Crop rotation design in view of soilborne pathogen dynamics

A methodological approach illustrated with *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *cepae*

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Thesis

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Abstract

During the last decades, agriculture went through an intensification process associated with an increased use of fossil fuel energy, which despite temporarily increasing yields often resulted in decreased overall sustainability. Crop rotation is considered a cornerstone of sustainable farming systems. The design of crop rotations is a complex process where several objectives should be combined. Models can support the design of crop sequences and help to reveal synergies and trade-offs among objectives. Despite their importance, pathogen dynamics are rarely taken into account in cropping system models, not in the least because quantitative information from classical crop rotation experiments to calibrate and evaluate the models is resource demanding, and therefore scarce.

The aim of this thesis was to develop a research approach where data (greenhouse pot experiments, microplot experiments, surveys on commercial farm fields) and model simulations were combined to identify crop sequences that minimize soilborne pathogen inoculum build up, and to subsequently include this information into models for designing sustainable crop rotations. The study was carried out based on two ecologically distinct and relevant pathogens in vegetable production systems: *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *cepae* (*Foc*).

Two aspects of the dynamics of *S. rolfsii* sclerotia were studied: survival after soil incorporation of green manures, and population changes under three cropping sequences. In pot experiments, sclerotia survival in soil after incorporation of a winter green manure and its decomposition during summer was generally lower than after summer green manure incorporation and decomposition during winter. The incorporation of various legume crops (black beans, cowpea, hairy vetch and lupines) allowed multiplication of sclerotia while various grasses (sudangrass, foxtail millet, oats and wheat) as well as sunhemp resulted in a reduction of sclerotia in the soil. The build-up of sclerotia populations in the microplots was dependent on the crop sequence. Multiplication in sweet pepper was greater after black oat than after onion or fallow.

The dynamics of *Foc* was studied at two different levels: multiplication in individual plants and population changes in different crop sequences. *Foc* colonized and multiplied in the root systems of 13 non-Allium plant species without inducing disease symptoms or growth retardation. These species thus constituted “reservoir-hosts” for *Foc*. The lowest *Foc* levels per g of dry weight of root were found in wheat, sunflower, cowpea and millet whereas the

highest *Foc* level was found in black bean. *Fusarium* pathogen dynamics was strongly affected by the cropping history in a particular field. *Fusarium* populations increased from transplant to harvest of onion when another onion crop had been planted in the same field during the previous winter, whereas *Fusarium* populations decreased when a winter green manure had been planted.

Pathogen dynamics in crop sequences was simulated by concatenating two simple models, the first one describing the build-up of the pathogen within a crop, and the second one describing the dynamic of the pathogen during the intercrop period. The simulations described differences among crop sequences and alternating cycles of increasing and decreasing soil pathogen populations, as well as differences at equilibrium populations related to host frequency and cropping history.

This thesis provides a methodological approach to the design of crop rotations and their effects on soil borne pathogen dynamics. The combination of data from controlled experiments, novel analytical tools (Bayesian analysis, modelling and simulation) and on-farm observations can lead to the identification of optimal crop rotations without extensive field experiments that require a lot of time, space and economic resources.

Key words: *Sclerotium rolfsii*, *Fusarium oxysporum* f.sp. *cepae*, soilborne pathogens, crop rotation, population dynamic models, simulation.

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Chapter 1

General introduction

1 Background of the study

1.1 Intensification and sustainability

During the last decades, major trends in agricultural farming systems were intensification and specialization of production, trends which are associated with an increased use of fossil fuel energy due to the intensive use of agrochemicals (fertilizers and pesticides), mechanization and irrigation. As a consequence of intensification and specialization, there has been an increase in soil erosion rates, a decrease in soil organic matter content and associated losses of soil structure and water holding capacity, an increase in pest and disease problems and biodiversity losses, and an increase in vulnerability to climate change and related extreme events (Altieri and Toledo 2011, Altieri et al. 2012, Dogliotti 2003, Kremen and Miles 2012, Tilman et al. 2012).

The high dependence on fossil fuel energy and the deterioration of ecosystem services makes many of these farming systems not sustainable (United Nations 2013). There is an urgent need for development of farming systems where farm productivity increases by drawing on internal ecological cycles, by reducing the dependency on non-renewable and external inputs and by optimizing the use of water and energy. Moreover, these strategies should also prevent further land and environment degradation and support farmers to achieve long-term goals of sustainability (Altieri et al. 2012, United Nations 2013).

1.2 Sustainable farming systems, soil health and soilborne diseases

Sustainability of farming systems depends on maintaining soil health, defined as the ability of a soil to sustain biological productivity, maintain environmental quality and promote plant and animal health (Doran and Zeiss 2000). Soil health is an ecological characteristic providing resistance to stress factors including disease outbreaks. Thus, suppressiveness to diseases could be seen as a manifestation of soil health (van Bruggen and Semenov 2000, van Bruggen and Termorshuizen 2003). Different soil environments have different management needs, and it should be assured that soil management practices introduced for example to improve soil fertility will also improve or maintain soil health (Abawi and Widmer 2000).

It is generally stated that soil organic matter may reduce incidence and severity of soilborne diseases by inducing suppressiveness. Several cultural practices like cropping sequences, green manures, cover crops, mulches, composts and animal manures affect soil organic matter and thus pathogens. Application of compost is widely reported to reduce soil-borne

diseases (Heather et al. 2006, Hoitink and Bohem 1999, Lumsden et al. 1983, Pereira et al. 1996), as well as incorporation of crop residues and biological soil disinfestation (Blok et al. 2000, Gamliel et al. 2000).

However, in some cases inter-crop activities like soil incorporation of a cover crop might increase disease incidence as was shown for pathogens with a wide host range such as *Sclerotium rolfsii* (Gilsanz et al. 2004, Jenkins and Averre, 1986) or facultative saprotrophic pathogens like *Phythium* spp (Manici et al. 2004). Crop residues in no-tillage small grain systems can be conducive to *Rhizoctonia solani* (Chung et al. 1988). Brassica species are effective for controlling Sclerotinia diseases on lettuce, whereas oats and broad beans are not (Pung et al. 2004).

1.3 Sustainable farming systems and crop rotation design

Sustainable farming systems have often been associated with relatively long crop rotations, in which the frequency of individual crop species is low and species diversity over time is high. An adequate crop rotation not only provides a regular supply of nutrients, maintains a good soil structure that enhances the water-holding capacity and allows for extensive root formation, reduces soil erosion and regulates disease, pest and weed outbreaks, but also spreads the workload more evenly over the seasons and provides food and income security to farmers. However, the design or selection of a particular crop rotation may be a trade-off between farmers' long-term objectives and ambitions at the whole farm level, local resource availability, climate, and shorter-term socio-economic conditions.

Models can support the design of crop sequences and help to reveal synergisms and trade-offs among objectives (Casagrande et al. 2010, Dogliotti et al. 2003, Dogliotti et al. 2004). As an example, the ROTAT model (Dogliotti et al. 2003) was designed to create all feasible crop rotations based on a list of crops and following well-defined quantitative agronomic rules. These agronomic rules are filters designed to eliminate undesirable crop sequences, and are built on previous research or on expert knowledge. One particular filter sets the "maximum cropping frequency of crop or groups of related crops", and aims to minimize the inoculum build-up of key soil-borne pathogens. However, the outcome of the model is highly sensitive to assumptions about pathogen population dynamics and yield loss (Dogliotti et al. 2005). Thus a deeper understanding of the interactions between cropping frequencies, inter-crop activities and soil-borne disease dynamics, expressed by means of quantitative models, is necessary to improve the ROTAT model and similar models (Bachinger and Zander 2007,

Schönhart et al. 2012) that contribute to the design of farming systems.

Soilborne pathogen dynamics in a crop rotation can be described by interlinked quantitative population models where different equations describe the increase and decline of the pathogen population during and after a crop and intercrop activity, respectively (Bailey and Gilligan 2004, Colbach et al. 1999, Mol et al. 1996, Tixier et al. 2006, van den Berg and Rossing 2005; van den Berg et al. 2006). However, model parameter estimation based on classical statistical experimental design requires much time and space and is often prohibitively costly (van den Berg et al. 2006). Thus new research strategies are required, where quantitative data generated under different experiments are combined by Bayesian calibration methods to estimate model parameters (Fabre et al. 2006, van den Berg et al. 2006).

2 Aim of the thesis

The general aim of this thesis was to explore a research approach where experimental data and model simulations are combined to explore crop sequences that minimize soilborne pathogen inoculum build up, to further include this information into models used for designing sustainable crop rotations.

Particular aims were:

- Provide a quantitative understanding of population dynamics of *S. rolfsii* and *Fusarium oxysporum* f.sp. *cepae* in vegetable production systems with a variety of crops and intercrops, by using simple mathematical models.
- Apply insight in population dynamics of soilborne pathogens for designing sustainable cropping systems.

3 Research strategy

To fulfil the objectives, first a conceptual model of soilborne pathogen dynamics was developed. Second, single land use activities were modelled following an approach built on simple mathematical models, where interlinked equations describe the increase and decline of the pathogen population during and after a crop and intercrop activity, respectively. Third

with the mathematical models calibrated for several crops and intercrop crop activities, multiple land use activities were simulated for soil pathogen dynamics in theoretical crop rotations (Figure 1). Data for model development were collected in several pot experiments in the greenhouse and microplot experiments in the field, and from farm surveys, where different crop sequences and intercrop activities were established, providing a wide range of different cropping system combinations.

A conceptual model of the soil – pathogen – crop system is presented in Figure 2. When starting a new crop, the pathogen population in the soil ("pathogen in soil") represents the initial inoculum density (P_i). While the crop grows, the pathogen infects the plants and multiplies leading to a build-up of the pathogen population and affecting plant growth (quantified by the "Biomass (yield)"). Infected plant tissue carrying the pathogen (*Pf*) decays ("pathogen in crop residues") and releases the infective pathogen units into the soil ("pathogen in soil"). Finally, the infective units remain in the soil waiting to start a new disease cycle, and are influenced by several soil processes affecting pathogen survival and infection. This whole process is influenced by different management practices (for example fallowing or green manure amendment) and the physical, chemical and biological environment.

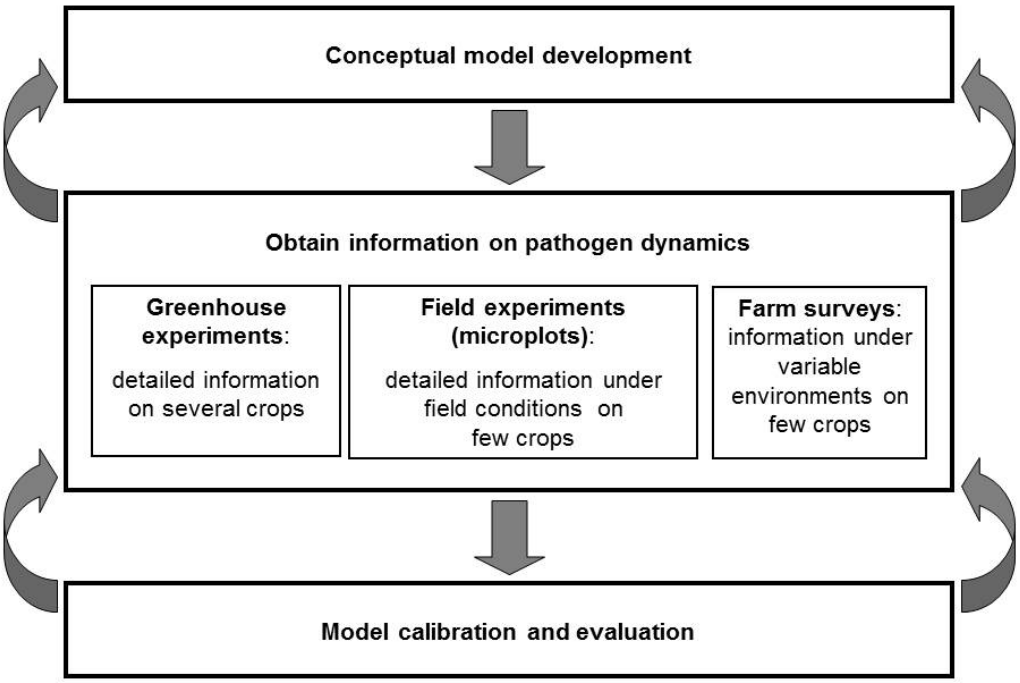

Figure 1. Outline of the research strategy followed in the present thesis.

Population models were defined for two soilborne pathogens of vegetables with different survival strategies and host ranges: *Sclerotium rolfsii* and *Fusarium oxysporum* f sp. *cepae* (*Foc*). *S. rolfsii* induces Southern blight on more than 500 plant species in over 100 plant families and produces sclerotia on the infected portions of the plant near the soil-air interface. The produced sclerotia are quickly released from the infected tissues and can survive in the soil from a few months to several years, depending on environmental conditions (Punja 1985, Xu et al. 2008). *Foc* is a soilborne pathogen of *Allium* species and *Asparagus officinalis*, which survives as macro- and micro-conidia in crop debris, as chlamydospores in the soil or as mycelium and conidia in colonized roots of non-*Allium* plant species without causing disease (Abawi and Lorbeer 1972, Brayford 1996). The colonized roots are the main source for inoculum build-up, and the release of the infective units into the soil is proportional to root decomposition.

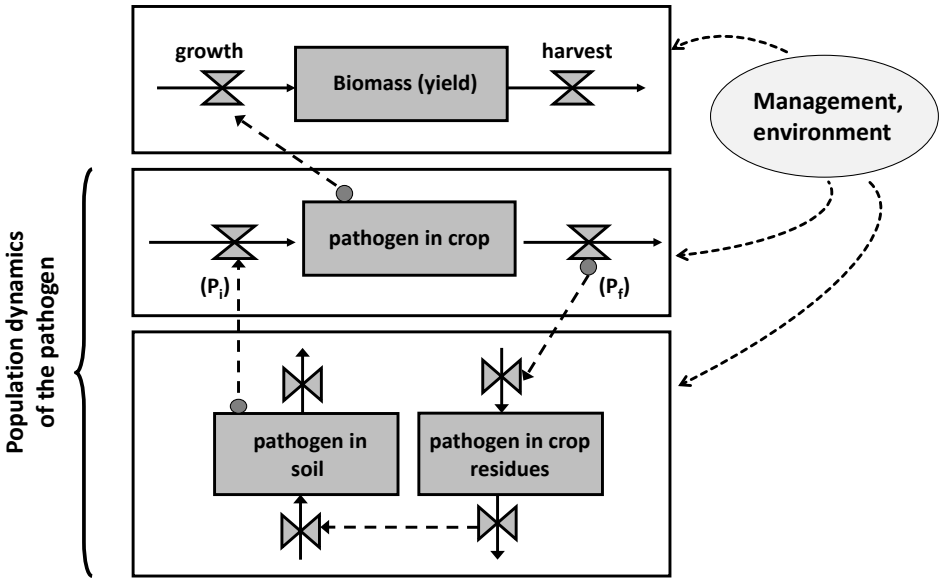

Figure 2. Conceptual model of soilborne pathogen dynamics, relating initial (P_i) and final (P_f) population densities and their relation with crop growth and yield loss.

4 Outline of the Thesis

4.1 Chapter 2

In this review chapter we first present the agronomic, economic and ecological reasons for crop rotation, followed by the main criteria for selecting the rotation crops and their sequence. We then describe the effects that rotation crops and their management have on plant disease development, and give examples of the contributions of different crop rotations

to disease management. Finally, the use of models as a tool in designing and evaluating crop sequences is outlined, where biophysical and socio-economic information is combined.

4.2 Chapter 3

In Chapter 3 our objective was to generate information on dynamics of sclerotia of *Sclerotium rolfsii* after soil incorporation of different crops and intercrops, and design and test a research approach where experimental data and model simulations are combined to explore crop sequences that minimize Southern blight incidence. To reach this goal the effects on *S. rolfsii* sclerotia dynamics of 17 green manure amendments and three cropping sequences were analysed in pot and microplot experiments, respectively. With the quantitative data obtained, two simple mathematical models were calibrated and combined to simulate soil sclerotia dynamics under different crop rotations.

4.3 Chapter 4

Chapter 4 was the first experimental work *on Fusarium oxysporum* f sp. *cepae* (*Foc*). Our aim was to generate information on the ability of *Foc* to multiply in the roots of different plant species and to propose a research approach where experimental data and model simulations are combined to explore crop sequences that minimize inoculum build-up. To reach this goal *Foc* multiplication rates in 13 plant species were tested in greenhouse experiments. Then in simple theoretical crop rotations, *Foc* build-up in reservoir-hosts was explored by using the experimental data to parameterize a population dynamics model.

4.4 Chapter 5

The objective of the second experimental study on *Foc* was to generate information on the pathogen dynamics for different crops and intercrops at field level and with the generated information validate the model proposed in Chapter 4. To reach this goal the effects of different crops and cropping sequences on *Foc* soil populations were surveyed in 35 Uruguayan commercial farm fields from 2009 to 2011, and measured in 2010 and 2011 in a microplot experiment conducted at the research station.

4.5 Chapter 6

In the General discussion the main results of the thesis are summarized, the research methodology discussed as well as some implications for future research.

References

Abawi, G.S. and Lorbeer, J.W. 1972. Several aspects of the ecology of *Fusarium oxysporum* f. sp. *cepae*. *Phytophatology* 62: 870-876.

Abawi, G. S. and Widmer, T. L. 2000. Impact of soil health management practices on soil-borne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology* 15:37-47.

Altieri, M. and Toledo, V.M. 2011. The agroecological revolution of Latin America: rescuing nature, securing food sovereignty and empowerment of peasants. *The Journal of Peasant Studies* 38: 587-612.

Altieri, M.A., Funes-Monzote, F.R., Petersen, P. 2012. Agroecologically efficient agricultural systems for smallholder farmers: contributions to food sovereignty. *Agronomy for Sustainable Development* 32: 1-13. doi: 10.1007/s13593-011-0065-6

Bailey, D.J. and Gilligan, C.A. 2004. Modeling and analysis of disease-induced host growth in the epidemiology of take-all. *Phytopathology* 94: 535 – 540.

Bachinger, J. and Zander, P. 2007. ROTOR, a tool for generating and evaluating crop rotations for organic farming systems. *European Journal of Agronomy* 26: 130 - 143

Blok, W.J., Lamers, J.G., Termorshuizen, A.J., Bollen, G.J. 2000. Control of soilborne plant pathogens by incorporating fresh organic amendments followed by tarping. *Phytopahtology* 90: 253 – 259.

Brayford, D. 1996. IMI Descriptions of Fungi and Bacteria No. 1263. *Fusarium oxysporum* f. sp. *cepae*. *Mycopathologia*, 133: 39 - 40.

Casagrande, M., Dogliotti, S., Groot, J.C.J., Aguerre, V., Abbas, A., Albin, A., Claassen, G.D.H., Chilibroste, P., Rossing W.A.H. 2010. Exploring options for sustainable farming systems development for vegetable family farmers in Uruguay using a modelling toolkit. In: 9th European IFSA Symposium, Building sustainable rural futures. The added value of systems approaches in times of change and uncertainty, Vienna, Austria, 04 - 07 July, 2010, pp. 463 – 469.

Chung, Y.R., Hoitink, H.A.H., Lipps, P.E. 1998. Interactions between organic matter decomposition level and soilborne disease severity. *Agriculture Ecosystems and Environment* 24: 183 – 193.

Colbach, N., Meynard, J-M, Duby, C., Huet, P. 1999. A dynamic model of the influence of rotation and crop management on the disease development of eyespot. Proposal of cropping systems with low disease risk. *Crop Protection* 18: 451 - 461.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2003. ROTAT, a tool for systematically generating crop rotations. *European Journal of Agronomy* 19: 239-250.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2004. Systematic design and evaluation of crop rotations enhancing soil conservation, soil fertility and farm income: a case study for vegetable farms in South Uruguay. *Agricultural Systems* 80: 277 - 302.

