2014. To evaluate the capacity of suppressiveness transference, soil samples were collected again on February 2015 and used to replace 10% of the volume of the soil in each pot. The experiments for evaluating the suppression to *R. solani* were installed one day after collecting soil samples and measurements of the density of *Fusarium* and *Trichoderma* started three weeks after each sampling.

An aliquot from the first soil sampling was submitted for chemical analysis at the Soil Testing Laboratory in the Soil Science Department of *Universidade Federal do Paraná*, and the results are shown in Table 3.3. The production systems were characterized for soil temperature at layer 0-13 cm from July 2014 to June 2015. Temperature measurements were taken once a month in the morning (8:00-9:00 a.m.) and afternoon (2:00-3:00 p.m.), with 4 portable skewers type thermometers for plot. The average temperature of the soil under ASP was 19.2 ± 0.07 °C, similar to the CO treatment (19.3 ± 0.11 °C). Both temperatures were lower than that observed for AP (19.9 ± 0.07 °C).

TABLE 3.3 - CHEMICAL CHARACTERIZATION OF THE EXPERIMENTAL SOIL COLLECTED ON AUGUST 2014

Characteristic	Unit	Production system/Nitrogen rate				
		AP/90	AP/180	ASP/90	ASP/180	CO/90
pH	$CaCl_2$	5.5	5.2	5.1	5.3	5.1
	SMP	6.8	6.6	6.8	6.9	6.4
Al^{+3}	$cmol_c/dm^3$	0	0	0.1	0	0
$H^{+}+Al^{+3}$	$cmol_c/dm^3$	2.7	3.2	2.7	2.5	3.7
Ca^{+2}	$cmol_c/dm^3$	2.8	2.8	2.1	1.9	2.9
Mg^{+2}	$cmol_c/dm^3$	1.3	1.1	0.6	0.6	1.1
K^{+}	$cmol_c/dm^3$	0.05	0.05	0.05	0.04	0.06
BS[a]	$cmol_c/dm^3$	4.1	4.0	2.8	2.5	4.1
P	mg/dm^3	29	26	15	14	19
OC[b]	g/dm^3	18	16	16	8	16
Ca/Mg		2.2	2.6	3.5	3.2	2.6

AP: Agropastoral system; ASP: Agrosilvopastoral system; CO: Control with not integrated crop. [a]Base saturation. [b]Organic carbon.

3.2.3 Soil suppressiveness to *Rhizoctonia solani*

A pot culture study was carried out with similar procedures as described in Samavat et al. (2014). Ten seeds of soybean cultivar NS 5909 RR were sowed in orifices 1 cm in diameter and 2 cm deep in each pot, which contained 1.5 liters of soil. Before closing the orifices, 0.6 g of *R. solani* inoculum was distributed on the soil surface of each pot (Figure 3.2). The pots were arranged randomly in a glasshouse of the *Universidade Federal do Paraná*, in Curitiba, Paraná State, Brazil.

A sample of each combination of production system and amount of N was used as control without infestation to check for possible natural occurrence of the pathogen in the soil. There was no incidence of symptoms in these pots in any evaluation, so they were not included in statistical analysis. The lot of seeds used in the experiments was collected in March 2014 and tested negative for the presence of *R. solani* using a blotter test without disinfecting the seeds' surface, indicating there was no influence from uncontrolled occurrences of the pathogen in the soil or seeds.

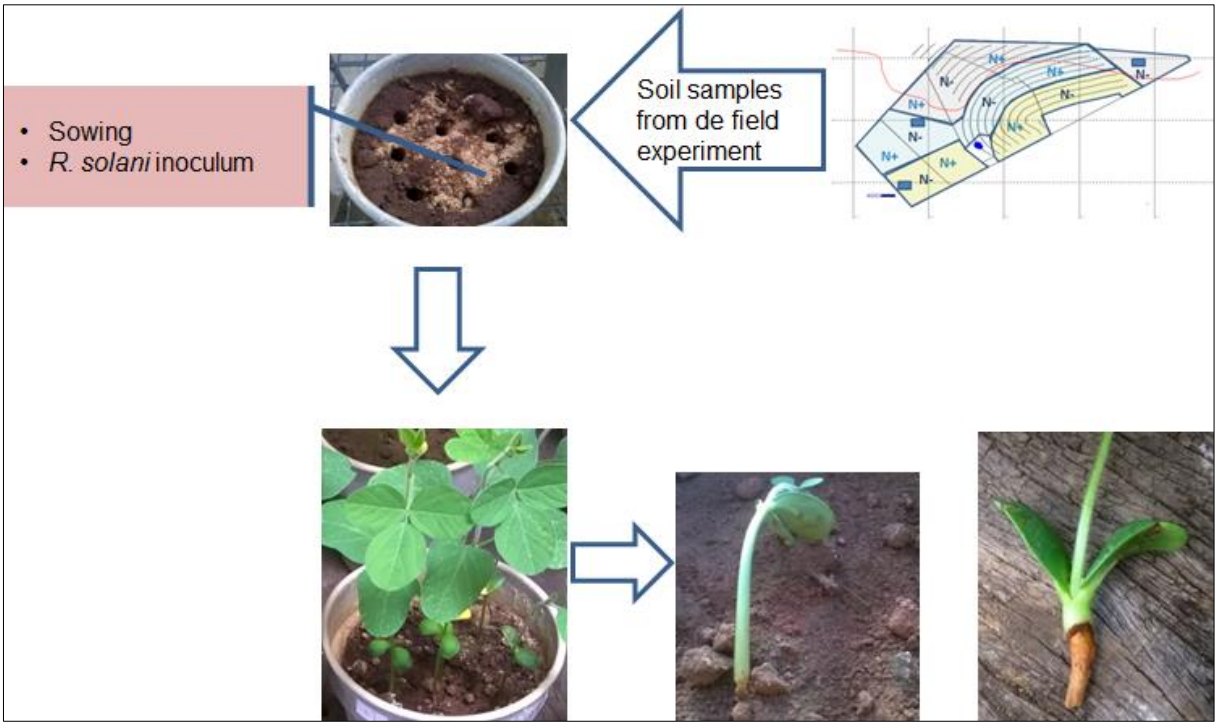
The number of germinated plants and plants showing symptoms caused by *R. solani* on the hypocotyl or main root was counted 35 days after sowing. Incidence per pot was calculated by the sum of the number of plants dead due to the incidence of the pathogen and plants with symptoms divided by the number of germinated plants. Seeds were sown for three consecutive times every 35 days without new infestation of the soil, thus providing three assessments of the disease incidence in each experimental unit (soil sample). A sample of plants with symptoms from each treatment was collected for isolation and confirmation of the pathogen in the laboratory.

After the third evaluation and without removal from the pots, the soil was autoclaved for one hour at 120 °C in two consecutive days and then re-infested with the pathogen. Three consecutive soybeans sowing were then carried out after two weeks, with the same measurements described before. The experiments were performed twice, with two different soil samples of experimental field, as described in section 3.2.2. The same prepared inoculum of *R. solani* was used for the first and second soil samplings and the amount of inoculum in the second sampling was adjusted to 0.8 g per pot to compensate for any loss of viability.

After these evaluations, 10% of the volume of the sterilized and re-infested soil in each pot of the first experiment was replaced by fresh soil samples collected from the same plots in the field. The soil in the pots was homogenized and the same

evaluations were performed again after three consecutive sowings, in order to assess the capacity of suppressiveness transference.

FIGURE 3.2 – PROCEDURES FOR THE SOIL SUPPRESSIVENESS TEST



Soil samples from the field experiment were collected and transferred to 1.5 liter pots, followed by pathogen infestation in the soil and three consecutive soybean sowings. 35 days after each sowing the incidence of damping-off was evaluated. SOURCE: the author (2016).

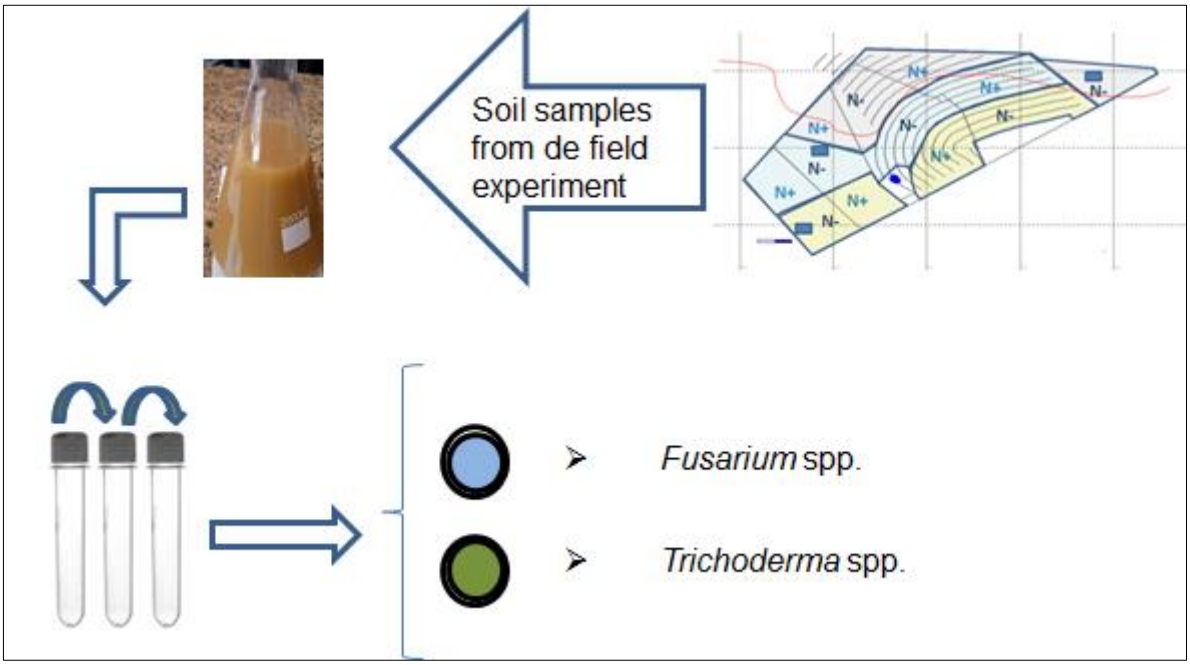
3.2.4 *Fusarium* and *Trichoderma* density

Population levels of cultivable *Fusarium* spp. and *Trichoderma* spp. in each soil sample were estimated by dilution plating on agar (PÉREZ-BRANDÁN et al., 2014; PRATT, 2008). An aliquot of 10 g of soil was diluted in 90 ml of sterile distilled water and stirred for 90 minutes at 170 RPM on an orbital flask shaker. Suspensions were diluted to a concentration of 10^{-3}, and 0.5 ml was then spread on petri dishes containing two different types of culture media with four plates per sample (Figure 3.3).

The culture media used were Nash and Snyder (1962) media to quantify *Fusarium* spp. and weak PDA (20% PDA + 80% agar-agar + 0.8 mL/L lactic acid) for *Trichoderma* spp. quantification. The proportions of PDA and lactic acid in the weak PDA medium were previous tested to favour the growth of *Trichoderma* without

bacterial growth. The plates were incubated in the dark at 22 to 26°C and evaluated after 5 days by visual quantification of colony forming units (CFU) per plate. The experiment was performed twice, with two different soil samples of experimental field, as described in section 3.2.2.

FIGURE 3.3 – PROCEDURES FOR THE TEST OF MICROORGANISMS DENSITY IN THE SOIL



SOURCE: the author (2016).

3.2.5 Data analysis

Statistical inferences of soil suppressiveness to *R. solani* was performed by fitting a generalized linear mixed model with binomial sampling distribution, logistic link, and random effects assigned to the experimental units. The statistical significance of experimental factors was assessed by likelihood ratio tests on a sequence of nested models defined by sequentially adding terms for: (I) single intercept, (II) nitrogen rates, (III) production system, and (IV) interaction between nitrogen rates and production system. Treatment comparisons were performed by contrasting the effects of production system for each nitrogen amount, with family-wise error rates of 0.05.

The effect of the pre-incubation of *R. solani* inoculum in the soil on the incidence of damping-off may differ between the three evaluations resulting from the three consecutive sowings. In particular, different results may be found for the first

evaluation (T1) because no re-infestation was done before the second (T2) and third (T3) evaluation times. For this reason, fitted models were used: Ma) without time effects, Mb) individual effects for T1, T2, and T3, and Mc) one effect for T1 and a common effect for T2 and T3. Maximized log-likelihood values (logLik), number of parameters, and the Akaike Information Criteria (AIC) guided the decision on whether and how the analysis of the three consecutive evaluations should be considered. A correlation coefficient between seed germination and proportion of plants with symptoms in each pot was computed to assess the influence of the *R. solani* inoculum.

Similar generalized linear mixed model structure was used for *Fusarium* and *Trichoderma* density analysis, with a Poisson distribution for the response variable and adding a random effect to account for measurements taken at the same experimental unit. Statistical analyses were performed using the R software (R CORE TEAM, 2015) and the add-on packages *latticeExtra* (SARKAR; ANDREWS, 2013), *lme4* (BATES et al., 2014), *reshape* (WICKHAM, 2007), *multcomp* (HOTHORN; BRETZ; WESTFALL, 2008), and *doBy* (HOJSGAARD; HALEKOH, 2014).

3.3 RESULTS

3.3.1 Soil suppressiveness to *Rhizoctonia solani*

The average proportions of plants showing symptoms in each assessment of the first experiment were 0.89 ± 0.06 (T1), 0.55 ± 0.06 (T2), and 0.54 ± 0.06 (T3). For the second experiment, the proportions were 0.97 ± 0.05, 0.81 ± 0.05, and 0.75 ± 0.05 for the T1, T2, and T3, respectively. The models that consider effect of evaluation times (models Mb and Mc) had similar fitting averages and were clearly better than model Ma, which does not consider evaluation times (Table 3.4). These results indicate the stable conditions for the second and third evaluation. Therefore, analysis was concentrated on the data from the T2 and T3, which were considered replications.

TABLE 3.4 - COMPARISON OF THE MODELS USED FOR DATA ANALYSIS OF SOIL SUPPRESSIVENESS TO *RHIZOCTONIA SOLANI*

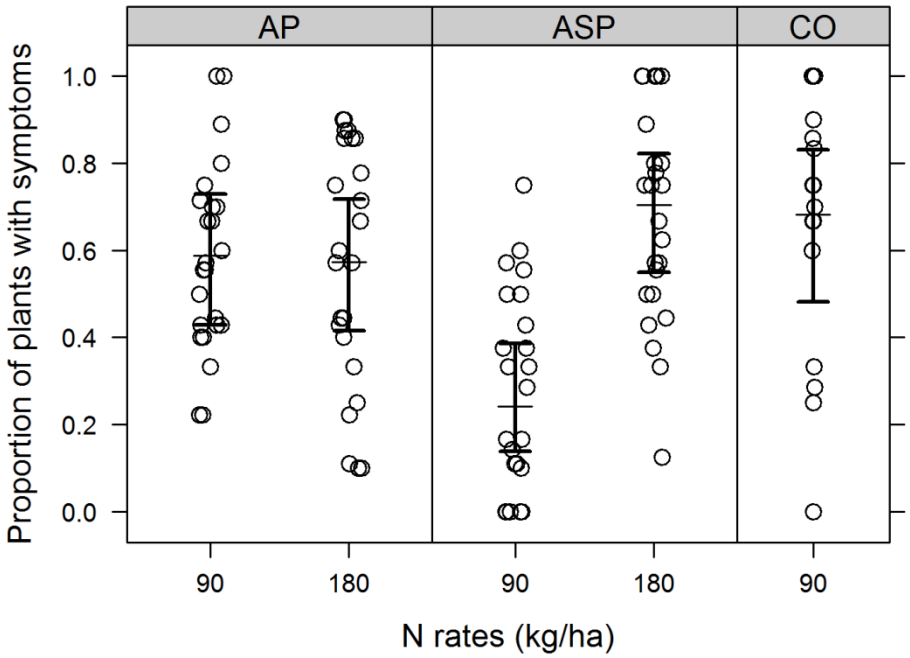
Model[1]	First experiment			Second experiment		
	logLik[2]	Df[3]	AIC[4]	logLik	Df	AIC
Ma	-325	7	664	-240	7	494
Mb	-284	9	585	-213	9	444
Mc	-284	8	584	-214	8	444

[1] Ma: without assessment time effect, Mb: one effect for each assessment time, Mc: one effect for the first assessment time and a common effect for second and third assessments. [2]Log-likelihood. [3]Degrees of freedom of the model. [4]Akaike Information Criterion.

The average ratio of germinated plants was 0.79 in first experiment and 0.76 in the second experiment. The correlation coefficients between germination and proportion of plants with symptoms for the first and second experiment were -0.17 and -0.18, respectively. These values indicate negligible effects of the inoculum on the germination of the soybean seeds.

Likelihood ratio tests indicated the model (IV) with interaction between N and production system effects, as the model of choice for the first experiment (Figure 3.4). The ASP treatment with 90 kg/ha of N resulted in at least one-half lower incidence of symptoms caused by *R. solani* than the other interactions between treatments.

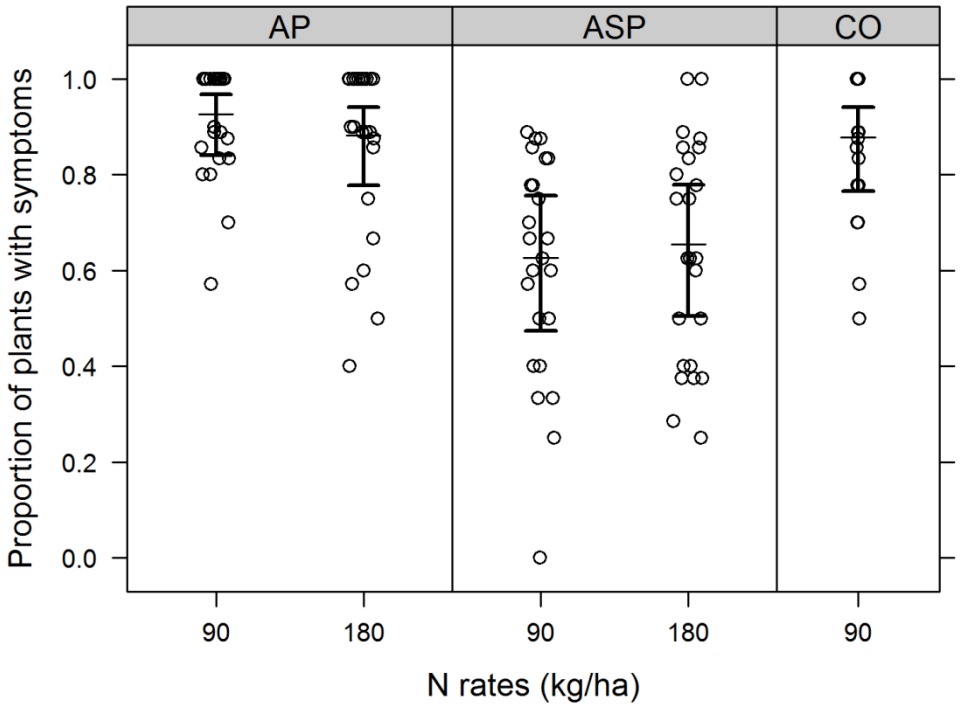
FIGURE 3.4 - PROPORTION OF SOYBEAN PLANTS WITH SYMPTOMS CAUSED BY *RHIZOCTONIA SOLANI* 35 DAYS AFTER SOWING, DEPENDING ON THE PRODUCTION SYSTEM AND AMOUNT OF NITROGEN, IN ARTIFICIALLY INFESTED SOIL COLLECTED IN AUGUST 2014



Bars show 95% confidence interval for the estimated averages. Horizontal line across the bar indicates estimated average. Points are the observed proportions in the experimental units. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with not integrated crop. Mean value of two consecutive assessments. Source: the author (2016).

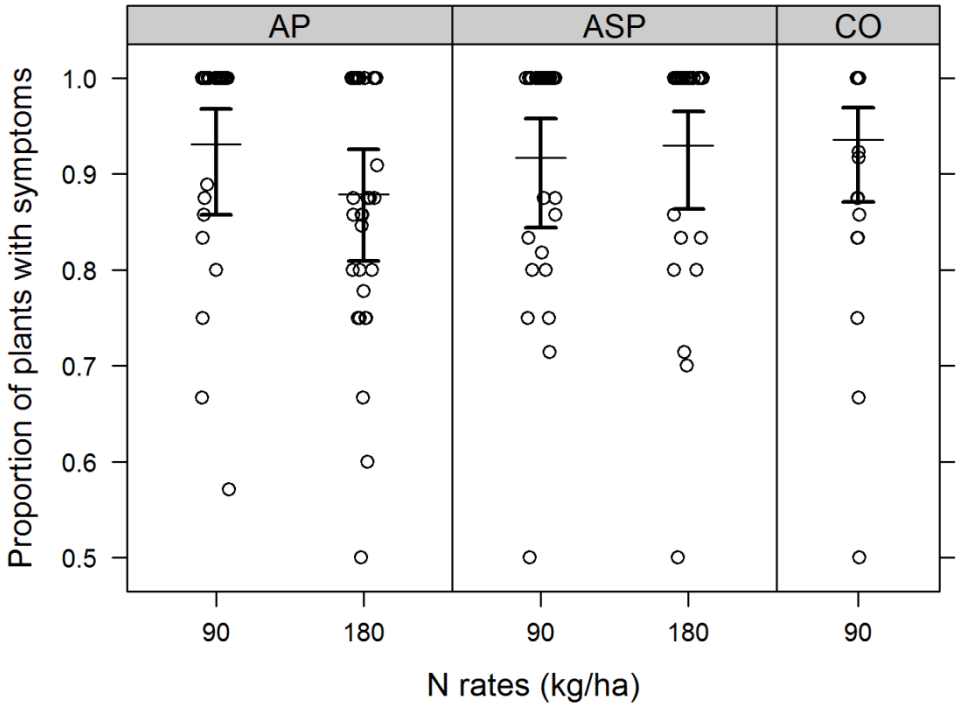
The model without interactions (III) was the model of choice for the second experiment. Disease incidence in ASP was approximately 0.7X of the other systems (Figure 3.5). There was no difference in the incidence of symptoms caused by *R. solani* among treatments in both experiments when the sterilized soil was evaluated (Figure 3.6). After substituting 10% of the volume of the infested soil with fresh soil, only production system effects were observed, with the lowest proportion of symptomatic plants in the ASP treatment (Figure 3.7), similar to what was observed for the second experiment with fresh soil.

FIGURE 3.5 - PROPORTION OF SOYBEAN PLANTS WITH SYMPTOMS CAUSED BY *RHIZOCTONIA SOLANI* 35 DAYS AFTER SOWING, DEPENDING ON THE PRODUCTION SYSTEM AND AMOUNT OF NITROGEN, IN ARTIFICIALLY INFESTED SOIL COLLECTED IN NOVEMBER 2014



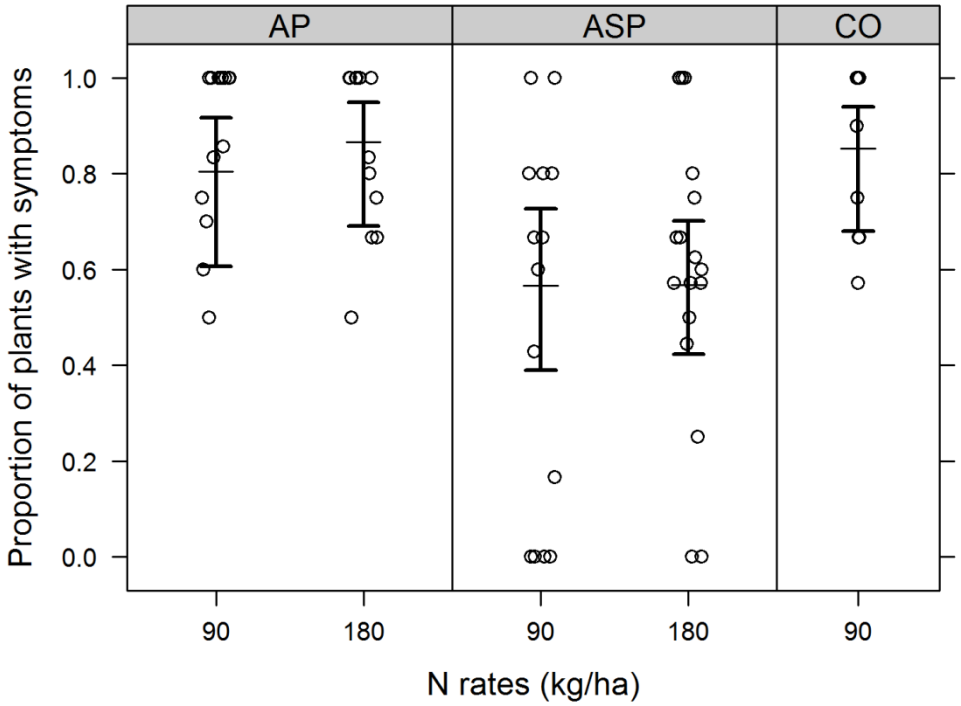
Bars show 95% confidence interval for the estimated averages. Horizontal line across the bar indicates estimated average. Points are the observed proportion in the experimental units. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with not integrated crop. Mean value of two consecutive assessments. Source: the author (2016).

FIGURE 3.6 - PROPORTION OF SOYBEAN PLANTS WITH SYMPTOMS CAUSED BY *RHIZOCTONIA SOLANI* 35 DAYS AFTER SOWING, DEPENDING ON THE PRODUCTION SYSTEM AND AMOUNT OF NITROGEN, IN STERILIZED AND ARTIFICIALLY INFESTED SOIL



Bars show 95% confidence interval for the estimated averages. Horizontal line across the bar indicates estimated average. Points are the observed proportion in the experimental units. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with not integrated crop. Mean values of two consecutive assessments in two independent experiments with soil samples collected on August and November 2014. Source: the author (2016).