Dogliotti, S., Van Ittersum, M.K., Rossing, W.A.H. 2005. A method for exploring sustainable development options at the farm scale: a case study for vegetable farms in South Uruguay. *Agricultural Systems* 86: 29 – 51.

Doran, J.W. and Zeiss, M.R. 2000. Soil health and sustainability: managing the biotic component of soil quality. *Applied Soil Ecology* 15: 3 – 11.

Fabre, F., Pierre, S., Dedryver, C.A., Plantegenest, M. 2006. Barley yellow dwarf risk assessment based on Bayesian modelling of aphid population dynamics. *Ecological Modelling* 193: 457 – 466.

Gamliel, A., Austerweil, M., Kirtzman, G. 2000. Non-chemical approach to soilborne pest management – organic amendments. *Crop Protection* 19: 847 – 853.

Gilsanz, J.C., Arboleya, J., Maeso, D., Paullier, J., Beyhaut, E., Labandera, C., Sanders, D.C., Hoyt, G.D. 2004. Evaluation of limited tillage and cover crop systems to reduce N use and disease population in small acreage vegetable farms. Mirror image projects in Uruguay and North Carolina, USA. In: *Acta Horticulturae* 638. Proceedings of XXVI International Horticulture Congress – Sustainability and Horticultural Systems. 163 – 169.

Heather, M.D., Stone, A.G., Dick, R.P. 2006. Compost and manure mediated impacts on soilborne pathogens and soil quality. *Soil Science Society of America Journal* 70: 347 – 358.

Hoitink, H.A.J. and Boehm, M.J. 1999. Biocontrol within the context of soil microbial communities: a substrate dependent phenomenon. *Annual Review of Phytopathology* 37: 427 – 446.

Jenkins, S.F. and Averre, C. 1986. Problems and progress in Integrated control of southern blight of vegetables. *Plant Disease* 70: 614 – 619.

Kremen, C. and Miles, A. 2012. Ecosystem services in biologically diversified versus conventional farming systems: benefits, externalities, and trade-offs. *Ecology and Society* 17: 40 http://www.ecologyandsociety.org/vol17/iss4/art40/

Lumsden, R.D., Lewis, J.A., Millner, P.D. 1983. Effect of composted sewage sludge on several soilborne pathogens and diseases. *Phytopathology* 73: 1543 – 1548.

Mol, L., Huisman, O.C., Scholte, K., Struik, P.C. 1996. Theoretical approach to the dynamics of the inoculum density of *Verticillium dahliae* in the soil: first test of a simple model. *Plant Pathology* 45: 192 – 204.

Pereira, J.C.R., Zambolim, L., Ribeiro do Vale, F.X., Chaves, G.M. 1996. Compostos orgânicos no controle de doenças de plantas. *Revisão Anual de Patología de Plantas* 4: 353 – 379.

Punja, Z.K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology* 23: 97-127.

Pung, H., Aird, P.L., Cross, S. 2004. The use of brassica green manure crops for soil improvements and soilborne disease management. 3rd Australasian soilborne disease Symposium, 8-11 February 2004. http://www.serveagresearch.com.au/publications/brassica-green-manure.pdf

Schönhart, M., Schmid, E., Schneider, U.A. 2012. CropRota – A crop rotation model to support integrated land use assessments. *European Journal of Agronomy* 34: 263 – 277.

Tillman, D., Cassman, K.G., Matson, P.A., Naylor, R., Polasky, S. 2012. Agricultural sustainability and intensive production practices. *Nature* 418: 671 – 677.

Tixier, P., Risède, J.M., Dorel, M., Malézieux, E. 2006. Modelling population dynamics of banana plant-parasitic nematodes: A contribution to the design of sustainable cropping systems. *Ecological Modelling* 198: 321 – 331.

United Nations. 2013. The future we want. Resolution adopted by the General Assembly, 123rd plenary meeting, 27 July 2012 (A/RES/66/288). Reissued for technical reasons on 17 January

2013. http://daccess-dds-ny.un.org/doc/UNDOC/GEN/N11/476/10/PDF/N1147610.pdf? OpenElement. Accessed on June12, 2013.

Van Bruggen, A.H.C. and Semenov, A.M. 2000. In search of biological indicators for soil health and disease suppression. *Applied Soil Ecology* 15: 13 – 24.

van Bruggen, A.H.C. and Termorshuizen, A.J. 2003. Integrated approaches to root disease management in organic farming systems. *Australasian Plant Pathology* 32: 141-156.

van den Berg, W. and Rossing, W.A.H. 2005. Generalized linear dynamics of a plant-parasitic nematode population and theeconomic evaluation of crop rotations. *Journal of Nematology* 37: 55 – 65.

van den Berg, W., Rossing, W.A.H., Grasman, J. 2006. Contest and scramble competition and the carry-over effect in *Globodera* spp. in potato-based crop rotations using an extended Ricker model. *Journal of Nematology* 38: 210 – 220.

Xu, Z., Gleason, M.L., Mueller, D.S., Esker, P.D., Bradley, C.A., Buck, J.W., Benson, D.M., Dixon, P.M., Monteiro, J.E.B.A. 2008. Overwintering of *Sclerotium rolfsii* and *S. rolfsii* var. *delphinii* in different latitudes of the United States. *Plant Disease* 92: 719 – 724.

Chapter 2

Management of soilborne diseases by crop rotation. A Review

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Abstract

Crop rotation is one of the oldest management strategies in agriculture with two main purposes: plant nutrition and pest management (weeds, pests and diseases, particularly soil borne diseases). Currently, conventional agriculture does not depend much on crop rotations because several of the biological processes promoted by this strategy have been replaced by external inputs, mainly synthetic fertilizers and pesticides. One of the consequences of crop rotation in farming systems is the mosaic of crops at farm and landscape levels. The size and complexity of the mosaics in space and time have a tremendous influence on the epidemic development of plant diseases and pests. In this review, we focus on the temporal variation in crops and its consequences for plant disease development, limiting our discussion to the effects on soilborne pathogens with inoculum spread over short distances. We first present the agronomic, economic and ecological reasons for crop rotation, followed by the main criteria for selecting the rotation crops and their sequence. We then describe the effects that rotation crops and their management have on plant disease development, and give examples of the contributions of different crop rotations to disease management. Finally, the use of models as a tool in designing and evaluating crop sequences is outlined, where biophysical and socio-economic information is combined.

1 Introduction

Crop rotation is one of the oldest management strategies in agriculture with two main purposes: plant nutrition and pest management (weeds, pests and diseases, particularly soil borne diseases). Currently, conventional agriculture does not depend so much on crop rotations because several of the biological processes promoted by this strategy, like nutrient cycling and pest suppression, have been replaced by external inputs mainly synthetic fertilizers and pesticides. Crop rotation is not only a long-term strategy for organic agriculture: it is a strong recommendation and a requirement in some countries. According to the International Federation for Organic Agriculture Movements (IFOAM) “organic agriculture is a production system that sustains the health of soils, ecosystems and people … relies on ecological processes, biodiversity and cycles adapted to local conditions, rather than the use of inputs”. The IFOAM Production Standards recommend, among other management strategies, “diverse and versatile crop rotation that includes green manure, legumes and deep rooting plants” (IFOAM 2006).

Here, the term crop rotation is used as a repeated planned sequence of crops with their associated management, which takes place in the same field (time dimension) and in the idealized case is reflected in space; each year all the crops of the sequence are present in the farm (space dimension) with more or less the same area (Figure 1). Crops could be either cash crops, feed crops, trap crops, cover crops, catch crops or green manure crops.

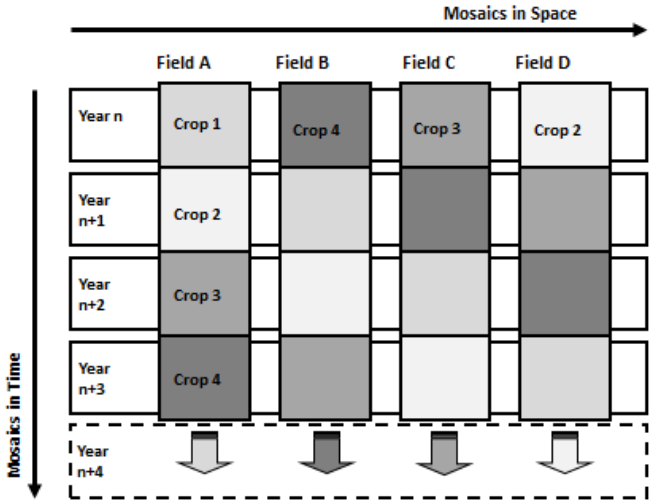


Figure 1. Simplified scheme of a crop rotation: all the crops are present in one particular year and at the end of one crop rotation cycle, all the crops have been present in each field, conferring diversity within (mosaics in time) and among fields (mosaics in space).

One of the consequences of crop rotation in farming systems is the mosaic of crops at farm and landscape levels. The larger the number of crops in a rotation, the smaller the fields in a particular farm, and the greater the agro-biodiversity at the landscape level. The size and complexity of the mosaics in space and time have a tremendous influence on the epidemic development of plant diseases and pests. Effects of the spatial distribution of crops on disease development are not discussed in this review.

Here, we focus on the temporal variation in crops and its consequences for plant disease development. The succession of a variety of crops can affect both foliar and root diseases. The effects on foliar diseases are, however, primarily determined by the spatial pattern in crops, especially when pathogen inoculum is spread over medium or large distances. We limit this chapter to effects on soilborne pathogens with local inoculum spread; this includes splash-dispersed soilborne pathogens that affect the lower stem and foliage besides root infecting pathogens.

Local spread and infection by soilborne pathogens is very much dependent on soil health (van Bruggen and Termorshuizen 2003), a term that is defined in the next section. Soil health contributes to ecosystem health including the ability to provide essential ecosystem services: **provisioning services** such as production of food, fibre and fresh water; **regulating services** such as climate, flood, disease, weed and pest control; **cultural services** such as spiritual, recreational, and cultural benefits; and **supporting services** such as nutrient cycling, soil formation and primary production maintaining the conditions for life on Earth (Millennium Ecosystem Assessment 2005). A healthy agroecosystem is considered to be a prerequisite for the ecological sustainability of the system. The sustainability of farming systems and rural communities has received ample emphasis at international forums since the Earth Summit in Rio de Janeiro in 1992 and was recently reinforced in the United Nations Conference on Sustainable Development Rio+20 in 2012 (United Nations 2013). Sustainability has often been associated with diversity, and in this respect, crop rotations have been a valuable tool in designing sustainable farming systems, as they provide diversity in space and over time. However, selection of a particular crop rotation may be a trade-off between an ideal crop sequence for ecological benefits and a practical sequence providing short-term market opportunities for economic gains.

In this chapter we first present the agronomic, economic and ecological reasons for crop rotation, followed by the main criteria for selecting the rotation crops and their sequence. We then describe the effects that rotation crops and their management have on plant disease

development, and give examples of the contributions of different crop rotations to disease management. Finally, the use of models as a tool in designing and evaluating crop sequences is outlined, where biophysical and socio-economic information is combined.

2 Reasons for crop rotation

As mineral fertilizers and pesticides became widely available in the past century, farming has become more and more specialized in many areas of the world, to the extent that only a few crops may be grown over extensive areas. Examples include rotation of corn and soybeans in the Midwest of the USA and monoculture of wheat in the Western States of the USA and Canada and in parts of France. The main reason for this trend has been economic: mechanization and bulk marketing supported by national subsidy schemes, which prompted scale enlargement as the first of two major farm development strategies. The second major strategy has been intensification through the increases of inputs per unit area, mainly fertilizers and pesticides and oil-based energy. These strategies contributed to an increased food supply but are increasingly seen as a major cause for concern due to negative side effects on social, economic and environmental indicators. Here, we mention environmental indicators such as leaching of nitrogen, phosphorous and pesticides in ground and surface water, a reduction in above- and below-ground biodiversity and soil erosion. To reverse the deterioration of agroecosystems, increasing numbers of farmers are trying to diversify their farming systems. This is especially true for organic farmers who have to rely on ecosystem services for crop production.

Organic farmers have many agronomic and economic reasons to use relatively long crop rotations, usually spanning a period of five to eight years (Table 1). First, an adequate crop rotation gives a **regular supply of nutrients** from a combination of active and stable soil organic matter and **maintenance of a good soil structure** that will enhance the water-holding capacity and allow extensive root formation necessary for a productive crop. Second, ground cover will **reduce erosion**, preventing loss of soil and associated nutrients and soil organic matter (SOM), and suppress weed growth. Although **management of pests and diseases** are also important benefits of crop rotation, this is often considered of secondary importance, although unjustified so from a scientific point of view. A variety of crops, each with their own management activity peaks, also **spreads the human labour inputs more evenly** over the seasons, even though the total workload may be increased. Finally, a multitude of crops **provide security** in case one of the crops fails due to unsuitable weather

conditions or pest and disease attack, guaranteeing a more stable income.

Table 1. Reasons for crop rotation and associated management in organic farms, and their contribution to maintain ecosystem services.*

Ecosystem services	Reasons	Associated management
Support: nutrient cycling	Balanced use of inorganic nutrients over time and in space	- Alternate crops with different nutrient extraction - Alternate crops with shallow and deep root systems - Include cover crops with BNF ability to catch atmospheric N - Include catch crops to extract / recycle the excess of nutrients
	Balanced return of inorganic nutrients back to soil	- Leave crop residues in the field (either incorporated or not) - Include green manure crops
Support: soil formation and water retention	Minimize soil erosion and soil physical structure deterioration	- Avoid fallow periods by planting cover crops, when possible combined with reduced tillage - Use of “biological tillage” by combining fibrous roots (e.g. from grasses) and deep tap roots (e.g. forage radish)
	Improve SOM and soil physical structure; to favour soil porosity, infiltration and water holding capacity	- Improve soil aggregates by favouring earthworm populations and fungi with their exudates (e.g.: glomalin from AMF) - Incorporate green manures with more recalcitrant residues (higher C/N ratio)
Regulation: weeds, pests and diseases	Prevent weed species accumulation	- Incorporate cover crops to substitute bare fallow periods - Use allelopathic properties of some crops - Use high crop density rates to increase crop competitive ability

Ecosystem services	Reasons	Associated management
Regulation: weeds, pests and diseases	Prevent insect pests and pathogen outbreaks	- Avoid consecutive crops from the same family or hosting same pest / disease - Take advantage of by products released by cover crops (e.g. saponins from oats, glucosinolates from brassicas) - Use trap crops when possible (mainly for management of plant parasitic nematodes) - Promote plant resistance (SAR) through enhancement of soil biology and balanced plant nutrition
Provision: food and fiber	Reduce crop failure risks and enhance potential income.	- Diversify farm activities and balanced labour distribution through the seasons, by combining different crops in rotation - All the above practices that improve rooting depth, water and nutrient uptake - All the above practices that minimize weeds, pests and diseases
Cultural: landscape identity	Demonstrate geo-morphological and culture-historical traits of geographic areas	- Choice crops that fit into the local biophysical context - Provide a diversity of crops - Visualize seasons

* Abbreviations: SOM= Soil Organic Matter , BNF= Biological Nitrogen Fixation , AMF= Arbuscular Mycorrhizal Fungi , SAR= Systemic Acquired Resistance.

Besides the above-mentioned reasons for crop rotation, there are **philosophical reasons**, which for many farmers counteract the increased overall workload needed to maintain a diverse farming system. These philosophical reasons can be found in the principles on which organic agriculture is based, mostly revolving around enhancing the sustainability of agroecosystems, in particular maintaining soil quality[1] and soil health[2] (IFOAM 2006).

In addition to geo-morphological soil type and climate, plants and their management are the main driving forces for soil biological processes and strongly affect soil biological composition, diversity and health (Hartmann et al. 2009). For a given soil, crop rotation is the main "driver" in selecting soil functional groups, and the differences in quality and quantity of root exudates and dead plant materials from successive plants lead to the maintenance of different biological communities in soil. The microorganisms in such communities can provide various ecological services. For example, the beneficial effects of *Azospirillum* spp. on sugar cane, rice or corn consist not only of biological nitrogen fixation (BNF) in the rhizosphere, but also bacterial auxin production resulting in increased soil exploration by roots and more efficient water and nutrient absorption (Dalla Santa et al. 2004). Diverse microbial communities are often associated with general root disease suppression through increased competition with the pathogen for resources or inhibition by antagonism and hyper-parasitism (Weller et al. 2002). Crop rotation, especially with plant species high in lignin or phenolic compounds, contributes to the creation of various micro-habitats that allow complex communities with different ecological functions to co-exist and foster a healthy, disease suppressive soil.

Crop rotation combined with no- or reduced tillage and the use of cover crops in between cash crops, can lead to a quantitative and qualitative improvement of soil organic matter (SOM). This strategy induces a higher saprophytic and mycorrhizal fungal biomass by increasing hyphae mat and associated extracellular exudates of polysaccharides and glycoproteins like glomalin from arbuscular mycorrhizal fungi (AMF). Both exudates and hyphae are responsible for soil macro-aggregate formation, for protecting plant-derived SOM

[1] "the capacity of a soil to function within ecosystem boundaries to sustain plant and animal productivity, maintain environmental quality, and promote plant and animal health" (Karlen et al. 1997).

[2] "capacity to support the production of food and fibre, to a level and with a quality sufficient to meet human requirements, together with continued delivery of other ecosystem services that are essential for maintenance of the quality of life for humans and the conservation of biodiversity" (Kibbelwhite et al. 2008).

and microbial organic matter (MOM) and for enhancing soil water infiltration and retention (Douds and Millner 1999, Scholberg et al. 2010). In conventional farms, no-till is combined with herbicide sprays to kill the cover crop and allow growth of a subsequent cash crop. As this is not possible in organic farms, no-till is unfortunately rarely used in those farms. Research is urgently needed to develop techniques and equipment, such as strip flaming or tillage, that allow the growth of a cash crop in the mulch of a previous cash or cover crop in organic farms.

If legume crops are used in the rotation, nutrient cycling is improved through BNF by symbiotic rhizobacteria (*Rhizobium* spp., *Sinorhizobium* spp., *Bradyrhizobium* spp.). Similarly, free-living nitrogen-fixing bacteria (*Beijerinckia fluminensis, Azotobacter paspali, Azospirillum* spp.) associated with non-legume plants like sugar cane, maize, or rice contribute to BNF, explaining the productivity of these crops under low input management (Baldani and Baldani 2005). The AMF-root-symbiosis increases phosphorus (P) uptake. AMF populations are favored when a cover crop like hairy vetch (*Vicia villosa*) or sorghum-sudangrass (*Sorghum* × *drummondi*) is included in a no tillage rotation, and decreased under non-mycorrhizal plants (like crucifers) or monoculture (Douds and Millner 1999).

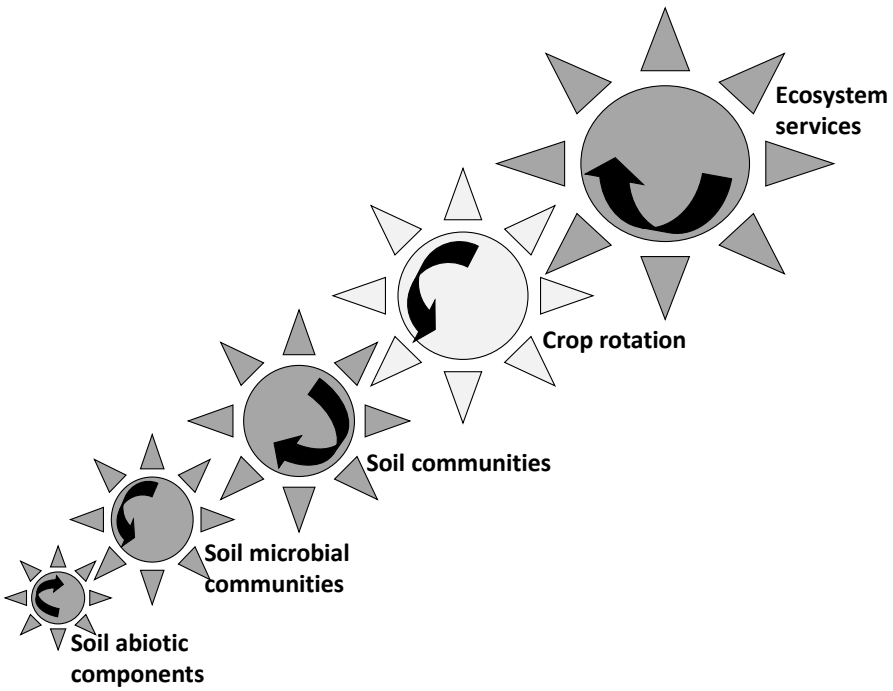


Figure 2. Interlocking cycles of abiotic components, soil microbial communities, soil communities, crops, and ecosystem services. Crop rotation mainly affects supporting and regulating ecosystem services (adapted from Millenium Assessment 2005).