FIGURE 3.7 - PROPORTION OF SOYBEAN PLANTS WITH SYMPTOMS CAUSED BY *RHIZOCTONIA SOLANI* 35 DAYS AFTER SOWING, DEPENDING ON THE PRODUCTION SYSTEM AND AMOUNT OF NITROGEN, IN STERILIZED AND ARTIFICIALLY INFESTED SOIL AFTER SUBSTITUTING 10% VOLUME OF SOIL OF EACH POT WITH FRESH SOIL COLLECTED FROM THE SAME FIELD PLOTS

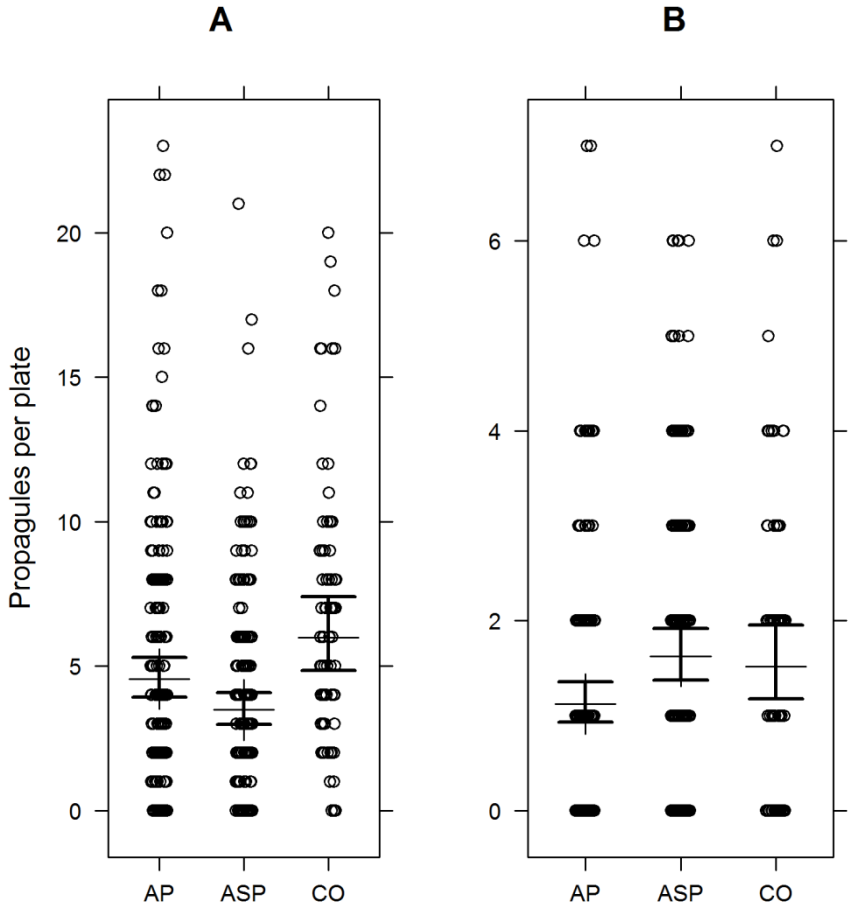


Bars show 95% confidence interval for the estimated averages. Horizontal line across the bar indicates estimated average. Points are the observed proportion in the experimental units. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with not integrated crop. Mean value of two consecutive assessments. Source: the author (2016).

3.3.2 *Fusarium* and *Trichoderma* density

Both experiments showed similar results for the density of the tested microorganisms, so the results were analysed together (Figure 3.8). N rates were not significant. *Fusarium* propagules retrieved in the ASP soil was approximately 0.7X and 0.6X of the amounts compared to AP and CO soils, respectively. *Trichoderma* propagules retrieved in the ASP soil was at approximately 1.5X the amounts compared to AP, but at similar amounts compared to CO.

FIGURE 3.8 - PROPAGULES OF *FUSARIUM* SPP. (A) AND *TRICHODERMA* SPP. (B) IN SOIL SAMPLES FROM DIFFERENT PRODUCTION SYSTEMS



Bars show 95% confidence interval for the estimated average count of propagules, and the horizontal line across the bar indicates the estimated average. Points are the observed proportion in the experimental units. Nº of propagules per plate X 2,000 = number of propagules per gram of soil. AP: agropastoral system; ASP: agrosilvopastoral system; CO: control with not integrated crop. Mean values of two experiments with soil samples collected on August and November 2014. Source: the author (2016).

3.4 DISCUSSION

Integrated Crop-Livestock Systems are systems with spatial and temporal interactions at different scales, with animals and farm crops within the same area, and aim to achieve synergism and emergent properties as a result of interactions among the soil, plants, animals, and the atmosphere (MORAES et al., 2014b). Some of these attributes were confirmed for the case study examined in this work. A lower incidence of soybean damping-off caused by *R. solani* (Figures 3.4, 3.5, 3.7), a lower density of

Fusarium spp. (Figure 3.8 A), and a higher density of *Trichoderma* spp. (Figure 3.8 B) in the soil of the agrosilvopastoral system were detected.

These results suggest that the ASP system has healthy soil (DORAN; ZEISS, 2000), which results in higher resiliency to disturbances or stresses, in line with discussions in Van Bruggen and Semenov (1999). The biological components of soil are critical to soil health (LARKIN, 2015), and suppressiveness is a relevant attribute of this characteristic (GARBISU; ALKORTA; EPELDE, 2011; VAN BRUGGEN et al., 2015).

As noted by Tsror (2010), effective management of *R. solani* requires the implementation of an integrated disease management approach, and within this approach cultural practices that decrease inoculum density in the soil are the most important measures. In the first experiment (first soil sampling), a lower incidence of symptoms caused by *R. solani* was observed in the lower N rate in ASP system (Figure 3.4). Interaction with N rates was not detected in the second soil sampling (Figure 3.5), nor in the experiment with 10% substituted soil (Figure 3.7), confirming the suppressiveness of the ASP soil to this pathogen.

In the ASP soil collected in the first experiment, a higher level of organic carbon was found in the 90 kg/ha N compared with the 180 kg/ha N (Table 3.3). The 90 kg/ha N treatment showed lower disease incidence, which is in accordance with Kühn et al. (2009), who observed higher C/N ratios in non-affected patches of sugar beet compared to disease-affected patches. These authors also observed hyphae growth of *R. solani* in fresh organic matter during the saprophytic phase of the pathogen, and discuss about the influence of soil organic matter on the disease occurrence.

Fresh or immature composts not only serve as a food base for biological control agents, but can also result in negative effects. Composts support saprophytic growth of plant pathogens and increase disease risk (BONANOMI et al., 2010; HOITINK; BOEHM, 1999), even if the green manure produces volatile compounds effective against the pathogen (YULIANTI; SIVASITHAMPARAM; TURNER, 2006). It is possible that higher amounts of fresh organic matter were produced in the 180 kg/ha N of ASP and this hypothesis may be supported the development of *R. solani* during the saprophytic phase (winter). The difference between disease incidences in the two N rates was only observed in the first experiment with soil samples collected during the pastoral phase in the winter.

Results of the first assessment (first sowing) were different from the subsequent ones (second and third) in a same experiment, as observed by the differences between the statistical models applied to evaluate pre-incubation of the pathogen in the soil (Table 3.4). These differences were probably because *R. solani* infestation in the soil was performed at the same time with the first sowing, when the pathogen was placed in direct contact with the seeds without previous interaction with soil and host. This is in accordance with other authors' observations (ASCENCION; LIANG; YEN, 2015; KOTSOU et al., 2004; LEWIS; LUMSDEN, 2001; YANGUI et al., 2008). Therefore, to provide the pathogen time to interact with the substrate prior to seed sowing increases the reliability of the results. Furthermore, Lebreton et al. (2004) and Anees et al. (2010) discuss the fact that the reduction in conduciveness (the opposite of suppressiveness) of the soil to a particular pathogen occurs after a few years of interactions among pathogen, host, and environment.

The absence of differences in disease incidence between treatments after soil sterilization and re-infestation (Figure 3.6) shows that biotic factors may be involved in suppressiveness to *R. solani*. Biological factors have been frequently associated with suppressiveness to several pathogens (BONILLA et al., 2012; GARBEVA; VAN VEEN; VAN ELSAS, 2004; LARKIN, 2015), although the main factor involved in soil suppressiveness may vary according to pathogen (BONANOMI et al., 2010; GARBEVA; VAN VEEN; VAN ELSAS, 2004). Microbial activity, (evaluated by the CO_2 evolution and fluorescein diacetate hydrolysis; cultivable bacterial, fungal, actinomycetes, protozoa, fluorescent *Pseudomonas* and *Fusarium* spp. communities) was the main factor related to suppressiveness to *R. solani* in an experiment contrasting biotic and abiotic factors in forest, pasture, fallowing, annual crops, perennial crops, and ploughed soils in São Paulo State, Brazil (GHINI; MORANDI, 2006).

When 10% of the soil volume in the pots was substituted by fresh soil, differences among treatments were observed again (Figure 3.7), showing the capacity of suppression transference by soil samples. These results are in accordance with Wiseman et al. (1996), who succeeded in transferring suppression to *R. solani* from one soil to another, as well as Oyarzun et al. (1998), who observed suppression to *Fusarium solani*, *Thielaviopsis basicola,* and *Aphanomyces euteiches* in pea culture.

The transfer of suppressiveness to *R. solani* by soil samples indicates that the suppressiveness is based on individual or select groups of microorganisms (WELLER

et al., 2002). *Trichoderma* spp. has been reported as antagonists of different pathogens (VERMA et al., 2007). The highest density of this microorganism observed in the ASP treatment compared to the AP treatment (Figure 3.8 B) contributes to the explanation of soil suppressiveness to *R. solani*, as the antagonism of *Trichoderma* spp. to *R. solani* is well documented (GROSCH et al., 2006; LEWIS; LUMSDEN, 2001; LORITO et al., 2010; PEREIRA et al., 2014).

Microorganisms in the soil can be in active, potentially active and dormant phases, and plate-count techniques can be used to access the cultivable active/potentially active phases, that represent about a half of the total microbial biomass and are also positively correlated with enzymes and respiratory activity in the soil (BLAGODATSKAYA; KUZYAKOV, 2013). These active/potentially active microbial populations are influenced by environmental changes such that imposed by the presence of herbivores (CARVALHO et al., 2010) and trees (NOTARO et al., 2014), while the total fungal community, including dormant phases, is almost uninfluenced by environmental changes promoted by cultivation practices (HAGN et al., 2003).

As previously suggested (CORRÊA et al., 2008; PÉREZ-BRANDÁN et al., 2014), there is a positive effect of *Trichoderma* on greater diversity of plant species, confirmed in the present work in the ASP treatment. Soil moisture is also known to be an important factor influencing *Trichoderma* spp. (INNOCENTI; ROBERTI; PIATTONI, 2015; JIN; HARMAN; TAYLOR, 1991). Although soil moisture was not recorded in the present work, the presence of trees in the ASP treatment and the maintenance of a greater quantity of residues on the soil of the CO (not grazed) may have contributed to maintenance of moisture in these systems. Evidence of this moisture maintenance is the average soil temperatures recorded in the field experiment: 19.2 ± 0.07 °C in ASP, which was similar to the CO treatment (19.3 ± 0.11 °C) and lower than that observed for AP (19.9 ± 0.07 °C).

Soil temperature varied among production systems mainly due to the presence or absence of grazing and trees. Comparing the average temperatures recorded in the soil of the evaluated fields in the present work with reported temperatures in the literature for *R. solani* (OLIVEIRA et al., 2014; OROZCO-AVITIA et al., 2013; TEWOLDEMEDHIN et al., 2006) and *Trichoderma* spp. (GUPTA; SHARMA, 2013; HAMZAH et al., 2012; SINGH et al., 2014), it is not possible to attribute the results of this work to changes in the soil temperature caused by differences in the assessed production systems.

The density of *Fusarium* spp. propagules was inversely proportional to the density of *Trichoderma* spp. propagules in the ASP treatment. The negative correlation between the population densities of these microorganisms in the soil has been reported with pathogenic *Fusarium* (KIM; KNUDSEN, 2013; SANT et al., 2010; SIVAN; CHET, 1989; SUÁREZ-ESTRELLA et al., 2007). The lower density of viable *Fusarium* propagules in the AP treatment compared to the CO treatment indicates that manure was more effective than crop residues in reducing *Fusarium* in the soil. The AP system presents lower residues due to grazing, but this activity results in manure distribution over the soil. Similar results were previous reported (SENECHKIN; VAN OVERBEEK; VAN BRUGGEN, 2014; SUN et al., 2015).

The chemical analysis of the experimental soils shows that the ASP system distinguishes itself from the others mainly due to its lower amounts of available calcium, magnesium, and phosphorus, probably due to the mineral absorption by the trees, since fertilization is the same in all treatments. These differences may have influenced the development of microorganisms as well as the development of cultivated plants. It should also be considered that soil gathering changes its structure, so it was not possible to observe the effects of soil structure in the results.

This is probably the first investigation addressing crop disease in ASP system, and future research should investigate the influence of such a complex production system in other diseases and epidemics.

3.5 CONCLUSION

The agricultural diversification promoted by agrosilvopastoral system in the region of Campos Gerais of Paraná State, Brazil, has the potential to suppress the soil-borne pathogen *Rhizoctonia solani*, reduce native cultivable *Fusarium*, and enhance beneficial microorganisms like *Trichoderma* in the soil.

ACKNOWLEDGEMENTS

The authors thank Dr. Augusto C. P. Goulart, of *Embrapa Agropecuária Oeste*, for providing the *R. solani* isolate used in this work. This work was supported by the SERVIAMBI project (Scientific Cooperation Term 21500.10/0008-2, signed between IAPAR and *Embrapa Florestas*) and also by grants of the project CNPq PROCAD

number 552334/2011-1 and CAPES.

REFERENCES

ALTIERI, M. A. The ecological role of biodiversity in agroecosystem. **Agriculture, Ecosystems and Environment**, v. 74, p. 19–31, 1999.

ANEES, M. et al. Biotic changes in relation to local decrease in soil conduciveness to disease caused by *Rhizoctonia solani*. **European Journal of Plant Pathology**, v. 126, n. 1, p. 29–41, 2010.

AOKI, T. et al. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex-*F. virguliforme* in North America and *F. tucumaniae* in South America. **Mycologia**, v. 95, n. 4, p. 660–684, 2003.

ASCENCION, L. C.; LIANG, W. J.; YEN, T. B. Control of *Rhizoctonia solani* damping-off disease after soil amendment with dry tissues of Brassica results from increase in Actinomycetes population. **Biological Control**, v. 82, p. 21–30, 2015.

BATES, D. et al. **lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-5**. Disponível em: <http://cran.r-project.org/package=lme4>. Acesso em: 25 ago. 2015.

BELL, L. W.; MOORE, A. D. Integrated crop-livestock systems in Australian agriculture: Trends, drivers and implications. **Agricultural Systems**, v. 111, p. 1–12, 2012.

BLAGODATSKAYA, E.; KUZYAKOV, Y. Active microorganisms in soil: Critical review of estimation criteria and approaches. **Soil Biology and Biochemistry**, v. 67, p. 192–211, 2013.

BONANOMI, G. et al. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. **Soil Biology & Biochemistry**, v. 42, n. 2, p. 136–144, 2010.

BONILLA, N. et al. Enhancing soil quality and plant health through suppressive organic amendments. **Diversity**, v. 4, n. 4, p. 475–491, 2012.

CARVALHO, P. C. DE F. et al. Managing grazing animals to achieve nutrient cycling and soil improvement in no-till integrated systems. **Nutrient Cycling in Agroecosystems**, v. 88, n. 2, p. 259–273, 2010.

CARVALHO, P. C. DE F. et al. Definições e terminologias para Sistema Integrado de Produção Agropecuária. **Revista Ciencia Agronomica**, v. 5, p. 1040–1046, 2014.

CORRÊA, C. A. et al. Efeito de rotações de cultura sob plantio direto sobre *Fusarium*

spp. e *Trichoderma* spp. em cultivos de feijoeiro irrigado. **Documentos IAC**, v. 85, p. 943–945, 2008.

DORAN, J. W.; ZEISS, M. R. Soil healht and sustainibility: managing the biotic component of soil quality. **Applied Soil Ecology**, v. 15, p. 3–11, 2000.

FAO. An international consultation on integrated crop-livestock systems for development: The way forward for sustainable production intensification. **Integrated Crop Management**, v. 13, n. 1, p. 1–79, 2010.

FAO. **FAOSTAT**. Disponível em: <http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E>. Acesso em: 25 ago. 2015.

FARIAS NETO, A. L. DE et al. Caracterização de genótipos de soja na região dos Cerrados quanto à reação à podridão vermelha da raiz, causada pelos fungos *Fusarium tucumaniae* e *Fusarium brasiliense*. **Revista Ceres**, v. 60, n. 2, p. 215–220, 2013.

FINLAY, R. The fungi in soil. In: VAN ELSAS, J.; JANSON, J.; TREVORS, J. (Eds.). . **Modern Soil Microbiology**. Boca Raton: CRC Press, 2007. p. 107–146.

GARBEVA, P.; VAN VEEN, J. A.; VAN ELSAS, J. D. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. **Annual Review of Phytopathology**, v. 42, n. 1, p. 243–270, 2004.

GARBISU, C.; ALKORTA, I.; EPELDE, L. Assessment of soil quality using microbial properties and attributes of ecological relevance. **Applied Soil Ecology**, v. 49, n. 1, p. 1–4, 2011.

GHINI, R.; MORANDI, M. A. B. Biotic and abiotic factors associated with soil suppressiveness to *Rhizoctonia solani*. **Scientia Agricola**, v. 63, n. 2, p. 153–160, 2006.

GONZÁLEZ GARCÍA, V.; PORTAL ONCO, M. A.; RUBIO SUSAN, V. Review. Biology and systematics of the form genus *Rhizoctonia*. **Spanish Journal of Agricultural Research**, v. 4, n. 1, p. 55–79, 2006.

GROSCH, R. et al. Fungal antagonists of the plant pathogen *Rhizoctonia solani*: selection, control efficacy and influence on the indigenous microbial community. **Mycological Research**, v. 110, n. 12, p. 1464–1474, 2006.

GUIMARÃES, G. B. et al. Geologia dos Campos Gerais. In: MELO, M.; MORO, R.; GUIMARÃES, G. (Eds.). . **Patrimônio Natural dos Campos Gerais do Paraná**. Ponta Grossa: Editora UEPG, 2007. p. 23–32.

GUPTA, V.; SHARMA, A. K. Assessment of optimum temperature of *Trichoderma harzianum* by monitoring radial growth and population dynamics in different compost manures under different temperature. **Okta Journal of Biosciences**, v. 1, n. 2, p. 151–157, 2013.

HAAN, C. D.; STEINFELD, H.; BLACKBURN, H. **Livestock & the environment: Finding a balance**. Disponível em: <http://www.fao.org/docrep/x5303e/x5303e00.htm>.

HAGN, A. et al. Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of *Trichoderma* spp. **Biology and Fertility of Soils**, v. 38, n. 4, p. 236–244, 1 ago. 2003.

HAMZAH, A. et al. Optimal physical and nutrient parameters for growth of *Trichoderma virens* UKMP-1M for heavy crude oil degradation. **Sains Malaysiana**, v. 41, n. 1, p. 71–79, 2012.

HOITINK, H. A. J.; BOEHM, M. J. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. **Annual Review of Phytopathology**, v. 37, p. 427–446, 1999.

HOJSGAARD, S.; HALEKOH, U. **doBy: Groupwise statisticas, LSmeans, linear contrasts, utilities. R package version 4.5-13**. Disponível em: <http://cran.r-project.org/package=doBy>. Acesso em: 28 ago. 2015.

HOTHORN, T.; BRETZ, F.; WESTFALL, P. Simultaneous inference in general parametric models. **Biometrical Journal**, v. 50, p. 346–363, 2008.

INNOCENTI, G.; ROBERTI, R.; PIATTONI, F. Biocontrol ability of *Trichoderma harzianum* strain T22 against *Fusarium* wilt disease on water-stressed lettuce plants. **BioControl**, v. 60, n. 4, p. 573–581, 2015.

JIN, X.; HARMAN, G. E.; TAYLOR, A. G. Conidial biomass and desiccation tolerance of *Trichoderma harzianum* produced at different medium water potentials. **Biological Control**, v. 1, n. 3, p. 237–243, 1991.

KIM, J. H. et al. Population structure of the *Gibberella fujikuroi* species complex associated with rice and corn in Korea. **Plant Pathology Journal**, v. 28, n. 4, p. 357–363, 2012.

KIM, T. G.; KNUDSEN, G. R. Relationship between the biocontrol fungus *Trichoderma harzianum* and the phytopathogenic fungus *Fusarium solani* f.sp. *pisi*. **Applied Soil Ecology**, v. 68, p. 57–60, 2013.

KINSBURSKY, R. S.; WEINHOLD, A. R. Influence of soil on inoculum density-disease incidence relationships of *Rhizoctonia solani*. **Phytopathology**, v. 78, n. 2, p. 127–130, 1988.

KOTSOU, M. et al. The effect of olive oil mill wastewater (OMW) on soil microbial communities and suppressiveness against *Rhizoctonia solani*. **Applied Soil Ecology**, v. 26, n. 2, p. 113–121, 2004.

KÜHN, J.; RIPPEL, R.; SCHMIDHALTER, U. Abiotic soil properties and the occurrence of *Rhizoctonia* crown and root rot in sugar beet. **Journal of Plant Nutrition and Soil**

Science, v. 172, n. 5, p. 661–668, 2009.

LARKIN, R. P. Soil health paradigms and implications for disease management. **Annual review of phytopathology**, v. 53, p. 199–221, 2015.

LEBRETON, L. et al. Changes in population structure of the soilborne fungus *Gaeumannomyces graminis* var. *tritici* during continuous wheat cropping. **Environmental Microbiology**, v. 6, n. 11, p. 1174–1185, 2004.

LEWIS, J. A.; LUMSDEN, R. D. Biocontrol of damping-off of greenhouse-grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp. **Crop Protection**, v. 20, n. 1, p. 49–56, 2001.

LORITO, M. et al. Translational research on *Trichoderma*: from 'omics to the field. **Annual review of phytopathology**, v. 48, p. 395–417, 2010.

MORAES, A. DE et al. Research on integrated crop-livestock systems in Brazil. **Revista Ciencia Agronomica**, v. 45, n. 5, p. 1024–1031, 2014a.

MORAES, A. DE et al. Integrated crop-livestock systems in the Brazilian subtropics. **European Journal of Agronomy**, v. 57, p. 4–9, 2014b.

NASH, S. M.; SNYDER, W. C. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. **Phytopathology**, v. 52, p. 567–572, 1962.

NOTARO, K. DE A. et al. Agroforestry systems, nutrients in litter and microbial activity in soils cultivated with coffee at high altitude. **Scientia Agricola**, v. 71, n. 2, p. 87–95, abr. 2014.

OLIVEIRA, A. C. C. DE et al. Influência da temperatura sobre isolados de *Rhizoctonia solani* (Kühn) obtidos em áreas produtoras de algodão nos Estados Brasileiros. **Bioscience Journal**, v. 30, n. SUPPL. 1, p. 119–130, 2014.

OROZCO-AVITIA, A. et al. Temperature effect on *Rhizoctonia solani* analyzed by microcalorimetry. **American Journal of Agricultural and Biological Sciences**, v. 8, n. 2, p. 162–166, 2013.

OYARZUN, P. J.; GERLAGH, M.; ZADOKS, J. C. Factors associated with soil receptivity to some fungal root rot pathogens of peas. **Applied Soil Ecology**, v. 10, n. 1–2, p. 151–169, 1998.

PEREIRA, J. L. et al. Analysis of *Phaseolus vulgaris* response to its association with *Trichoderma harzianum* (ALL-42) in the presence or absence of the phytopathogenic fungi *Rhizoctonia solani* and *Fusarium solani*. **PLoS ONE**, v. 9, n. 5, p. 1–14, 2014.

PÉREZ-BRANDÁN, C. et al. Soybean fungal soil-borne diseases: a parameter for measuring the effect of agricultural intensification on soil health. **Canadian Journal of Microbioogy**, v. 60, n. 2, p. 73–84, 2014.

PONTES, L. DA S. et al. Relationship between sward height and herbage mass for integrated crop-livestock systems with trees. **Grassland Science**, v. 63, n. 1, p. 29–35, jan. 2017.

PRATT, R. G. Fungal population levels in soils of commercial swine waste disposal sites and relationships to soil nutrient concentrations. **Applied Soil Ecology**, v. 38, n. 3, p. 223–229, 2008.

R CORE TEAM. **R: A language and environment for statistical computing.**Viena, 2015. Disponível em: <http://www.r-project.org/>

RYSCHAWY, J. et al. Mixed crop-livestock systems: an economic and environmental-friendly way of farming? **Animal**, v. 6, n. 10, p. 1722–1730, 2012.

SALTON, J. C. et al. Integrated crop-livestock system in tropical Brazil: Toward a sustainable production system. **Agriculture, Ecosystems and Environment**, v. 190, p. 70–79, 2014.

SAMAVAT, S. et al. Application of new bioformulations of *Pseudomonas aureofaciens* for biocontrol of cotton seedling damping-off. **Journal of Plant Protection Research**, v. 54, n. 4, p. 335–339, 2014.

SANT, D. et al. Effect of *Trichoderma asperellum* strain T34 on *Fusarium* wilt and water usage in carnation grown on compost-based growth medium. **Biological Control**, v. 53, n. 3, p. 291–296, 2010.

SARKAR, D.; ANDREWS, F. **latticeExtra: Extra graphical utilities based on Lattice. R package version 0.6-26**, 2013. Disponível em: <http://cran.r-project.org/package=latticeExtra>. Acesso em: 25 ago. 2015

SENECHKIN, I. V.; VAN OVERBEEK, L. S.; VAN BRUGGEN, A. H. C. Greater *Fusarium* wilt suppression after complex than after simple organic amendments as affected by soil pH, total carbon and ammonia-oxidizing bacteria. **Applied Soil Ecology**, v. 73, p. 148–155, 2014.

SINGH, A. et al. Optimal physical parameters for growth of *Trichoderma* species at varying pH , temperature and agitation. **Virology & Mycology**, v. 3, n. 1, p. 1–7, 2014.

SIVAN, A.; CHET, I. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. **Phytopathology**, v. 79, n. 2, p. 198–203, 1989.

STOCKDALE, E. A.; BROOKES, P. C. Detection and quantification of the soil microbial biomass – impacts on the management of agricultural soils. **Journal of Agricultural Science**, v. 144, p. 285–302, 2006.

SUÁREZ-ESTRELLA, F. et al. Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. *melonis*. **Crop Protection**, v. 26, n. 1, p. 46–53, 2007.

SUN, R. et al. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. **Soil Biology & Biochemistry**, v. 88, p. 9–18, 2015.

TEWOLDEMEDHIN, Y. T. et al. Characterization of *Rhizoctonia* spp. recovered from crop plants used in rotational cropping systems in the Western Cape province of South Africa. **Plant Disease**, v. 90, n. November, p. 1399–1406, 2006.

THORNTON, P. K.; HERRERO, M. Integrated crop-livestock simulation models for scenario analysis and impact assessment. **Agricultural Systems**, v. 70, n. 2–3, p. 581–602, 2001.

TSROR, L. Biology, epidemiology and management of *Rhizoctonia solani* on potato. **Journal of Phytopathology**, v. 158, n. 10, p. 649–658, 2010.

VAN BRUGGEN, A. H. C. et al. Soil health indicators and *Fusarium* wilt suppression in organically and conventionally managed greenhouse soils. **Applied Soil Ecology**, v. 86, p. 192–201, 2015.

VAN BRUGGEN, A. H. C.; SEMENOV, A. M. A new approach to the search for indicators of root disease suppression. **Australasian Plant Pathology**, v. 28, p. 4–10, 1999.

VAN DER HEIJDEN, M. G. A.; WAGG, C. Soil microbial diversity and agro-ecosystem functioning. **Plant and Soil**, v. 363, n. 1–2, p. 1–5, 2013.

VERMA, M. et al. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. **Biochemical Engineering Journal**, v. 37, n. 1, p. 1–20, 2007.

WELLER, D. M. et al. Microbial populations responsible for specific soil suppressiveness to plant pathogens. **Annual Review of Phytopathology**, v. 40, n. 1, p. 309–348, 2002.

WESTPHAL, A. et al. Contributions of *Fusarium virguliforme* and *Heterodera glycines* to the disease complex of sudden death syndrome of soybean. **PLoS ONE**, v. 9, n. 6, p. 1–13, 2014.

WICKHAM, H. Reshaping data with the reshape package. **Journal of Statistical Software**, v. 21, n. 12, p. 1–20, 2007.

WISEMAN, B. M. et al. Suppression of *Rhizoctonia solani* anastomosis group 8 in Australia and its biological nature. **Soil Biology & Biochemistry**, v. 28, n. 6, p. 727–732, 1996.

WRATHER, A. et al. Effect of diseases on soybean yield in the top eight producing countries in 2006. **Plant Health Progress**, 2010.

YANGUI, T. et al. Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. **Crop**

Protection, v. 27, n. 2, p. 189–197, 2008.

YULIANTI, T.; SIVASITHAMPARAM, K.; TURNER, D. W. Saprophytic growth of *Rhizoctonia solani* Kühn AG2-1 (ZG5) in soil amended with fresh green manures affects the severity of damping-off in canola. **Soil Biology & Biochemistry**, v. 38, n. 5, p. 923–930, 2006.

ZHANG, H. et al. Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. **PLoS ONE**, v. 7, n. 2, p. 1–14, 2012.

4 MICROCLIMATE IN PRODUCTION SYSTEM CONTAINING TREES ENHANCES POWDERY MILDEW SEVERITY

ABSTRACT

Although integrated crop-livestock systems (ICLSs) including trees are being studied more intensely during the last two decades, epidemiology of plant diseases in these production systems is still poorly studied. Microclimatic modifications due to the system adopted may influence diseases. This work evaluated powdery mildew (PM) severity on oats (*Avena strigosa* and *A. sativa*) and on soybean (*Glycine max*), and the microclimate inside the soybean canopy. Evaluations were done in a long-term experiment with three production systems: agropastoral (AP), agrosilvopastoral (ASP) and not integrated crop (CO), with two rates of nitrogen (N) for AP and ASP. Winter pastures in the experimental area are composed by black oat or white oat intercropped with annual reygrass (*Lolium multiflorum*). Corn (*Zea mays*) and soybean are cultivated in alternated summer crops. Eucalyptus (*Eucalyptus dunnii*) and silver oak (*Grevillea robusta*) were planted in the ASP system eighth years before evaluations of this work started, with an average of 4.5 m between trees and 14 m between rows. The PM on oats in ASP was about 20X more severe than in the others systems. The PM on soybean differed comparing the systems but was more severe in ASP, with at least 4X more than that observed in the others. In agreement, conidia of *Microsphaera diffusa* retrieved in spore traps in ASP were consistently higher than in the others systems. ASP showed shorter leaf wetness duration, higher daylight relative humidity (RH), and lower temperature amplitude. Multiple regression analysis showed leaf wetness duration and daylight RH were the characteristics most related to PM on soybean. In conclusion, microclimate in ASP system enhances PM severity compared to AP and CO.

Key-words: *Blumeria graminis. Microsphaera diffusa*. Integrated crop-livestock system. Agroforestry. Shadow. Leaf wetness duration.

RESUMO

Embora sistemas integrados de produção agropecuária (SIPAs) incluindo árvores serem estudados mais intensamente durante as últimas duas décadas, a epidemiologia de doenças de plantas nesses sistemas de produção é ainda pouco estudada. Modificações microclimáticas devido ao sistema adotado podem influenciar doenças. Este trabalho avaliou a severidade de oídio em aveia (*Avena strigosa* e *A. sativa*) e em soja (*Glycine max*), e o microclima dentro do dossel da soja. As avaliações foram realizadas em um experimento de campo de longa duração com três sistemas de produção: agropastoril (AP), agrosilvipastoril (ASP) e parcela de lavoura não integrada (CO), com dois níveis de nitrogênio (N) para AP e ASP. As pastagens de inverno na área experimental são compostas por aveia preta ou aveia branca consorciadas com azevém anual (*Lolium multiflorum*). Milho (*Zea mays*) e soja são cultivados no verão em anos alternados. Eucalipto (*Eucalyptus dunnii*) e grevílea (*Grevillea robusta*) foram plantados no sistema ASP oito anos antes das avaliações deste trabalho iniciarem, com uma média de 4,5 m entre árvores e 14 m entre fileiras. O oídio em aveia no ASP foi cerca de 20X mais severo que nos outros sistemas. O oídio em soja diferiu entre os sistemas, mas foi mais severo no ASP, com pelo menos 4X mais que o observado nos demais. Em concordância, a quantidade de conídios de *Microsphaera diffusa* recuperada em armadilhas caça-esporos no ASP foi consistentemente maior que nos outros sistemas. ASP apresentou menor duração do molhamento foliar, maior umidade relativa (UR) diurna e menor amplitude de temperatura. Análise de regressão múltipla revelou que a duração do molhamento foliar e a UR diurna foram as características mais relacionadas com o oídio na soja. Em conclusão, o microclima em sistema ASP aumenta a severidade de oídio comparado com AP e CO.

Palavras-chave: *Blumeria graminis*. *Microsphaera diffusa*. Integração lavoura-pecuária. Agrofloresta. Sombra. Duração do molhamento foliar.

4.1 INTRODUCTION

Integrated systems of agricultural production, including crop yield and animals grazing in the same area, are longstanding production systems and considered the main form of agricultural land use in the world (BELL; MOORE, 2012; HAAN; STEINFELD; BLACKBURN, 1997; THORNTON; HERRERO, 2001). These production systems aim to achieve synergy and emergent properties as the result of interactions among soil, plant, animal and atmosphere (MORAES et al. 2014a), and are included in the definition of Integrated Crop-Livestock System (ICLS) (CARVALHO et al., 2014; FAO, 2010; MORAES et al., 2014a). Examples of the benefits attributed to ICLS include reduction in costs and risks, increase in the efficiency of land and machinery use, increased diversity, greenhouse gas mitigation, reduction in plant diseases, and weed incidence and increased profitability and incomes (ALTIERI, 1999; BELL; MOORE, 2012; CARVALHO et al., 2010; RYSCHAWY et al., 2012).

Most common ICLSs in the subtropical region of Brazil comprise rotation or succession of summer crops, such as soybean (*Glycine max*), corn (*Zea mays*), bean (*Phaseolus vulgaris*) or rice (*Oryza sativa*) with annual winter pastures, such as black oat (*Avena strigosa*) and annual ryegrass (*Lolium multiflorum*), alone or mixed (MORAES et al., 2014b). The inclusion of tree species in such production systems is still not common (MORAES et al., 2014a), and there is a lack of knowledge on how the mixing plant species including trees affects the environment and its consequences on crops (MALÉZIEUX et al., 2009).

The systems can lead to environment modifications, which can increase or decrease plant diseases, depending on the particular requirement of the organisms (MATSON, 1997). Besides that general affirmation, little is known about the influence of different arrangements of ICLS in plant disease occurrence or progress, particularly when the forestry component is added (GAMBLE et al., 2014; SCHROTH et al., 2000). The presence of trees in a production system can implies in a drastic modification of the microclimate, including light intensity, temperature and relative humidity (RH) and its consequences on dew formation and leaf wetness duration (DIETZ et al., 2007; LUO; GOUDRIAAN, 2000).

In a meta-analysis to evaluate effects of agroforestry on pest, disease and weed control (PUMARIÑO et al., 2015), only one paper (KOECH; WHITBREAD, 2006) was about disease. This work showed lower bean rust inside an alley cropping than

outside them, and results were related to microclimate. Knowledge on how different arrangements of ICLSs influence microclimate and the occurrence of diseases in cultivated plants can guide better management practices in diversified environments (MATSON, 1997).

Powdery mildew (PM) is one of the most important disease for a variety of summer and winter crops (DEAN et al., 2012; GLAWE, 2008; KANG; MIAN, 2010; TWOMEY et al., 2015). The microclimate is relevant for PM and several microclimatic characteristics have been studied. Particularly, leaf wetness and RH are important components for spore germination and infection.

PMs can be divided in two groups according to their ability to germinate on free water: one group with germination in water comparable to that on the leaf surface and the other showing poor germination (SIVAPALAN, 1993). The author also observed that growth and conidia production of *Blumeria graminis* (DC.) Speer was lower after conidia that originated the pustules were in contact with free water. Earlier results (GRAINGER, 1947; MANNERS; HOSSAIN, 1963) suggest that a saturated atmosphere provides optimum conditions for spore germination in *B. graminis* if care is taken to exclude liquid water. The production of conidia by pustules of *B. graminis* which had been wetted with a few drops of water was reduced less than a half (WARD; MANNERS, 1974). If pustules were kept for a second day after wetting, the sporulation returned to normal, so there seemed to be no permanent damage on germination for wetting. In soybean eight hours of leaf wetness was the ideal period to the progress of PM (*Microsphaera diffusa* Cooke & Peck) in Brazil (ALVES et al., 2009).

Leaf wetness occurs predominantly due precipitation/irrigation or dew formation (ROWLANDSON et al., 2015). Since precipitation does not occur every day but dew is frequent in humid tropical regions (DIETZ et al., 2007), dew plays an important role in the leaf wetness. Experimental and observational results shown that duration and amount of dew is higher on the exposed than on covered surfaces (DIETZ et al., 2007; LUO; GOUDRIAAN, 2000).

Radiation also has great influence on PM. Shaded environment increased severity of *Erysiphe necator* on grapevine and this result was related to the reduction of ultraviolet (UV) radiation and temperature provided by the shadow (AUSTIN; WILCOX, 2012). PM on *Poa* and *Festuca* spp. have been reported with greater severity in shady environment (SMILEY; DERNOEDEN; CLARKE, 1992). High light intensity reduced germination and hyphal growth of *Sphaerotheca macularis* f. sp. *fragariae* on

strawberry (AMSALEM et al., 2006). Mycelial growth of *Uncinula necator* was negatively affected by UV B and that shaded environment was more favorable for spore germination and mycelial growth of the pathogen (WILLOCQUET et al., 1996). Cucumber plants grown under red light were more resistant to *Sphaerotheca fuliginea* while plants grown under other monochromatic spectra were less resistant than plants grown under white light (WANG et al., 2010).

Although *B. graminis* develops in a wide range of temperature, the optimum temperature for spore germination is near 20 ºC (CHEREWICK, 1944; MANNERS; HOSSAIN, 1963). Better development of *M. diffusa* on soybean plants was observed at temperatures of 23 and 24 ºC in Brazil, depending on the cultivar (ALVES et al., 2009), in accordance with previous reported (MIGNUCCI, 1989).

Based on the general affirmation that ICLS can reduce plant diseases, and on the lack of studies on the mechanisms governing these effects (MALÉZIEUX et al., 2009), the current study focused on assessing the influence of two ICLSs arrangements on the PM severity on oats and soybean. Densities of conidia of *M. diffusa* in the air, and microclimatic characteristics that can be helpful to understand epidemiology of this disease were also recorded. The integration between crop and livestock (agropastoral system - AP) and integration of crop, livestock and forestry (agrosilvopastoral system - ASP) were compared with a control system (not integrated crop - CO) in a long term ICLS experiment installed in the Ponta Grossa municipality, in the Brazilian subtropics.

4.2 MATERIALS AND METHODS

4.2.1 Description of the evaluated agricultural systems

The main field experiment (EXP 1) consisted of an ICLS long term experiment installed in 2006 within an experimental station belonging to the *Instituto Agronômico do Paraná* (IAPAR), in Ponta Grossa municipality, Paraná State, Brazil (25°07´S; 50°03´W; and 953m asl) (Figure 3.1). This municipality is located in Campos Gerais of Paraná region. The climate according to Köppen classification is Cfb mesothermal humid subtropical.

EXP 1 has treatments arranged in an incomplete factorial with 3 replications considered in randomized blocks. Experimental factor one is production system with

three levels: integration between crop and livestock (agropastoral - AP), integration of crop, livestock and trees (agrosilvopastoral - ASP) and a control with not integrated crop (CO). Experimental factor 2 is amount of nitrogen (N) applied as urea on cover in total area about 40 days after sowing of the winter pasture, with two levels: 90 kg/ha (in all production systems) and 180 kg/ha (in AP and ASP systems). The lower dose of N is the normally applied on winter pastures in the Region, and the upper dose was included in the original experiment expecting to partially compensate the reduction in the forage growth due the light interception by trees.

The winter grazing in all production systems consists of black oat and annual ryegrass intercropped. Cattle grazing occur for an uninterrupted period of 90 to 120 days every winter in AP and ASP systems, and stocking rate in each plot is managed to maintain the pasture with about 20 cm height. The summer crop in all production systems consists of soybean and corn on alternated crop seasons. Summer crops and winter pastures are established by direct seeding (no tillage). Chemical fertilization and pesticides used are listed in Tables 3.1 and 3.2 (Chapter 3).

The tree component of the ASP is composed of eucalyptus (*Eucalyptus dunnii*) and silver oak (*Grevillea robusta*) alternated in single rows, with an average of 4.5 m between trees and 14 m between rows. Pink pepper (*Schinus terebinthifolius*) was included in the original experiment but was cut during the summer of 2013 in order to decrease shading level by trees. The decrease in light availability for the understory (crops and grazing) vegetation calculated as the difference between two ceptometers, compared to the treeless systems was 53 ± 1.5% in 2014 and 56 ± 0.9% in 2015 winters (PONTES et al., 2017). Each plot has an area of about one hectare, except CO plots, which were established within the AP plots in 2010 and sized at 100 m².

PM on oat was also evaluated in another experiment (EXP 2) in the same experimental station, containing only production system factor with two levels: AP and ASP. Other differences of EXP 2 are: white oat (*A. sativa*) instead black oat is intercropped with annual ryegrass and the tree component of the ASP is *Eucalyptus benthamii* in single rows, with 2.5 m between trees and 16 m between rows planted in 2011. There was no fungicide spraying in oat plots. During the soybean cultivation, an area of at least 560 m^2 of each AP and ASP plots and the total area of CO plots were note sprayed for allow the disease evaluation.

4.2.2 Disease evaluation

Disease severity on oats was evaluated once a year in 2014 and 2015 winters, before cattle grazing begins when the plants were in stages 37-39 of the Zadoks scale (ZADOKS; CHANG; KONZAK, 1974). EXP 1 was cultivated with black oat cultivar PIR 161 and EXP 2 was cultivated with white oat cultivar IPR 61. Three samples of at least 30 leaves per plot were taken, collecting the third leaf from the base of the plant. The severity of PM was evaluated by estimating the percentage leaf area with symptoms on the upper surface of the leaves, using a standard area diagram with 12 levels varying from 0.4 to 37 % (PETERSON; CAMPBELL; HANNAH, 1948). PM was evaluated in black oat cultivar IPR 161 and white oat cultivar IPR 61.

PM on soybean (cultivar BMX Apollo RR) leaves was assessed weekly from sixth until thirteenth week after sowing, V6 until R5.4 stage of Fehr and Caviness scale (FEHR; CAVINESS, 1977) in the 2014/2015 crop season. Five samples with six trifoliate leaves of upper, middle and lower sections of the canopy were collected in each plot each time. In order to evaluate effect of distance from trees on PM severity, the five samples collected in the ASP were distributed in five transects between tree rows. Transects 1 and 5 were 2.5 m from trees (edges of alley cropping), transects 2 and 4 were 5 m from trees and transect 3 was 7 m from trees (middle of alley cropping). Severity was assessed on the basis of percentage of leaf area expressing symptoms using a standard area diagram with eight levels varying from 0.6 to 60 % (MATTIAZZI, 2003).

One spore trap (REIS; SANTOS, 1985) per plot with a microscope slide with vaseline was used to assess the amount of *M. diffusa* spores in the air (Figure 4.1). Glasses were changed weekly during the same period when disease severity was evaluated on soybean leaves, and at least three samples of 28 mm^2 were evaluated in each one. Traps were allocated 1.2 m from the ground.

FIGURE 4.1 – SPORE TRAP

SOURCE: the author (2016).

4.2.3 Microclimatic evaluation

Microclimatic characteristics were evaluated during the 2014/2015 crop season inside the soybean canopy. Leaf wetness duration was estimated with the aid of a leaf wetness sensor S-LWA-M003® (Onset Computer Corporation) per treatment installed 0.25 m from de ground (Figure 4.2 a). The sensors were calibrated in the field through visual assessments of the beginning and the end time of leaf wetness, noting up the values measured by the sensors in these times, as recommended in the instructions manual. Evaluation was performed with one-minute interval for 71 consecutive days starting on January 13 (soybean plants at the V6 stage of Fehr and Caviness scale).

Air temperature, RH and light intensity were recorded with the aid of a temperature/relative-humidity/light data logger U12-012® (Onset Computer Corporation) per treatment (Figure 4.2 b). Sensors were fixed at 0.25 m from the ground and protected from the rain with a plastic cover. Evaluation was carried out with five minutes interval during 77 consecutive days beginning on January 7 (soybean plants in the V5 stage of Fehr and Caviness scale).

FIGURE 4.2 – LEAF WETNESS SENSOR (a) AND TEMPERATURE/RELATIVE-HUMIDITY/LIGHT DATA LOGGER (b)

SOURCE: the author (2016).

4.2.4 Data analysis

For the PM severity on oats no statistical analyses were applied. For the PM severity on soybean analyses were performed assuming a Gaussian linear mixed effects model for transformed severities. Due to the presence of zeros, a two parameter Box-Cox transformation given by $y(\lambda1,\lambda2) = (y+\lambda2)^{\lambda1-1}/\lambda1$, when $\lambda1 \neq 0$ or $y(\lambda1,\lambda2) = \log(y+\lambda2)$, when $\lambda1=0$, with $\lambda1$ and $\lambda2$ estimated by maximum likelihood using the function boxcoxfit of the add-on package *geoR* (RIBEIRO JR; DIGGLE, 2015) was used to meet the normality distribution of the assumed model. Analysis of the density of spores in the air was performed by fitting a generalized linear mixed effect model with Poisson sampling distribution. In both analyses statistical significance of experimental factors was assessed by likelihood ratio tests on a sequence of all possible nested models including experimental factors (production system and N rates) and time (weeks after seeding). Treatment comparisons were performed by contrasting effects of production system (N rates was not significant) in each time with familywise error rates of 0.05.

Microclimatic characteristics inside the soybean canopy at each production system were plotted showing averages per week. A multiple regression using a linear model with marginal hypothesis selection of predictors was performed. This analysis considered all production systems combined. The disease severity on soybean was the dependent variable, and the independent variables were leaf wetness duration, light intensity, night and daylight temperature, and night and daylight RH. Statistical analysis were performed using the R software (R CORE TEAM, 2015) and the add-on packages *latticeExtra* (SARKAR; ANDREWS, 2013), *lme4* (BATES et al., 2014), and *multcomp* (HOTHORN; BRETZ; WESTFALL, 2008).

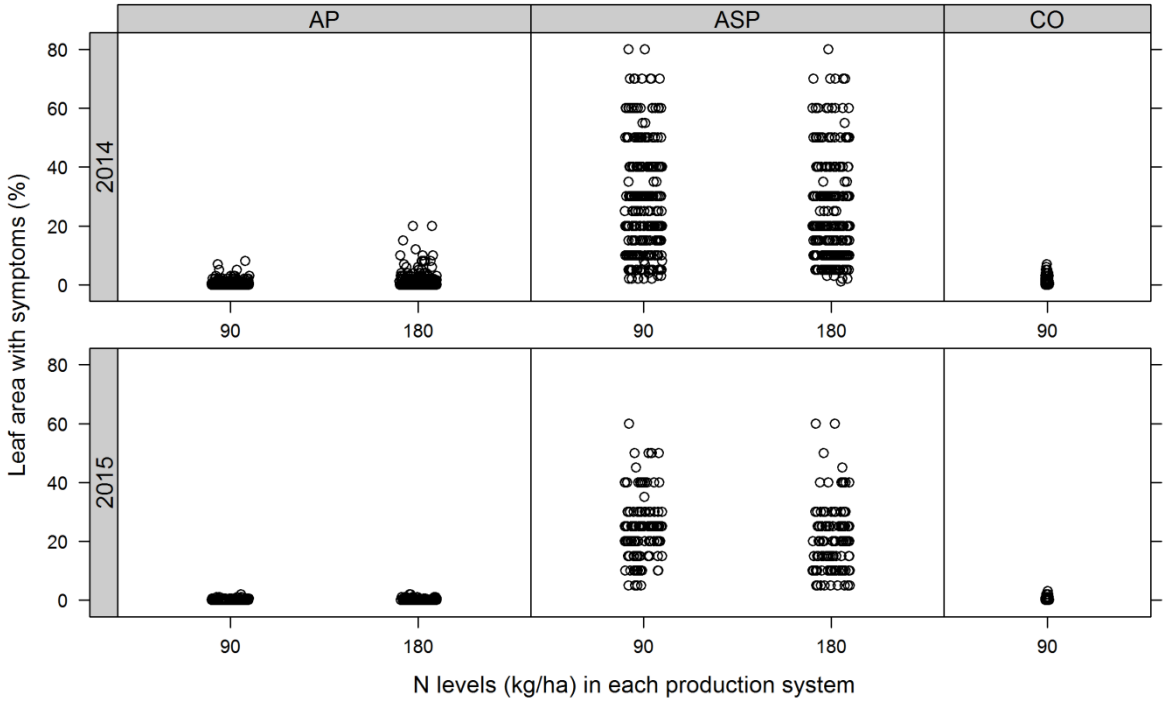
4.3 RESULTS

4.3.1 Powdery mildew severity on oats

No statistical analyses were necessary for the PM severity on oats because of the overwhelming evidence of the difference between ASP and others treatments in the two experiments and two years evaluated (Figures 4.3 and 4.4). In the EXP 1 (black oat) maximum severity in the ASP treatment was 80 and 60 % of the evaluated leaf area with symptoms in 2014 and 2015 respectively. And in the EXP 2 (white oat) maximum severity in the ASP treatment was 63 and 30 % in 2014 and 2015 respectively (Table 4.1).

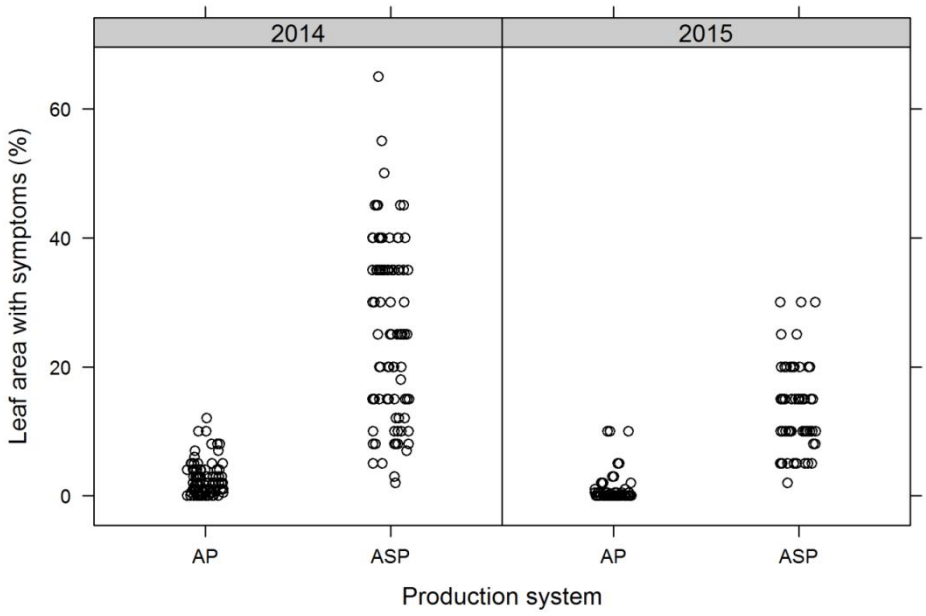
There was no effect of N rate or its interaction with production system. Table 4.1 shows the range and average values of severity in each treatment for each experiment and season. Comparing the results by blocks and samples (data not shown) the same consistent results were found.

FIGURE 4.3 - SEVERITY OF POWDERY MILDEW ON BLACK OAT (CULTIVAR IPR 161) AT EXP 1 IN 2014 AND 2015



AP: agropastoral system, ASP: agrosilvopastoral system, CO: not integrated crop. N rate: kg/ha of nitrogen as urea on winter pasture. Points are the observed proportions in the experimental units. Source: the author (2016).

FIGURE 4.4 - SEVERITY OF POWDERY MILDEW ON WHITE OAT (CULTIVAR IPR 61) AT EXP 2 IN 2014 AND 2015



AP: agropastoral system, ASP: agrosilvopastoral system. Points are the observed proportions in the experimental units. Source: the author (2016).

TABLE 4.1 - RANGE AND MEAN VALUES OF POWDERY MILDEW SEVERITY ON OATS IN TWO EXPERIMENTS AND TWO YEARS

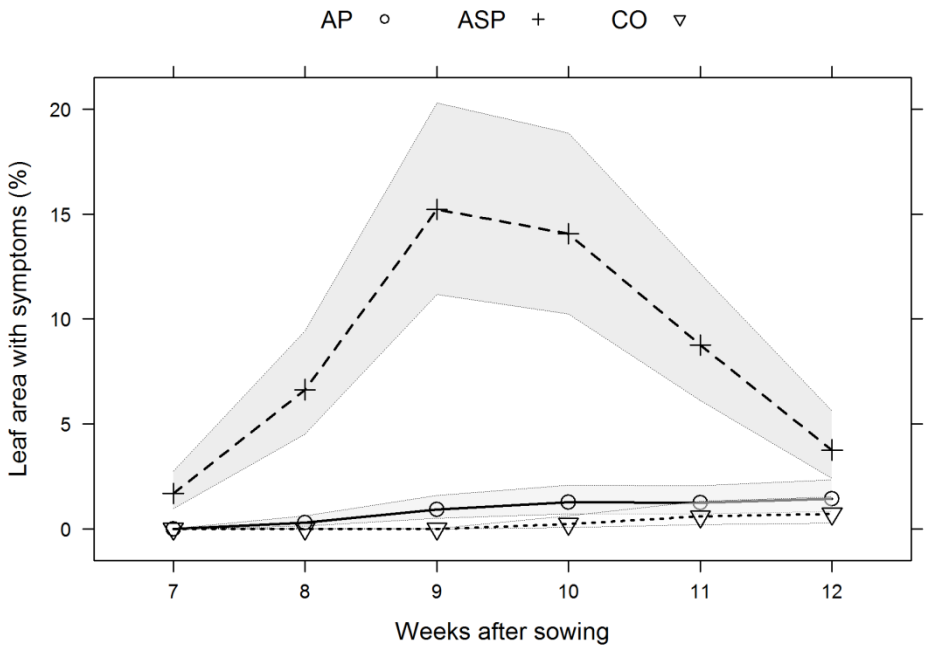
System	N level	2014			2015		
		Min.	Mean	Max.	Min.	Mean	Max.
EXP 1							
AP	90	0	0.4	8	0	0.1	2
ASP	90	2	28.5	80	5	24.4	60
CO	90	0	0.6	7	0	0.2	3
AP	180	0	1.3	20	0	0.1	2
ASP	180	1	23.2	80	5	20.3	60
EXP 2							
AP	---	0	2.4	12	0	1.0	8
ASP	---	7	25.4	63	4	13.1	30

AP: agropastoral system, ASP: agrosilvopastoral system, CO: not integrated crop. N rate: kg/ha of nitrogen as urea on winter pasture. EXP 1: black oat IPR 161, EXP 2: white oat IPR 61.