To summarize, crop rotations are implemented for a large number of reasons, primarily agronomic and economic reasons, but also for their contributions to soil health and agroecosystem sustainability. Plants are the main drivers for shifting and diversifying soil communities; so, the choice of crops will affect soil functional groups and basic ecological services. These services, such as water and nutrient cycling (supporting services) and disease suppression (regulating services), have a profound influence on the agronomic performance of the crops produced in a rotation. Organic farmers value the ecological consequences of crop rotation because they depend on the services that enhance crop yields and economic stability (Figure 2).

3 Choice of rotation crops and their sequence

Crop rotations determine farming system characteristics such as crop yields, soil erosion, occurrence of pests, diseases and weeds, and dynamics of nitrogen and energy fluxes including labour. These in turn reflect overall agro ecosystem health. More than one crop rotation could be suitable for a particular farming system, and the final choice will depend on farm constraints and farmers' preferences. It is quite common to have more than one crop rotation in a particular farm, so that the farm resources are optimized, e.g. water for irrigation, soil type, etc. (Figure 3).

Even though farmers implement crop rotations on their farms, the selected crops and their sequence are not always sustainable in the long term. One sequence may be good for market demands, but very demanding of labour and conducive to SOM decline. Another sequence could be excellent in preventing nematode problems, but may not generate sufficient income or may not be feasible for crop residue management if the proper equipment is lacking.

In this section some criteria for selecting crops are presented and organized largely according to the ecosystem services presented in Table 1, first focusing on nutrient cycling, next on soil formation, followed by weed, pest and disease regulation, and finally food and fibre provision. This will then lead into the next section, which will be focused on plant disease management by crop rotation.

3.1 Nutrient cycling

Nutrient retention and recycling can be optimized by alternating crops with different nutrient uptake or ability for BNF, different decomposition rates (due to phenological stage, C:N ratio and other aspects of tissue composition), or different residue management (soil incorporation or not). Besides the nutrient contribution through decomposition of crop debris, about one third of crop assimilates is released through the roots into the soil and directly feeds the soil and the micro-organisms therein (Marschener 1995).

Including legumes once every 3 years has been advocated in order to provide sufficient nitrogen (N) to the system. The amount of N fixed is mainly determined by the leguminous crop species, the presence of compatible strains of N fixing bacteria and the amount of N available in soil. The amount of N fixed can range from about 60 to 200 kg N per hectare and year. A major constraint, however, to BNF is the great susceptibility to many root and foot diseases. As many of these pathogens have a wide host range attacking many different

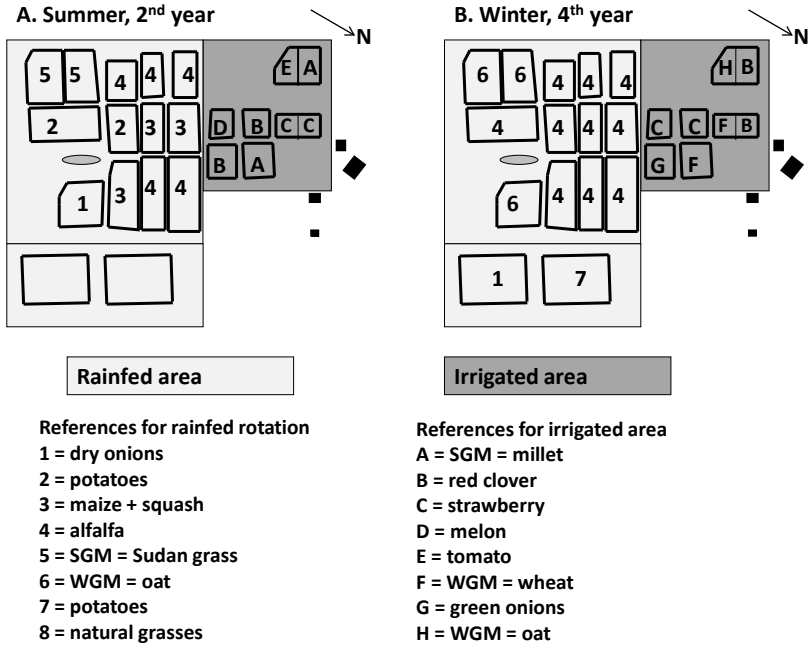


Figure 3. One irrigated and one rainfed eight-year crop rotation implemented on a temperate vegetable farm to optimize water use efficiency. A. Summer 2nd year = crops installed during the summer period of the second year of the rotations, B. Winter 4th year = crops installed during the winter period of the fourth year of the rotations.

Irrigated rotation: strawberry – green onions – Summer green manure (SGM, millet) – table beets – winter green manure (WGM, wheat) – tomatoes – WGM (oat) – melon – WGM (wheat) – SGM (sudangrass) – strawberry – red clover. Rainfed rotation: potatoes – SGM (sudangrass)– dry onions / garlic – WGM (oat) – squash / maize – WGM (wheat) – potatoes – four years of alfalfa.

legumes and sometimes also non-legumes, legume health management is a complex issue with many unresolved problems. These pose a great challenge to organic production systems.

Legumes like lupine (*Lupinus* spp.) and pigeon pea (*Cajanus cajan*) not only contribute with BNF, but also improve P bioavailability because of excreted root organic acids (Drinkwater and Snapp 2007). However, utilization of N released by legume crops can be a bottleneck if nutrient release is not synchronized with crop demand of the subsequent crop. For example, leguminous cover crops such as clovers (*Trifolium* spp.) have low C:N ratios and can release large amounts of N short after soil incorporation, therefore their use may result in excessive N leaching especially on sandy soils (Scholberg et al. 2010). Moreover, high nitrogen contents in subsequent crops may induce susceptibility to various root pathogens (van Bruggen and Termorshuizen 2003). To prevent excessive N availability and leaching, mixtures of grasses and legumes can be used in which more biomass with higher C:N ratio is produced, delaying mineralization and N release as shown for organic tomato production systems in California when either rye (*Secale cereale*) or triticale (*Secale cereale* x *Triticum durum*) plus common vetch (*Vicia sativa*), field pea (*Pisium sativum*) or bell bean (*Vicia faba*) were used (Madden et al. 2004).

When the aim is to reduce the risk of potential nutrient leaching losses between two cash crops, “nutrient catch crops” are planted. Deep-rooted and fast growing crops such as rye (*Secale cereale*), forage radish (*Raphanus sativus* var. *niger*) or other brassicas and sun hemp (*Crotalaria juncea*) can effectively acquire nutrients (specially N, but also P and micronutrients) from deep soil layers and make them more readily available for subsequent crops when killed and incorporated or left as a mulch (Scholberg et al. 2010).

3.2 Soil formation

Minimize soil erosion and deterioration of soil physical structure

If soil physical properties are the main constraint, species with fibrous and deep roots and with more recalcitrant residues (higher C:N ratio) are preferred as rotational crops, while N inputs can be provided by other sources. For example, in temperate vegetable production on clay soils, the inclusion of grasses as green manures in the crop rotation, either as winter cover crops like black oats (*Avena strigosa*), wheat (*Triticum aestivum*), barley (*Hordeum vulgare*) and triticale (*Secale cereale x Triticum durum*) or summer cover crops such as sorghum (*Sorghum* spp.), millet (*Setaria italica*) and Italian ryegrass (*Pennisetum glaucum,*

P. americanum) has proven to be effective in controlling soil erosion and improving porosity and apparent soil density (Dogliotti 2003). Similarly, a mixture of sunflower (*Helianthus annuus*) and sorghum (*Sorghum* spp.) or millet (*Setaria italica*) is a better option than sunflower alone. Sunflower residues degrade quite fast and so the mulch layer, leading to erosion because the soil is soon exposed to the effects of rainfall and wind.

Leaving crop residues in the field can also contribute to improvement of soil structure through improving SOM. For example, after harvesting the ears, sweet corn crops can provide substantial amounts of residues (stalks and leaves) that can be chopped and incorporated into the soil, preferably combined with manure to avoid N immobilization because of the high C:N ratio of the residue. The inclusion of forage radish (*Raphanus sativus* var. *niger*) as a previous crop can help in managing soil compaction problems. Its root system can penetrate plow pans or other layers of compacted soil and leave root channels, which are colonized by the following crop (Williams and Weil 2004). The improved soil structure obtained in this way will result in better root penetration and aeration and reduces the risk of root rots such as those caused by *Phytophthora* species (Workneh et al. 1993).

Improve water use efficiency

Water use efficiency is a big challenge for farmers, especially in the case of rain-fed cropping systems. Water use efficiency can be enhanced by increasing SOM and soil aggregate stability, by improving soil physical properties, and by minimizing water losses due to evaporation. One example is the inclusion of a cover crop of forage radish and rye prior to a soybean crop. The cover crop supplies a mulch layer that limits evaporation from the soil surface early in the season and increases infiltration, while provides pathways for roots to obtain water from the subsoil (Williams and Weil, 2004). This can again contribute to a reduction in root diseases caused by soilborne pathogens like *Phytophthora* and *Pythium* species (van Bruggen and Termorshuizen 2003).

3.3 Regulation: Weeds

One of the main constraints in organic farming is competition by weeds, which can be minimized by combining several strategies. Diversified crop rotations have a greater variety of factors affecting weed populations: light, water and nutrient conditions affect weed growth depending on the specific crops; tillage and cultivation affect their death and germination time; and crop residues affect physical, chemical and biological soil characteristics, which in turn affect weed growth. Densely planted cover crops between cash crops can suppress

weeds through direct competition and allelopathy, the release of phytotoxins from both root exudates and decomposing residues. Some crops known for their ability to release phytotoxic compounds are sorghum, sunflower, crotalaria, red clover, several brassicas, rye, oats and black oats, triticale and barley (Liebman et al. 2001).

In organic reduced tillage systems in southern Brazil, summer annual weeds in soybeans or maize crops are suppressed by previously cropping a mixture of rye (*Secale cereale*), fodder radish (*Raphanus* spp.) and vetch (*Vicia* spp.). Apart from other beneficial effects, the crop mixture suppresses weeds by releasing phytotoxins: B-phenyllactic acid, B-hydroxybutiric acid and various benzoxazolinone compounds from rye, and glucosinolate from fodder radish. Contact of crop plants with the toxins could be avoided by placing crop seeds below the allelopathic zone, 5-10 cm from the mulch (Altieri et al. 2008). A five-year rotation of maize-soybeans-maize-oat plus clover-forage for hay can be effective in reducing weed populations because weeds are continuously challenged by changing soil disturbance patterns and resource competition (Liebman et al. 2001).

3.4 Regulation: Pests and diseases

Crop rotation is most effective against arthropod pests that do not disperse over great distances and/or that overwinter in or near host crop fields, such as the Colorado potato beetle (*Leptinotarsa decemlineata*) and onion maggot (*Delia antiqua*). Also a minimum distance to last years' crops has to be observed to avoid short distance moving. For example Colorado potato beetles can walk up to 100 m to find new potato crops. Crop rotation can also improve overall plant resistance to insect pests through its effects on soil quality and health (Zehnder et al. 2007). Rotation with glucosinolate containing brassica crops can contribute to pest management through biofumigation, even though attention should be paid to N release from decomposing plant material to avoid N excess in early growth stages of the new crop.

Crop rotation combined with reduced tillage and straw mulch can suppress some insects. For example in potato crops with straw mulch, Colorado potato beetle is suppressed and aphid infestation and virus *Potato Virus Y* (PVY) incidence are reduced, probably through a combination of reduced host-finding ability, increased predation from natural enemies and less soluble N in potato foliage (Zehnder et al. 2007). However, organic mulching can enhance other pests like ants and cutworms so the whole system should be assessed carefully.

Crop rotations and the particular sequence of the crops in a rotation also have profound effects on phytophagous nematodes and plant pathogens. Examples of these effects and management practices to control plant diseases and nematodes are presented in the next section.

4 Crop rotation and disease development

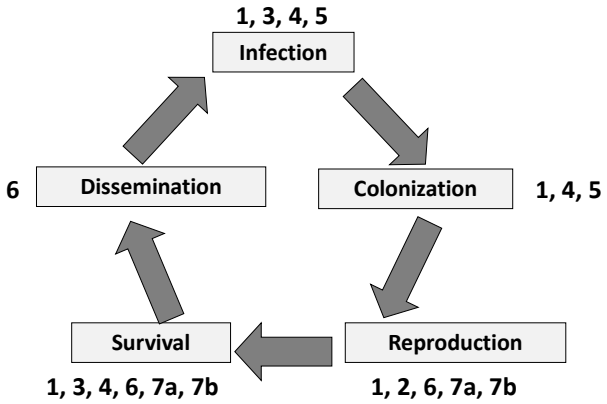
Plant disease development is a dynamic process involving living entities (the plant host and the pathogen) and the environment (biotic and abiotic components), summarized in the "disease triangle" concept. Crop rotation affects each component of the disease triangle, and can be planned in such a way that each component is minimized or in the case of the host even eliminated. Indeed, some growers opt not to grow a particular crop, because they cannot sufficiently control one or more diseases affecting that crop, for example potatoes, which are often severely affected by late blight on organic farms. Although the simplest strategy is to eliminate the host, a decline in pathogen populations can also be achieved by crop rotation. Crop rotation contributes in pathogen reduction by antibiosis, parasitism, predation, or release of toxic compounds from crop residues or root exudates. Also promotes an unfavourable environment for pathogen development by changing the nutritional status or resistance response of the host plant, or by improving soil physical properties.

In this section, we will analyze crop rotation effects on the different phases of the disease cycle: host infection and colonization, pathogen reproduction, survival and dissemination (Figure 4). General criteria for designing crop sequences to manage plant diseases will be highlighted, taking the properties and functions of some plant species and families and some farming practices into account.

4.1 Effect of rotational crops on the disease cycle phases

Infection

The infection process consists on bacterial multiplication or fungal spore germination on the host, appressorium formation, and plant penetration. The process is affected by the presence of the host, toxic compounds from previous or current allelopathic crops and by competitors for carbon, energy, nutrients and attachment sites. For example, some oat (*Avena sativa*) cultivars produce saponins in response to *Pyrenophora* pathogen attack (Bahramnejad et al. 2008). Similarly, particular wheat cultivars stimulate the growth of fluorescent Pseudomonads



1) Non host crop; 2) Trap crop; 3) Allelopathic crops; 4) Rotational crops that increase soil competitors; 5) Rotational crops that stimulate plant promoters (PGPR, AMF, etc.); 6) Crop residue decomposition; 7a) Tillage with soil inversion, 7b) Tillage without soil inversion

Figure 4. Disease cycle phases and the effects of various crop rotation practices (indicated by numbers) on each step in the disease cycle.

known for producing antibiotics and siderophores and successfully competing with other microorganisms when iron is scarce. Wheat can also increase non-pathogenic *Fusarium oxysporum*, which competes for the same niche as the pathogenic strains (Mazzola 2007). Also, rotations that increase AMF can contribute to plant protection by reducing pathogen infection sites.

Colonization

Colonization involves the pathogen growth in the plant and the initiation of plant tissue damage. Colonization can be interrupted through mechanisms triggered by trap crops and by rotational crops that increase soil competitors and plant growth promoters. For example, *Meloidogyne* spp. fails to develop galls in *Crotolaria spectabilis* roots or develop very slowly resulting in abortion of giant cells[3] in *Tagetes patula* (Winoto Suatmadji 1969; Wang et al. 2002).

Some cereals like wheat attract and promote the growth of bacterial species in their rhizosphere, in particular *Pseudomonas* spp. and *Bacillus* spp. that are antagonistic to a wide range of root pathogens and may also induce systemic resistance (Kloepper et al. 2004).

[3] Giant cells are *Meloidogyne* spp. feeding sites, induced by synchronous mitosis without cell division, resulting in multinucleate large cells.

Induced systemic resistance (ISR) is the activation of plant defence mechanisms without direct interaction between the resistance–inducing microorganism and the pathogen, and involves jasmonate and ethylene signals among others (Raaijmakers et al. 2008). For example *Bacillus pumilus* limits colonization of *Fusarium oxysporum* f.sp. *pisi* on pea roots (*Pisum sativum*) by inducing cell wall barriers containing callose and phenolic compounds (Kloepper et al. 2004).

Reproduction

Crop residue decomposition can affect reproduction and survival of some necrotrophic pathogens. On the one hand, some pathogens like *Pythium* spp. and *Rhizoctonia solani* can multiply in decomposing debris, leading to increased inoculum density and disease risk if a crop is planted too soon after residue incorporation. On the other hand, disease risks can be reduced when a pathogen is outcompeted by enhanced biological activity during residue decomposition.

Trap crops can attract nematodes, but do not allow their reproduction. *Crotalaria* spp., *Mucuna* spp., *Tagetes* spp. and some brassicas can be used in a crop rotation for this purpose. The effectiveness of the different trap crops is variable, which can be attributed to differences in pedo-climatic conditions, nematode races, and trap crop cultivars; thus local responses to particular trap crops should be checked. For example, fodder radish (*Raphanus sativus*) and white mustard (*Sinapis alba*) are grown in Northern Europe before sugar beets to reduce *Heterodera schachtii* populations. Although the nematode invades the brassica roots, their sexual differentiation is disrupted. This results in very low densities of female populations in the next generation, causing a significant decrease in nematode populations and thus low infection in the next sugar beet crop (Matthiesen and Kirkegaard 2006).

Most of the plant-parasitic nematodes suppressed by *Crotalaria* spp. are sedentary endoparasitic nematodes. In the USA *C. spectabilis* roots can be colonized by juveniles of *Meloidogyne* spp. but these fail to form galls, whereas in South Africa *M. hapla* fail to reproduce (Wang et al. 2002). *C. juncea* roots can be infected by *Rotylenchulus reniformis,* but the nematode does not develop or reproduce. However, sometimes – especially under tropical conditions – the life cycle is only delayed (Wang et al. 2002).

Marigolds (*Tagetes* spp.) are effective in controlling polyphagous nematodes like *Pratylenchus* spp., *Meloidogyne* spp. and *Tylenchorhynchus* spp., even though not all marigold species are equally effective for all nematodes in all soil types; they are usually

more effective in sandy than in clay soils. The effectiveness of *Tagetes* species against *Pratylenchus* spp. decreases in the order *T. patula, T. erecta* and *T. minuta.* A biennial rotation of *T. patula* with susceptible host crops can keep *Pratylenchus* spp. at a low density, with the exception of *P. thornei* on heavy soil (Winoto Suatmadji 1969). In Florida, USA, *T. erecta* is resistant to *M. arenaria* whereas *T. minuta* is not, but both are resistant to *M. incognita* and *M. javanica* (Kruegger et al. 2007). The nematicidal effect is related to the level of α-terthiophene and other related compounds found inside marigold roots. However, these compounds are only active when the marigolds are growing because they are chemically altered by near-UV light and become inactive when plants are taken out of the soil. Even though *T. patula* can be colonized by nematodes, the roots present small and necrotic lesions with one-three dead or twisted *Pratylenchus penetrans*, or few *Meloidogyne hapla* galls where giant cells are slowly formed and the nematodes ultimately die while giant cells abort (Winoto Suatmadji 1969). Despite the beneficial effects of marigolds in controlling nematodes, their use is restricted to intensive cropping systems (like vegetables and flowers) and to tropical/sub-tropical regions because of agronomical constraints: small seeds, slow germination rates in temperate regions, high light requirements, frost sensitivity of some cultivars, and finally no economic return of secondary products (Winoto Suatmadji 1969).

Survival

It is well known that growing the same crop or crops within one family repeatedly results in the build-up of soilborne pathogen populations, thus rotation is at the basis of disease management by pathogen starving in the absence of host. In addition, pathogen survival is challenged by soil antagonistic communities and toxic compounds released during crop residue decomposition.

If legumes such as beans and soybeans are used for grain production, leguminous green manure crops should be avoided as the preceding crop, despite the beneficial effects on soil properties, since some soilborne pathogens can be shared. For example, when beans (*Phaseolus vulgaris*) are the commercial crop, a preceding hairy vetch crop is very effective in suppressing *Thielaviopsis basicola,* but increases the damage caused by the lesion nematode *Pratylenchus penetrans* (Abawi and Widmer 2000). Similarly, some species of *Crotalaria* reduce *Meloidogyne* populations, but increase *Pratylenchus* spp. (Wang et al. 2002), while *Mucuna aterrina* and Lima bean (*Phaseolus lunatus*) increase populations of *Fusarium oxyxporum* f.sp. *phaseoli*. Instead of rotation with these legume crops, lesion nematode problems and the associated root disease complex (*Fusarium oxyxporum* f. sp. *phaseoli*, *R. solani*, *Pythium ultimum*, *T. basicola*) can better be controlled with sudangrass

or ryegrass (grasses), or rape seed (brassicas). A three-year crop rotation of sweet corn – table beets – beans is effective for the management of both the bean root disease complex and the beet cyst nematode (*Heterodera schachtii*), since in this sequence two consecutive years without host plant of both the lesion and the cyst nematode are achieved (Abawi and Widmer 2000).

Pathogen survival is also influenced by toxic compounds released during crop residue decomposition of Sudangrass and sorghum hybrids, oats, rye and Brassicaceae, among others. Epidermal cells of sudangrass (*Sorghum sudanense*), the hybrid sorghum – sudangrass (*S. bicolor* x *S. sudanense*) and sudangrass hybrids (*S. sudanense* x *S. sudanense*) contain dhurrin (cyanoglucoside) and isothyocianates (ITC), which are hydrolyzed into hydrogen cyanide when the tissue is damaged. Dhurrin levels vary in the course of crop development, with higher contents during the first developmental stages. So after the crop is chopped and either incorporated in the soil or left as mulch, these compounds are released into the soil, with toxic properties against nematodes, fungi and plants. For example, lettuce is highly susceptible to the northern root knot nematode (*Meloidogyne hapla*), but rotation with sudangrass is effective in reducing nematode populations and damage. The best results are found with the incorporation of one - two month old sudangrass crops, but since lettuce is also sensitive to the phytotoxic compounds released by sudangrass, lettuce can be planted only three-four weeks after crop residue incorporation (Abawi and Widmer 2000).

Oat shoots are known to produce different antimicrobial compounds when plants are attacked by pathogens or decomposed, and because of this are used in rotation with other crops to reduce pathogen build-up. Among these compounds are saponins (avenacoside A and B which are converted into 26-desglucoavenacoside A and B), flavonoids (flavone (5)-C-glycosides), phytoalexins and avenanthramides (hydroxycinnamic acid amide) that have antimicrobial and anti-nematode activity (Bahraminejad et al. 2008). For example, *Gaeumannomyces graminis* var. *tritici* incidence and populations of root lesion nematodes *Pratylenchus thornei* are reduced when wheat is preceded by oats. Additionally saponins obtained from oat shoots inhibit in vitro growth of *Pyrenophora* species except *P. avenae* (pathogenic to oats) suggesting some specificity in the antimicrobial properties (Bahraminejad et al. 2008).

'Biofumigation' is the beneficial use of *Brassica* green manures that release isothiocyanates. Isothiocyanates (ITCs) are natural toxic compounds, produced after hydrolysis of aliphatic and aromatic glucosinolates (GLS), common secondary plant compounds of diverse

Brassicaceae. GLS and the hydrolytic enzyme myriosinase, coexist in plant cells although physically separated, so that only after tissue break-down hydrolysis takes place releasing isothiocyanates and other potentially toxic compounds such as organic cyanides, oxazolidinethiones, nitriles, epinitriles and ionic thiocyanates, that have either fungistatic or biocidal properties. The types of GLS present in the tissues are quite constant within species, but vary considerably between species, e.g., *Brassica napus* predominately has non-ITC liberating glucosinolates while *B. juncea* mostly has ITC-liberating glucosinolates. Also, total GLS concentration changes with environmental conditions and crop age, generally declining after flower onset (Matthiessen and Kirkegaard 2006). The effectiveness of biofumigation will be affected by the selection of the brassica species, which determine the concentration and type of GLS produced, the amount of biomass produced, fineness of fragments and its soil incorporation, and factors that favour hydrolysis of the GLS (neutral pH, moderate temperature, high soil water content). Better results are observed in light soil with lower SOM than on heavy soils rich in SOM. In Australia, biofumigation with Indian mustard (*B. juncea*) is effective in reducing *Ralstonia solanacearum* populations in the soil, lowering disease severity in a following tobacco crop. Elsewhere, biofumigation with *B. juncea* has also suppressed nematodes (*Meloidogyne chitwoodi, Tylenchus semipenetrans*) and fungi (*Sclerotinia minor, Rhizoctonia solani)* (Matthiessen and Kirkegaard 2006).

Dissemination

The spread of the pathogen from an inoculum source to a host can be affected by crop residue decomposition. For example, rain splash dissemination of *Colletotrichum* spp. causal agent of strawberry anthracnose, is restricted when recalcitrant residues of the previous crop such as straw, which decomposes slowly, are left on the soil surface. Similarly, *Cercospora arachidicola* (teleomorph = *Mycosphaerella arachidis*) dispersal from overwintering stroma in the soil is limited due to wheat residues left on the surface, and leaf spot of peanut is suppressed (Cantonwine et al. 2007). The “Frijol Tapado” system, a traditional management method of web blight of beans caused by *Thanatephourus cucumeris* (anamorph: *Rhizoctonia solani*) is another nice example. Famers broadcast bean seeds into selected plant species, mainly broadleaf weeds, after the weeds are cut to form a mulch layer. The beans germinate through the mulch layer, which apart from avoiding weed proliferation and conservation of soil moisture prevents soil splashing, the most important means of dissemination of inoculum causing bean web blight (Thurston 1990).

4.2 Crop sequences and associated management

Including a non-host in the rotation scheme results in a decline in the pathogen population, since the pathogen cannot infect the non-host plant and therefore its reproduction and survival are affected. Ideally, the host is reintroduced in the crop sequence when the population is low enough to cause insignificant economic losses. This goal is quite easy to obtain for those pathogens with a specific or narrow host range and low mobility. Depending on their survival ability, the period without hosts may need to range from one to three years to more than five, or even 10 years and more. For example the beet cyst nematode (*Heterodera schachtii*) can be managed with a rotation with three years without beets, whereas white rot caused by *Sclerotium cepivorum* needs 10 years without onion, garlic or other members of the *Alliaceae* (Abawi and Widmer 2000) (Figure 5).

Crop rotation should be combined with other practices for successful disease management. Among this practices are: adequate soil and field preparation to promote residue decomposition, facilitate seed germination and avoid standing water in the field; and the use of healthy plant material. But also tillage system, residue management and soil organic amendments modify the crop environment, either by enhancing or reducing the potential of the rotational crops to control a particular disease.

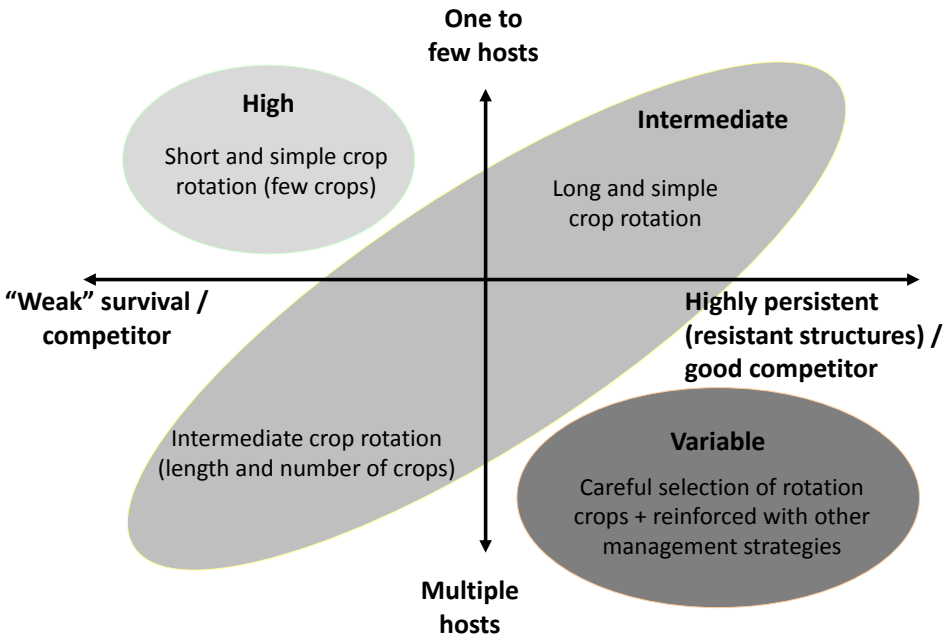


Figure 5. Effectiveness of crop rotation in disease management depending on frequency of hosts and survival characteristics of the pathogen.

Tillage

The interactive effects of tillage practices and a particular crop rotation on crop growth are specific to the pathogen-soil-crop-environment combination, and individual factors responsible for the effects are difficult to discern. A reduced tillage maize-maize-sugar beet rotation has been conducive to the development of *R. solani* root rot on sugar beet (Bhure et al. 2009). Plowing reduces root rot incidence and increases sugar yields, since the pathogen survives better in undisturbed upper soil layers. In contrast, in a wheat-oat-sugar beet rotation the practice of intercropping and incorporation of mustard with wheat reduces root rot incidence on sugar beet, compensating for the negative effects of reduced tillage (Bhure et al. 2009).

The interactive effect of tillage depends on the number of hosts, reservoir hosts (on which they can survive without causing damage) and non-hosts in the sequence. But also depends on the survival strategy and survival period of the pathogen. For example, when soil inversion is practiced after growing wheat infected with *Pseudocercosporella herpotrichoides*, the causal agent of eye spot, disease risk on the last host crop is lower in a non host (sunflower) – host (wheat) – host (wheat) sequence than in a host (wheat) - non host (canola) – host (wheat) sequence. This is because the pathogen can survive two to three years on host residue and soil inversion retrieves the pathogen buried at the end of the first crop (Colbach and Meynard 1995). In general, at least two years of a non-host are needed for a reduction in disease in a host crop, if the pathogen survives two to three years in the absence of its host.

Pathogen distribution in the field also can be affected by the interaction of crop sequence and tillage. Lettuce drop, caused by *Sclerotinia minor*, generally occurs in an aggregated pattern when lettuce is grown in monoculture, but rotation with broccoli combined with tillage not only reduces disease incidence, but also changes the pattern to become more random (Hao and Subbarao 2006). Apparently, soil mixing due to tillage combined with death due to isothiocyanate production from broccoli debris rearranges sclerotia of *S. minor* both horizontally and vertically, disrupting the aggregated pattern found in monoculture lettuce.

Finally, it is also important to consider the long-term effects of soil management practices on earthworm populations. In no- or minimum tillage soils earthworm populations will increase tremendously (Riley et al. 2008) contributing to the management of soilborne diseases, as explained below.

Crop residue management

Crop residues, either incorporated or left on the surface, contribute to the build-up of general suppression. General suppression is attained by the increase of total soil microbial and faunal, especially earthworm, activity, and competition in response to the carbon and other nutrients added. Residue decomposition may also release antimicrobial compounds to some pathogens. For example decomposition of canola reduces the viability of *Cochliobolus sativus* spores and consequently common root rot incidence in cereals (Bailey and Lazarovits 2003; Raaijmakers et al. 2008).

Surface residue retention can favor filamentous fungi and actinomycetes, among them *Trichoderma* spp. and *Streptomyces* spp., both playing important roles in controlling soilborne pathogens and degrading organic residues. Several *Trichoderma* species are known for their antifungal activity towards several fungal and oomycetous pathogens (*Fusarium*, *Rhizoctonia*, *Alternaria*, *Ustilago*, *Venturia*, *Colletotrichum*, *Pythium* and *Phytophthora*). Some strains may promote plant growth directly. The mechanisms of action used by *Trichoderma* (competition, antibiosis, parasitism, or systemic-induced resistance) are influenced by concentration and availability of nutrients within the residues and soil organic matter (Vinale et al. 2008).

Earthworm populations are also favored by surface crop residues and considerably speed up their decomposition, thus reducing residue borne pathogens (Stephens et al. 1994 a, b). At the same time their effects on microbial life reduce soil borne diseases as shown for *Rhizoctonia solani* on wheat (Clapperton et al. 2001) and *Fusarium oxysporum* f. sp. *asparagi* and *F. proliferatum* on asparagus (*Asparagus officinalis*), *Verticillium dahliae* on eggplant (*Solanum melongena*), and *F. oxysporum* f. sp. *lycopersici* Race 1 on tomato (Elmer 2009).

Leaving crop residues on the soil surface may, however, also help pathogen survival, as is the case for *Macrophomina phaseolina* causing charcoal rot of soybeans, *Fusarium* sp. causing root and crown rot on maize and *R. solani* causing root and crown rot on sugar beet. Therefore burying the residues for accelerating substrate decomposition may be a recommended practice for specific situations (Bailey and Lazarovits 2003, Raaijmakers et al. 2008), although this practice can compromise other benefits of no- or reduced tillage. In particular, micro- and macrofaunal communities and fungi that provide various services to the agro-ecosystem survive better under no tillage systems. Pathogens with strong competitive saprotrophic ability such as *Rhizoctonia* and *Pythium* species may multiply in decomposing

plant debris and cause damping-off if the next crop is planted within three weeks after residue incorporation. Thus, trade-offs between management practices in the short and long term should be considered when designing crop rotations and associated management practices.

Interactions of rotation, soil type, and environment

The effect of a particular crop sequence on a particular disease can also depend on soil type (sand, loam, clay) and organic amendment, giving inconsistent results in experiments with the same crop sequence and target pathogen. For example, sclerotinia stem rot (SSR) on soybeans caused by *Sclerotinia sclerotiorum* can be managed by rotation with non-hosts like maize, but this is sometimes not enough to control the disease. In Québec, Canada, three years of maize followed by one year of soybeans reduced the incidence of SSR only in combination with compost amendment on sandy loam soil. However the same rotation was conducive to disease development on clay loam soil, probably due to the higher water retention and a denser crop canopy on the latter soil, both factors favouring SSR (Rousseau et al. 2006).

Despite the disease suppressive potential of different plant families or species, not all crop sequences are equally effective in all environments, and local research is needed in order to find out the best or the most feasible combination. Even though the same pathogens constrain crop production in different regions, the selected crops for the rotation may be different for each region, not only for their effect on disease management but also for their economic returns. For example, strawberry is a high-value crop. In New York State, USA, strawberry replant disorder (SRD) associated with several pathogens (*Rhizoctonia* spp., *Fusarium* spp., *Pythium* spp.), nematodes (*Pratylenchus* spp.) and physiological disorders is a serious constraint. Research showed that SRD could be managed by a crop sequence of kale (*Brassica oleracea*) - sweet corn (*Zea mays*) - rye (*Secale cereale*), selected from 12 plant species tested in five different crop rotations. The improvement in performance of the strawberry crop was associated with changes in soil microbiology and to a lesser extent with weed populations (Seiges and Pritts 2006). On the other hand, in Connecticut, USA, two nematode problems (*Pratylenchus penetrans, Meloidogyne hapla*) and black root rot (*Rhizoctonia fragariae*) were managed by rotating out of strawberry for two years with sorghum-sudangrass (*Sorghum bicolor* x *S. sudanense* Triple “S”) and saia oat (*Avena strigosa*) (La Mondia, 1999). In Dahu, Taiwan, where *M. incognita*, *M. hapla*, *P. coffeae* and *P. penetrans* are dominant plant-parasitic nematodes in strawberries, corn and bare fallow are recommended as the most appropriate rotation strategy for nematode management,

while rice (*Oryza sativa*) is considered a less suitable rotation crop because of its low economic returns (Chen and Tsay 2006).

4.3 Examples of crop rotation effects on disease development

Crop rotation affects the incidence and severity of different diseases, as well as the dynamics of the associated the soilborne pathogens. Within each environment and pathosystem, or disease - crop combination, a number of mechanisms leading to disease suppressiveness are involved. Some of these mechanisms are total or partial resistance of the crop to pathogen infection or colonization, release of pathogen toxicity compounds by crop decomposition, higher microbial competition by enhancement of total soil biology, among others (Table 2). In many cases one or more soilborne diseases are reduced on the same crop as a result of longer rotations. For example, in the Sustainable Agriculture Farming Systems (SAFS) experiment in California, USA, where conventional, low-input and organic cropping systems were compared, the main differences in tomato root disease severity, caused by a complex of pathogens, were due to rotation length rather than the type of farming system (Figure 6). The main pathogen was *Pyrenochaeta lycopersici*, the causal agent of tomato corky root. It forms microsclerotia, which are released from decomposing tomato debris in one to two years. Thus, in a two-year rotation of tomato and wheat, the inoculum density in soil is maximal during the growing season of the next tomato crop, while in a four-year rotation most microsclerotia die before the next tomato crop due to natural biological control.

Most of the examples found in the literature and presented here involve relatively short rotations of not more than four years, and simple schemes with few crops and focus on one pathosystem. Long-lasting effects of crop rotation on overall soil health have frequently not been addressed despite the relevant impact on farming system performance and sustainability.

Also, crop sequence design has multiple effects on soil ecosystem functioning, including the regulation of many soilborne diseases on the crops grown. In designing a rotation for disease management at least the main potential disease problems among the crops in the sequence should be considered, since sometimes a particular crop sequence with associated management can reduce one pathogen but increase another. For example, while trying to control apple replant disease, *Brassica napus* seed meal reduced *Rhizoctonia solani* but increased *Pythium* spp (Mazzola 2007). Similarly, while nematode problems in strawberry,

caused by *Meloidogyne incognita*, *M. hapla*, *Pratylenchus coffeae* and *P. penetrans*, were controlled by rotation with taro (*Colocasia esculena*), *Phytophthora* problems increased in strawberries (Chen and Tsay 2006).

Table 2. Examples of crop rotations reported in the literature, including their effects on particular pathogens and the mechanisms involved.