4.3.2 Powdery mildew severity on soybean and spores density in the air

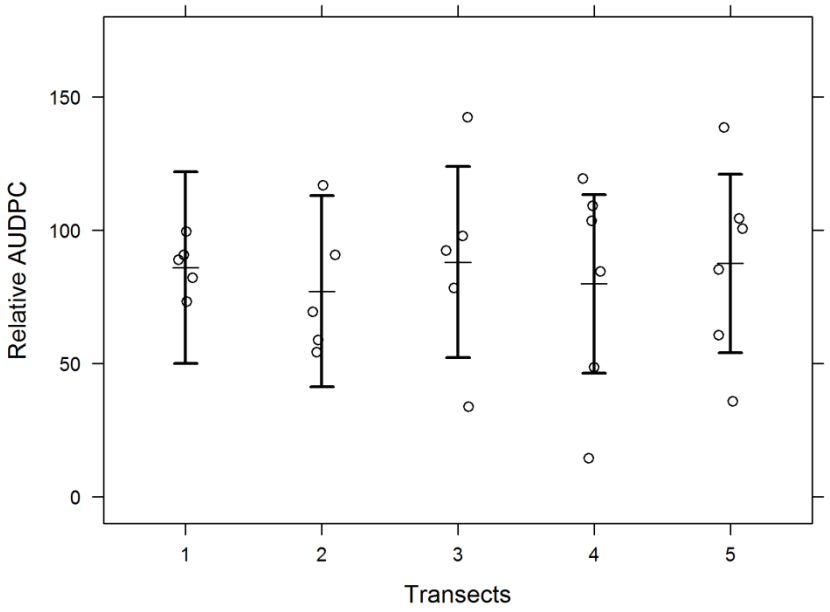
Disease incidence on soybean was first observed at week eight after sowing in the AP and ASP, and only at week 11 in the CO treatment. Differences between production systems were observed from week eight until the final evaluation, with the highest severity in ASP system and also larger difference between ASP and the others (Figure 4.5). Maximum severity observed in ASP and CO was 27.2% and 3.3%, respectively, at week 11 after seeding (R5.1 stage of Fehr and Caviness scale). Maximum severity in AP treatment was 6.7% at week 12. There was no differences of PM severity among the five transects between tree rows (Figure 4.6) along of evaluated time.

FIGURE 4.5 - POWDERY MILDEW SEVERITY ON SOYBEAN (CULTIVAR BMX POTÊNCIA RR), FOR WEEK, IN THREE PRODUCTION SYSTEMS



AP: agropastoral, ASP: agrosilvopastoral, CO: not integrated crop. Bands show the 95% confidence interval for the estimated averages. For statistical analysis data were transformed using a two parameter box-cox transformation (see item 4.2.4, data analysis). Source: the author (2016).

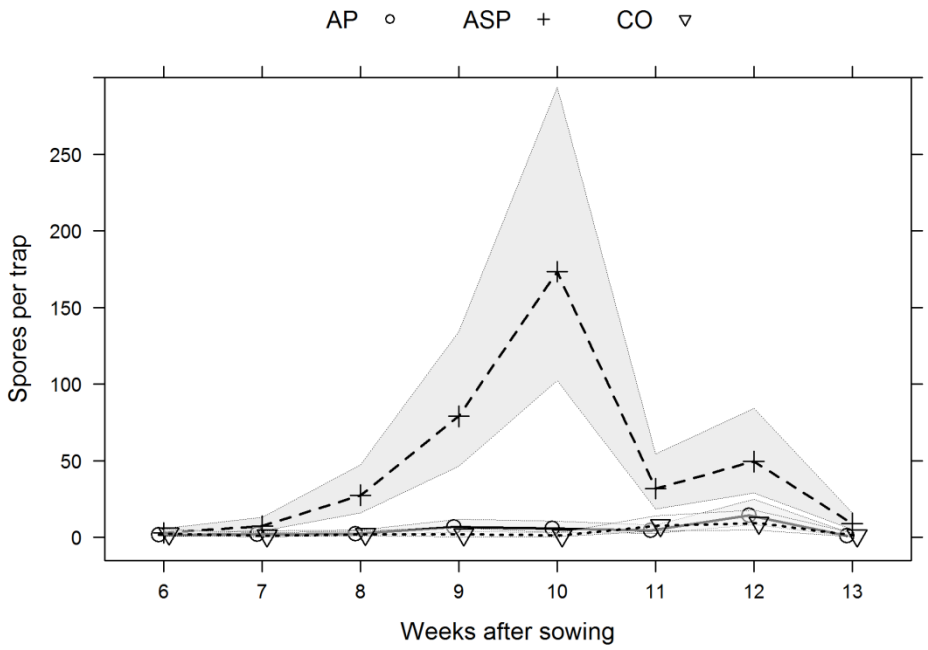
FIGURE 4.6 - RELATIVE AREA UNDER DISEASE PROGRESS CURVE (AUDPC) OF POWDERY MILDEW SEVERITY ON SOYBEAN AT 5 TRANSECTS INSIDE THE ALLEY CROPPING IN THE AGROSILVOPASTORAL (ASP) SYSTEM



Transects 1 and 5: 2.5 m from trees (edges of alley cropping), transects 2 and 4: 5 m from trees, and transect 3: 7 m from trees (center of alley cropping). Bars show 95% confidence interval for the estimated averages. Points are the observed proportions in the experimental units. Source: the author (2016).

Spore traps in all treatments showed spores in the first evaluation and it was not possible to know when spores arrived in each production system. Higher density of *M. diffusa* spores in the air was consistently observed in the ASP treatment compared to the others (Figure 4.7), even before the symptoms of disease. Maximum number of spores per evaluated area in the microscope glass in ASP was 540 at week 10 after sowing (R4 stage of Fehr and Caviness scale). Maximum in CO was 29 spores at week 11, and maximum in AP was 54 at week 12.

FIGURE 4.7 - DENSITY OF *MICROSPHAERA DIFFUSA* SPORES IN SPORE TRAPS, FOR WEEK, IN THREE PRODUCTION SYSTEMS

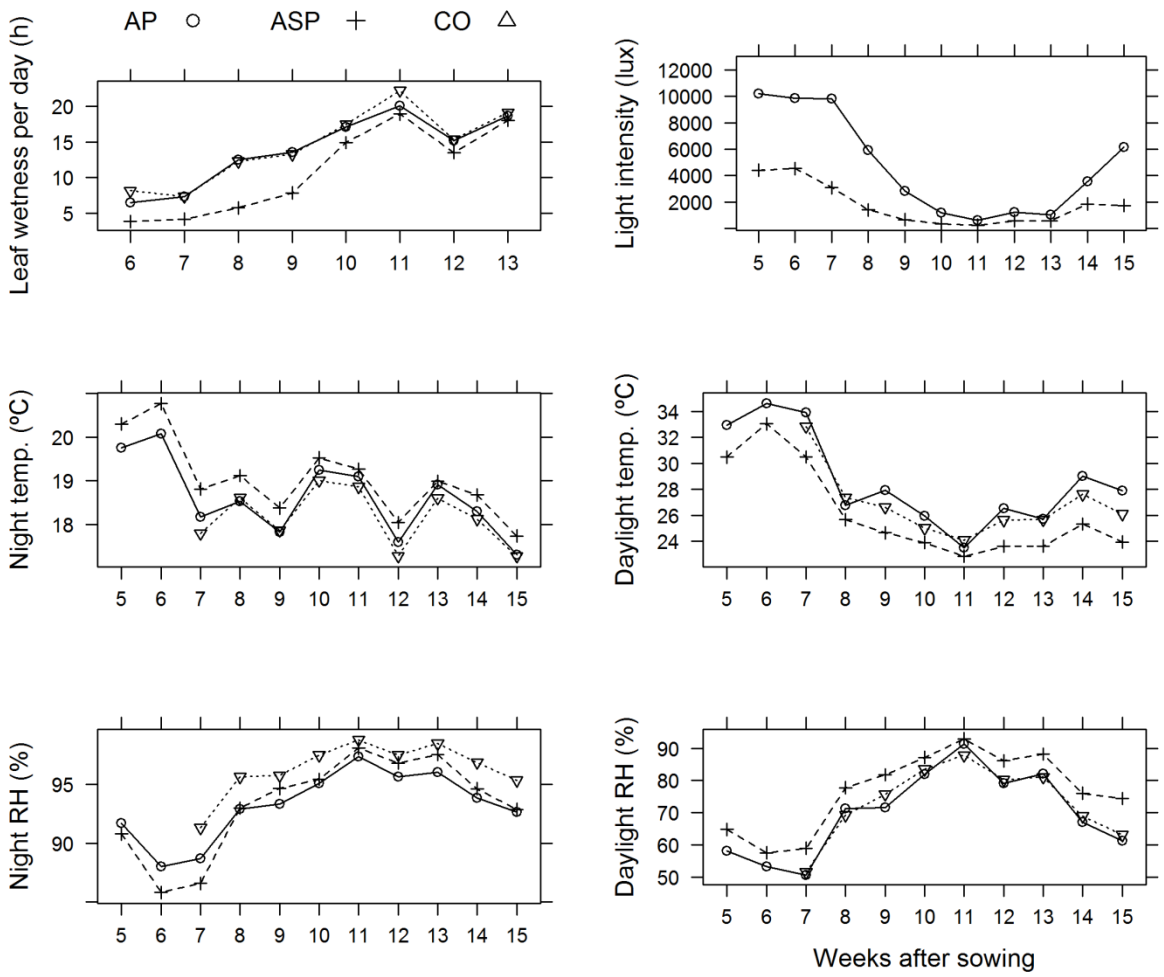


AP: agropastoral, ASP: agrosilvopastoral, CO: not integrated crop. Bands show 95% confidence interval for the estimated averages. Source: the author (2016).

4.3.3 Microclimatic characteristics

The mean leaf wetness duration recorded in the soybean canopy at ASP was 10.9 hours/day, lower than in the other treatments along entire evaluated period (Figure 4.8). Differences of leaf wetness duration between AP and CO treatments were less frequent and intense. Average light intensity recorded in ASP was 0.4X compared to that in the AP. Nocturnal temperature recorded in the ASP (mean of 19 ºC) was consistently higher compared to that in the others treatments, while the daylight temperature in ASP (mean of 26.5 ºC) was always lower compared to that in the others, and the differences between AP and CO treatments were less frequent and intense (Figure 4.8).

FIGURE 4.8 - MICROCLIMATIC CHARACTERISTICS INSIDE THE SOYBEAN CANOPY IN THREE PRODUCTION SYSTEMS



AP: agropastoral, ASP: agrosilvopastoral, and CO: not integrated crop. Points show the mean value for week. Source: the author (2016).

RH during the night in ASP (mean of 93.4 %) was always lower than that in CO, but similar to that recorded in the AP. Daylight RH in ASP (mean of 73.4 %) was always higher compared to that in the others, and there were no differences between AP and CO treatments (Figure 4.8). The estimated parameters from multiple regression between microclimate and PM severity on soybean showed that leaf wetness duration and daylight RH were the most related with the disease severity (Table 4.2).

TABLE 4.2 - PARAMETER ESTIMATION USING MULTIPLE REGRESSION BETWEEN POWDERY MILDEW SEVERITY (%) IN SOYBEAN LEAVES (DEPENDENT VARIABLE), AND MICROCLIMATIC VARIABLES AT FIELD IN 2014/2015 CROP SEASON, WITH DATA OF ALL PRODUCTION (AGROPASTORAL, AGROSILVOPASTORAL AND NOT INTEGRATED) SYSTEMS COMBINED

Independent variables	Estimated parameters	Standard errors	F Sta.	p-value
Leaf wetness duration (h)	-1.3150	0.4382	9.0048	0.0121
Light intensity (lux)	0.0008	0.0009	0.8107	0.3872
Night temperature (ºC)	1.1880	1.9270	0.3799	0.5502
Daylight temperature (ºC)	1.8810	1.9160	0.9640	0.3472
Night relative humidity (%)	0,0601	0.8977	0.0045	0.9478
Daylight relative humidity (%)	1.1650	0.6045	3.7160	0.0801

4.4 DISCUSSION

In this work it was detected a higher PM severity, caused by both *B. graminis* and *M. diffusa,* in the ASP (afforested) system when compared to AP and CO (non-afforested) systems. Microclimate recorded in the field showed that this production system favors PM severity. Despite agricultural diversification promoted by ICLSs have benefits already well documented by several authors (ALTIERI, 1999; BELL; MOORE, 2012; CARVALHO et al., 2010; RYSCHAWY et al., 2012), the influence of the systems in different diseases must be considered to plan strategies of management.

Higher PM severity was recorded in the ASP system than that in AP and CO independent of N rate, year and evaluated host. This result shows that the presence of trees in the production system makes the environment more favorable to this disease, confirming current knowledge (DIETZ et al., 2007; LUO; GOUDRIAAN, 2000; ROWLANDSON et al., 2015). The microclimatic characteristics that favored PM in the present work are also in accordance with discussed in Aust and Hoyningen-Huene review (1986).

It was expected a low severity of PM on soybean at the center of the crop alleys, due to low tree-crop interface (SCHROTH et al., 2000), but this effect was not observed in this work, maybe because of the low spacing (14 m) between rows of trees. This indicates that a larger spacing between rows can be considered in the ASP system that contains eucalyptus and silver oak, with the objective of minimizes the deleterious effect of covering of trees on crops and pastures.

Shorter duration of leaf wetness in ASP system was consistently observed over an eight-week evaluation, confirming previous observation inside the bean canopy in a *Leucaena leucocephala* alley (KOECH; WHITBREAD, 2006). Leaf wetness was also the microclimatic characteristic more related with PM on soybean (Table 4.2). This may be a key factor for increased PM severity in this production system, as the leaf wetness has been shown to be harmful to conidial germination of species causing PM (ALVES et al., 2009; GRAINGER, 1947; MANNERS; HOSSAIN, 1963; SCHNATHORST, 1965; SIVAPALAN, 1993). Higher temperature was observed overnight at the ASP system, which explains lower dew by condensation (AGAM; BERLINER, 2006; DIETZ et al., 2007; HUGHES; BRIMBLECOMBE, 1994). These results show that the presence of trees in a production system is an important factor in reducing the duration of leaf wetness promoted by dew on crops and forages cultivated among them.

Dew is a very common and natural phenomenon where humid air condenses on a substrate and transforms into liquid water (BEYSENS, 1995) when the surface temperature is lower than or equal to the dew-point temperature (AGAM; BERLINER, 2006). The conditions necessary for a heavy dewfall are clear skies and dry air above for maximum radiative heat loss, high absolute humidity near the surface and some turbulent mixing to bring fresh moist air down to the ground (HUGHES; BRIMBLECOMBE, 1994; ROWLANDSON et al., 2015). Higher amount of dew was observed on top leaves of rice in uncovered plants than in three different durations of artificial covering of plants during the night (LUO; GOUDRIAAN, 2000). And more dewfall was observed on the uppermost surface canopy in a tropical forest, where radiative heat losses resulted in lower temperatures of the leaves surface (DIETZ et al., 2007). In the present study the presence of trees in the ASP system modified the microclimate enough to reduce dew formation.

In addition to providing shorter leaf wetness, temperature changes provided by trees in ASP may have contributed directly to greater PM severity. It was demonstrated that low variation in temperature favors *M. diffusa* (ALVES et al., 2009; PAP; RANKOVC; MASIREVIC, 2013; PHILLIPS, 1984), while more variation disfavor it. In the present work it was observed smaller temperature changes in ASP, with higher nighttime and lower daytime temperatures. The average daytime temperature observed in ASP (26.5 °C) was lower than in the other treatments and closer to the best temperatures reported for *M. diffusa* in Brazil (ALVES et al., 2009).

High RH is necessary for spore germination in PM, but liquid water on plant surface is not desirable (GRAINGER, 1947; MANNERS; HOSSAIN, 1963; PAP; RANKOVC; MASIREVIC, 2013). The ASP system showed higher RH during the day compared to AP and CO systems. Although PM is largely independent in terms of moisture, especially in the early stages of conidial germination (PAP; RANKOVC; MASIREVIC, 2013), the high daylight RH may be favored spore germination.

The lower light intensity in the ASP system probably influenced PM severity in both species of oat and in soybean. This is in accordance with others works that appointed higher PM severity in shaded environments (AUSTIN; WILCOX, 2012; SMILEY; DERNOEDEN; CLARKE, 1992) and in plants exposed to lower radiation (AMSALEM et al., 2006; WILLOCQUET et al., 1996). Therefore, the overall characteristics of the microclimate in the ASP in this study favored the PM.

Besides the natural environment competition between trees and crops or pastures for light and nutrients, e.g., increased PM severity provided by the environment can contribute to a reduction in grain production and in the availability of fodder for livestock. On the other hand, removing the infested leaves by the livestock can be considered as a disease control measure. It can be interesting when the grain production is the objective after cattle grazing, in a double purpose cereal. In this work soybean and oat plots were not sprayed, and the PM severity expressed in ASP system reached levels that justify control measures. Public, cooperative and particular research institutes on soybean in the subtropical region of Brazil recommend to start the chemical (spraying) control when severity reach 20 % of leaf area with symptoms (REUNIÃO..., 2009). Powdery mildew was considered as an important disease for the top ten soybean-producing countries (WRATHER et al., 2001).

As the same specie causing PM on oat is also responsible for PMs in wheat and barley, the cultivation of these species in afforested setting provides an extra difficulty. PM is one of the most prevalent wheat diseases, with damages reaching 34 % in moderate infestation (MWALE; CHILEMBWE; ULUKO, 2014), although low (4 %) leaf area covered can reduce transpiration and light assimilation (RABBINGE; JORRITSMA; SCHANS, 1985). This can reduce productivity and increase the need for control in these environments. In general the control of PM should be intensified whenever a crop that has this disease with a potentially causing loss is grown in alley cropping.

These results can be extrapolated, albeit cautiously, to other biotic or abiotic damages that are favored by shorter periods of leaf wetness, lower temperature range, higher RH and limited solar radiation. It should be investigated, for example, whether and how microclimatic characteristics of agroforestry influence the occurrence and severity of insect pests, germination and growth of weeds, frost damage in plants, among others. Recent works have demonstrated that agroforestry serve as a refuge and source of beneficial insects and even birds and bats that suppress pest insects (HARTERREITEN-SOUZA et al., 2014; MAAS; CLOUGH; TSCHARNTKE, 2013; POCH; SIMONETTI, 2013). But the diversity of crop species in an agro-ecosystem has a much less predictable effect on microbial pathogens than on insects (MATSON, 1997).

About diseases, fortunately PM seems to be an exception among overall plant diseases concerning with leaf wetness duration (AUST; HOYNINGEN-HUENE, 1986). Long periods of leaf wetness favors other important pathogens as *Phakopsora* (NARVÁEZ et al., 2010), *Puccinia* (BARRERA; HOY; LI, 2012), *Drechslera* and *Septoria* (SHANER, 1981), for example. Others evaluations are in course at the experimental area where the present work was done, as suppressiveness to and surviving of soil-borne pathogens, severity of corn and soybean diseases, and also the efficiency of native microorganisms to control pathogens. These works are showing advantages of ASP system in suppressing soil-borne pathogens and reducing some diseases of soybean and corn. Previous work (YAMOAH; BURLEIGH, 1990) showed reduced proportion of infected leaves with rust and number of uredinia per plant in maize cultivated in *Sesbania sesban* alley cropping.

Plant breeding for crops and pastures has been made along decades focused on full sun environments. Certainly great efforts will be necessary for its adaptation for covered/shaded ambient, like in alley cropping. This effort will depend on the adoption and importance of these production systems in the near future, although this practice seems not to have great potential to be more broadly implemented in the next decade (WEZEL et al., 2014). For this reason it is recommended to consider others arrangements of trees in the ASP system, especially with larger distances between trees or tree rows, when the crop and/or forage cultivated inside them are susceptible to PM.

4.5 CONCLUSION

Powdery mildew in oats and soybean in afforested production system is more severe than in non-afforested ones, influenced by the microclimate.

ACKNOWLEDGEMENTS

This work was supported by the SERVIAMBI project (Scientific Cooperation Term 21500.10/0008-2, signed between IAPAR and *Embrapa Florestas*) and also by grants of the project CNPq PROCAD number 552334/2011-1 and CAPES.

REFERENCES

AGAM, N.; BERLINER, P. R. Dew formation and water vapor adsorption in semi-arid environments - A review. **Journal of Arid Environments**, v. 65, n. 4, p. 572–590, 2006.

ALTIERI, M. A. The ecological role of biodiversity in agroecosystem. **Agriculture, Ecosystems and Environment**, v. 74, p. 19–31, 1999.

ALVES, M. DE C. et al. Intensidade de oídio *Microsphaera diffusa* Cke. & Pk. em cultivares de soja [*Glycine max* (L.) Merr.] sob diferentes temperaturas e períodos de molhamento foliar. **Ciência e Agrotecnologia**, v. 33, p. 1926–1930, 2009.

AMSALEM, L. et al. Effect of climatic factors on powdery mildew caused by *Sphaerotheca macularis* f. sp. *fragariae* on strawberry. **European Journal of Plant Pathology**, v. 114, n. 3, p. 283–292, 2006.

AUST, H.-J.; HOYNINGEN-HUENE, J. V. Microclimate in relation to epidemics of powdery mildew. **Annual Review of Phytopathology**, v. 24, p. 491–510, 1986.

AUSTIN, C. N.; WILCOX, W. F. Effects of sunlight exposure on grapevine powdery mildew development. **Phytopathology**, v. 102, n. 9, p. 857–866, 2012.

BARRERA, W.; HOY, J.; LI, B. Temperature and leaf wetness effects on infection of sugarcane by *Puccinia melanocephala*. **Journal of Phytopathology**, v. 160, n. 6, p. 294–298, 2012.

BATES, D. et al. **lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-5**. Disponível em: <http://cran.r-project.org/package=lme4>. Acesso em: 25 ago. 2015.

BELL, L. W.; MOORE, A. D. Integrated crop-livestock systems in Australian agriculture: Trends, drivers and implications. **Agricultural Systems**, v. 111, p. 1–12, 2012.

BEYSENS, D. The formation of dew. **Atmospheric Research**, v. 39, p. 215–237, 1995.

CARVALHO, P. C. DE F. et al. Managing grazing animals to achieve nutrient cycling and soil improvement in no-till integrated systems. **Nutrient Cycling in Agroecosystems**, v. 88, n. 2, p. 259–273, 2010.

CARVALHO, P. C. DE F. et al. Definições e terminologias para Sistema Integrado de Produção Agropecuária. **Revista Ciencia Agronomica**, v. 5, p. 1040–1046, 2014.

CHEREWICK, W. Studies on the biology of *Erysihe graminis* D. C. **Canadian Journal Research**, v. 22C, p. 52–86, 1944.

DEAN, R. et al. The Top 10 fungal pathogens in molecular plant pathology. **Molecular Plant Pathology**, v. 13, n. 4, p. 414–430, 2012.

DIETZ, J. et al. Vertical patterns and duration of surface wetness in an old-growth tropical montane forest, Indonesia. **Flora**, v. 202, n. 2, p. 111–117, 2007.

FAO. An international consultation on integrated crop-livestock systems for development: The way forward for sustainable production intensification. **Integrated Crop Management**, v. 13, n. 1, p. 1–79, 2010.

FEHR, W. R.; CAVINESS, C. E. **Stages of soybean development**. Ames: [s.n.].

GAMBLE, J. D. et al. Establishment and early productivity of perennial biomass alley cropping systems in Minnesota, USA. **Agroforestry Systems**, v. 88, n. 1, p. 75–85, 2014.

GLAWE, D. A. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. **Annual Review of Phytopathology**, v. 46, n. 1, p. 27–51, 2008.

GRAINGER, J. The ecology of *Erysiphe graminis* DC. **Transactions of the British Mycological Society**, v. 31, n. 1–2, p. 54–65, 1947.

HAAN, C. D.; STEINFELD, H.; BLACKBURN, H. **Livestock & the environment: Finding a balance**. Disponível em: <http://www.fao.org/docrep/x5303e/x5303e00.htm>.

HARTERREITEN-SOUZA, É. S. et al. The role of integrating agroforestry and vegetable planting in structuring communities of herbivorous insects and their natural enemies in the Neotropical region. **Agroforestry Systems**, v. 88, n. 2, p. 205–219, 2014.

HOTHORN, T.; BRETZ, F.; WESTFALL, P. Simultaneous inference in general parametric models. **Biometrical Journal**, v. 50, p. 346–363, 2008.

HUGHES, R. N.; BRIMBLECOMBE, P. Dew and guttation: formation and environmental significance. **Agricultural and Forest Meteorology**, v. 67, n. 3–4, p. 173–190, 1994.

KANG, S.-T.; MIAN, M. A. R. Genetic map of the powdery mildew resistance gene in soybean PI 243540. **Genome**, v. 53, n. 5, p. 400–405, 2010.

KOECH, E. K.; WHITBREAD, R. Incidence and severity of bean rust (*Uromyces appendiculatus*) in alleys between *Leucaena* hedgerows in Kenya. **New Forests**, v. 31, n. 3, p. 385–402, 2006.

LUO, W.; GOUDRIAAN, J. Measuring dew formation and its threshold value for net radiation loss on top leaves in a paddy rice crop by using the dewball: a new and simple instrument. **International Journal of Biometeorology**, v. 44, n. 4, p. 167–171, 2000.

MAAS, B.; CLOUGH, Y.; TSCHARNTKE, T. Bats and birds increase crop yield in tropical agroforestry landscapes. **Ecology Letters**, v. 16, n. 12, p. 1480–1487, 2013.

MALÉZIEUX, E. et al. Mixing plant species in cropping systems: concepts, tools and models. A review. **Agronomy for Sustainable Development**, v. 29, p. 43–62, 2009.

MANNERS, J.; HOSSAIN, S. Effects of temperature and humidity on conidial germination in *Erysiphe graminis*. **Transactions of the British Mycological Society**, v. 46, n. 2, p. 225–234, 1963.