Crop rotation and pathosystem	Results	Mechanisms involved	References
Rotation: canola - wheat - soybean Pathogen / disease: *Gaeumannomyces graminis* var. *tritici* / wheat take-all root rot	*G. graminis* var. *tritici* present at a level below economic impact after a year of canola (infections start later in the season, so high disease incidence but low severity) Effect limited to one season of wheat after canola. One year out of three of canola also good for preventing Phoma black leg and Sclerotinia stem rot of canola	Biofumigation: hydrolysis of glucosinolates from decaying canola tissues affects *G.graminis* var. *tritici*. Effect on soilborne antagonists remained unknown	Cunfer et al. 2006.
Rotation: a) maize-maize-sugar beet, b) maize-wheat-(mustard) sugar beet, c) wheat-(mustard) oat-sugar beet Pathogen: *Rhizoctonia solani* AG 2-2IIIB Disease: sugar beet root and crown rot Factors studied: sugar beet cultivar, crop rotation, and soil tillage (plowing vs reduced tillage)	Combination of variety and previous crop (non host) contributes to inoculum reduction and less disease Plowing reduced disease incidence when maize - sugar beet (both hosts) is planted Intercrop and incorporation of mustard reduces disease incidence, and compensates reduced tillage	Genetic (partial) resistance and non hosts *R.solani* survives better in upper soil layers Biofumigation + enhancement of soil biology leads to *Rhizoctonia* spp. supressiveness	Buhre et al. 2009.
Rotation: a) Sugarcane – legumes (soybean or peanut) b) sugarcane monoculture Pathogen: *Pratylenchus zeae* *Meloidogyne javanica*	Rotation reduces almost 80% of the population of *Pratylenchus zeae* and 50% of the total plant parasitic nematodes population	Non-host crop	Stirling 2008

Crop rotation and pathosystem	Results	Mechanisms involved	References
Rotation: a) Lettuce (2 crops)–strawberry b) Brocoli (2 crops)–strawberry (lettuce and broccoli crop residues incorporated after harvest) Pathogen: *Verticillium dahliae* *Pyhtium* spp. Disease: strawberry Verticillium wilt	Disease incidence and severity was 12 -24% and 22 - 36% lower, respectively, in broccoli rotated compared with lettuce rotated fields After the second broccoli crop, *V. dahliae* densities in organic fields decreased 47% to 25%, and were lower than in lettuce rotated fields. Crop rotation had no consistent effect on the inoculum densities of *Pythium* spp.	Consistent results in reduction of *V. dahliae* population may be due to an increase in antagonist microflora Inconsistent results on *Pythium* spp. populations may be due to complex interactions between crop rotation and soil type, management practices, etc.	Njoroge et al. 2009
Rotation: lettuce – broccoli / fallow a) LLLL b) LFLF c) BBLL d) BLBL Pathogen / disease: *Sclerotinia minor* / lettuce drop	Sclerotia population and lettuce drop decreased in rotations with broccoli, remained constant in LFLF and increased in lettuce monoculture. BBLL and BLBL treatments were similar, suggesting that the number of broccoli crops was critical for reducing *S. minor* sclerotia in soil. Distribution pattern was aggregated in monoculture and randomized in lettuce-broccoli rotations.	Non-host crop Other mechanisms derived from broccoli crop residue decomposition could affect sclerotia survival.	Hao and Subbarao 2006, Hao et al. 2003
Rotation: Winter wheat - peanut (*Arachis hypogaea*) Pathogen: *Cercospora arachidicola* (teleomorph = *Mycosphaerella arachidis*) Disease: leaf spot of peanut	The presence of wheat residue is partly responsible for the early leaf spot suppression in strip-tilled fields. Without crop rotation of at least 1 year, no suppression was	Cover crop residue interferes with primary inoculum dispersal from overwintering stroma in the soil to the plant tissues.	Cantonwine et al. 2007.

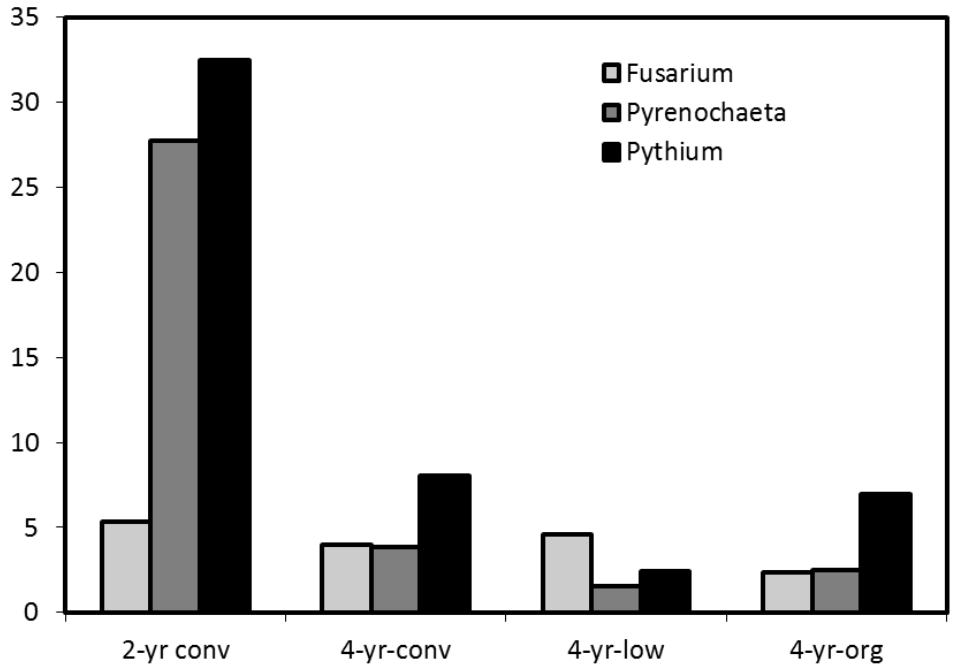


Figure 6. Disease severity of *Fusarium*, *Pyrenochaeta* and *Pythium* root rot on tomato in conventional treatments with a two- or four- year rotation, and low-input or organic treatments with a four-year rotation; values are mean percentages of main root tips infected (*Fusarium oxysporum* f.sp. *radici-lycopersici* and *Pythium ultimum*) or of the maximum potential number of lesions per root (*Pyrenochaeta lycopersici*) in 1995, 1996 and 1997 combined. The two–year rotation was significantly (P=0.05) different from the four-year rotations for *Pythium* and *Pyrenochaeta* root rots, not for *Fusarium* root rot (modified from van Bruggen and Termorshuizen 2003).

5 From analysis to designing crop sequences: Modelling

Management of soil biota cannot be seen in isolation from the management of the entire agricultural ecosystem. Farmers continuously assess their system's performance and make adjustments as part of decision-making at the field level, which is affected by their objectives and ambitions at the whole farm level, local resource availability, climate, and socio-economic conditions. Thus, crop rotations or crop sequences have consequences and are affected by drivers at different organizational, time and spatial scales (Thenail et al. 2009). To a certain extent multiple stakeholders also affect crop rotations, as the farmer aims to balance the demand from market parties, legislative constraints and family aspirations. If analysing existing rotations or designing new ones, the multi-stakeholder, multi-scale and multi-objective nature needs to be taken into account.

Models are a powerful tool to deal with complex temporal interactions in crop rotations and spatial heterogeneity on farms. Models can also help to structure the thinking of researchers when considering critical experiments and of farmers during re-design of their cropping systems. Following Tittonell (2008) we distinguish four roles for models: Describing, Explaining, Exploring and Designing, the DEED cycle, which constitute sequential steps in research for development, aimed at redesigning systems. Here we describe each of these four roles and provide an illustration.

5.1 Describing

The aim in the descriptive phase of modelling is to map out salient elements of reality, without necessarily paying attention to causes and effects. Databases derived from census, maps (i.e. geo-referenced data) and correlation and regression analyses all contribute to creating a view on reality. Questions here could be: which are the predominant crop sequences on a farm or in a region; did sequences change in the course of time; and which variables are correlated with the observed crop sequences and their changes? Data on crop sequences are scarce, since they require substantial organization and monitoring. However, in industrialized countries in order to account for potential environmental impacts increase, data are slowly becoming available. These database enable studies on the occurrence of crops and management practices in space and time (Thenail et al. 2009), providing insight in the degree of variation among farms. A recent statistical analysis of activities on more than 50,000 farms from 1990 to 2003 in Europe suggested that increased regional farm diversity can be a strategy through which regions in Europe can adapt to unfavourable conditions, such as higher temperatures and associated droughts (Reidsma and Ewert 2008).

5.2 Explaining

Explanatory models are aimed at describing and further elucidating potential causes underlying observed phenomena. Depending on the level at which the causative processes or phenomena are described, models may be denoted as summary-oriented or mechanistic. Summary models do not ‘dig deep’ into process descriptions, may be quantitative or expert-opinion based and usually have an applied aim: helping better decision-making. Examples are the scoring system for the risk of nematode reproduction and damage in cropping systems (Molendijk and Korthals 2005), simulation of nematode dynamics using relations between initial and final population densities (so-called P_i-P_f relations) (van der Berg et al. 2006) and various soil organic matter dynamics models. Mechanistic models describe

processes in the time-space domain, and different levels of detail may be represented, as well as different domains of knowledge. Non-linear models have been developed to understand the essential dynamics of plant disease epidemics (Gilligan 2002), nematode dynamics (Tixier et al. 2008), weed dynamics (Liebman et al. 2001) and crop growth and soil water and nutrient dynamics (Carberry et al. 2002). Model complexity easily gets out of hand when processes are added just to improve model performance. Increasingly, models are seen as tools for learning, and transparency and conciseness are then essential features.

For quantitative models to function, their parameters need to be calibrated on data collected from the systems being studied. A specific problem arises when such data are collected in different types of experiments. Multi-year trials are costly, so data on soilborne pathogens often originate from pot experiments, and where possible complemented with microplots and observations in farmer fields. Combination of information for parameter estimation constitutes a major challenge for the future.

5.3 Exploring

In this research mode, explanatory models are used to assess what-if questions or delineate the potential solutions from optimization. Often explorations follow the phase of explanation. Exploration also represents the first step towards changing crop rotations in a phase of farm re-design. In such cases it is not sufficient to address soil health and disease management only, but new indicators need to be brought into the analysis that take into account social, economic and wider agronomic concerns. Different models can be distinguished: models to *generate* crop rotations or crop sequences, models aimed at *evaluation* of the generated crop rotations or crop sequences, and finally models aimed at *selecting* interesting alternatives. Such explorations may be done at the cropping system level, but may also be scaled up to farm or regional levels (see recent reviews by Janssen and van Ittersum 2007; Rossing et al. 2007). A specific aspect of the multi-attribute evaluation of rotations is that not all knowledge is quantitative and formal. Expertise and judgment are important sources of knowledge as well (Dogliotti 2003). Recently, fuzzy logic approaches were applied to cropping systems evaluation to combine quantitative and qualitative knowledge (Saddok et al. 2009). Selection of interesting alternatives concerns identification of those rotations that outperform others in at least one objective. All other rotations can be discarded as inferior because at least one other rotation exists that performs better in at least one objective. The potential number of combinations is daunting, and this criterion allows a reduction to those rotations that merit further discussion (de Voil et al. 2006).

5.4 Designing

While in the explorative phase a range of alternatives is identified to assess the "window of opportunity", the design phase concerns the actual choice of a rotation and its implementation on a farm. Models can play a role here by visualizing the consequences of particular crop sequences, and providing more detail on aspects that could not be taken into account during explorations, such as the outcomes of particular investment strategies. Very few instances have been reported where the full cycle from description to design has been completed. The APSIM crop model (Carberry et al. 2002) has been used with farmers in this way, describing a farmer's crop yields in the past based on weather and soil data and management information, exploring alternative crops for the next season and selecting the best fitting one. Redesign of entire rotations in collaboration with farmers has been implemented in Uruguay using a tool named ROTAT to generate all feasible crop rotations and later the crop rotations were combined with a range of production techniques to create a wide variety of alternative production activities at the field scale (Dogliotti et al. 2013). A mixed integer linear programming model (MILP), named Farm Images, was developed to allocate production activities to a farm with land units differing in soil quality, while maximizing or minimizing socio-economic and environmental objectives, subject to constraints at the farm level. However, the outcome of the model is highly sensitive to assumptions on pathogen population dynamics and yield loss (Dogliotti 2005), so a conceptual model relating initial and final pathogen population densities (Figure 7) was proposed and based on it simple quantitative mathematical models are tested to improve the Farm Images model (this thesis).

6 Concluding remarks

Crop rotation delivers numerous ecological services as affected by several microbial processes, which confer "insurance" to agro-ecosystem disturbances, among others plant diseases. Not all the benefits of a crop rotation will be realized during the first few years after its initiation.

For SOM, there is a "build-up" process that takes several cycles to achieve stationary levels under different farming systems, as demonstrated in several experiments around the world (Körschens 2006, Rothamsted Research 2006). The same applies for suppressiveness to soilborne pathogens, pests and weeds. As an example, the analysis of many years of take-all incidence culminated in the development of the hypothesis of 'take-all decline', where

severe symptoms are often seen in rotations with high frequency of wheat, but less commonly in continuous wheat (Rothamsted Research 2006).

Long term experiments addressing the whole system and not only disease management, even though important, are scarce. Short-term experiments and associated simple crop rotation schemes provide useful information regarding the main principles involved in disease suppression, but other processes and interactions have been omitted.

To address the positive effects of crop rotation on the whole system more attention should be paid to and efforts invested in long-term experiments and on-farm research, where local conditions as well as farmers' knowledge related to crop management can be combined. New analytical tools (meta-analysis, modelling, simulation) will help in progressing towards a better understanding and design of crop rotations for improving agro-ecosystem health and thus disease suppressiveness.

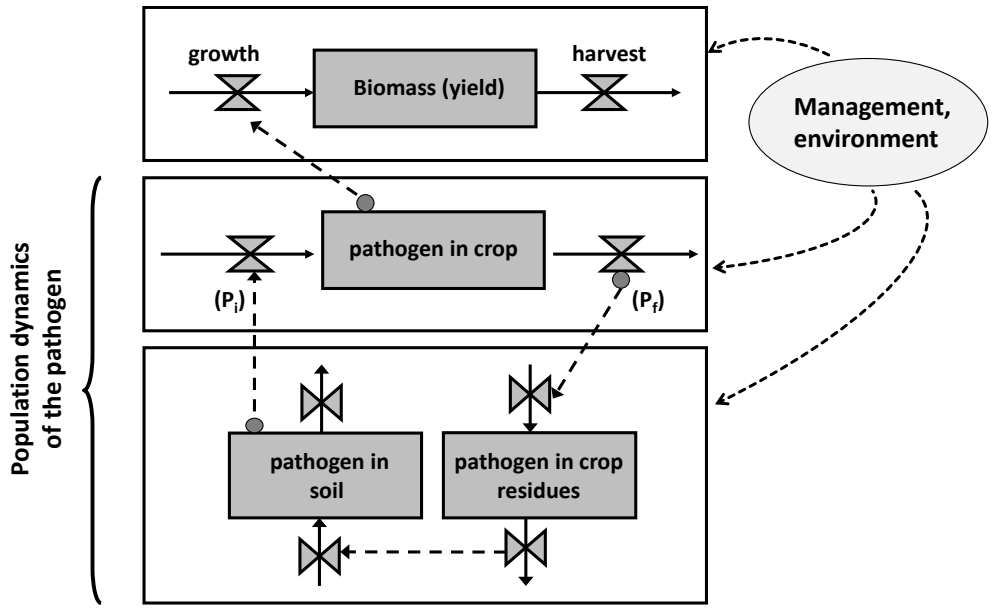


Figure 7. Conceptual model of soilborne pathogen population dynamics, relating initial (P_i) and final (P_f) population densities and their relation with yield losses.

References

Abawi, G.S. and Widmer, T. L. 2000. Impact of soil health management practices on soil-borne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology* 15:37-47.

Altieri, M.A., Lovato, P.M., Lana, M., Bittencourt, H. 2008. Testing and scaling-up agroecologically based organic no tillage systems for family farmers in southern Brazil. Pages 662-665 In: Proceedings of the Second Scientific Conference of the International Society of Organic Agriculture Research (ISOFAR), Vol II. Neuhoff, D. et al., eds. University of Bonn, Bonn. http://orgprints.org/12338/1/Altieri_12338_ed.doc.

Bahraminejad, S., Asenstorfer, R.E., Riley, I.T., Schultz, C.J. 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.*). Journal of Phytopathology* 156:1-7.

Bailey, K.L. and Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendments. *Soil and Tillage Research* 72:169-180.

Baldani, J.I. and Baldani, V.L. 2005. History on the biological nitrogen fixation research in graminaceous plants: special emphasis on the Brazilian experience. *Annais da Academia Brasileira da Ciência* 77:549-579.

Buhre, C., Kluth, C., Bürcky, K., Märländer, B., Varrelmann, M. 2009. Integrated control of root and crown rot in sugar beet: Combined effects of cultivar, crop rotation, and soil tillage. *Plant Disease* 93:155-161.

Cantonwine, E.G., Culbreath, A.K., Stevenson, K.L. 2007. Effects of cover crop residue and preplant herbicide on early leaf spot of peanut. *Plant Disease* 91:822-827.

Carberry, P.S., Hochman, Z., McCown, R.L., Dalgliesh, N.P., Foale, M.A., Poulton, P.L., Hargreaves, J.N.G., Hargreaves, D.M.G., Cawthray, S., Hillcoat N., Robertson, M.J. 2002. The FARMSCAPE approach to decision support: farmers', advisers', researchers' monitoring, simulation, communication and performance evaluation. *Agricultural Systems* 74:141-177.

Chen, P. and Tsay, T.T. 2006. Effect of crop rotation on *Meloidogyne* spp. and *Pratylenchus* spp. populations in strawberry fields in Taiwan. *Journal of Nematology* 38:339-344.

Clapperton, M.J., Lee, N.O., Binet, F., Conner, R.L. 2001. Earthworms indirectly reduce the effects of take-all (*Gaeumannomyces graminis* var. *tritic*i) on soft white spring wheat (*Triticum aestivum* cv. Fielder). *Soil Biology and Biochemistry* 33:1531-1538.

Colbach, N. and Meynard, J.M. 1995. Soil tillage and eye spot: influence of crop residue distribution on disease development and infection cycle. *European Journal of Plant Pathology* 101:601-611.

Cunfer, B.M., Buntin, G.D., Phillips, D.V. 2006. Effect of crop rotation on take-all of wheat in double-cropping systems. *Plant Disease* 90:1161-1166.

Dalla Santa, O.R., Fernández Hernández, R., Michelena Alvarez, G.L., Ronzelli Jr., P., Soccol, C.R. 2004. *Azospirillum* sp. inoculation in wheat, barley and oats seeds greenhouse experiments. *Brazilian Archives of Biology and Technology* 47:843-850.

deVoil, P., Rossing, W.A.H., Hammer, G.L. 2006. Exploring profit – sustainability trade-offs in cropping systems using evolutionary algorithms. *Environmental Modelling and Software* 21:1368-1374.

Dogliotti, S. 2003. Exploring options for sustainable development of vegetable farms in South

Uruguay. Ph.D diss. Wageningen University, Wageningen.

Dogliotti, S., Garcia, M.C., Peliffo, S., Dieste, J.P., Pedemonte, A.J., Bacigalupe, G.F., Scarlato, M., Alliaume, F., Alvarez, J., Chiappe, M., Rossing, W.A.H. 2013. *Agricultural Systems*. http://dx.doi.org./10.1016/j.agsy.2013.02.009.

Douds, D.D., and Millner, P.D. 1999. Biodiversity of arbuscular mycorrhizal fungi in agroecosystems. *Agriculture Ecosystems and Environment* 74:77-93.

Drinkwater, L.E. and Snapp, S.S. 2007. Nutrients in agroecosystems: rethinking the management paradigm. *Advances in Agronomy* 92:163-186. doi: 10.1016/S0065-2113(04)92003-2.

Edel. V., Steinberg, C., Gautheron, N., Alabouvette, C. 1997. Populations of nonpathogenic *Fusarium oxysporum* associated with roots of four plant species compared to soilborne populations. *Phytopathology* 87: 693-697.

Elmer, W.H. 2009. Influence of earthworm activity on soil microbes and soilborne diseases of vegetables. *Plant Disease* 93:175-179.

Gilligan, C.A. 2002. An epidemiological framework for disease management *Advances in Botanical Research* 38:1-64.

Hao, J.J., Subbarao, K.V., Koike, S.T. 2003. Effects of broccoli rotation on lettuce drop caused by *Sclerotinia minor* and on the population density of sclerotia in soil. *Plant Disease* 87:159-166.

Hao, J.J. and Subbarao, K.V. 2006. Dynamics of lettuce drop incidence and *Sclerotinia minor* inoculum under varied crop rotations. *Plant Disease* 90:269-278.

Hartmann, A., Schmid, M., van Tuinen, D., Berg, G. 2009. Plant-driven selection of microbes. *Plant and Soil* 321: 235-257. doi: 10.1007/s11104-008-9814-y.

IFOAM. 2006. The IFOAM norms for organic production and processing. Version 2005. Corrected version January 2009. http://www.ifoam.org/about_ifoam/standards/norms/norm_documents_library/Norms_ENG_V4_20090113.pdf.

Janssen, S. and van Ittersum, M.K. 2007. Assessing farm innovations and responses to policies: A review of bio-economic farm models. *Agricultural Systems* 94:622-636.

Karlen, D.L., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F., Schuman, G.E. 1997. Soil quality: a concept, definition, and framework for evaluation. *Soil Science Society of America Journal* 61:4-10.

Kibblewhite, M.G., Ritz, K., Swift, M.J. 2008. Soil health in agricultural systems. *Philosophical Transactions of the Royal Society of London Series B-Biological Sciences* 363:685-701.

Kloepper, J.W., Ryu, C.-M., Zhang, S. 2004. Induced systemic resistance and promotion of plant growth by *Bacillus* spp. Phytopathology 94:1259-1266.

Körschens, M. 2006. The importance of long-term field experiments for soil science and environmental research – a review. *Plant Soil and Environment* 52 (Special Issue):1-8

La Mondia, J.A. 1999. Influence of rotation crops on the strawberry pathogens *Pratylenchus penetrans*, *Meloidogyne hapla*, and *Rhizoctonia fragariae*. *Journal of Nematology* 31(4S):650-655.

Liebman, M., Mohler, C.L. Staver, C.P. 2001. Ecological Management of Agricultural Weeds. Cambridge Univ. Press, Cambridge, UK.

Madden, N.M., Mitchell, J.P., Lanini, W.T., Cahn, M.D., Herrero, E.V., Park, S., Temple, S.R., van Horn, M. 2004. Evaluation of conservation tillage and cover crop systems for organic processing tomato production, *HortTechnology* 14:243-250.

Marschner, H. 1995. Mineral nutrition of higher plants (2nd ed.). Academic Press, London, UK.

Matthiessen, J.N. and Kirkegaard, J.A. 2006. Biofumigation and Enhanced Biodegradation: Opportunity and Challenge in Soilborne Pest and Disease Management. *Critical Reviews in Plant Sciences* 25:235-265.

Mazzola, M. 2007. Manipulation of rhizosphere bacterial communities to induce suppressive soils. *Journal of Nematology* 39:213-220.

Millennium Ecosystem Assessment (MA). 2005. Accessed on July, 30th 2009 at http://www.maweb.org/en/index.aspx.

Molendijk, L.P.G. and Korthals, G.W. 2005. Nematode control strategies in The Netherlands. *Acta Horticulturae* 698:83-88.

Njoroge, S.M.C., Kabir, Z., Martin, F.N., Koike, S.T., Subbarao, K.V. 2009. Comparison of crop rotation for Verticillium wilt management and effect on *Pythium* species in conventional and organic strawberry production. *Plant Disease* 93:519-527.

Raaijmakers, J.M., Paulitz, T.C., Steinberg, C., Alabouvette, C., Moënne-Loccoz, Y. 2008. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* 321:341-361. doi 10.1007/s11104-008-9568-6.

Reidsma, P. and Ewert, F. 2008. Regional Farm diversity can reduce vulnerability of food production to climate change. *Ecology and Society* 13:38. [online] http://www.ecologyandsociety.org/vol13/iss1/art38/.

Riley, H., Pommeresche, R., Eltun, R., Hansen, S., Korsaeth, A. 2008. Soil structure, organic matter and earthworm activity in a comparison of cropping systems with contrasting tillage, rotations, fertilizer levels and manure use. *Agriculture Ecosystems and Environment* 124:275-284.

Rossing, W.A.H., Zander, P., Josien, E., Groot, J.C.J., Meyer, B.C., Knierim, A. 2007. Integrative modeling approaches for analysis of impact of multifunctional agriculture: a review for France, Germany and The Netherlands. *Agriculture Ecosystems and Environment* 120:41-57.

Rothamsted Research. 2006. Guide to the Classical and other Long-term Experiments, Datasets and Sample Archive. http://www.rothamsted.bbsrc.ac.uk/resources/LongTermExperiments.pdf

Rousseau, G., Rioux, S., Dostaler D. 2006. Multivariate effects of plant canopy, soil physico-chemistry and microbiology on Sclerotinia stem rot of soybean in relation to crop rotation and urban compost amendment. *Soil Biology and Biochemistry* 38:3325-3342.

Sadok, W., Angevin, F., Bergez, J.E., Bockstaller, C., Colomb, B., Guichard, L., Reau, R., Messean, A., Dore, T. 2009. MASC, a qualitative multi-attribute decision model for ex ante assessment of the sustainability of cropping systems. *Agronomy for Sustainable Development* 29:447-461.

Scholberg, J.M.S., Dogliotti, S., Leoni, C., Cherr, C.M., Zotarelli, L., Rossing, W.A.H. 2010. Cover crops for sustainable agrosystems in the Americas. Pages 23-58. In: Genetic Engeneering, Biofertilization, Soil Quality and Organic Farming. Lichtfouse, E. (Ed.) *Sustainable Agriculture Reviews*, Vol. 4. Springer.

Seiges, A.N. and Pritts, M. 2006. Cover crop rotations alter soil microbiology and reduce replant disorders in strawberry. *HortScience* 41:1303-1308.

Stephens, P.M., Davoren, C.W., Ryder, M.H., Doube, B.M. 1994. Influence of the earthworms *Aporrectodea rosea* and *Aporrectodea trapezoides* on *Rhizoctonia solani* disease of wheat seedlings and the interaction with a surface mulch of cereal-pea straw. *Soil Biology Biochemistry* 26:1285-1287.

Stephens, P.M., Davoren, C.W., Ryder, M.H., Doube, B.M., Correll, R.L. 1994. Field evidence for reduced severity of Rhizoctonia bare-patch disease of wheat, due to the presence of the earthworms *Aporrectodea rosea* and *Aporrectodea trapezoides*. *Soil Biology Biochemistry* 26:1495-1500.

Stirling, G.R. 2008. The impact of farming systems on soil biology and soilborne diseases: examples from the Australian sugar and vegetable industries – The case for better integration of sugarcane and vegetable production and implications for future research. *Australasian Plant Pathology* 37:1-18.

Thenail, C., Joannon, A., Capitaine, M., Souchère, V., Mignolet, C., Schermann, N., Di Pietro, F., Pons, Y., Gaucherel, C., Viaud, V., Baudry, J. 2009. The contribution of crop-rotation organization in farms to crop-mosaic patterning at local landscape scales. *Agriculture. Ecosystems and. Environment* 131:207-219.

Thurston, H.D. 1990. Plant disease management practices of traditional farmers. *Plant Disease* 74:96-102.

Tittonell, P.A. 2008. Msimu wa Kupanda: targeting resources within diverse, heterogenous and dynamic farming systems of East Africa. Ph.D. diss. Wageningen University, Wageningen.

Tixier, P., Salmon, F., Chabrier C., Quénéhervé, P. 2008. Modelling pest dynamics of new crop cultivars: The FB920 banana with the *Helicotylenchus multicinctus–Radopholus similis* nematode complex in Martinique. *Crop Protection* 27: 1427-1431.

United Nations. 2013. The future we want. Resolution adopted by the General Assembly, 123rd plenary meeting, 27 July 2012 (A/RES/66/288). Reissued for technical reasons on 17 January 2013. http://daccess-dds-ny.un.org/doc/UNDOC/GEN/N11/476/10/PDF/N1147610.pdf?OpenElement. Accessed on June12, 2013.

van Bruggen, A.H.C. and Termorshuizen, A.J. 2003. Integrated approaches to root disease management in organic farming systems. *Australasian Plant Pathology* 32: 141 - 156

van den Berg, W. ,W.A.H. Rossing, J. Grasman, 2006. Contest and scramble competition and the carry-over effect in *Globodera* spp. in potato-based crop rotations using an extended Ricker model. *Journal of Nematology* 38: 210-220.

Vinale, F., Sivasithamparam, K., Ghisalberti, E.L., Marra, R., Woo, S L., Lorito, M. 2008. Trichoderma–plant–pathogen interactions. *Soil Biology and Biochemistry* 40: 1–10

Wang, K.H., Sipes, B.S., Schmitt, D.P. 2002. *Crotalaria* as a cover crop for nematode management: A review. *Nematropica* 32:35-57.

Weller, D.M., Raaijmakers, J.M., McSpadden Gardener, B.B.; Thomashow, L.S. 2002. Microbial populations responsible for specific soil suppressiveness to plant pathogens. *Annual Review of*

Phytopathology 40:309–48

Wiggins, B.E. and Kinkel, L.L. 2005. Green manures and crop sequences influence alfalfa root rot and pathogen inhibitory activity among soil-borne streptomycetes. *Plant and Soil* 268: 271–283. doi: 10.1007/s11104-004-0300-x

Williams, S. M. and Weil, R.R. 2004. Crop cover root channels may alleviate soil compaction effects on soybean crop. *Soil Science Society of America Journal* 68:1403–1409.

Winoto Suatmadji, R. 1969. Studies on the effect of Tagetes species on plant parasitic nematodes. Ph.D diss. Wageningen University, Wageningen.

Workneh, F., A.H.C. van Bruggen, L.E. Drinkwater, C. Shennan. 1993. Relationships between soil variables and corky root and phytophthora root rot of tomatoes in organic and conventional farms. *Phytopathology* 83:581–589.

Zehnder, G., Gurr, G M., Kühne, S., Wade, M. R., Wratten, S. D., Wyss , E. 2007. Arthropod pest management in organic crops. *Annual Review of Entomology* 52:57–80. doi: 10.1146/annurev.ento.52.110405.091337.

Chapter 3

Sclerotium rolfsii dynamics in soil as affected by crop sequences

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Abstract

Crop rotation has been used for the management of soilborne diseases for centuries, but has not often been planned based on scientific knowledge. Our objective was to generate information on *Sclerotium rolfsii* dynamics under different crop or intercrop activities, and design and test a research approach where simple experiments and the use of models are combined to explore crop sequences that minimize Southern blight incidence.

The effect of seventeen green manure (GM) amendments on sclerotia dynamics was analysed in greenhouse and field plot experiments during two years. The relative densities of viable sclerotia 90 days after winter GM (WGM) incorporation were generally lower than after summer GM (SGM) incorporation, with average recovery values of 60 and 61% for WGM in the field, 66 and 43% for WGM in the greenhouse, and 162 to 91 % for SGM in the greenhouse, in 2009 and 2010 respectively. Sclerotia survival on day *d* after GM amendment was described by the model $S_f = S_i \exp(-b*d)$, relating initial (S_i) and final (S_f) sclerotia densities. Relative decay rates of the sclerotia (b) in SGM amended soil were largest for alfalfa (0.0077 ± 0.0031 day^{-1}) and sudangrass (0.0072 ± 0.0030 day^{-1}). In WGM amended soil, the largest b values were for oat (0.0096 ± 0.0024 day^{-1}), wheat (0.0090 ± 0.0024 day^{-1}) and alfalfa (0.0087 ± 0.0023 day^{-1}).

The effect of three cropping sequences (sweet pepper – fallow, sweet pepper – black oat and sweet pepper – onion) on sclerotia dynamics was analysed in microplot experiments, and the data were used to calibrate the model $P_f = P_i / (\alpha + \beta P_i)$, relating initial ($P_i$) and final ($P_f$) sclerotia densities. Median values for the relative rate of population increase at low P_i ($1/\alpha$, dimension less) and the asymptote ($1/\beta$, number of viable sclerotia in 100 g of dry soil) were 8.22 and 4.17 for black oat (BO), 1.13 and 8.64 for onion (O), and 6.26 and 17.93 for sweet pepper (SwP).

By concatenating the two models, sclerotia population dynamics under several crop sequences were simulated. At steady state, the sequence SwP-O-Fallow-BO resulted in the lowest long-term sclerotia density (7.09 sclerotia/100 g soil), and SwP-Fallow in the highest (17.89 sclerotia/100 g soil). The developed methodology facilitates the selection of a limited number of rotation options to be tested in farmers' fields.

Keywords:
Sclerotium rolfsii, population dynamics, simulation model, crop rotation, green manure amendment

1 Introduction

Crop rotation has been used for the management of soilborne diseases (SBD) for centuries, but has not often been planned based on scientific knowledge. Crops in the rotation should be selected in such a way that the probabilities for pathogens to infect and colonize the host are minimized. If a non-host is included in the rotation scheme, the pathogen cannot infect the non-host plant and therefore its reproduction and survival are affected, resulting in a decline in the pathogen population. Ideally, the host is reintroduced in the crop sequence when the population is low enough not to cause economic losses. Depending on the pathogen's survival ability, the period without hosts may need to range from one to more than 10 years. In terms of the environment, crop rotation combined with an adequate soil and field preparation and appropriate tillage system can promote soil health thereby contributing to the management of SBD (van Bruggen and Semenov 1999). Crop residues or green manure amendments, either incorporated or left on the surface, can contribute to the build-up of general suppression by increasing total soil microbial and faunal activity and competition in response to the carbon and other nutrients added (Bailey and Lazarovits 2003, Bulluck and Ristaino 2002, Grünwald et al. 2000, Liu et al. 2007). Residue decomposition may also release antimicrobial compounds to some pathogens (Bailey and Lazarovits 2003, Njoroge et al. 2008, Raaijmakers et al. 2009, Smolinska 2000).

Crop rotations are at the core of farming systems, and should accomplish several objectives, not only SBP management. Models can support the design of crop sequences and help to reveal synergisms and trade-offs among objectives (Casagrande et al. 2010, Dogliotti et al. 2003, Dogliotti et al. 2004). Despite their importance, pathogen dynamics are rarely taken into account in cropping system models, or at best are introduced as forcing functions influencing the state of the system without being affected by the system (Tixier et al. 2006). Pathogen dynamics in a crop sequence can be described by interlinked quantitative population models where different equations describe the increase and decline of the pathogen population during and after a crop and intercrop activity, respectively (Colbach et al. 1999, Leoni et al. 2013, Mol et al. 1996, Tixier et al. 2006, van der Berg and Rossing 2005).

Sclerotium rolfsii Sacc.(telomorph: *Athelia rolfsii* (Cruzi) Tu and Kimbrough) is a widespread soil borne pathogen in vegetable production areas. It causes Southern blight, a disease which affects more than 500 plant species in over 100 plant families. The disease can be a severe problem, especially in warm regions. *S. rolfsii* produces sclerotia on the infected

portions of the plant near the soil line. The sclerotia can survive in the soil from a few months to several years, depending on environmental conditions (Punja 1985, Xu et al. 2008), and are the primary inoculum source for disease development. After germination, *S. rolfsii* hyphae produce oxalic acid and pectinolytic and cellulolytic enzymes which kill and disintegrate host tissues, starting a new infection (Le 2011, Punja 1985). In South Uruguay (34° S and 56° E, average altitude 44 m above sea level) *S. rolfsii* is a big constraint not only for summer crops, but also for slow-growing winter crops, like onions and garlic, that are harvested in December- January.

Management of Southern blight requires integrated strategies that either reduce the sclerotia population in the soil or prevent the infection of host plants. Crop sequences and organic amendments have been reported to contribute to Southern blight management (Bulluck and Ristaino 2002, Jenkins and Averre 1986, Liu et al. 2007, Punja 1985, Rodriguez-Kabana et al. 1994, Stapleton et al. 2010, Taylor and Rodriguez-Kabana 1999b). However, to design proper crop sequences for disease management, it is necessary to obtain a quantitative understanding of the effects of cropping frequencies and intercrop activities (e.g. the use of green manures) on pathogen dynamics.

In this study the effects on *S. rolfsii* sclerotia dynamics of 17 green manure amendments and three cropping sequences were analyzed in pot and microplot experiments, respectively. With the quantitative data obtained, two simple population models were calibrated and combined to simulate soil sclerotia dynamics under different crop rotations. Our objective was to generate information on sclerotia dynamics after soil incorporation of different crops and intercrops, and design and test a research approach where experimental data and model simulations are combined to explore crop sequences that minimize Southern blight incidence.

2 Materials and methods

2.1 *S. rolfsii* isolate and inoculum production

The *S. rolfsii* isolate was obtained from diseased onions (*Allium cepa* L.) collected at “Centro Regional Sur” Research Station of the Faculty of Agronomy in Canelones, Uruguay, in December 2008. After purification, the isolate was transferred onto PDA slants and incubated at 25 ºC till sclerotia were produced, after which the tubes were stored at 4 ºC.

To produce inoculum for the experiments, 5 plugs on the edge of a 10 day-old colony of *S. rolfsii* were transferred into a 1 litre flask with previously autoclaved oat kernels (50 g oat kernels + 75 ml distilled water, autoclaved for 10 minutes on two successive days). The flasks were incubated at room temperature for two months to allow sclerotia development. After two months, the sclerotia were removed from the substrate by sieving and air dried for 24 hr under sterile conditions. The sclerotia were stored at 4 ºC and used within 2 weeks after production.

2.2 Population dynamics in GM-amended soil – Pot experiments

During 2009 and 2010, six pot experiments were carried out for assessing population dynamics of *S. rolfsii* in green manure amended soil at INIA Las Brujas Research Station, Canelones, Uruguay. Of the six pot experiments four were conducted in a greenhouse and two in the field. In the greenhouse we assessed the effect of eight summer green manures (SGM) and nine winter green manures (WGM), while in the field only WGM were evaluated.

Green manure crops were grown in 2.5 m x 4.0 m plots on a Typic-Vertic Argiudolls, with a silt clay loam texture and pH 6.72, with 1.43 % organic carbon, 0.2% total N, 0.06 µg/g P_2O_5, and 0.06 meq/100 g K_2O. SGM were planted in December 2008 and 2009, and harvested in April 2009 and March 2010, respectively. WGM were planted in May 2009 and 2010, and harvested in October of the same years. Alfalfa (*Medicago sativa*), a perennial feed crop, was sown in April 2007, harvested and incorporated into soil on the same dates as the SGM and WGM. No fertilizers or pesticides were applied. Above-ground biomass was collected from one representative plot per treatment. The species of SGM and WGM planted, their sowing rates, dry matter content and C/N ratios at harvest were standard for the region and are presented in Table S1 of the supplementary material. Plant biomass and soil were checked for the presence of natural *S. rolfsii* with the methanol assay described below, and the pathogen was not found.

Fresh top soil (0.2 m depth) collected from the centre of each GM plot was sieved through a 1 cm mesh sieve and mixed with sterilized river sand (2/3 soil; 1/3 sand by volume). The soil-sand mixture was amended with the chopped GM above ground biomass at the amounts indicated in Table S1. The amounts (kg dry matter ha^{-1}) were similar to the biomass incorporated into the soil by the farmers in the area. One hundred ml of each soil+sand+GM mixture were inoculated with 25 sclerotia of *S. solfsii* (except for SGM in 2010 when 20 sclerotia were used), and put into panty-nylon sock bags (Bulluck and Ristaino 2002). Each

bag was buried at 10 cm depth in a 1.5 L plastic pot filled with the same soil+sand+GM mixture, one bag per pot. Pots with soil+sand and pots with soil+sand+GM without *S. rolfsii* sclerotia were used as controls. For the four experiments in the greenhouse, the pots were kept in the greenhouse and watered twice a month by replacing the lost water. Air temperature and relative humidity were recorded with a data logger. For the two pot experiments in the field, the pots were buried in a pit in the field so that the nylon bags were placed 10 cm deep. The site was kept without vegetation and rain-fed. Air temperature, air relative humidity, rain and evapotranspiration (Class A pan evaporation) data were collected with an automatic meteorological station (data available at http://www.inia.org.uy/online/site/692646I1.php and in Table S3 of the supplementary material), and soil temperature was measured in bare soil at 10 cm depth with a soil probe. The treatments (soil+sand+GM mixtures and the un-amended control) were randomly arranged for each of the four sampling dates with 3 replications for SGM in 2009, and 4 replications for SGM in 2010 and WGM in 2009 and 2010 in both greenhouse and field experiments.