MATSON, P. A. Agricultural intensification and ecosystem properties. **Science**, v. 277, p. 504–509, 1997.

MATTIAZZI, P. **Efeito do oídio (*Micrhosphaera diffusa* Cooke & Peck) na produção e duração da área foliar sadia da soja**. [s.l.] Universidade de São Paulo, 2003.

MIGNUCCI, J. Powdery mildew. In: SINCLAIR, J.; BACKMAN, P. (Eds.). . **Compendium of soybean diseases**. 3. ed. Saint Paul: American Phytopathological Society, 1989. p. 19–21.

MORAES, A. DE et al. Integrated crop-livestock systems in the Brazilian subtropics. **European Journal of Agronomy**, v. 57, p. 4–9, 2014b.

MORAES, A. DE et al. Research on integrated crop-livestock systems in Brazil. **Revista Ciencia Agronomica**, v. 45, n. 5, p. 1024–1031, 2014a.

MWALE, V. M.; CHILEMBWE, E. H. C.; ULUKO, H. C. Wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*): Damage effects and genetic resistance developed in wheat (*Triticum aestivum*). **International Research Journal of Plant Science**, v. 5, n. 1, p. 1–16, 2014.

NARVÁEZ, D. F. et al. Effects of surface wetness periods on development of soybean rust under field conditions. **Plant Disease**, v. 94, n. 2, p. 258–264, 2010.

PAP, P.; RANKOVC, B.; MASIREVIC, S. Effect of temperature, relative humidity and light on conidial germination of oak powdery mildew (*Microsphaera alphitoides* Griff. et Maubl.) under controlled conditions. **Archives of Biological Sciences**, v. 65, n. 3, p. 1069–1077, 2013.

PETERSON, R.; CAMPBELL, A.; HANNAH, A. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. **Canadian Journal Research**, v. 26C, n. 5, p. 496–500, 1948.

PHILLIPS, D. V. Stability of *Microsphaera diffusa* and the effect of powdery mildew on yield of soybean. **Plant Disease**, v. 68, n. 11, p. 953–956, 1984.

POCH, T. J.; SIMONETTI, J. A. Ecosystem services in human-dominated landscapes: Insectivory in agroforestry systems. **Agroforestry Systems**, v. 87, n. 4, p. 871–879, 2013.

PONTES, L. DA S. et al. Relationship between sward height and herbage mass for integrated crop-livestock systems with trees. **Grassland Science**, v. 63, n. 1, p. 29–35, jan. 2017.

PUMARIÑO, L. et al. Effects of agroforestry on pest, disease and weed control: A meta-analysis. **Basic and Applied Ecology**, v. 16, n. 7, p. 573–582, 2015.

RABBINGE, R.; JORRITSMA, I. T. M.; SCHANS, J. Damage components of powdery mildew in winter-wheat. **Netherlands Journal Of Plant Pathology**, v. 91, p. 235–247, 1985.

R CORE TEAM. **R: A language and environment for statistical computing.**Viena, 2015. Disponível em: <http://www.r-project.org/>

REIS, E. M.; SANTOS, H. P. DOS. População de *Helminthosporium sativum* no ar quantificado através de uma armadilha tipo cata-vento. **Fitopatologia Brasileira**, v. 10, p. 515–519, 1985.

REUNIÃO... **Indicações Técnicas para a Cultura da Soja no Rio Grande do Sul e em Santa Catarina 2009/2010**. Passo Fundo: Embrapa Trigo, 2009.

RIBEIRO JR, P.; DIGGLE, P. **geoR: Analysis of Geostatistical Data. R package version 1.7-6**. Disponível em: <http://www.leg.ufpr.br/geoR>. Acesso em: 25 ago. 2015.

ROWLANDSON, T. et al. Reconsidering leaf wetness duration determination for plant disease management. **Plant Disease**, v. 99, n. 3, p. 310–319, 2015.

RYSCHAWY, J. et al. Mixed crop-livestock systems: an economic and environmental-friendly way of farming? **Animal**, v. 6, n. 10, p. 1722–1730, 2012.

SARKAR, D.; ANDREWS, F. **latticeExtra: Extra graphical utilities based on Lattice. R package version 0.6-26**, 2013. Disponível em: <http://cran.r-project.org/package=latticeExtra>. Acesso em: 25 ago. 2015

SCHNATHORST, W. C. Environmental relationships in the powdery mildews. **Annual Review of Phytopathology**, v. 3, p. 343–366, 1965.

SCHROTH, G. et al. Pests and diseases in agroforestry systems of the humid tropics. **Agroforestry Systems**, v. 50, p. 199–241, 2000.

SHANER, G. Effect of environment on fungal leaf blights of small grains. **Annual Review of Phytopathology**, v. 19, n. 1, p. 273–296, 1981.

SIVAPALAN, A. Effects of water on germination of powdery mildew conidia. **Mycological Research**, v. 97, n. 1, p. 71–76, 1993.

SMILEY, R. W.; DERNOEDEN, P. H.; CLARKE, B. B. **Compendium of Turfgrass Diseases**. 2. ed. [s.l.] APS PRESS, 1992.

THORNTON, P. K.; HERRERO, M. Integrated crop-livestock simulation models for scenario analysis and impact assessment. **Agricultural Systems**, v. 70, n. 2–3, p. 581–602, 2001.

TWOMEY, M. C. et al. Development of partial ontogenic resistance to powdery mildew in hop cones and its management implications. **PLoS ONE**, v. 10, n. 3, p. 1–24, 2015.

WANG, H. et al. Light quality affects incidence of powdery mildew, expression of defence-related genes and associated metabolism in cucumber plants. **European Journal of Plant Pathology**, v. 127, n. 1, p. 125–135, 2010.

WARD, S. V; MANNERS, J. G. Environmental effects on the quantity and viability of conidia produced by *Erysiphe graminis*. **Transactions of the British Mycological Society**, v. 62, n. 1, p. 119–128, 1974.

WEZEL, A. et al. Agroecological practices for sustainable agriculture. A review. **Agronomy for Sustainable Development**, v. 34, n. 1, p. 1–20, 2014.

WILLOCQUET, L. et al. Effects of radiation, especially ultraviolet B, on conidial germination and mycelial growth of grape powdery mildew. **European Journal of Plant Pathology**, v. 102, n. 5, p. 441–449, 1996.

WRATHER, J. A. et al. Soybean disease loss estimates for the top ten soybean-producing countries in 1998. **Canadian Journal of Plant Pathology**, v. 23, p. 115–121, 2001.

YAMOAH, C. F.; BURLEIGH, J. R. Alley cropping *Sesbania sesban* (L) Merill with food crops in the highland region of Rwanda. **Agroforestry Systems**, v. 10, n. 2, p. 169–181, 1990.

ZADOKS, J. C.; CHANG, T. T.; KONZAK, C. F. A decimal code for the growth stages of cereals. **Weed Research**, v. 14, n. 6, p. 415–421, 1974.

5 INTEGRATED CROP-LIVESTOCK SYSTEM IN THE BRAZILIAN SUBTROPICS REDUCES THE SURVIVAL PERIOD OF *SCLEROTINIA SCLEROTIORUM* SCLEROTIA

ABSTRACT

White mold caused by *Sclerotinia sclerotiorum* is an important plant disease that attacks hundreds of plant species of different botanical families. Sclerotia produced by the pathogen are the central component of epidemics as they are the primary long-term survival structures responsible for the inocula maintenance in the soil for one or more years, depending mainly on soil biology and depth of sclerotial burial. Integrated crop-livestock systems (ICLSs) are sustainable options for agriculture diversification. Several studies show its benefits, however there are few studies about the influence of different ICLS arrangements on plant diseases. This work investigated the influence of two arrangements of ICLS: agropastoral (AP) and agrosilvopastoral (ASP) compared to not integrated crop (CO) in the survival period of *Sclerotinia sclerotiorum* sclerotia in the field, buried and on the soil surface. Besides that, microorganisms collected in the experimental field were tested as antagonists for *S. sclerotiorum*. The study was conducted in a long-term ICLS experiment installed in 2006 that contains two experimental factors: production systems with three levels (AP, ASP and CO), and nitrogen (N) doses on the wither pasture with two levels: 90 kg/ha (for all productions systems) and 180 kg/ha (for AP and ASP). Sclerotia of a native *S. sclerotiorum* strain of the experimental field were multiplied in laboratory, put in the field and evaluated periodically during 24 months when buried and during 13 months when on the soil surface. The experiment with buried sclerotia was in duplicate. Number of recovered sclerotia, carpogenic germination, apothecia production, miceliogenic germination and colonization by *Trichoderma* in not germinated sclerotia were evaluated. Microorganisms grown in not germinated sclerotia were tested as antagonists for capogenic germination of the native and two exogenous strains of *S. sclerotiorum*. Sclerotia left on the soil surface showed low survival period than the buried sclerotia and survived for 13 months. When buried the survival period was lower in the ASP than in the AP system, but interacting with the N doses in the first experiment. When on the soil surface the survival period was lower in the AP system than in the CO and ASP. The native *S. sclerotiorum* sclerotia germinated less than the exogenous strains after treated with the native microorganisms tested as antagonists. These results shown a reduction in the survival period of *Sclerotinia sclerotiorum* sclerotia promoted by ICLSs.

Key-words: *Trichoderma*. Agropastoral system. Agrosilvopastoral system. Suppressiveness. Soil health. White mold.

RESUMO

O mofo-branco causado por *Sclerotinia sclerotiorum* é uma importante doença de planta que ataca centenas de espécies vegetais de diferentes famílias botânicas. Os escleródios produzidos pelo patógeno são o componente central das epidemias porque são estruturas primárias de sobrevivência que mantém o inoculo no solo por um ou mais anos, dependendo principalmente da biologia do solo e da profundidade de enterro. Sistemas integrados de produção agropecuária (SIPAs) são opções sustentáveis para a diversificação da produção agrícola. Diversos estudos comprovam efeitos benéficos, entretanto existem poucos estudos sobre a influência de diferentes arranjos de SIPA nas doenças de plantas. Este trabalho investigou a influência de dois arranjos de SIPA: agropastoril (AP) e agrosilvipastoril (ASP), comparados com parcelas de lavoura não integrada (CO) no período de sobrevivência de escleródios de *Sclerotinia sclerotiorum* no campo, enterrados e na superfície do solo. Além disso, microrganismos coletados na área experimental foram testados como antagonistas de *S. sclerotiorum*. O estudo foi conduzido num experimento de SIPAs de longa duração instalado em 2006 que contem dois fatores: sistema de produção com três níveis (AP, ASP e CO), e doses de nitrogênio (N) sobre a pastagem de inverno, com dois níveis: 90 kg/ha (para todos os sistemas de produção) e 180 kg/ha (para AP e ASP). Escleródios de um isolado de *S. sclerotiorum* nativo da área experimental foram multiplicados em laboratório, colocados no campo e avaliados periodicamente durante 22 meses quando enterrados e durante 13 meses quando sobre a superfície do solo. O experimento com escleródios enterrados foi realizado em duplicata. Número de escleródios recuperados, germinação carpogênica, produção de apotécios, germinação miceliogênica e colonização por *Trichoderma* nos escleródios não germinados foram avaliados. Microrganismos crescidos em escleródios não germinados foram também testados como antagonistas da germinação carpogênica do isolado nativo e dois isolados exógenos de *S. sclerotiorum*. Escleródios deixados na superfície do solo tiveram sua sobrevivência reduzida em relação aos enterrados e sobreviveram por 13 meses. Quando enterrados, o período de sobrevivência foi menor no sistema ASP em comparação com o AP, porém interagindo com as doses de N no primeiro experimento. Quando na superfície do solo, o período de sobrevivência de escleródios foi mais curto no sistema AP do que no CO e ASP. A germinação de escleródios do isolado nativo do patógeno foi menor que dos isolados exógenos depois de tratados com microrganismos nativos. Esses resultados mostram uma redução na sobrevivência de escleródios de *S. sclerotiorum* promovida por SIPAs.

Palavras-chave: *Trichoderma*. Sistema agropastoril. Sistema agrosilvipastoril.. Supressividade. Saúde do solo. Mofo branco.

5.1 INTRODUCTION

White mold (WM) is an ancient and important plant disease, caused by *Sclerotinia sclerotiorum* (Lib.) de Bary, one of the most devastating and cosmopolitan plant pathogen (BOLTON; THOMMA; NELSON, 2006), hosted by more than 500 species of 75 botanical families (BOLAND; HALL, 1994; SAHARAN; MEHTA, 2008). The disease results in variable productivity damages that may be as high as 100% (GRAU, 1989; KAMAL et al., 2016; MEYER et al., 2014; WRATHER et al., 2001).

Sclerotia produced by the pathogen are the central component of epidemics as they are the primary long-term survival structures and survive in the soil for at least one, with references of more than eight years, depending on several factors as the soil biology, chemical and physical characteristics, the presence of susceptible plants, depth of sclerotial burial in the soil and soil atmosphere (ADAMS; AYERS, 1979; COLEY-SMITH; COOKE, 1971; REIS; TOMAZINI, 2005; SAHARAN; MEHTA, 2008; WU; SUBBARAO, 2008). Infection can occur either by myceliogenic or carpogenic germination of sclerotia, although for *S. sclerotiorum* ascospores released in the air after carpogenic germination is the more important inoculum (ABAWI; GROGAN, 1979). Sclerotia germinates carpogenically producing apothecia with ascospores that are released in the air and infect aerial tissues of the plants promoting cell collapse (AGRIOS, 1997; BOLTON; THOMMA; NELSON, 2006).

The decline in population of sclerotia in the soil can be predominantly attributed to their germination or decomposition by microorganism (ALEXANDER; STEWART, 1994; BEN-YEPHET; GENIZI; SITI, 1993; MERRIMAN, 1976; SHARMA et al., 2015; WILLIAMS; WESTERN, 1965). With information provided by grower's experience (ADAMS; AYERS, 1979), sclerotia of *S. sclerotiorum* survive in nature for about 4 to 5 years. But do not germinate while buried to more than 4 cm depth (WU; SUBBARAO, 2008). The survival period of sclerotia was about 12 month on the soil surface in a no till system with soybean (*Glycine max*) and black oat (*Avena strigosa*) succession in the North of Rio Grande do Sul State of Brazil where the climate according to Köppen classification is Cfa, humid subtropical (BRUSTOLIN et al., 2016). When buried to 10 cm depth in the same region they survived for 36 months (REIS; TOMAZINI, 2005).

Although influence of crop rotation on sclerotia survival have been yet investigated (BEN-YEPHET; GENIZI; SITI, 1993; GRACIA–GARZA et al., 2002; MUELLER; PEDERSEN; HARTMAN, 2002; ROUSSEAU; RIOUX; DOSTALER, 2007;

SAHARAN; MEHTA, 2008), extrapolations on longevity between different types of sclerotia and soils cannot be made (MERRIMAN, 1976; MERRIMAN et al., 1979). And there is lack of knowledge about the influence of integrated crop-livestock systems (ICLS) on the survival of sclerotia. ICLSs including crops and animals grazing in the same area are longstanding production systems and considered the main form of agricultural land use in the world (BELL; MOORE, 2012; HAAN; STEINFELD; BLACKBURN, 1997; THORNTON; HERRERO, 2001). Studies in this area have been intensified in recent years due to the benefits these systems bring to the farmer, including reductions in plant diseases and weed incidence (ALTIERI, 1999; BALBINO et al., 2011; BELL; MOORE, 2012; FRANZLUEBBERS, 2007). However, the system can lead to environment modifications, which can increase or decrease plant diseases, depending on the particular requirement of the organisms (MATSON, 1997).

The main objective of this study was to compare the survival period of sclerotia of *S. sclerotiorum* in two arrangements of ICLS - with and without trees, compared to a control with not integrated crop, in a long term field experiment. Effects of two rates of nitrogen (N) applied in the soil was also evaluated. Sclerotia survival was evaluated at 8-10 cm depth in the soil and on the soil surface. Additionally, microorganisms grown in non-germinated sclerotia were tested in vitro as antagonists to sclerotia germination.

5.2 MATERIALS AND METHODS

5.2.1 Description of the evaluated agricultural systems

The field experiment evaluated consists of an ICLS long-term experiment installed in 2006 in Ponta Grossa municipality, Paraná State, Brazil. The experiment has two distinct soil classes that were separated by blocks: Dystric Cambisol (one block) and Dystric Ferralsol (two blocks). The climate according to Köppen classification is Cfb, mesothermal humid subtropical.

The experiment has treatments arranged in an incomplete factorial with 3 replications considered in randomized blocks. Experimental factor one is production system with three levels: integration between crop and livestock (agropastoral system - AP), integration of crop, livestock, and trees (agrosilvopastoral system - ASP), and a control with not integrated crop (CO). Experimental factor 2 is amount of N applied as urea on cover in total area about 40 days after sowing of the winter pasture, with two

levels: 90 kg/ha (in all production systems) and 180 kg/ha (in AP and ASP systems). The 90 kg/ha dose of N is the normally applied on winter pastures in the Region, and the 180 kg/ha dose was included in the original experiment expecting to partially compensate the reduction in the forage growth due the light interception by trees.

All production systems have summer crops of soybean and corn (Zea mays) on alternated crop seasons. The winter grazing in all production systems consists of black oat and annual ryegrass (*Lolium multiflorum*) intercropped. Summer crops and winter pastures are established by direct seeding (no tillage). Chemical fertilization and pesticides used are listed in Tables 3.1 and 3.2 (Chapter 3). Cattle grazing occur for an uninterrupted period of 90 to 120 days every winter in AP and ASP systems, and stocking rate is managed to maintain the pasture with 20 cm height.

The tree component of the ASP is composed of eucalyptus (*Eucalyptus dunnii*) and silver oak (*Grevillea robusta*) alternated in single rows, with an average of 4.5 m between trees and 14 m between rows. Pink pepper (*Schinus terebinthifolius*) was included in the original experiment but was cut during the summer of 2013 in order to decrease shading level by trees. The decrease in light availability for the understory (crops and grazing) vegetation calculated as the difference between two ceptometers, compared to the treeless systems was 53 ± 1.5% in 2014 and 56 ± 0.9% in 2015 winters (PONTES et al., 2017). Each plot has an area of about one hectare, except CO plots, which were established within the AP plots in 2010 and sized at 100 m².

5.2.2 Multiplication of sclerotia and installation of the field experiments

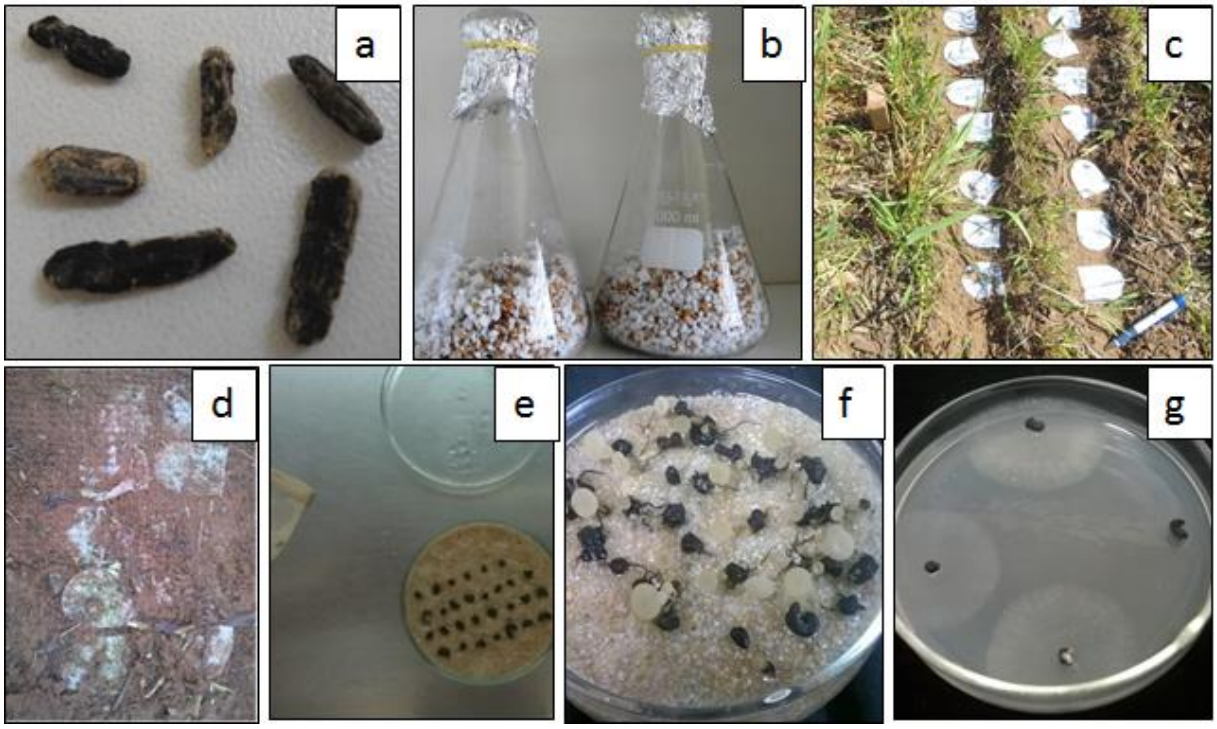
Sclerotia were collected from soybean stems at the field experiment on March 2013, cultivated in potato dextrose agar (PDA) and multiplied according to Sansford and Coley-Smith (1992). Fragments of seven-day colonies of the pathogen were transferred to one liter Erlenmeyer's flasks containing autoclaved wheat grains and perlite. After about five weeks of incubation, during which the flasks were manually shaken every 3-4 days to dislodge the sclerotia, the substrate was rinsed and transferred to a tray and dried for 3 days at room temperature. Sclerotia were then manually separated and classified with the help of sieves to obtain sclerotia of 2-4 mm diameter, and stored at room temperature for about four weeks until placed in the field (Figure 5.1 a-b).

The sclerotia were placed in groups of 30 in polyester bags and distributed in the field experiment in two ways: buried at 8-10 cm depth and on the soil surface (Figure 5.1 c-d). The experiment with buried sclerotia was performed twice. First experiment was installed on August 2013 and evaluated monthly from third to 14th month. Second experiment was installed on December 2014 and evaluated bimonthly from second to 24th month. The experiment with sclerotia on the soil surface was installed once, on December 2014 and evaluated monthly from first to 13th month.

At evaluation times one bag of sclerotia in the soil surface, and two bags of sclerotia buried were collected per plot and disinfested in 1% sodium hypochlorite for 90 seconds, double washed in sterile distilled water (SDA), placed on moistened sand previous washed, autoclaved and sieved of one millimeter, diameter inside 90 mm petri dishes (Figure 5.1 e) and incubated with 15 ± 1 ºC and 12 hours photoperiod provided by fluorescent lamps. Disinfectant concentration and duration of disinfestation were chosen after a previous test with several concentrations and periods submitted to disinfectant with sclerotia of the same *S. sclerotiorum* strain used in the experiment, and after buried for three months.

During incubation period, SDA was refilled in the dishes every three to four days to maintain the sand completely saturated. After 60 days of incubation the number of recovered sclerotia and carpogenic germination were evaluated considering germinated the sclerotium with one or more stipes with the length equal or higher of the less diameter of sclerotium (Figure 5.1 f). Additionally, the number of apothecia per sclerotia was counted considering that all stipes were capable to form apothecia, and three or more stipes were counted as three. Sclerotia not germinated carpogenically were transferred to PDA to evaluate myceliogenic germination and the presence of *Trichoderma* (Figure 5.1 g).

FIGURE 5.1 - SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* COLLECTED FROM SOYBEAN STEMS (a), MULTIPLIED IN WHEAT-PERLITE SUBSTRATE AFTER CULTURED ON PDA (b), BURIED IN THE FIELD (c) OR LEFT ON THE SOIL SURFACE (d) INSIDE POLYESTER BAGS, TRANSFERRED TO MOISTENED SAND TO GERMINATE AFTER DISINFESTED (e), SHOWING APOTHECIA (f), AND GERMINATING MICELIOGENICALLY (g)



SOURCE: the author (2016).

5.2.3 Test of candidates for biological control agent

Microorganisms yielded in sclerotia not germinated on PDA and not also colonized for *Trichoderma* were recovered and stored on PDA, if fungus, and on nutrient broth agar (NBA), if bacteria and tested as biological control agents (BCA) candidates. Thirty newly produced sclerotia of the same strain tested for survival in the field (S1) were placed in petri dishes containing the BCA candidates, with three replications, and left for 72 hours at room temperture, with manual agitation twice a day to enhance the contact between sclerotia and BCA candidate. Negative controls with PDA and NBA, and positive controls with commercial *Trichoderma*, native *Trichoderma* collected at the field experiment and fluazinan were also evaluated.

After the incubation period, sclerotia were transferred to polyester bags and burried 10 cm depth in an orchard of peach and persimmon. After 30 days sclerotia were collected, disinfested and evaluated for carpogenic germination as described in

section 5.2.2. Thirteen candidates in this test (one fungus from CO, six funguses and two bacteria from AP, and four funguses from ASP) were tested again with the same and two more *S. sclerotiorum* strains (S2 and S3) collected from soybean plants 35 km far from the field where S1 was collected. The same evaluation was performed after 30 days buried in a soybean field.

5.2.4 Data analysis

The number of recovered sclerotia and the carpogenic germination were assessed in relation to the total amount of sclerotia placed in the soil in each polyester bag. Number of apothecia was assessed by the sum of stipes produced in each sample, considering only three apothecia for sclerotia with three or more. Miceliogenic germination and the presence of *Trichoderma* were assessed in relation to the amount of sclerotia recovered but not germinated carpogenically.

Statistical inferences of sclerotia recovering and germination were performed by fitting a generalized linear model with binomial sampling distribution and logistic link. The model included effect of blocs, treatments (production systems and N rates) alone and combined and time effect. The statistical significance of experimental factors was assessed by ANOVA and linear hypothesis (multiple comparisons) by the false discovery rate (fdr) method with error rates of 0.05.

Similar generalized linear structure was used for number of apothecia produced, with a Poisson distribution for the response variable. Statistical analyses were performed using the R software (R CORE TEAM, 2015) and the add-on packages *doBy* (HOJSGAARD; HALEKOH, 2014), *latticeExtra* (SARKAR; ANDREWS, 2013) and *multcomp* (HOTHORN; BRETZ; WESTFALL, 2008).