The density of viable sclerotia in each pot was determined by the methanol procedure (Rodriguez-Kabana et al. 1980). On days 0, 30, 60 and 90, the nylon bags were retrieved from the pots. The soil inside each nylon bag was carefully removed and air dried for a few days. After 3 to 7 days, the soil was sieved through 2 mm mesh, spread evenly over a paper towel in a perforated pan, moistened with 1% aqueous methanol solution, sealed with a polyethylene bag and incubated at 27°C for 3 days. On the third day, the number of germinated sclerotia visible on the soil surface was counted and expressed as the number of viable sclerotia in 100 g dry soil. The relative density (RD) of viable sclerotia for each GM amendment was calculated as: RD_d (%) = (S_d number of viable sclerotia on day *d* / S_0 number of viable sclerotia on day 0) * 100, where *d* was 30, 60 or 90 days.

The dynamics of viable sclerotia in GM amended soil was described by a negative exponential decay model:

$$Sf = Si\ e^{-bd} \quad (1)$$

where *Sf* number of viable sclerotia in 100 g dry soil on day *d*, *Si* = initial number of viable sclerotia in 100 g dry soil on day 0, *b* = sclerotia relative decay rate (day^{-1}). Data for all recovery dates were used to estimate parameter values, but the RD is reported only for the 90-day period, the usual time frame for GM decomposition under field conditions.

2.3 Population dynamics under different crop sequences – Microplot experiment

To study the effect of crop sequences on *S. rolfsii* sclerotia dynamics in the soil, a microplot experiment was installed in April 2009 at the INIA Las Brujas Research Station (34° 40' S., 56° 20' W., 32 m over sea level). The soil in the experiment was a Typic-Vertic Argiudolls, with a clay loam texture (34% sand, 28% silt, 38% clay) and pH 6.21, with 1.76% organic carbon, 0.16% total N, 36 µg/g P_2O_5, and 1.14 meq/100 g K_2O. A total of 21 microplots arranged in three blocks were installed in a grass field with no history of Southern blight in the previous 5 years. Each 2.5m x 2.0m microplot was delimited by a steel frame 50 cm high, inserted 30 cm into the ground. Rows between plots were under 2 m wide permanent grass.

Uniform starting conditions were created by growing a black oat (*Avena strigosa*) crop in all microplots from May to November 2009. In January 2010 four plots were inoculated with *S. rolfsii* sclerotia (7 g of inoculum, approx. 8900 sclerotia), and three plots remained un-inoculated in each block. The day after inoculation, a sweet pepper (*Capsicum annuum*) crop was planted in each plot (23 plantlets per plot, 0.4 m x 0.5 m plant spacing). In April 2010, sweet pepper was harvested and followed by one of three planting options, resulting in three cropping sequences: black oat - sweet pepper – fallow – sweet pepper (BO - SwP – F - SwP), black oat - sweet pepper – black oat - sweet pepper (BO - SwP – BO - SwP), and black oat - sweet pepper – onion - sweet pepper (BO - SwP - O – SwP) (Table 1). All plots were managed according to integrated crop management practices for the region. Air temperature, relative humidity, precipitation and soil evapotranspiration (Class A pan evaporation) were registered with an automatic meteorological station (data available at http://www.inia.org.uy/online/site/692646I1.php). The summary of the data is presented in Table S4 of the supplementary material.

S.rolfsii soil populations were assessed 10 days after BO sowing in 2009, the day after SwP transplanting and the day of SwP harvesting in 2010 and 2011. Top soil samples (0-15 cm) of 500 g were collected from each microplot and divided into three or four subsamples. The soil samples were air-dried and sieved and then subjected to the 1% methanol assay (Rodriguez-Kabana et al., 1980) to quantify the density of *S.rolfsii* sclerotia per 100 g of dry soil.

The dynamics of *S.rolfsii* sclerotia in soil planted with sweet pepper, black oat or onion was described by equation (2) relating soil sclerotia densities at the start (*Pi*) and the harvest (*Pf*) of each crop, expressed in (number of viable sclerotia in 100 g dry soil):

Table 1. Microplot experiment set-up.

Crop sequences 2009-2011 [a]	Number of plots [b]		
	Total	Naturally infested [c]	Inoculated [d]
BO - SwP – F - SwP	9	3	6
BO - SwP — BO - SwP	6	3	3
BO – SwP - O - SwP	6	3	3

BO: black oat (*Avena strigosa*), SwP: sweet pepper (*Capsicum annum*), F: fallow, O: onion (*Allium cepa*) [b] Plots were arranged in three blocks [c] Un-inoculated plots but with a natural *S. rolfsii* sclerotia population. [d] Inoculated plots. Inoculation was in January 2010 with *S. rolfsii* sclerotia produced on oat kernels and air dried (7 grams of inoculum/plot, approx. 8900 sclerotia).

$$Pf = Pi/(\propto + \beta Pi) \quad (2)$$

where *1/α* represents the slope at the origin and *1/β* the horizontal asymptote (van den Berg and Rossing 2005). Soil sclerotia densities estimated at SwP at harvest in 2010 and at SwP transplant and harvest in 2011 were used jointly to estimate model parameters (Table 3).

2.4 Statistical analyses

All data analyses were carried out in R, version 2.12.2 (R Development Core Team 2011), with different packages. ANOVA analyses were performed with the “agricolae” package (de Mendiburu 2012).

The sclerotia relative decay rate *b* of equation (1) was estimated with the "lme4" package (Bates et al. 2011) by analyzing the observed numbers of viable sclerotia in 100 g dry soil using a Poisson linear mixed model that accounted for possible overdispersion in the counts (Gelman and Hill 2007). The initial number of viable sclerotia in equation (1) was treated as a fixed parameter that was allowed to vary across combinations of crops and years. The decay rate was allowed to vary across crops and randomly across years. The lme4 specification of the model was:

S_d ~ crop*year + crop:day + (0 + day_ | year) + (1 | obs)

The type of pot experiment (greenhouse or field) in WGM was accounted for by replacing the factor year by the combination of year and type of experiment. We report the crop-dependent

decay rates and their associated standard errors.

Parameters *α* and *β* for each crop in equation (2) were estimated by Bayesian non-linear least-squares regression after log transformation of the data with uniform (flat) prior distributions U(1, 50) for *1/α* and U(0.1, 10) for *1/β* using the DE-MC_{ZS} algorithm (ter Braak and Vrugt 2008) with three parallel Markov chains and 33333 generations. The posterior distribution was represented by 1000 samples (draws) obtained by thinning the second half of each chain. The median of these samples for *1/α* and *1/β* was selected as the best estimate, since it was more stable than the mean, but we report the mean and its associated standard error as well. Finally, an F-test were carried out to check if *1/α* and *1/β* estimates differed depending on the crop or on the preceding crop.

2.5 Population dynamics– Simulations for different crop sequences

Using the calibrated models, the population dynamics of *S. rolfsii*, in terms of sclerotia per 100 g of soil, were simulated in theoretical crop sequences with sweet pepper as the main cash crop by concatenating equations (2) and (1), describing the crop or intercrop (fallow, GM decomposition) phase respectively. The result calculated from one equation constituted the input for the other and so forth (Figure 1).

Calculations were repeated for a total of 6 cycles when stationary sclerotia densities were reached. Runs were initialized to cover the range of situations commonly found in vegetable fields in Southern Uruguay, i.e. 1 to 15 viable sclerotia per 100 g dry soil (Gilsanz et al. 2004). For these simulations it was assumed that: (i) at the start of a crop all available sclerotia in the soil are infective and colonize the new crop, (ii) sclerotia decay occurs only during GM decomposition or fallow periods, and not during the growth phase of any crop, and (iii) no sclerotia are produced during the winter periods when temperatures are too low for the pathogen to colonize the host and multiply on it.

To assess the consequences of uncertainty in model parameters, model simulations were made assuming model parameters to be stochastic, resulting in a probability distribution of viable sclerotia in the soil. Stochastic model output was generated by 1000 random draws from the probability distributions of the parameters. For b, draws were made from normal distributions with estimates of the means and standard errors as shown in Table 2. Random vaues for *1/α* and *1/β* were obtained from the 1000 stored samples representing their posterior distribution. The final numbers of sclerotia per cropping sequence were

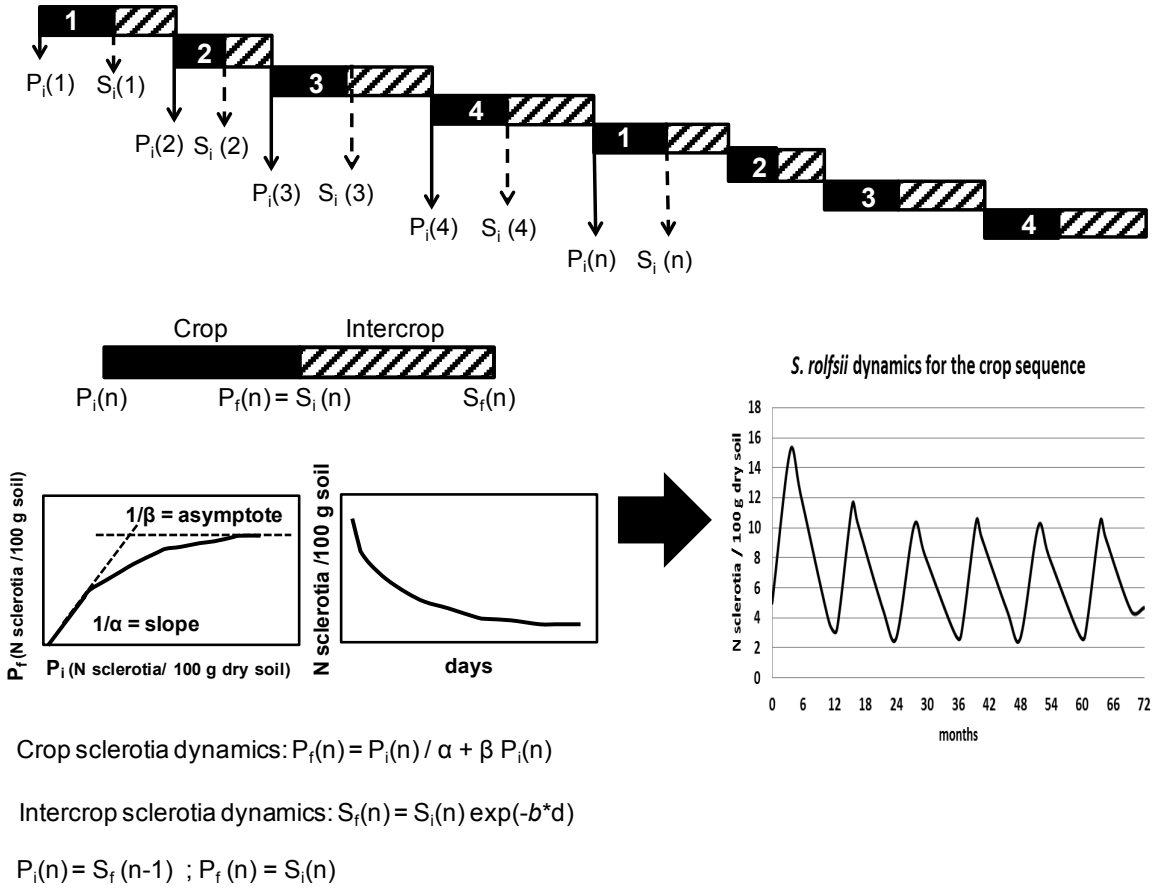

Figure 1. Schematic representation of a crop sequence and the processes involved in soil sclerotia dynamics for *Sclerotium rolfsii*: pathogen multiplication during crop growth and pathogen survival during the intercrop period.

summarized in boxplots. Simulations were run in the R environment (R Development Core Team 2011). The results, the estimated mean of *b*, and median values of *1/α* and *1/β*, were compared to the outcome of the model with fixed parameters.

3 Results

3.1 Population dynamics in GM-amended soil – Pot experiments

In none of the non-inoculated controls with un-amended soil+sand or those with GM amended soil, sclerotia of *S. rolfsii* were recovered, indicating that neither the soil nor the incorporated GM were contaminated with native sclerotia. In the inoculated treatments

sclerotia recovery (number of sclerotia in 100 g dry soil) differed depending on GM type and time after soil amendment on days 60 and 90 (ANOVA significant at p=0.05), but not on day 0. Thus the decline in sclerotia por 100 g of soil relative to day 0 could be analysed statistically.

Sclerotial densities relative to the number of sclerotia recovered on day 0 varied significantly (Figure S1 of the supplementary material). After 90 days relative densities in un-amended soil were always less than 100%, with recovery values of 49% and 73% for WGM in the field, 61% and 42% for WGM in the greenhouse, and 96% and 77% for SGM in the greenhouse, in 2009 and 2010, respectively. In amended soil, the incorporation of GM resulted in relative densities both lower and higher than 100%, in the latter case suggesting saprophytic growth resulting in formation of new sclerotia. As for un-amended soil, the relative densities of viable sclerotia 90 days after WGM incorporation (end of January) were generally lower than after SGM (end of June) incorporation, with average recovery values of 60 and 61% for WGM in the field, 66 and 43% for WGM in the greenhouse, and 162 to 91 % for SGM, in 2009 and 2010 respectively.

Despite the fact that environmental conditions in the greenhouse (Table S2) were similar for the two years, sclerotia recovery was not consistent from year to year across GM amendments. In general, relative densities were higher in 2009 than in 2010, except for sun hemp, wheat and forage radish (Figure S1 A and B). In the field experiment, the relative density of recovered sclerotia was more variable than in the greenhouse. Field sclerotia recovery in 2009 was greatest after oat, wheat, forage radish and white lupine amendments, whereas in 2010 this was the case after black oat, red clover, blue lupine, hairy vetch and alfalfa amendments (Figure S1 C). The temperatures and relative air humidity were similar in the two years, but rainfall was much greater and pan evaporation was lower in 2009 than in 2010 (Table S3), possibly affecting differentially biomass decomposition for the various crops.

The relative decay rate of the sclerotia in SGM amended soil was largest for alfalfa (0.0077 ± 0.0031 day^{-1}) and sudangrass (0.0072 ± 0.0030 day^{-1}), the only two amendments that were significantly (p< 0.05) different from un-amended soil, which had a relative decay rate of 0.0037 ± 0.0032 day^{-1}. The smallest relative decay rate of sclerotia was found in cowpea-amended soil (0.0021 ± 0.0032 day^{-1}) (Table 2). In WGM-amended soil, only hairy vetch did not differ from un-amended soil, and the largest relative decay rates were for oat (0.0096 ± 0.0024 day^{-1}), wheat (0.0090 ± 0.0024 day^{-1}) and alfalfa (0.0087 ± 0.0023 day^{-1}) (Table 2).

Table 2. Relative decay rate (day^{-1}) of *Sclerotium rolfsii* sclerotia and standard errors in soil amended with summer green manure (SGM) or winter green manure (WGM).

	Relative decay rate			Relative decay rate	
SGM	Estimate	s.e.	WGM	Estimate	s.e.
Sudangrass (*Sorghum* × *drummondi*)	**0.0071** [a]	0.0030	Black Oat(*Avena strigosa*)	**0.0071**	0.0023
Foxtail millet (*Setaria italica*)	0.0040	0.0028	Oat (*Avena byzantina*)	**0.0096**	0.0024
Corn (*Zea mays*)	0.0046	0.0029	Wheat (*Triticum aestivum*)	**0.0090**	0.0024
Sunflower (*Heliantus annus*)	0.0033	0.0031	Forage Radish (*Brassica napus*)	**0.0054**	0.0022
Black beans *(Phaseolus vulgaris*)	-0.0044	0.0030	Red clover (*Trifolium pratense*)	**0.0048**	0.0023
Cowpea (*Vigna unguiculata*)	0.0021	0.0032	Blue Lupine (*Lupinus angustifolius*)	**0.0072**	0.0023
Sunnhemp (*Crotalaria juncea*)	0.0042	0.0029	White Lupine (*Lupinus albus*)	**0.0059**	0.0023
Alfalfa (*Medicago sativa*)	**0.0077**	0.0032	Hairy vetch (*Vicia villosa*)	0.0031	0.0023
Un-amended soil	0.0037	0.0032	Alfalfa (*Medicago sativa*)	**0.0088**	0.0023
			Un-amended soil	0.0035	0.0024

[a] Bold values were statistically different ($p<0.05$) from un-amended soil, within the column.

Table 3. Average, standard error, minimum and maximum *Sclerotium rolfsii* soil population (number of viable sclerotia in 100 g dry soil) for the different crop sequences in the microplots, determined by the methanol assay.

Crop sequence [a]		**Sampling date**			
		Feb 2010 [b-]	May 2010 [c]	Feb 2011 [c]	June 2011 [c]
BO-SwP-F-SwP	Average (s.e.)	4.86 (1.18) [d]	0.74 (0.22)	1.53 (0.23)	4.77 (0.58)
	Min - Max	0.50 – 12.07	0.20 – 2.33	0.45 – 2.47	2.05 – 6.91
BO-SwP-BO-SwP	Average (s.e.)	6.56 (1.29) [e]	1.59 (0.53)	2.48 (0.43)	13.58 (2.63)
	Min - Max	2.50 – 11.99	0.00 – 3.36	0.71 – 3.97	4.14 – 23.17
BO-SwP-O-SwP	Average (s.e.)	6.05 (1.18) [e]	0.52 (0.23)	0.99 (0.24)	3.07 (0.55)
	Min - Max	1.87 – 8.95	0.81 – 2.11	0.26 – 1.85	1.73 – 5.17
	LSD [f]	4.60	1.24	1.35	8.27

[a] BO: black oat, SwP: Sweet Pepper, F: Fallow, O: Onion. [b] *S. rolfsii* sclerotia population measured after artificial inoculation with sclerotia produced on oat kernels and air dried. Data from inoculated and un-inoculated microplots. [c] May 2010: sclerotia population after SwP harvest and before installing BO or O, Feb. 2011: sclerotia population after harvesting BO and O and before SwP transplant, June 2011: sclerotia population after SwP harvest. [d]Average of nine microplots (six inoculated and three un-inoculated) [e] Average of six microplots (three inoculated and three un-inoculated) [f] Least significant differences (LSD) at P= 0.05

3.2 Population dynamics under different crop sequences – Microplot experiment

Immediately after inoculation with lab-produced sclerotia and before planting of sweet pepper

in February 2010, a large variation in sclerotial density was observed among plots, but there were no differences among the intended crop sequences (Table 3). The variation in sclerotial density was likely due to natural infestation in some of the microplots. At the end of the sweet pepper crop (May 2010) the sclerotia populations had decreased in all microplots, but there was still a wide range of sclerotial densities. Measurements of May 2010, February 2011, and June 2011 were used to estimate the parameters for the dynamics described in equation (2).

The relationships between the initial populations (*Pi*) and final populations (*Pf*) constituted saturation curves that were adequately described by equation (2) (Figure 2). The asymptote was clearly highest for sweet pepper, indicating that sweet pepper, which was grown in summer, resulted in the greatest multiplication of the pathogen. On the winter crops, black oats and onions, the sclerotia hardly multiplied, so that the asymptotes were much smaller.