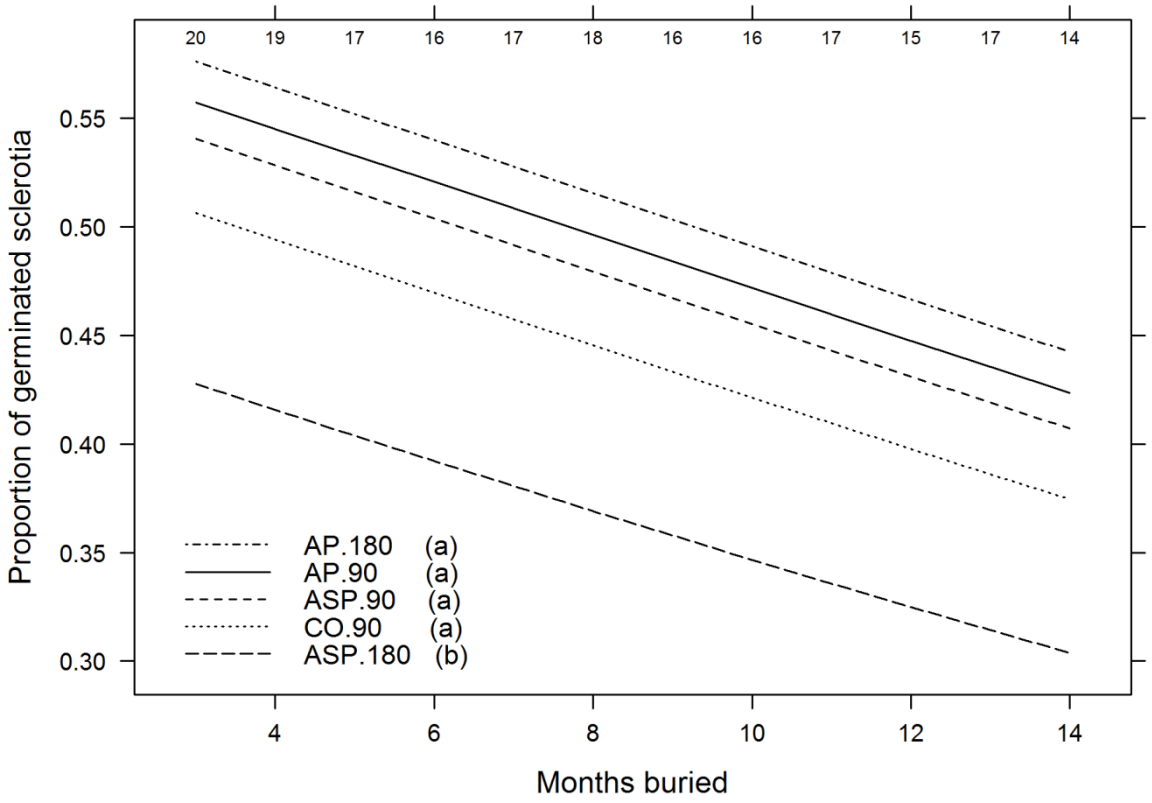
5.3 RESULTS

5.3.1 Sclerotia recovering and carpogenic germination

Sclerotia germination declined over the months in all experiments, following the decline in sclerotia recovered from the soil, although evaluations had not been carried until germination reach zero in buried sclerotia. In the first experiment with

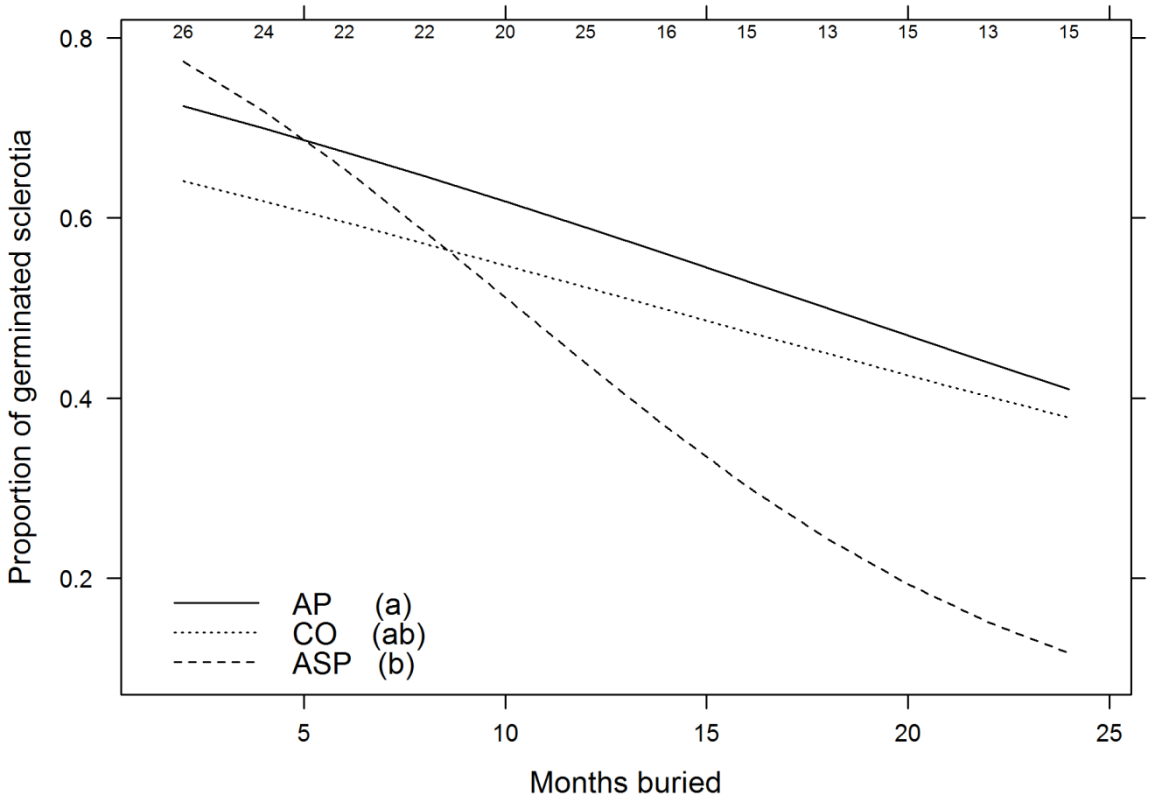
buried sclerotia, interaction between production system and N rate was observed and ASP with higher N rate promoted a reduction in sclerotia germination in relation to all others combinations between production system and N rate (Figure 5.2). In the second experiment with buried sclerotia interaction between production system and time was observed. The ASP system reduced the sclerotia germination when compared to AP, but not different of CO. (Figure 5.3).

FIGURE 5.2 – PROPORTIONS OF CARPOGENIC GERMINATION OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* ACCORDING TO A QUASIBINOMIAL MODEL, AFTER BURIED ON AUGUST 2013 IN THREE PRODUCTION SYSTEMS WITH TWO N DOSES



AP: agropastoral, ASP: agrosilvopastoral, CO: not integrated crop. 90 and 180 are the amount of nitrogen (kg/ha) applied on the winter pasture. Treatments followed by the same letter are not different according to the fdr method. Carpogenic germination was assessed in relation to the total number of sclerotia left in each polyester bag in the field. Numbers in the top are the mean sclerotia recovered each month. Source: the author (2016).

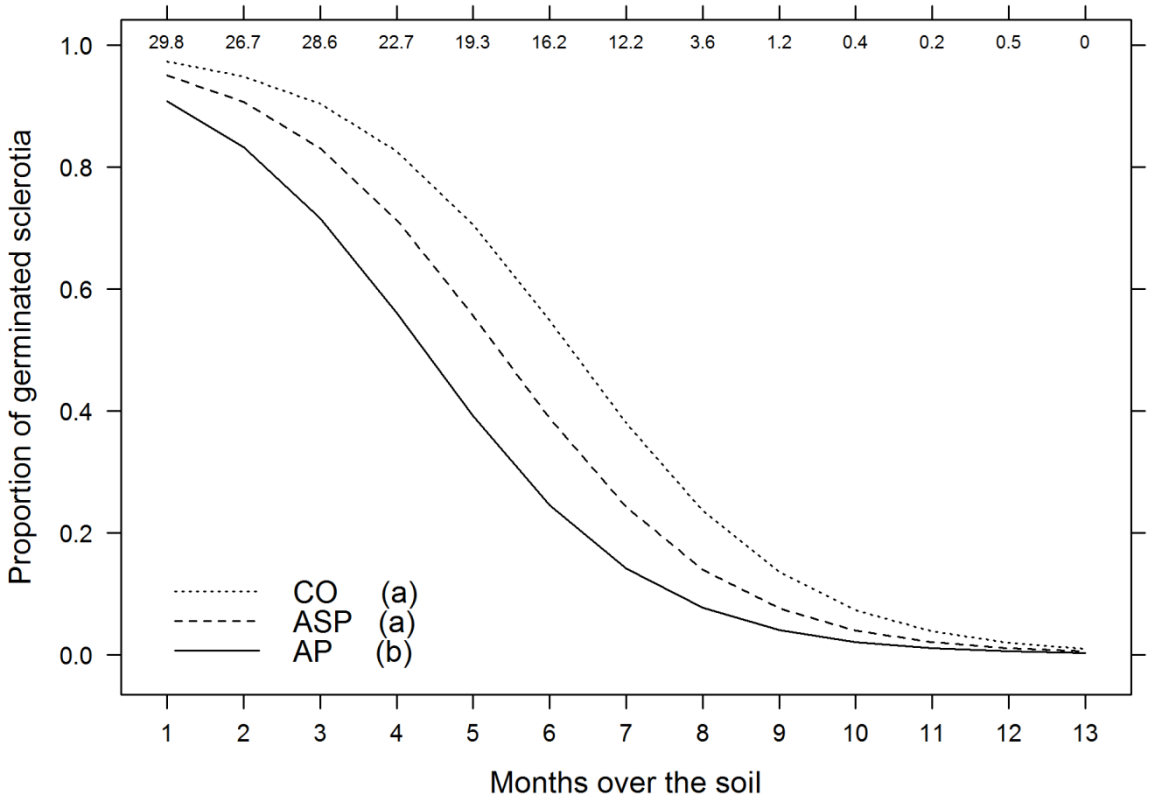
FIGURE 5.3 – PROPORTIONS OF CARPOGENIC GERMINATION OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* ACCORDING TO A QUASIBINOMIAL MODEL, AFTER BURIED ON DECEMBER 2014 IN THREE PRODUCTION SYSTEMS



AP: agropastoral, ASP: agrosilvopastoral, CO: not integrated crop. Treatments followed by the same letter are not different according to the fdr method. Carpogenic germination was assessed in relation to the total number of sclerotia left in each polyester bag in the field. Numbers in the top are the mean sclerotia recovered each month. Source: the author (2016).

For sclerotia left on the soil surface germination reached zero at 10th 12th and 13th month in AP, ASP and CO, respectively, when no more sclerotia was recovered from the soil. Only production system effect was observed and AP system reduced the sclerotia survival compared to the CO and ASP (Figure 5.4). Sclerotia on the soil surface showing apothecia were frequently observed in the field during the evaluation period.

FIGURE 5.4 – PROPORTIONS OF CARPOGENIC GERMINATION OF SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* ACCORDING TO A QUASIBINOMIAL MODEL, AFTER LEFT ON THE SOIL SURFACE OF THREE PRODUCTION SYSTEMS ON DECEMBER 2014



AP: agropastoral, ASP: agrosilvopastoral, CO: not integrated crop. Treatments followed by the same letter are not different according to the fdr method. Carpogenic germination was assessed in relation to the total number of sclerotia left in each polyester bag in the field. Numbers in the top are the mean sclerotia recovered each month. Source: the author (2016).

In both second buried and superficial experiment a delay to germination decline was observed in the first months while in the first experiment it was not evident, probably because the two first months were not evaluated. Initial proportions of sclerotia germinated carpogenically was lower in the first experiment with buried sclerotia, and similar in the second and superficial experiments, these ones installed at the same time and after the first one.

Carpogenic germination of sclerotia in relation to the total amount of sclerotia left in the soil (Figures 5.2-5.4) was mainly explained by the number of recovered sclerotia than by the proportion of recovered sclerotia that germinated (Table 5.1). Number of apothecia per treatment (Table 5.1) showed very similar results that carpogenic germination in all experiments with buried and superficial sclerotia.

TABLE 5.1 – PROPORTIONS OF RECOVERED SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* FROM THE SOIL, CARPOGENICALLY GERMINATED SCLEROTIA, AND NUMBER OF APOTHECIA PER EVALUATION

	First experiment with buried sclerotia					
Treatment[1]	Rec[2]		Carp[3]		Apo[4]	
AP.180	0.61	a	0.92	ns	39	a
AP.90	0.61	a	0.90		37	a
ASP.90	0.58	a	0.88		35	a
CO.90	0.55	a	0.90		33	ab
ASP.180	0.46	b	0.85		28	b
	Second experiment with buried sclerotia					
	Rec		Carp		Apo	
AP	0.76	a	0.93	ns	45	a
CO	0.74	ab	0.91		40	ab
ASP	0.64	b	0.92		37	b
	Experiment with sclerotia on the soil surface					
	Rec		Carp		Apo	
CO	0.81	a	0.81	ns	33	a
ASP	0.74	a	0.72		26	ab
AP	0.51	b	0.72		22	b

[1]AP: agropastoral system, ASP: agrosilvopastoral system, CO: control with not integrated crop; 90 and 180 are the N rates in kg/ha on the winter pasture. [2]Proportion of recovered sclerotia in relation to the total sclerotia left in the soil for each evaluation. [3]Carpogenic germination in relation to the recovered sclerotia. [4]Apothecia per sample; three or more apothecia were counted as three. Numbers followed by the same letter in the columns for each experiment are not different according to the fdr method.

5.3.2 Miceliogenic germination

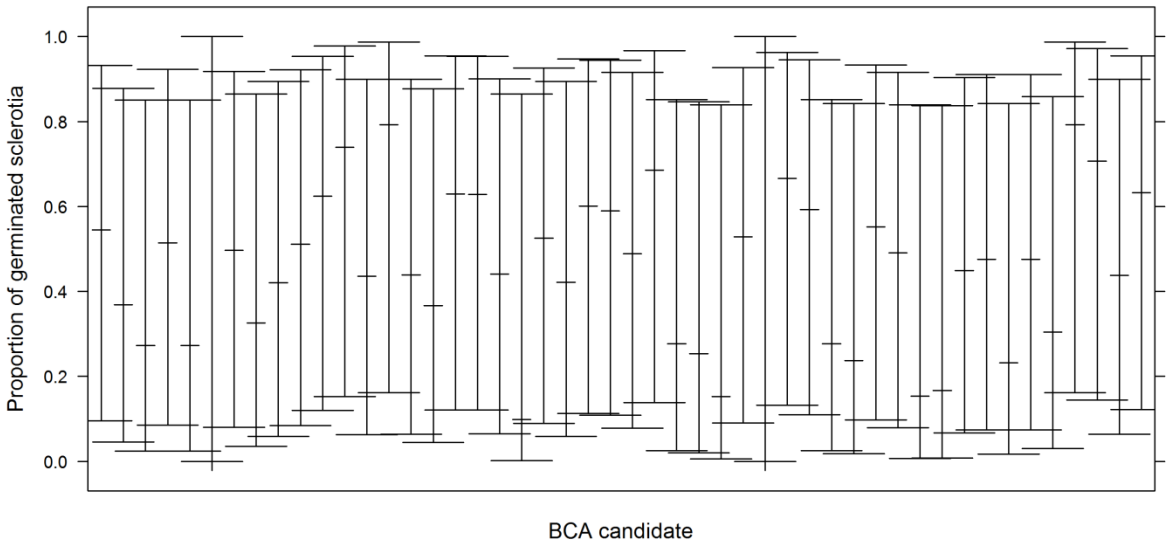
There were no differences between treatments for miceliogenic germination and *Trichoderma* colonization in any experiment. With carpogenically and miceliogenically germinations (total germination) summed, results were similar to that of only carpogenic germination in all experiments (data not shown). For buried sclerotia, the proportions that germinated miceliogenically were 0.86, 0.87 and 0.85 for AP, ASP and CO in the first experiment and 0.60, 0.56 and 0.81 in the second experiment, respectively, and the proportions of sclerotia colonized with *Trichoderma* were 0.55, 0.57 and 0.56 for AP, ASP and CO in the first experiment and 0.42, 0.41 and 0.53 in the second experiment, respectively. For sclerotia left on the soil surface, the proportions that germinated miceliogenically were 0.69, 0.62 and 0.68 for AP, ASP

and CO, respectively, and the proportions of sclerotia colonized with *Trichoderma* were 0.62, 0.53 and 0.60 for AP, ASP and CO, respectively.

5.3.3 Efficiency of candidates for biological control

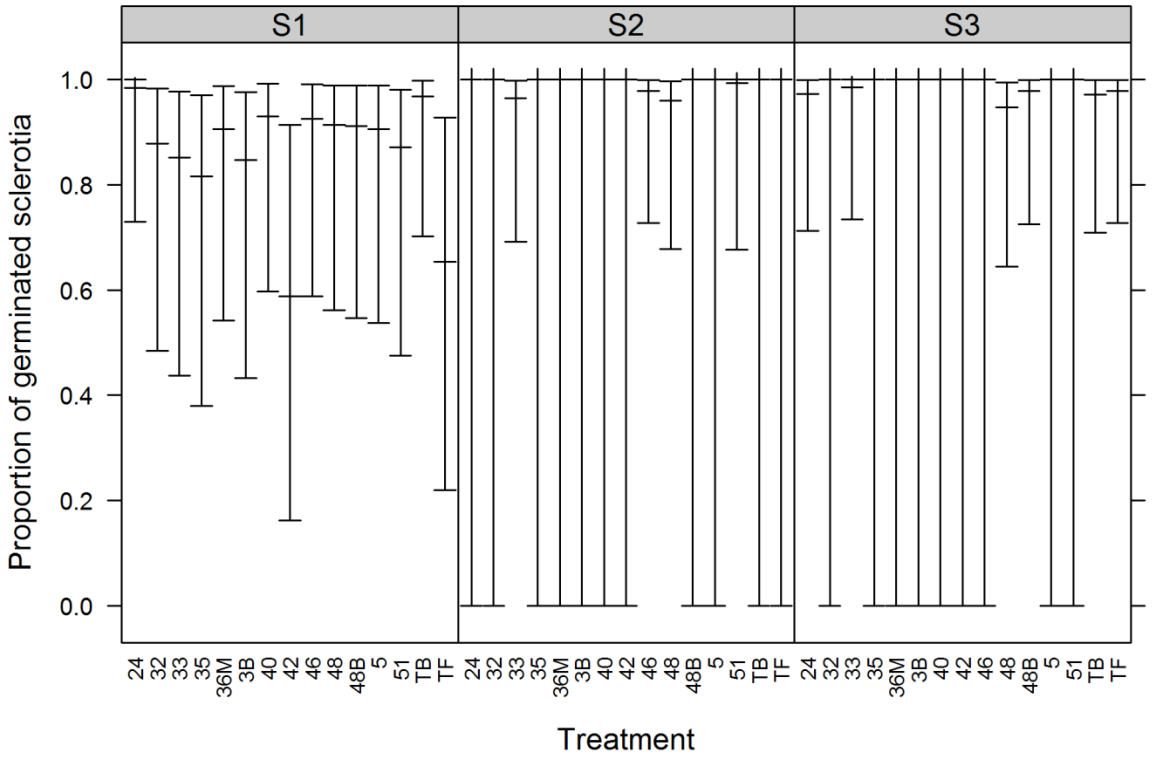
A total of 52 BCA candidates were recovered from sclerotia that do not germinate carpogenically: 21 from AP, 23 from ASP and 8 from CO. No one controlled S1 strain in the first test (Figure 5.5). The same result was observed with the 13 chosen candidates toked to the second test with two more *S. sclerotiorum* strains (Figure 5.6). Only pathogen strain was significant in this second test, without interaction with BCA candidate or production system were candidates were collected. Carpogenic germination of S1 strain was lower than of exogenous strains (Figure 5.7).

FIGURE 5.5 - PROPORTIONS OF GERMINATED SCLEROTIA OF *SCLEROTINIA SCLEROTIORUM* TREATED WITH 52 BCA CANDIDATES AND BURIED FOR 30 DAYS



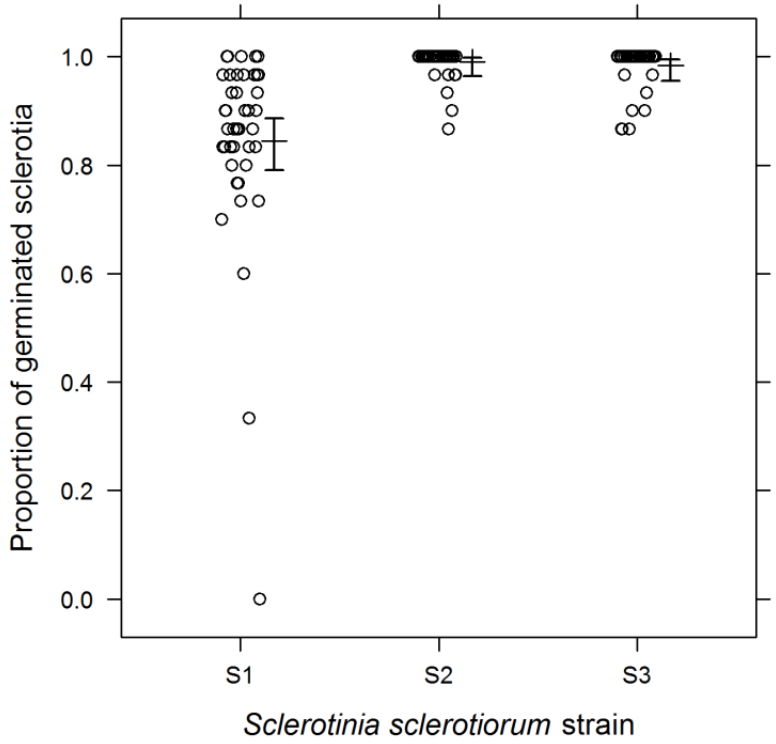
Bars show the 95% confidence interval for the estimated proportions of germinated sclerotia. The two last levels in axis x are the negative control with potato dextrose agar and positive control with commercial *Trichoderma*, respectively. Source: the author (2016).

FIGURE 5.6 - PROPORTIONS OF GERMINATED SCLEROTIA OF THREE STRAINS OF *SCLEROTINIA SCLEROTIORUM* TREATED WITH 13 BCA CANDIDATES AND BURIED FOR 30 DAYS



Bars show the 95% confidence interval for the estimated proportions of germinated sclerotia. TB and TF are the negative control with nutrient broth agar and potato dextrose agar, respectively. Source: the author (2016).

FIGURE 5.7 – DATA AND PROPORTIONS OF GERMINATED SCLEROTIA OF THREE STRAINS OF *SCLEROTINIA SCLEROTIORUM* TREATED WITH 13 BCA CANDIDATES AND BURIED FOR 30 DAYS



Bars show the 95% confidence interval for the estimated proportions of germinated sclerotia. Points are the observed proportions in the experimental units. Source: the author (2016).

5.4 DISCUSSION

ICLSs are referred as advantageous production systems when compared to not integrated crop, by, among others benefits, reduction in plant diseases (BELL; MOORE, 2012; FRANZLUEBBERS, 2007). The present study partly confirmed this statement, as the survival period of sclerotia of *S. sclerotiorum* was lower on the soil surface of AP system than on the CO. In addition, ASP (the more complex productive arrange in this wok) reduced the survival period of buried sclerotia when compared to AP and CO. This characteristic of ICLSs may be referred as a consequence of the soil health, a desirable characteristic of production systems (DORAN; ZEISS, 2000; GARBISU; ALKORTA; EPELDE, 2011) and may be assessed by the interaction between pathogens and native microorganisms in the environment (BONILLA et al., 2012; PÉREZ-BRANDÁN et al., 2014). This was observed in the present work by the

less germination of sclerotia of the native pathogen strain than of the exogenous strains. Advantages of ASP system in reducing damping-off caused by the soil-borne pathogen *Rhizoctonia solani* was previous observed in the same field experiment evaluated in this work (see chapter 3 of this thesis).

The reduction in the number of sclerotia recovered was the main reason for germination decline, both on the soil surface and buried, in accordance with Ben-Yephet et al. (1993). These authors observed that the germinability of recovered sclerotia was not affected during a seven year evaluation, while the number and size of recovered sclerotia declined. And despite the recovering rates of sclerotia after 11 month varied between 21 to 50 % depending on the depth buried (5 to 15 cm), 98 to 100 % of control sclerotia kept in plastic bags in the dark at room temperature were still viable (ALEXANDER; STEWART, 1994).

Results of the present study together with the literature show that decline of sclerotia in the soil are the main reason for the inoculum reduction. This indicates also that accessing the proportions of recovered sclerotia is sufficient to evaluate the survival period of sclerotia of *S. sclerotiorum*, and the additional work of evaluate carpogenic germination is frequently not necessary. The decline in population of sclerotia in the soil can be predominantly attributed to their germination or decomposition by microorganism, both influenced by physical and chemical characteristics of the soil and atmosphere (MERRIMAN, 1976; SHARMA et al., 2015; WILLIAMS; WESTERN, 1965).

The survival period of sclerotia was shorter on the soil surface than buried, confirming previous report (REIS; TOMAZINI, 2005). This result is especially important in Brazil, where no-tillage (direct sowing into the previous crop stubble) is adopted in about 70 % of the total cultivated area (DERPSCH et al., 2010), allowing the majority of sclerotia to relay on the soil surface. Even that is difficult to predict longevity of sclerotia under different conditions (MERRIMAN, 1976; MERRIMAN et al., 1979), the mean survival period observed for sclerotia in the present study confirmed previous report that sclerotia kept on the soil surface in Brazil lost their viability after 12 months (BRUSTOLIN et al., 2016). The till system and crop succession in the control plots in the present work were very similar to that evaluated for the above mentioned authors, but in a different geographical region. These results confirms also a previous report of similarity in the rates of sclerotia decline at four fields located in two different regions

with different annual temperature, rainfall, soil type and crop sequence (BEN-YEPHET; GENIZI; SITI, 1993).

Among the ICLSs arrangements tested, AP allows more light to reach the soil surface, due to absence of trees and because of the cattle grazing. This may favored the carpogenic germination of sclerotia while in the field since soil covering reduces apothecia germination and consequent sclerotia degradation (SUN; YANG, 2000; VENTUROSO et al., 2013). This explains the faster decline in recovering sclerotia able to germinate of the soil surface from AP system when compared to CO and ASP. Besides this, stocking rates and the consequent manure deposition on the soil in AP were greater than in ASP, since the pasture height regulated the stocking rates and the presence of trees reduced pasture grow in ASP, as demonstrated by the evaluations of sward height and herbage mass in the same field experiment (PONTES et al., 2017).

Apothecia arising from sclerotia on the soil surface were frequently observed in the field during the evaluation period, mainly during the autumn and begin of winter. Carpogenic germination is favored after passing through a cold period, near to 10 to 15 °C (COLEY-SMITH; COOKE, 1971). Although soil moisture is also a critical factor in germination and production of apothecia (MILA; YANG, 2008; SUN; YANG, 2000), the region where this study was done has well distributed rain along the year and all treatments promoted a permanent soil covering, avoiding soil dryness.

ASP is the arrangement that presents higher vegetable diversity in this study, and with the higher amount of N applied, the reduction in crop and forage growth by light interception promoted by trees was partly compensated (PONTES et al., 2017), influencing also the stocking rate. This may explain the low number of recovered sclerotia able to germinate from this interaction with production system and N rate in the first experiment with buried sclerotia. However, in the second experiment interaction with N rate was not observed and ASP reduced germination compared to AP. As sclerotia were buried to 8-10 cm depth and light did not influence germination (WU; SUBBARAO, 2008), the interaction with soil microorganisms was probably the main influence in the survival period (SHARMA et al., 2015). This is in accordance with reported by Cezar et al. (2015), that agroforestry showed similar microbiological indicators that an area under natural regeneration for 10 years, explained to richness of plant species and vegetation diversity. Besides that, the commonly reported BCA *Trichoderma* spp. was found to be more abundant in ASP than in AP treatment in this

same field experiment (see chapter 3 of this thesis), also explaining the low survival of sclerotia in ASP system.

The *S. sclerotiorum* strain collected from the field experiment (S1) showed lower germination than the two exogenous strains (S2 and S3) after treated with the BCA candidates. Diversity among different isolates of *S. sclerotiorum* was already reported (SHARMA et al., 2013). As the BCA candidates were collected from S1 sclerotia and both S1 and BCA candidates collected from the same long-term experiment, a previous interaction occurred between then, explaining the lower germination of S1. Long-term experiments are particularly valuable for allow to assess the cumulative impacts of agricultural intensification on soil health (CARVALHO et al., 2010; RODRIGUES et al., 2016). In this case study, interactions between soil-born pathogen and microbiota may be contributed to limit disease. This is in accordance with previous work that observed decline in *Rhizoctonia solani* (ANEES et al., 2010) and *Gaeumannomyces graminis* (LEBRETON et al., 2004) populations after prolonged interaction with pathogen and native microorganisms in the soil, suggesting the development of specific antagonists inside the disease patches in response to the pathogen.

Biological control occurs naturally in the field. In a broad view, agricultural intensification, for increase biodiversity and equilibrium of the system result in disease reduction. Even knowing that the amount of data in the present work limits the extrapolation of these finds, this is the first study showing reduction in the survival of *S. sclerotiorum* sclerotia promoted by ICLSs.

5.5 CONCLUSION

The agricultural diversifications promoted by ICLSs in the Brazilian subtropics have the potential to reduce the survival period of *S. sclerotiorum* sclerotia in the soil. The decline of sclerotia in the soil is the main reason for the inoculum reduction.

ACKNOWLEDGEMENTS

This work was supported by the SERVIAMBI project (Scientific Cooperation Term 21500.10/0008-2, signed between IAPAR and *Embrapa Florestas*) and also by grants of the project CNPq PROCAD number 552334/2011-1 and CAPES.

REFERENCES

ABAWI, G. S.; GROGAN, R. G. Epidemiology of diseases caused by *Sclerotinia* species. **Phytopathology**, v. 69, n. 8, p. 899–904, 1979.

ADAMS, P. B.; AYERS, W. A. Ecology of *Sclerotinia* species. **Phytopathology**, v. 69, n. 8, p. 896–899, 1979.

AGRIOS, G. N. **Plant pathology**. 4. ed. San Diego: Academic Press, 1997.

ALEXANDER, B. J. R.; STEWART, A. Survival of sclerotia of *Sclerotinia* and *Sclerotium* spp. in New Zealand horticultural soil. **Soil Biology and Biochemistry**, v. 26, n. 10, p. 1323–1329, 1994.

ALTIERI, M. A. The ecological role of biodiversity in agroecosystem. **Agriculture, Ecosystems and Environment**, v. 74, p. 19–31, 1999.

ANEES, M. et al. Biotic changes in relation to local decrease in soil conduciveness to disease caused by *Rhizoctonia solani*. **European Journal of Plant Pathology**, v. 126, n. 1, p. 29–41, 2010.

BALBINO, L. C. et al. Evolução tecnológica e arranjos produtivos de sistemas de integração lavoura-pecuária-floresta no Brasil. **Pesquisa Agropecuaria Brasileira**, v. 46, n. 10, p. 1–12, 2011.

BELL, L. W.; MOORE, A. D. Integrated crop-livestock systems in Australian agriculture: Trends, drivers and implications. **Agricultural Systems**, v. 111, p. 1–12, 2012.

BEN-YEPHET, Y.; GENIZI, A.; SITI, E. Sclerotial survival and apothecial production by *Sclerotinia sclerotiorum* folowing outbreaks of lettuce drop. **Phytopathology**, v. 83, n. 5, p. 509–513, 1993.

BOLAND, G. J.; HALL, R. Index of plant hosts of *Sclerotinia sclerotiorum*. **Canadian Journal of Plant Pathology**, v. 16, p. 93–108, 1994.

BOLTON, M. D.; THOMMA, B. P. H. J.; NELSON, B. D. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. **Molecular Plant Pathology**, v. 7, n. 1, p. 1–16, 2006.

BONILLA, N. et al. Enhancing soil quality and plant health through suppressive organic amendments. **Diversity**, v. 4, n. 4, p. 475–491, 2012.

BRUSTOLIN, R. et al. Longevity of *Sclerotinia sclerotiorum* sclerotia on the soil surface under field conditions. **Summa Phytopathologica**, v. 42, n. 2, p. 172–174, jun. 2016.

CARVALHO, P. C. DE F. et al. Managing grazing animals to achieve nutrient cycling

and soil improvement in no-till integrated systems. **Nutrient Cycling in Agroecosystems**, v. 88, n. 2, p. 259–273, 2010.

CEZAR, R. M. C. et al. Soil biological properties in multistrata successional agroforestry systems and in natural regeneration. **Agroforestry Systems**, v. 89, p. 1035–1047, 2015.

COLEY-SMITH, J. R.; COOKE, R. C. Survival and germination of fungal sclerotia. **Annual Review of Phytopathology**, v. 9, p. 65–92, 1971.

DERPSCH, R. et al. Current status of adoption of no-till farming in the world and some of its main benefits. **International Journal of Agricultural and Biological Engineering**, v. 3, n. 1, p. 1–25, 2010.

DORAN, J. W.; ZEISS, M. R. Soil healht and sustainibility: managing the biotic component of soil quality. **Applied Soil Ecology**, v. 15, p. 3–11, 2000.

FRANZLUEBBERS, A. J. Integrated crop-livestock systems in the southeastern USA. **Agronomy Journal**, v. 99, n. 2, p. 361–372, 2007.

GARBISU, C.; ALKORTA, I.; EPELDE, L. Assessment of soil quality using microbial properties and attributes of ecological relevance. **Applied Soil Ecology**, v. 49, n. 1, p. 1–4, 2011.

GRACIA–GARZA, J. A. et al. Influence of crop rotation and tillage on production of apothecia by *Sclerotinia sclerotiorum*. **Canadian Journal of Plant Pathology**, v. 24, n. 2, p. 137–143, jun. 2002.

GRAU, C. R. *Sclerotinia* stem rot. In: SINCLAIR, J. B.; BACKMAN, P. (Eds.). . **Compendium of soybean diseases**. 3. ed. Minnesota: APS Press, 1989. p. 47–48.

HAAN, C. D.; STEINFELD, H.; BLACKBURN, H. **Livestock & the environment: Finding a balance**. Disponível em: <http://www.fao.org/docrep/x5303e/x5303e00.htm>.

HOJSGAARD, S.; HALEKOH, U. **doBy: Groupwise statisticas, LSmeans, linear contrasts, utilities. R package version 4.5-13**. Disponível em: <http://cran.r-project.org/package=doBy>. Acesso em: 28 ago. 2015.

HOTHORN, T.; BRETZ, F.; WESTFALL, P. Simultaneous inference in general parametric models. **Biometrical Journal**, v. 50, p. 346–363, 2008.

KAMAL, M. M. et al. Biology and biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary in oilseed Brassicas. **Australasian Plant Pathology**, v. 45, n. 1, p. 1–14, 2016.

LEBRETON, L. et al. Changes in population structure of the soilborne fungus *Gaeumannomyces graminis* var. *tritici* during continuous wheat cropping. **Environmental Microbiology**, v. 6, n. 11, p. 1174–1185, 2004.

MATSON, P. A. Agricultural intensification and ecosystem properties. **Science**, v. 277, p. 504–509, 1997.

MERRIMAN, P. R. Survival of sclerotia of *Sclerotinia sclerotiorum* in soil. **Soil Biology and Biochemistry**, v. 8, n. 5, p. 385–389, 1976.

MERRIMAN, P. R. et al. Survival of sclerotia of *Sclerotinia sclerotiorum* and effects of cultivation practices on disease. **Soil Biology and Biochemistry**, v. 11, n. 6, p. 567–570, 1979.

MEYER, M. C. et al. **Ensaios cooperativos de controle químico de mofo branco na cultura da soja: safras 2009 a 2012.Documentos**: 345. Londrina: [s.n.]. Disponível em: <http://ainfo.cnptia.embrapa.br/digital/bitstream/item/101371/1/Ensaios-cooperativos-de-controle-quimico-de-mofo-branco-na-cultura-da-soja-safras-2009-a-2012.pdf>.

MILA, A. L.; YANG, X. B. Effects of fluctuating soil temperature and water potential on sclerotia germination and apothecial production of *Sclerotinia sclerotiorum*. **Plant Disease**, v. 92, n. 1, p. 78–82, 2008.

MUELLER, D. S.; PEDERSEN, W. L.; HARTMAN, G. L. Effect of crop rotation and tillage system on *Sclerotinia* stem rot on soybean. **Canadian Journal of Plant Pathology**, v. 24, n. 4, p. 450–456, dez. 2002.

PÉREZ-BRANDÁN, C. et al. Soybean fungal soil-borne diseases: a parameter for measuring the effect of agricultural intensification on soil health. **Canadian Journal of Microbioogy**, v. 60, n. 2, p. 73–84, 2014.

PONTES, L. DA S. et al. Relationship between sward height and herbage mass for integrated crop-livestock systems with trees. **Grassland Science**, v. 63, n. 1, p. 29–35, jan. 2017.

R CORE TEAM. **R: A language and environment for statistical computing.**Viena, 2015. Disponível em: <http://www.r-project.org/>

REIS, E. M.; TOMAZINI, S. L. Viabilidade de escleródios de *Sclerotinia sclerotiorum* em duas profundidades no solo. **Summa Phytopathologica**, v. 31, n. 1, p. 97–99, 2005.

RODRIGUES, M. et al. Legacy phosphorus and no tillage agriculture in tropical oxisols of the Brazilian savanna. **Science of The Total Environment**, v. 542, p. 1050–1061, jan. 2016.

ROUSSEAU, G.; RIOUX, S.; DOSTALER, D. Effect of crop rotation and soil amendments on *Sclerotinia* stem rot on soybean in two soils. **Canadian Journal of Plant Science**, v. 87, p. 605–614, 2007.

SAHARAN, G. S.; MEHTA, N. ***Sclerotinia* diseases of crop plants: Biology, ecology and disease management**. [s.l.] Springer, 2008. v. 53

SANSFORD, C. E.; COLEY-SMITH, J. R. Production and germination of sclerotia of *Sclerotinia sclerotiorum*. **Plant Pathology**, v. 41, p. 154–156, 1992.

SARKAR, D.; ANDREWS, F. **latticeExtra: Extra graphical utilities based on Lattice. R package version 0.6-26**, 2013. Disponível em: <http://cran.r-project.org/package=latticeExtra>. Acesso em: 25 ago. 2015

SHARMA, P. et al. Genetic diversity and morphological variability of *Sclerotinia sclerotiorum* isolates of oilseed Brassica in India. **African Journal of Microbiology Research**, v. 7, n. 18, p. 1827–1833, 2013.

SHARMA, P. et al. *Sclerotinia sclerotiorum* (Lib.) de Bary causing *Sclerotinia* rot in oilseed Brassicas : a review. **Journal of Oilseed Brassica**, v. 6, n. Special, p. 1–44, 2015.

SUN, P.; YANG, X. B. Light, temperature, and moisture effects on apothecium production of *Sclerotinia sclerotiorum*. **Plant Disease**, v. 84, n. 12, p. 1287–1293, 2000.

THORNTON, P. K.; HERRERO, M. Integrated crop-livestock simulation models for scenario analysis and impact assessment. **Agricultural Systems**, v. 70, n. 2–3, p. 581–602, 2001.

VENTUROSO, L. DOS R. et al. Produção de soja e germinação carpogênica de *Sclerotinia sclerotiorum* sob diferentes coberturas de solo. **Semina:Ciencias Agrarias**, v. 34, n. 2, p. 615–626, 2013.

WILLIAMS, G. H.; WESTERN, J. H. The biology of *Sclerotinia trifoliorum* Erikss. and other species of sclerotium-forming fungi. **Annals of Applied Biology**, v. 56, p. 261–258, 1965.

WRATHER, J. A. et al. Soybean disease loss estimates for the top ten soybean-producing countries in 1998. **Canadian Journal of Plant Pathology**, v. 23, p. 115–121, 2001.

WU, B. M.; SUBBARAO, K. V. Effects of soil temperature, moisture, and burial depths on carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor*. **Phytopathology**, v. 98, n. 10, p. 1144–1152, 2008.

6 GENERAL CONCLUSIONS

The agropastoral system in the Brazilian subtropics has the potential to reduce the soil-borne pathogen *Sclerotinia sclerotiorum* and native *Fusarium* spp.

The agrosilvopastoral (ASP) system in this region has the potential to reduce the soil-borne pathogens *Sclerotinia sclerotiorum* and *Rhizoctonia solani*. This last one influenced by the soil microbiota. The ASP system also reduced the native *Fusarium* spp. In contrast, powdery mildew caused by *Blumeria graminis* and *Microsphaera diffusa* in ASP system with low (14 m) spacing between tree rows is more severe than in non-afforested systems, influenced by the microclimate.

7 FINAL CONSIDERATIONS

The general affirmation that integrated crop-livestock systems (ICLSs) reduce plant diseases was partly confirmed in this study. However, results presented here confirm what Matson (1997) said about 20 years ago: "*crop diversification can either encourage or inhibit pathogen growth, depending on the particular requirements of the organism*". Soil-borne pathogens studied were reduced by the system intensification, while powdery mildew was enhanced with afforestation.

It is important to remember also that this study covered only a small part of plant diseases, and the Erysiphaceae pathogens studied here have peculiar microclimatic requirements. Beside this, the low spacing between trees (high density of plants) that favored powdery mildew in this study is not a standard recommendation for agrosilvopastoral or agroforestry systems.

The comparative epidemiology of several others important plant diseases need still to be studied in different arrangements of agricultural diversification and in different Regions. This means that, depending on the interaction between the production system and the pathogen requirements, positive, negative or not significant influence on plant disease can be observed. A general concept on plant diseases in ICLSs (if possible) will be built with the consensus of several studies pointing out different directions.

GENERAL REFERENCES

ABAWI, G. S.; GROGAN, R. G. Epidemiology of diseases caused by *Sclerotinia* species. **Phytopathology**, v. 69, n. 8, p. 899–904, 1979.

ADAMS, P. B.; AYERS, W. A. Ecology of *Sclerotinia* species. **Phytopathology**, v. 69, n. 8, p. 896–899, 1979.

AGAM, N.; BERLINER, P. R. Dew formation and water vapor adsorption in semi-arid environments - A review. **Journal of Arid Environments**, v. 65, n. 4, p. 572–590, 2006.

AGRIOS, G. N. **Plant pathology**. 4. ed. San Diego: Academic Press, 1997.

ALEXANDER, B. J. R.; STEWART, A. Survival of sclerotia of *Sclerotinia* and *Sclerotium* spp. in New Zealand horticultural soil. **Soil Biology and Biochemistry**, v. 26, n. 10, p. 1323–1329, 1994.

ALTIERI, M. A. The ecological role of biodiversity in agroecosystem. **Agriculture, Ecosystems and Environment**, v. 74, p. 19–31, 1999.

ALVES, M. DE C. et al. Intensidade de oídio *Microsphaera diffusa* Cke. & Pk. em cultivares de soja [*Glycine max* (L.) Merr.] sob diferentes temperaturas e períodos de molhamento foliar. **Ciência e Agrotecnologia**, v. 33, p. 1926–1930, 2009.

AMSALEM, L. et al. Effect of climatic factors on powdery mildew caused by *Sphaerotheca macularis* f. sp. *fragariae* on strawberry. **European Journal of Plant Pathology**, v. 114, n. 3, p. 283–292, 2006.

ANEES, M. et al. Biotic changes in relation to local decrease in soil conduciveness to disease caused by *Rhizoctonia solani*. **European Journal of Plant Pathology**, v. 126, n. 1, p. 29–41, 2010.

AOKI, T. et al. Sudden-death syndrome of soybean is caused by two morphologically and phylogenetically distinct species within the *Fusarium solani* species complex-*F. virguliforme* in North America and *F. tucumaniae* in South America. **Mycologia**, v. 95, n. 4, p. 660–684, 2003.

ASCENCION, L. C.; LIANG, W. J.; YEN, T. B. Control of *Rhizoctonia solani* damping-off disease after soil amendment with dry tissues of Brassica results from increase in Actinomycetes population. **Biological Control**, v. 82, p. 21–30, 2015.

AUST, H.-J.; HOYNINGEN-HUENE, J. V. Microclimate in relation to epidemics of powdery mildew. **Annual Review of Phytopathology**, v. 24, p. 491–510, 1986.

AUSTIN, C. N.; WILCOX, W. F. Effects of sunlight exposure on grapevine powdery mildew development. **Phytopathology**, v. 102, n. 9, p. 857–866, 2012.

BALBINO, L. C. et al. Evolução tecnológica e arranjos produtivos de sistemas de integração lavoura-pecuária-floresta no Brasil. **Pesquisa Agropecuaria Brasileira**, v. 46, n. 10, p. 1–12, 2011.

BARRERA, W.; HOY, J.; LI, B. Temperature and leaf wetness effects on infection of sugarcane by *Puccinia melanocephala*. **Journal of Phytopathology**, v. 160, n. 6, p. 294–298, 2012.

BATES, D. et al. **lme4: linear mixed-effects models using Eigen and S4. R package version 1.1-5**. Disponível em: <http://cran.r-project.org/package=lme4>. Acesso em: 25 ago. 2015.

BELL, L. W.; MOORE, A. D. Integrated crop-livestock systems in Australian agriculture: Trends, drivers and implications. **Agricultural Systems**, v. 111, p. 1–12, 2012.

BEN-YEPHET, Y.; GENIZI, A.; SITI, E. Sclerotial survival and apothecial production by *Sclerotinia sclerotiorum* folowing outbreaks of lettuce drop. **Phytopathology**, v. 83, n. 5, p. 509–513, 1993.

BEYSENS, D. The formation of dew. **Atmospheric Research**, v. 39, p. 215–237, 1995.

BLAGODATSKAYA, E.; KUZYAKOV, Y. Active microorganisms in soil: Critical review of estimation criteria and approaches. **Soil Biology and Biochemistry**, v. 67, p. 192–211, 2013.

BOLAND, G. J.; HALL, R. Index of plant hosts of *Sclerotinia sclerotiorum*. **Canadian Journal of Plant Pathology**, v. 16, p. 93–108, 1994.

BOLTON, M. D.; THOMMA, B. P. H. J.; NELSON, B. D. *Sclerotinia sclerotiorum* (Lib.) de Bary: biology and molecular traits of a cosmopolitan pathogen. **Molecular Plant Pathology**, v. 7, n. 1, p. 1–16, 2006.

BONANOMI, G. et al. Identifying the characteristics of organic soil amendments that suppress soilborne plant diseases. **Soil Biology & Biochemistry**, v. 42, n. 2, p. 136–144, 2010.

BONILLA, N. et al. Enhancing soil quality and plant health through suppressive organic amendments. **Diversity**, v. 4, n. 4, p. 475–491, 2012.

BRUSTOLIN, R. et al. Longevity of *Sclerotinia sclerotiorum* sclerotia on the soil surface under field conditions. **Summa Phytopathologica**, v. 42, n. 2, p. 172–174, jun. 2016.

CARVALHO, P. C. DE F. et al. Managing grazing animals to achieve nutrient cycling and soil improvement in no-till integrated systems. **Nutrient Cycling in Agroecosystems**, v. 88, n. 2, p. 259–273, 2010.

CARVALHO, P. C. DE F. et al. Definições e terminologias para Sistema Integrado de

Produção Agropecuária. **Revista Ciencia Agronomica**, v. 5, p. 1040–1046, 2014.

CEZAR, R. M. C. et al. Soil biological properties in multistrata successional agroforestry systems and in natural regeneration. **Agroforestry Systems**, v. 89, p. 1035–1047, 2015.

CHEREWICK, W. Studies on the biology of *Erysihe graminis* D. C. **Canadian Journal Research**, v. 22C, p. 52–86, 1944.

CLARKSON, J. P. et al. Ascospore release and survival in *Sclerotinia sclerotiorum*. **Mycological research**, v. 107, n. 2, p. 213–222, 2003.

COLEY-SMITH, J. R.; COOKE, R. C. Survival and germination of fungal sclerotia. **Annual Review of Phytopathology**, v. 9, p. 65–92, 1971.

CORRÊA, C. A. et al. Efeito de rotações de cultura sob plantio direto sobre *Fusarium* spp. e *Trichoderma* spp. em cultivos de feijoeiro irrigado. **Documentos IAC**, v. 85, p. 943–945, 2008.

DANIELSON, G. A.; NELSON, B. D.; HELMS, T. C. Effect of *Sclerotinia* stem rot on yield of soybean inoculated at different growth stages. **Plant Disease**, v. 88, n. 3, p. 297–300, 2004.

DEAN, R. et al. The Top 10 fungal pathogens in molecular plant pathology. **Molecular Plant Pathology**, v. 13, n. 4, p. 414–430, 2012.

DENNIS, C.; WEBSTER, J. Antagonistic properties of species-groups of *Trichoderma*: III. Hyphal interaction. **Transactions of the British Mycological Society**, v. 57, n. 3, p. 363–369, 1971.

DERPSCH, R. et al. Current status of adoption of no-till farming in the world and some of its main benefits. **International Journal of Agricultural and Biological Engineering**, v. 3, n. 1, p. 1–25, 2010.

DIETZ, J. et al. Vertical patterns and duration of surface wetness in an old-growth tropical montane forest, Indonesia. **Flora**, v. 202, n. 2, p. 111–117, 2007.

DORAN, J. W.; ZEISS, M. R. Soil healht and sustainibility: managing the biotic component of soil quality. **Applied Soil Ecology**, v. 15, p. 3–11, 2000.

FAO. An international consultation on integrated crop-livestock systems for development: The way forward for sustainable production intensification. **Integrated Crop Management**, v. 13, n. 1, p. 1–79, 2010.

FAO. **FAOSTAT**. Disponível em: <http://faostat3.fao.org/faostat-gateway/go/to/browse/Q/QC/E>. Acesso em: 25 ago. 2015.

FARIAS NETO, A. L. DE et al. Caracterização de genótipos de soja na região dos Cerrados quanto à reação à podridão vermelha da raiz, causada pelos fungos

Fusarium tucumaniae e *Fusarium brasiliense*. **Revista Ceres**, v. 60, n. 2, p. 215–220, 2013.

FEHR, W. R.; CAVINESS, C. E. **Stages of soybean development**. Ames: [s.n.].

FINLAY, R. The fungi in soil. In: VAN ELSAS, J.; JANSON, J.; TREVORS, J. (Eds.). . **Modern Soil Microbiology**. Boca Raton: CRC Press, 2007. p. 107–146.

FRANZLUEBBERS, A. J. Integrated crop-livestock systems in the southeastern USA. **Agronomy Journal**, v. 99, n. 2, p. 361–372, 2007.

GAMBLE, J. D. et al. Establishment and early productivity of perennial biomass alley cropping systems in Minnesota, USA. **Agroforestry Systems**, v. 88, n. 1, p. 75–85, 2014.

GARBEVA, P.; VAN VEEN, J. A.; VAN ELSAS, J. D. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease suppressiveness. **Annual Review of Phytopathology**, v. 42, n. 1, p. 243–270, 2004.

GARBISU, C.; ALKORTA, I.; EPELDE, L. Assessment of soil quality using microbial properties and attributes of ecological relevance. **Applied Soil Ecology**, v. 49, n. 1, p. 1–4, 2011.

GHINI, R.; MORANDI, M. A. B. Biotic and abiotic factors associated with soil suppressiveness to *Rhizoctonia solani*. **Scientia Agricola**, v. 63, n. 2, p. 153–160, 2006.

GLAWE, D. A. The powdery mildews: A review of the world's most familiar (yet poorly known) plant pathogens. **Annual Review of Phytopathology**, v. 46, n. 1, p. 27–51, 2008.

GODFRAY, H. C. J. et al. The future of the global food system. **Philosophical Transactions: Biological Sciences**, v. 365, n. 1554, p. 2769–2777, 2010.

GONZÁLEZ GARCÍA, V.; PORTAL ONCO, M. A.; RUBIO SUSAN, V. Review. Biology and systematics of the form genus *Rhizoctonia*. **Spanish Journal of Agricultural Research**, v. 4, n. 1, p. 55–79, 2006.

GRACIA–GARZA, J. A. et al. Influence of crop rotation and tillage on production of apothecia by *Sclerotinia sclerotiorum*. **Canadian Journal of Plant Pathology**, v. 24, n. 2, p. 137–143, jun. 2002.

GRAINGER, J. The ecology of *Erysiphe graminis* DC. **Transactions of the British Mycological Society**, v. 31, n. 1–2, p. 54–65, 1947.

GRAU, C. R. *Sclerotinia* stem rot. In: SINCLAIR, J. B.; BACKMAN, P. (Eds.). . **Compendium of soybean diseases**. 3. ed. Minnesota: APS Press, 1989. p. 47–48.

GROSCH, R. et al. Fungal antagonists of the plant pathogen *Rhizoctonia solani*:

selection, control efficacy and influence on the indigenous microbial community. **Mycological Research**, v. 110, n. 12, p. 1464–1474, 2006.

GUIMARÃES, G. B. et al. Geologia dos Campos Gerais. In: MELO, M.; MORO, R.; GUIMARÃES, G. (Eds.). . **Patrimônio Natural dos Campos Gerais do Paraná**. Ponta Grossa: Editora UEPG, 2007. p. 23–32.

GUPTA, V.; SHARMA, A. K. Assessment of optimum temperature of *Trichoderma harzianum* by monitoring radial growth and population dynamics in different compost manures under different temperature. **Okta Journal of Biosciences**, v. 1, n. 2, p. 151–157, 2013.

HAAN, C. D.; STEINFELD, H.; BLACKBURN, H. **Livestock & the environment: Finding a balance**. Disponível em: <http://www.fao.org/docrep/x5303e/x5303e00.htm>.

HAGN, A. et al. Fungal diversity in agricultural soil under different farming management systems, with special reference to biocontrol strains of *Trichoderma* spp. **Biology and Fertility of Soils**, v. 38, n. 4, p. 236–244, 1 ago. 2003.

HAMZAH, A. et al. Optimal physical and nutrient parameters for growth of *Trichoderma virens* UKMP-1M for heavy crude oil degradation. **Sains Malaysiana**, v. 41, n. 1, p. 71–79, 2012.

HAO, J. J.; SUBBARAO, K. V; DUNIWAY, J. M. Germination of *Sclerotinia minor* and *S. sclerotiorum* sclerotia under various soil moisture and temperature combinations. **Phytopathology**, v. 93, n. 4, p. 443–450, 2003.

HARTERREITEN-SOUZA, É. S. et al. The role of integrating agroforestry and vegetable planting in structuring communities of herbivorous insects and their natural enemies in the Neotropical region. **Agroforestry Systems**, v. 88, n. 2, p. 205–219, 2014.

HENSON, J. M.; BUTLER, M. J.; DAY, A. W. The dark side of the mycelium: melanins of phytopathogenic fungi. **Annual Review of Phytopathology**, v. 37, n. 1, p. 447–471, set. 1999.

HERRERO, M. et al. Smart investments in sustainable food production: revising mixed crop-livestock systems. **Science**, v. 327, n. 2010, p. 821–824, 2010.

HOITINK, H. A. J.; BOEHM, M. J. Biocontrol within the context of soil microbial communities: a substrate-dependent phenomenon. **Annual Review of Phytopathology**, v. 37, p. 427–446, 1999.

HOJSGAARD, S.; HALEKOH, U. **doBy: Groupwise statisticas, LSmeans, linear contrasts, utilities. R package version 4.5-13**. Disponível em: <http://cran.r-project.org/package=doBy>. Acesso em: 28 ago. 2015.

HOTHORN, T.; BRETZ, F.; WESTFALL, P. Simultaneous inference in general

parametric models. **Biometrical Journal**, v. 50, p. 346–363, 2008.

HUANG, H. C.; KOZUB, G. C. Monocropping to sunflower and decline of *sclerotinia* wilt. **Botanical Bulletin of Academia Sinica**, v. 32, p. 163–170, 1991.

HUGHES, R. N.; BRIMBLECOMBE, P. Dew and guttation: formation and environmental significance. **Agricultural and Forest Meteorology**, v. 67, n. 3–4, p. 173–190, 1994.

INNOCENTI, G.; ROBERTI, R.; PIATTONI, F. Biocontrol ability of *Trichoderma harzianum* strain T22 against *Fusarium* wilt disease on water-stressed lettuce plants. **BioControl**, v. 60, n. 4, p. 573–581, 2015.

JANVIER, C. et al. Soil health through soil disease suppression: Which strategy from descriptors to indicators? **Soil Biology and Biochemistry**, v. 39, n. 1, p. 1–23, 2007.

JIN, X.; HARMAN, G. E.; TAYLOR, A. G. Conidial biomass and desiccation tolerance of *Trichoderma harzianum* produced at different medium water potentials. **Biological Control**, v. 1, n. 3, p. 237–243, 1991.

KAMAL, M. M. et al. Biology and biocontrol of *Sclerotinia sclerotiorum* (Lib.) de Bary in oilseed Brassicas. **Australasian Plant Pathology**, v. 45, n. 1, p. 1–14, 2016.

KANG, S.-T.; MIAN, M. A. R. Genetic map of the powdery mildew resistance gene in soybean PI 243540. **Genome**, v. 53, n. 5, p. 400–405, 2010.

KEMEN, E.; JONES, J. D. G. Obligate biotroph parasitism: can we link genomes to lifestyles? **Trends in Plant Science**, v. 17, n. 8, p. 448–457, ago. 2012.

KIM, J. H. et al. Population structure of the *Gibberella fujikuroi* species complex associated with rice and corn in Korea. **Plant Pathology Journal**, v. 28, n. 4, p. 357–363, 2012.

KIM, T. G.; KNUDSEN, G. R. Relationship between the biocontrol fungus *Trichoderma harzianum* and the phytopathogenic fungus *Fusarium solani* f.sp. *pisi*. **Applied Soil Ecology**, v. 68, p. 57–60, 2013.

KINSBURSKY, R. S.; WEINHOLD, A. R. Influence of soil on inoculum density-disease incidence relationships of *Rhizoctonia solani*. **Phytopathology**, v. 78, n. 2, p. 127–130, 1988.

KOECH, E. K.; WHITBREAD, R. Incidence and severity of bean rust (*Uromyces appendiculatus*) in alleys between *Leucaena* hedgerows in Kenya. **New Forests**, v. 31, n. 3, p. 385–402, 2006.

KOHN, L. M. A monographic revision of the genus *Sclerotinia*. **Mycotaxon**, v. 9, n. 2, p. 365–444, 1979.

KOTSOU, M. et al. The effect of olive oil mill wastewater (OMW) on soil microbial

communities and suppressiveness against *Rhizoctonia solani*. **Applied Soil Ecology**, v. 26, n. 2, p. 113–121, 2004.

KÜHN, J.; RIPPEL, R.; SCHMIDHALTER, U. Abiotic soil properties and the occurrence of *Rhizoctonia* crown and root rot in sugar beet. **Journal of Plant Nutrition and Soil Science**, v. 172, n. 5, p. 661–668, 2009.

LARKIN, R. P. Soil health paradigms and implications for disease management. **Annual review of phytopathology**, v. 53, p. 199–221, 2015.

LEBRETON, L. et al. Changes in population structure of the soilborne fungus *Gaeumannomyces graminis* var. *tritici* during continuous wheat cropping. **Environmental Microbiology**, v. 6, n. 11, p. 1174–1185, 2004.

LEWIS, J. A.; LUMSDEN, R. D. Biocontrol of damping-off of greenhouse-grown crops caused by *Rhizoctonia solani* with a formulation of *Trichoderma* spp. **Crop Protection**, v. 20, n. 1, p. 49–56, 2001.

LINK, V. H.; JOHNSON, K. B. White Mold (*Sclerotinia*). **The Plant Health Instructor**, 2007.

LIU, B.; GLENN, D.; BUCKLEY, K. *Trichoderma* communities in soils from organic, sustainable, and conventional farms, and their relation with Southern blight of tomato. **Soil Biology & Biochemistry**, v. 40, p. 1124–1136, 2008.

LIU, Y.; PAUL, V. H. Studies on the germination of scierotia of *Sclerotinia sclerotiorum*. **Journal of Plant Diseases and Protection**, v. 114, n. 1, p. 14–19, 2007.

LORITO, M. et al. Translational research on *Trichoderma*: from 'omics to the field. **Annual review of phytopathology**, v. 48, p. 395–417, 2010.

LUO, W.; GOUDRIAAN, J. Measuring dew formation and its threshold value for net radiation loss on top leaves in a paddy rice crop by using the dewball: a new and simple instrument. **International Journal of Biometeorology**, v. 44, n. 4, p. 167–171, 2000.

MAAS, B.; CLOUGH, Y.; TSCHARNTKE, T. Bats and birds increase crop yield in tropical agroforestry landscapes. **Ecology Letters**, v. 16, n. 12, p. 1480–1487, 2013.

MALÉZIEUX, E. et al. Mixing plant species in cropping systems: concepts, tools and models. A review. **Agronomy for Sustainable Development**, v. 29, p. 43–62, 2009.

MANNERS, J.; HOSSAIN, S. Effects of temperature and humidity on conidial germination in *Erysiphe graminis*. **Transactions of the British Mycological Society**, v. 46, n. 2, p. 225–234, 1963.

MARÍN-GUIRAO, J. I. et al. Effect of *Trichoderma* on horticultural seedlings' growth promotion depending on inoculum and substrate type. **Journal of Applied Microbiology**, v. 121, n. 4, p. 1095–1102, out. 2016.

MASTOURI, F.; BJÖRKMAN, T.; HARMAN, G. E. Seed treatment with *Trichoderma harzianum* alleviates biotic, abiotic, and physiological stresses in germinating seeds and seedlings. **Phytopathology**, v. 100, n. 11, p. 1213–1221, 2010.

MATSON, P. A. Agricultural intensification and ecosystem properties. **Science**, v. 277, p. 504–509, 1997.

MATTIAZZI, P. **Efeito do oídio (*Micrhosphaera diffusa* Cooke & Peck) na produção e duração da área foliar sadia da soja**. [s.l.] Universidade de São Paulo, 2003.

MC LEAN, D. M. Role of dead flower parts in infection of certain crucifers by *Sclerotinia sclerotiorum* (Lib.) D By. **Plant Disease Reporter**, v. 42, n. 5, p. 663–666, 1958.

MERRIMAN, P. R. Survival of sclerotia of *Sclerotinia sclerotiorum* in soil. **Soil Biology and Biochemistry**, v. 8, n. 5, p. 385–389, 1976.

MERRIMAN, P. R. et al. Survival of sclerotia of *Sclerotinia sclerotiorum* and effects of cultivation practices on disease. **Soil Biology and Biochemistry**, v. 11, n. 6, p. 567–570, 1979.

MEYER, M. C. et al. **Ensaios cooperativos de controle químico de mofo branco na cultura da soja: safras 2009 a 2012.Documentos**: 345. Londrina: [s.n.]. Disponível em: <http://ainfo.cnptia.embrapa.br/digital/bitstream/item/101371/1/Ensaios-cooperativos-de-controle-quimico-de-mofo-branco-na-cultura-da-soja-safras-2009-a-2012.pdf>.

MIGNUCCI, J. Powdery mildew. In: SINCLAIR, J.; BACKMAN, P. (Eds.). . **Compendium of soybean diseases**. 3. ed. Saint Paul: American Phytopathological Society, 1989. p. 19–21.

MILA, A. L.; YANG, X. B. Effects of fluctuating soil temperature and water potential on sclerotia germination and apothecial production of *Sclerotinia sclerotiorum*. **Plant Disease**, v. 92, n. 1, p. 78–82, 2008.

MILLER, A. W.; PALLARDY, S. G. Resource competition across the crop-tree interface in a maize-silver maple temperate alley cropping stand in Missouri. **Agroforestry Systems**, v. 53, n. 3, p. 247–259, 2001.

MORAES, A. DE et al. Research on integrated crop-livestock systems in Brazil. **Revista Ciencia Agronomica**, v. 45, n. 5, p. 1024–1031, 2014a.

MORAES, A. DE et al. Integrated crop-livestock systems in the Brazilian subtropics. **European Journal of Agronomy**, v. 57, p. 4–9, 2014b.

MUELLER, D. S.; PEDERSEN, W. L.; HARTMAN, G. L. Effect of crop rotation and tillage system on *Sclerotinia* stem rot on soybean. **Canadian Journal of Plant Pathology**, v. 24, n. 4, p. 450–456, dez. 2002.

MWALE, V. M.; CHILEMBWE, E. H. C.; ULUKO, H. C. Wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*): Damage effects and genetic resistance developed in wheat (*Triticum aestivum*). **International Research Journal of Plant Science**, v. 5, n. 1, p. 1–16, 2014.

NARVÁEZ, D. F. et al. Effects of surface wetness periods on development of soybean rust under field conditions. **Plant Disease**, v. 94, n. 2, p. 258–264, 2010.

NASH, S. M.; SNYDER, W. C. Quantitative estimations by plate counts of propagules of the bean root rot *Fusarium* in field soils. **Phytopathology**, v. 52, p. 567–572, 1962.

NOTARO, K. DE A. et al. Agroforestry systems, nutrients in litter and microbial activity in soils cultivated with coffee at high altitude. **Scientia Agricola**, v. 71, n. 2, p. 87–95, abr. 2014.

OLIVEIRA, A. C. C. DE et al. Influência da temperatura sobre isolados de *Rhizoctonia solani* (Kühn) obtidos em áreas produtoras de algodão nos Estados Brasileiros. **Bioscience Journal**, v. 30, n. SUPPL. 1, p. 119–130, 2014.

OROZCO-AVITIA, A. et al. Temperature effect on *Rhizoctonia solani* analyzed by microcalorimetry. **American Journal of Agricultural and Biological Sciences**, v. 8, n. 2, p. 162–166, 2013.

OYARZUN, P. J.; GERLAGH, M.; ZADOKS, J. C. Factors associated with soil receptivity to some fungal root rot pathogens of peas. **Applied Soil Ecology**, v. 10, n. 1–2, p. 151–169, 1998.

PANSTRUGA, R.; SPANU, P. D. Powdery mildew genomes reloaded. **New Phytologist**, v. 202, n. 1, p. 13–14, 2014.

PAP, P.; RANKOVC, B.; MASIREVIC, S. Effect of temperature, relative humidity and light on conidial germination of oak powdery mildew (*Microsphaera alphitoides* Griff. et Maubl.) under controlled conditions. **Archives of Biological Sciences**, v. 65, n. 3, p. 1069–1077, 2013.

PEREIRA, J. L. et al. Analysis of *Phaseolus vulgaris* response to its association with *Trichoderma harzianum* (ALL-42) in the presence or absence of the phytopathogenic fungi *Rhizoctonia solani* and *Fusarium solani*. **PLoS ONE**, v. 9, n. 5, p. 1–14, 2014.

PÉREZ-BRANDÁN, C. et al. Soybean fungal soil-borne diseases: a parameter for measuring the effect of agricultural intensification on soil health. **Canadian Journal of Microbioogy**, v. 60, n. 2, p. 73–84, 2014.

PETERSON, R.; CAMPBELL, A.; HANNAH, A. A diagrammatic scale for estimating rust intensity on leaves and stems of cereals. **Canadian Journal Research**, v. 26C, n. 5, p. 496–500, 1948.

PHILLIPS, D. V. Stability of *Microsphaera diffusa* and the effect of powdery mildew on yield of soybean. **Plant Disease**, v. 68, n. 11, p. 953–956, 1984.

POCH, T. J.; SIMONETTI, J. A. Ecosystem services in human-dominated landscapes: Insectivory in agroforestry systems. **Agroforestry Systems**, v. 87, n. 4, p. 871–879, 2013.

PONTES, L. DA S. et al. Relationship between sward height and herbage mass for integrated crop-livestock systems with trees. **Grassland Science**, v. 63, n. 1, p. 29–35, jan. 2017.

PRATT, R. G. Fungal population levels in soils of commercial swine waste disposal sites and relationships to soil nutrient concentrations. **Applied Soil Ecology**, v. 38, n. 3, p. 223–229, 2008.

PUMARIÑO, L. et al. Effects of agroforestry on pest, disease and weed control: A meta-analysis. **Basic and Applied Ecology**, v. 16, n. 7, p. 573–582, 2015.

RABBINGE, R.; JORRITSMA, I. T. M.; SCHANS, J. Damage components of powdery mildew in winter-wheat. **Netherlands Journal Of Plant Pathology**, v. 91, p. 235–247, 1985.

R CORE TEAM. **R: A language and environment for statistical computing.**Viena, 2015. Disponível em: <http://www.r-project.org/>

REIS, E. M.; SANTOS, H. P. DOS. População de *Helminthosporium sativum* no ar quantificado através de uma armadilha tipo cata-vento. **Fitopatologia Brasileira**, v. 10, p. 515–519, 1985.

REIS, E. M.; TOMAZINI, S. L. Viabilidade de escleródios de *Sclerotinia sclerotiorum* em duas profundidades no solo. **Summa Phytopathologica**, v. 31, n. 1, p. 97–99, 2005.

REUNIÃO... **Indicações Técnicas para a Cultura da Soja no Rio Grande do Sul e em Santa Catarina 2009/2010**. Passo Fundo: Embrapa Trigo, 2009.

RIBEIRO JR, P.; DIGGLE, P. **geoR: Analysis of Geostatistical Data. R package version 1.7-6**. Disponível em: <http://www.leg.ufpr.br/geoR>. Acesso em: 25 ago. 2015.

RODRIGUES, M. et al. Legacy phosphorus and no tillage agriculture in tropical oxisols of the Brazilian savanna. **Science of The Total Environment**, v. 542, p. 1050–1061, jan. 2016.

ROUSSEAU, G.; RIOUX, S.; DOSTALER, D. Effect of crop rotation and soil amendments on *Sclerotinia* stem rot on soybean in two soils. **Canadian Journal of Plant Science**, v. 87, p. 605–614, 2007.

ROWLANDSON, T. et al. Reconsidering leaf wetness duration determination for plant disease management. **Plant Disease**, v. 99, n. 3, p. 310–319, 2015.

RYSCHAWY, J. et al. Mixed crop-livestock systems: an economic and environmental-

friendly way of farming? **Animal**, v. 6, n. 10, p. 1722–1730, 2012.

SAHARAN, G. S.; MEHTA, N. ***Sclerotinia*** **diseases of crop plants: Biology, ecology and disease management**. [s.l.] Springer, 2008. v. 53

SALTON, J. C. et al. Integrated crop-livestock system in tropical Brazil: Toward a sustainable production system. **Agriculture, Ecosystems and Environment**, v. 190, p. 70–79, 2014.

SAMAVAT, S. et al. Application of new bioformulations of *Pseudomonas aureofaciens* for biocontrol of cotton seedling damping-off. **Journal of Plant Protection Research**, v. 54, n. 4, p. 335–339, 2014.

SANSFORD, C. E.; COLEY-SMITH, J. R. Production and germination of sclerotia of *Sclerotinia sclerotiorum*. **Plant Pathology**, v. 41, p. 154–156, 1992.

SANT, D. et al. Effect of *Trichoderma asperellum* strain T34 on *Fusarium* wilt and water usage in carnation grown on compost-based growth medium. **Biological Control**, v. 53, n. 3, p. 291–296, 2010.

SARKAR, D.; ANDREWS, F. **latticeExtra: Extra graphical utilities based on Lattice. R package version 0.6-26**, 2013. Disponível em: <http://cran.r-project.org/package=latticeExtra>. Acesso em: 25 ago. 2015

SCHNATHORST, W. C. Environmental relationships in the powdery mildews. **Annual Review of Phytopathology**, v. 3, p. 343–366, 1965.

SCHROTH, G. et al. Pests and diseases in agroforestry systems of the humid tropics. **Agroforestry Systems**, v. 50, p. 199–241, 2000.

SENECHKIN, I. V.; VAN OVERBEEK, L. S.; VAN BRUGGEN, A. H. C. Greater *Fusarium* wilt suppression after complex than after simple organic amendments as affected by soil pH, total carbon and ammonia-oxidizing bacteria. **Applied Soil Ecology**, v. 73, p. 148–155, 2014.

SHANER, G. Effect of environment on fungal leaf blights of small grains. **Annual Review of Phytopathology**, v. 19, n. 1, p. 273–296, 1981.

SHARMA, P. et al. Genetic diversity and morphological variability of *Sclerotinia sclerotiorum* isolates of oilseed Brassica in India. **African Journal of Microbiology Research**, v. 7, n. 18, p. 1827–1833, 2013.

SHARMA, P. et al. *Sclerotinia sclerotiorum* (Lib.) de Bary causing *Sclerotinia* rot in oilseed Brassicas : a review. **Journal of Oilseed Brassica**, v. 6, n. Special, p. 1–44, 2015.

SINGH, A. et al. Optimal physical parameters for growth of *Trichoderma* species at varying pH , temperature and agitation. **Virology & Mycology**, v. 3, n. 1, p. 1–7, 2014.

SIVAN, A.; CHET, I. The possible role of competition between *Trichoderma harzianum* and *Fusarium oxysporum* on rhizosphere colonization. **Phytopathology**, v. 79, n. 2, p. 198–203, 1989.

SIVAPALAN, A. Effects of water on germination of powdery mildew conidia. **Mycological Research**, v. 97, n. 1, p. 71–76, 1993.

SMILEY, R. W.; DERNOEDEN, P. H.; CLARKE, B. B. **Compendium of Turfgrass Diseases**. 2. ed. [s.l.] APS PRESS, 1992.

STOCKDALE, E. A.; BROOKES, P. C. Detection and quantification of the soil microbial biomass – impacts on the management of agricultural soils. **Journal of Agricultural Science**, v. 144, p. 285–302, 2006.

SUÁREZ-ESTRELLA, F. et al. Antagonistic activity of bacteria and fungi from horticultural compost against *Fusarium oxysporum* f. sp. *melonis*. **Crop Protection**, v. 26, n. 1, p. 46–53, 2007.

SUN, P.; YANG, X. B. Light, temperature, and moisture effects on apothecium production of *Sclerotinia sclerotiorum*. **Plant Disease**, v. 84, n. 12, p. 1287–1293, 2000.

SUN, R. et al. Bacterial diversity in soils subjected to long-term chemical fertilization can be more stably maintained with the addition of livestock manure than wheat straw. **Soil Biology & Biochemistry**, v. 88, p. 9–18, 2015.

TANAKA, D. L.; KARN, J. F.; SCHOLLJEGERDES, E. J. Integrated crop/livestock systems research: practical research considerations. **Renewable Agriculture and Food Systems**, v. 23, n. 1, p. 80–86, 2008.

TEWOLDEMEDHIN, Y. T. et al. Characterization of *Rhizoctonia* spp. recovered from crop plants used in rotational cropping systems in the Western Cape province of South Africa. **Plant Disease**, v. 90, n. November, p. 1399–1406, 2006.

THORNTON, P. K.; HERRERO, M. Integrated crop-livestock simulation models for scenario analysis and impact assessment. **Agricultural Systems**, v. 70, n. 2–3, p. 581–602, 2001.

TSROR, L. Biology, epidemiology and management of *Rhizoctonia solani* on potato. **Journal of Phytopathology**, v. 158, n. 10, p. 649–658, 2010.

TURKINGTON, T. K.; MORRAL, R. A. A. Use of petal infestation to forecast *Sclerotinia* stem rot of canola: the influence of inoculum variation over the flowering period and canopy density. **Phytopathology**, v. 83, n. 6, p. 682–689, 1993.

TWOMEY, M. C. et al. Development of partial ontogenic resistance to powdery mildew in hop cones and its management implications. **PLoS ONE**, v. 10, n. 3, p. 1–24, 2015.

VAN BRUGGEN, A. H. C. et al. Soil health indicators and *Fusarium* wilt suppression

in organically and conventionally managed greenhouse soils. **Applied Soil Ecology**, v. 86, p. 192–201, 2015.

VAN BRUGGEN, A. H. C.; SEMENOV, A. M. A new approach to the search for indicators of root disease suppression. **Australasian Plant Pathology**, v. 28, p. 4–10, 1999.

VAN DER HEIJDEN, M. G. A.; WAGG, C. Soil microbial diversity and agro-ecosystem functioning. **Plant and Soil**, v. 363, n. 1–2, p. 1–5, 2013.

VENTUROSO, L. DOS R. et al. Produção de soja e germinação carpogênica de *Sclerotinia sclerotiorum* sob diferentes coberturas de solo. **Semina:Ciencias Agrarias**, v. 34, n. 2, p. 615–626, 2013.

VERMA, M. et al. Antagonistic fungi, *Trichoderma* spp.: panoply of biological control. **Biochemical Engineering Journal**, v. 37, n. 1, p. 1–20, 2007.

WANG, H. et al. Light quality affects incidence of powdery mildew, expression of defence-related genes and associated metabolism in cucumber plants. **European Journal of Plant Pathology**, v. 127, n. 1, p. 125–135, 2010.

WARD, S. V; MANNERS, J. G. Environmental effects on the quantity and viability of conidia produced by *Erysiphe graminis*. **Transactions of the British Mycological Society**, v. 62, n. 1, p. 119–128, 1974.

WELLER, D. M. et al. Microbial populations responsible for specific soil suppressiveness to plant pathogens. **Annual Review of Phytopathology**, v. 40, n. 1, p. 309–348, 2002.

WESTPHAL, A. et al. Contributions of *Fusarium virguliforme* and *Heterodera glycines* to the disease complex of sudden death syndrome of soybean. **PLoS ONE**, v. 9, n. 6, p. 1–13, 2014.

WEZEL, A. et al. Agroecological practices for sustainable agriculture. A review. **Agronomy for Sustainable Development**, v. 34, n. 1, p. 1–20, 2014.

WICKHAM, H. Reshaping data with the reshape package. **Journal of Statistical Software**, v. 21, n. 12, p. 1–20, 2007.

WILLETTS, H. J.; WONG, J. A.-L. The biology of *Sclerotinia sclerotiorum*, *S.trifoliorum*, and *S.minor* with emphasis on specific nomenclature. **The Botanical Review**, v. 46, p. 101–165, 1980.

WILLIAMS, G. H.; WESTERN, J. H. The biology of *Sclerotinia trifoliorum* Erikss. and other species of sclerotium-forming fungi. **Annals of Applied Biology**, v. 56, p. 261–258, 1965.

WILLOCQUET, L. et al. Effects of radiation, especially ultraviolet B, on conidial germination and mycelial growth of grape powdery mildew. **European Journal of**

Plant Pathology, v. 102, n. 5, p. 441–449, 1996.

WISEMAN, B. M. et al. Suppression of *Rhizoctonia solani* anastomosis group 8 in Australia and its biological nature. **Soil Biology & Biochemistry**, v. 28, n. 6, p. 727–732, 1996.

WRATHER, A. et al. Effect of diseases on soybean yield in the top eight producing countries in 2006. **Plant Health Progress**, 2010.

WRATHER, J. A. et al. Soybean disease loss estimates for the top ten soybean-producing countries in 1998. **Canadian Journal of Plant Pathology**, v. 23, p. 115–121, 2001.

WRIGHT, I. A. et al. Integrating crops and livestock in subtropical agricultural systems. **Journal of the Science of Food and Agriculture**, v. 92, n. 5, p. 1010–1015, 2012.

WU, B. M.; SUBBARAO, K. V. Effects of soil temperature, moisture, and burial depths on carpogenic germination of *Sclerotinia sclerotiorum* and *S. minor*. **Phytopathology**, v. 98, n. 10, p. 1144–1152, 2008.

YAMOAH, C. F.; BURLEIGH, J. R. Alley cropping *Sesbania sesban* (L) Merill with food crops in the highland region of Rwanda. **Agroforestry Systems**, v. 10, n. 2, p. 169–181, 1990.

YANGUI, T. et al. Control of damping-off caused by *Rhizoctonia solani* and *Fusarium solani* using olive mill waste water and some of its indigenous bacterial strains. **Crop Protection**, v. 27, n. 2, p. 189–197, 2008.

YULIANTI, T.; SIVASITHAMPARAM, K.; TURNER, D. W. Saprophytic growth of *Rhizoctonia solani* Kühn AG2-1 (ZG5) in soil amended with fresh green manures affects the severity of damping-off in canola. **Soil Biology & Biochemistry**, v. 38, n. 5, p. 923–930, 2006.

ZADOKS, J. C.; CHANG, T. T.; KONZAK, C. F. A decimal code for the growth stages of cereals. **Weed Research**, v. 14, n. 6, p. 415–421, 1974.

ZHANG, H. et al. Population analysis of the *Fusarium graminearum* species complex from wheat in China show a shift to more aggressive isolates. **PLoS ONE**, v. 7, n. 2, p. 1–14, 2012.