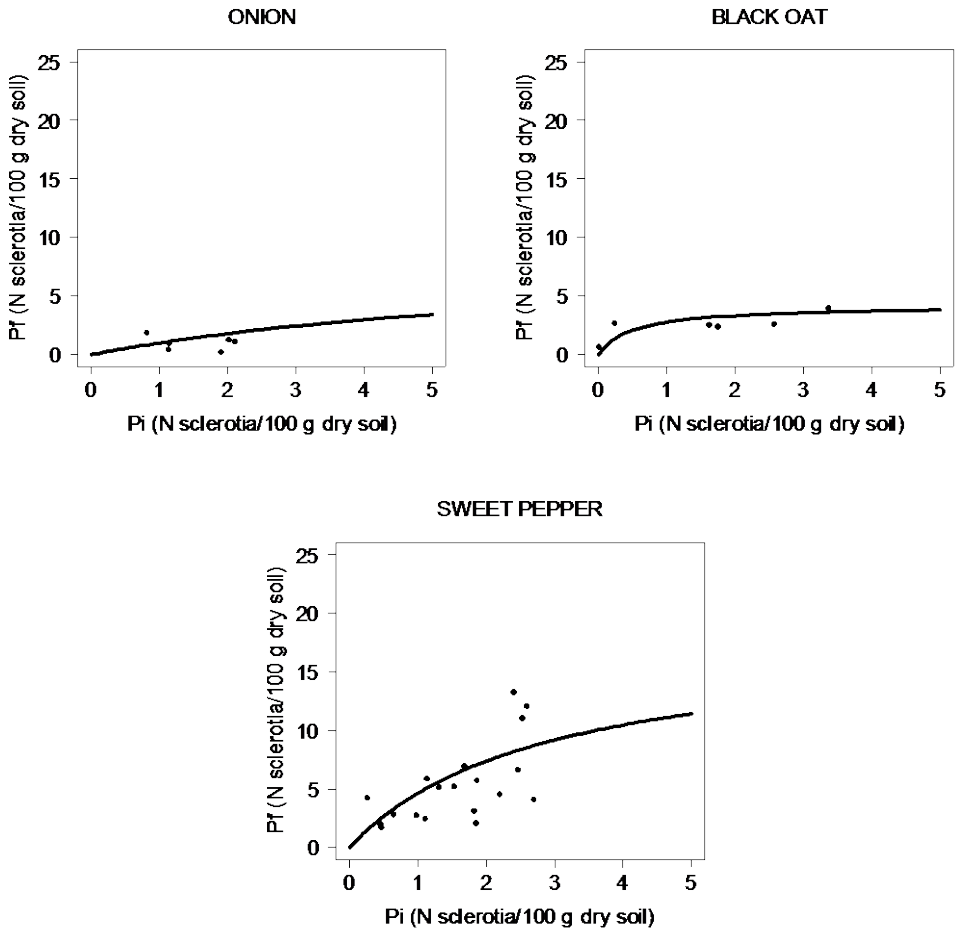

Figure 2. Relation between final (P_f) and initial (P_i) *Sclerotium. rolfsii* soil population (viable sclerotia in 100 g of dry soil) for onion, black oat and sweet pepper. Dots are measured values in the microplot experiment, and the line represents the fitted model $P_f = P_i / (\alpha + \beta P_i)$.

Table 4. Estimated parameters 1/*α* and 1/*β* (number of viable sclerotia in 100 g dry soil) for *Sclerotium rolfsii* sclerotia dynamics in soil, under different cropping sequences.

Crop	Previous crop / activity	1/α slope at the origin			1/β asymptote			AP [a]
		Median	Mean	s.e.	Median	Mean	s.e.	
Onion 2010	Sweet	1.13	2.39	2.48	8.64	14.98	14.95	0.14
Black oat 2010	pepper	8.22	7.79	1.79	4.17	5.69	5.79	0.27
Sweet pepper 2011	Onion	6.16	6.25	2.18	11.35	16.58	13.25	0.26
	Black oat	7.74	7.44	1.80	31.39	31.32	18.85	0.33
	Fallow	5.84	6.05	1.72	11.73	15.63	9.92	0.23
	All	6.26	6.48	1.70	17.93	21.30	10.69	0.28

[a] Acceptance Probability. AP reference rates are 0.4 for *d* = 1 and 0.28 for *d* = 5, where *d* is the number of estimated parameters of the posterior distribution (ter Braak and Vrugt 2008).

The fit to equation (2) was better for black oats and sweet pepper than for onion, as indicated by the acceptance probabilities (Table 4).The rates of population increase at low P_i (median values for 1/*α*), were larger for black oat (8.22) than for onion (1.13).Values for the asymptote (1/*β*), were greater for onion (8.64 viable sclerotia in 100 g of dry soil) than for black oat (4.17 viable sclerotia in 100 g of dry soil) (F-test, p>0,05) (Table 4).

For the sweet pepper crops grown in 2011 the sclerotia population densities as well as the estimates of the population parameters 1/α and 1/β did not depend on the previous crop (F-test, p>0.05; Table 4). Combining the information from all the plots, the rate of population increase at low P_i (1/*α*) was 6.26 and the asymptote (1/*β*) was 17.93 viable sclerotia in 100 g dry soil (Table 4).

3.3 Population dynamics – Simulations for different crop sequences

Deterministic simulations. In the sequences Sweet pepper – Fallow (SwP-F), Sweet pepper - Black Oat (SwP-BO) and Sweet pepper- Onion (SwP-O) the steady state of the system was generally reached after three cycles, irrespective of the initial inoculum density. The only exception was SwP-F at low initial inoculum density ($P_{i(0)}$ = 1 sclerotia in 100 g dry soil), which needed six cycles to reach equilibrium (Figure 3 A, B and C). The maximum population densities at steady state after the various crop sequences were generally below those after the SwP-F sequences (Figure 3 and Table 5).The sequence SwP-F resulted in the highest soil sclerotia density, with 18 viable sclerotia in 100 g dry soil.

Table 5. Simulated P_i - P_f values (number of viable sclerotia in 100 g dry soil) for the first sweet pepper crop in the sequence, when steady states were reached.

	Deterministic[a]		Stochastic[b]	
	P_i	P_f	P_i	P_f
Crop sequence			Median (Min. – Max.)	
SwP – F [c]	6.94	17.89	5.23 (0.00 – 173.86)	10.07 (0.00 – 45.80)
SwP – O	2.76	10.50	2.25 (0.09 – 36.24)	7.20 (0.31 – 34.73)
SwP – BO	2.55	9.95	2.30 (0.78 – 23.04)	8.20 (3.09 – 31.76)
SwP-O-SwP-BO	2.71	10.38	2.28 (0.69 – 20.69)	7.53 (3.02 – 26.41)
SwP-BO-SwP-O	2.83	10.69	2.34 (0.67 – 22.93)	7.30 (2.61 – 30.15)
SwP-O-F-BO	1.62	7.09	1.67 (0.33 – 19.01)	6.33 (1.60 – 25.13)

[a] Parameters were fixed single estimates [b] Parameters were described by their distributions [c] SwP: Sweet Pepper, O: Onion, BO : Black oat, F: Fallow

For the sequences SwP-O-F-BO, SwP-O-SwP-BO and SwP-BO-SwP-O steady states were reached after 2 cycles (4 years) irrespective of initial soil *S.rolfsii* population density (Figure 3 D, E and F). At equilibrium, the sequence SwP-O-F-BO resulted in 30% lower initial soil inoculum density (2 viable sclerotia in 100 g dry soil) than the sequences SwP-O-SwP-BO and SwP-BO-SwP-O (3 viable sclerotia in 100 g dry soil) (Table 5).

Stochastic simulations. When stochasticity was included for the parameters, long term simulations of Swp-F resulted in a median population density of 10 viable sclerotia in 100 g dry soil, with a variation between 0 and 46 viable sclerotia in 100 g dry soi l (Table 5). All other sequences resulted in lower median population densities. The median population densities after stochastic simulations were generally lower than those after deterministic

simulations. However, after stochastic simulations the ranges of the values were quite wide and skewed right, as indicated in the boxplot with the dots above the whisker (Figure 4). This variation reflects the uncertainty of the *S. rolfsii* population dynamics, and seems a better representation of the dynamics in farm fields.

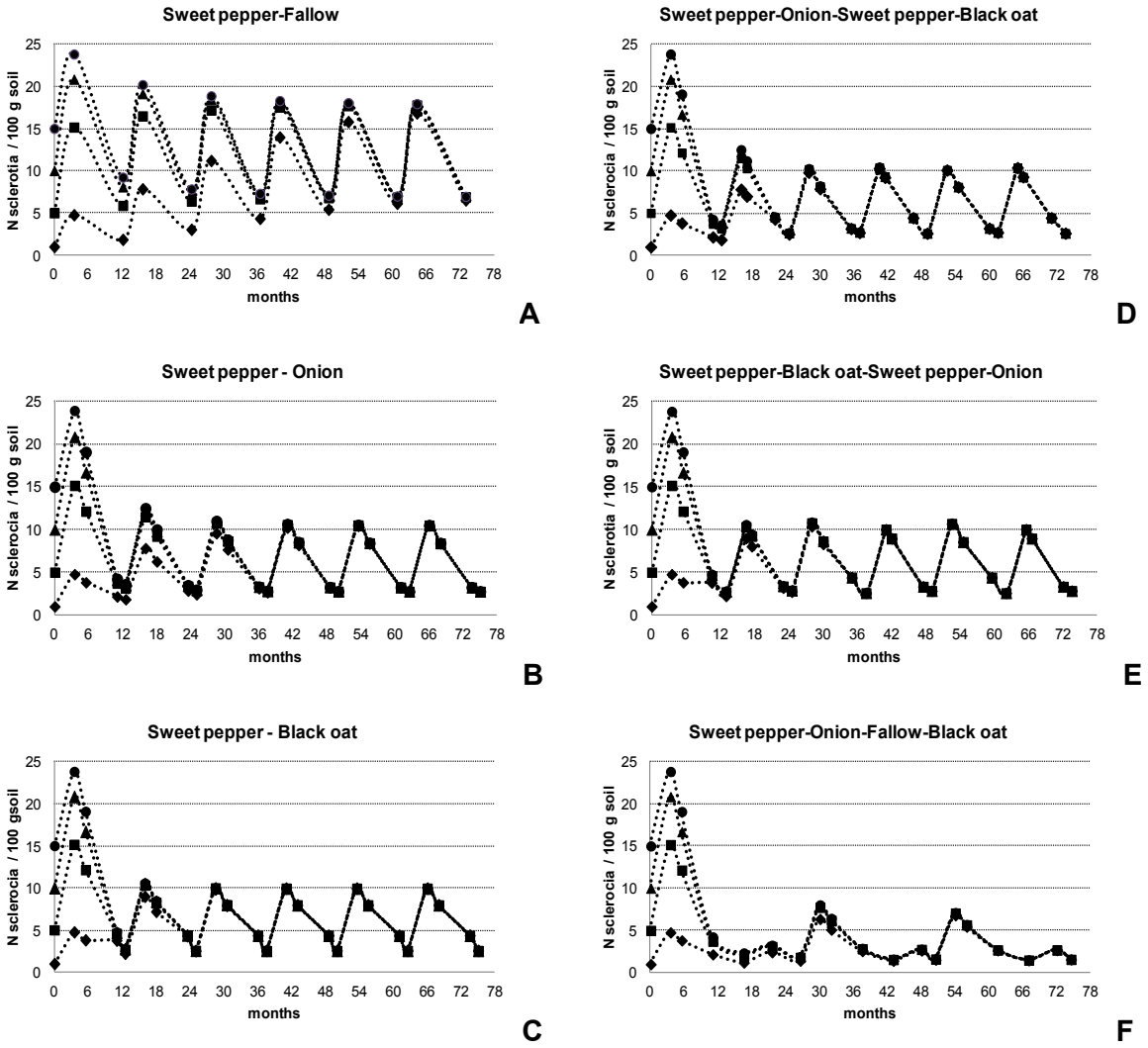

Figure 3. Dynamics of *Scleortium rolfsii* sclerotia soil population (number of viable scleortia in 100 g dry soil) for different crop sequences, after deterministic simulation over six years with fixed parameters: mean of the relative decay rate *b* in the equation $S_f = S_i * \exp(-b*d)$, and median of the slope at origin *1/α* and the asymptote *1/β* in the equation $P_f = P_i / (\alpha + \beta P_i)$. Symbols are estimated P_i values for each crop: diamonds - $P_{i(0)}$ = 1 sclerotia/100 g dry soil, squares - $P_{i(0)}$ = 5 sclerotia / 100 g dry soil, triangles - $P_{i(0)}$ = 10 sclerotia / 100 g dry soil, and dots - $P_{i(0)}$ = 15 sclerotia / 100 g dry soil.

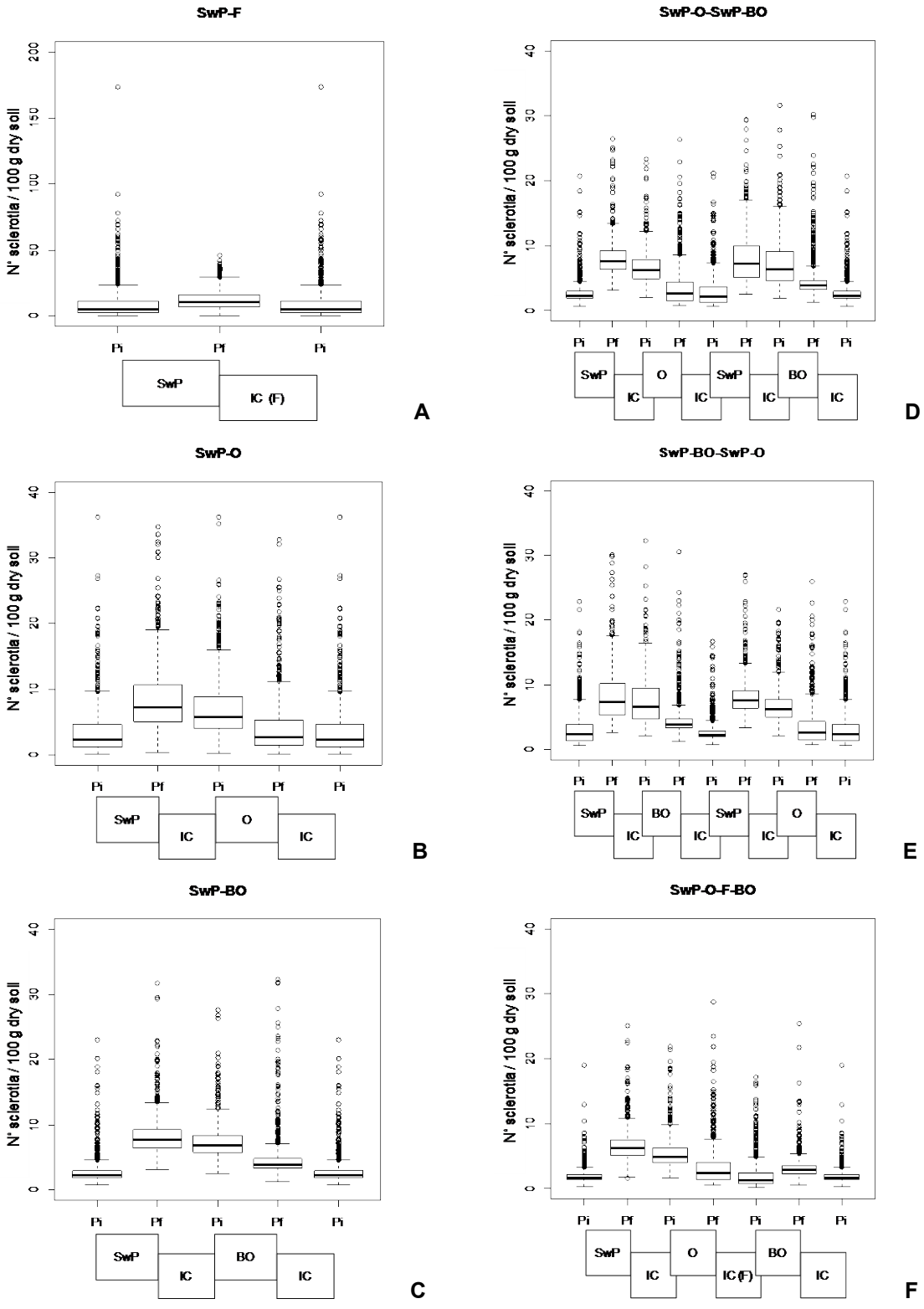

Figure 4. Boxplot of the P_i-P_f values (number of viable sclerotia in 100 g dry soil) for the different crops in the sequence, when steady states were reached after stochastic simulation. P_f values were calculated with equation (2) ($P_f = P_i / \alpha + \beta P_i$) and P_i with equation (1) ($P_{,i} = S_f$, $S_f = S_i$ * exp(-b*d)). The boxes along the x axis represent the crop or intercrop activity. SwP: Sweet pepper crop, BO: Black oat crop, O: Onion crop, F: Fallow, IC: intercrop period.

4 Discussion

4.1 Population dynamics in GM-amended soil

The first objective of this study was to quantify the dynamics of sclerotia of *S. rolfsii* after the soil incorporation of various cash and green manure crops. The main outcome was that certain crops allowed multiplication of sclerotia while others resulted in a reduction of the sclerotia densities. In particular, the incorporation of the residues of various legume crops (black beans, cowpea, lupins and hairy vetch) resulted in the highest sclerotia densities. On the other hand, various grasses (sudangrass, foxtail millet, and in some cases oat and wheat) as well as sunhemp resulted in a reduction of sclerotia in the soil.

Differences in sclerotia populations may have been caused by saprophytic growth and sclerotia formation in crop debris of some crops and not in others. In addition, crop decomposition can release compounds that stimulate sclerotia germination and colonization of available substrate (Flores-Moctezuma et al. 2006), and secondary sclerotia can be produced after eruptive germination (Punja and Grogan 1981, Punja et al. 1984). For example, Beute and Rodriguez-Kabana (1981) found that the incorporation of peanut stems increased the density of *S. rolfsii* sclerotia. However, an increase in inoculum density does not necessarily increase the inoculum potential. For example, wheat straw increased *S. rolfsii* inoculum density but not Southern blight disease incidence (Ferguson and Shew 2001).

In addition to a decrease in inoculum potential, a decrease in sclerotia density has been observed after addition of residues of various grasses which may release toxic chemical compounds during decomposition. Epidermal cells of sudangrass contain dhurrin (cyanoglucoside) and isothyocianates, which are hydrolyzed into hydrogen cyanide when the tissue is damaged. Oat shoots produce different antimicrobial compounds upon decomposition, among them saponins, flavonoids, phytoalexins and avenanthramides (hydroxycinnamic acid amide), some of them also produced by poaceous species like wheat (Bahraminejad et al. 2008, Stapleton et al. 2010). The rind of the sclerotia is permeable to gases and water, and biotoxic compounds can diffuse into and out of the sclerotia (Smolinska 2000).

The deleterious effect of green manure amendments on sclerotia can be enhanced when soil temperatures are increased. Stapleton et al. (2010) reported that residues of poaceous species (oat, wheat, barley, sudangrass) when combined with soil solarization were more effective in reducing *S. rolfsii* survival. Indeed, we found greater sclerotia decay rates after

incorporation of WGM biomass when temperatures were higher. Flores-Moctezuma et al. (2006) mentioned that *Parthenium hysterophorus* when incorporated into soil increased sclerotial populations, whereas when the residues were combined with solarization the number of *S. rolfsii* sclerotia and disease incidence on onions decreased.

In addition to indirect effects of environmental conditions on sclerotia survival, direct effects of temperature and moisture on survival of *S. rolfsii* in soil have been demonstrated (Beute and Rodríguez-Kábana 1981, Martins et al. 2010, Punja 1985, Xu et al. 2008). This was also the case for sclerotia of other pathogens (Lodha et al. 2003, Wu et al. 2008). In our greenhouse experiments soil moisture was maintained by regular irrigation of the pots. Thus, lower final sclerotial densities after incorporation of WGM at the end of January compared to those after SGM incorporation in June could be explained by increased sclerotial decay at higher soil temperatures during December-January in Uruguay. This is consistent with decreased survival of *S. rolfsii* at temperatures above 20^{o}C (Beute and Rodríguez-Kábana 1981).

Without incorporation of green manures in the field experiment, the relative density of recovered sclerotia was 49% and 73% for 2009 and 2010 respectively. Soil temperatures were similar in both years, above 20 ˚C after WGM incorporation, but 2010 was much dryer than 2009 (Table S3). So soil moisture rather than temperature may have contributed to differences in sclerotia recovery between these two years. This is in accordance with previous reports where *S. rolfsii* sclerotia survival was highest under dry conditions (Beute and Rodríguez-Kábana 1981, Martins et al. 2010, Punja 1985). Also for other soilborne pathogens survival is negatively affected by wet heat, as in warm humid regions (Wu et al. 2008) or as extensively demonstrated by soil solarization (Katan and DeVay 1991).

Sclerotia survival was adequately described by a negative exponential model (equation 1) which implies a constant daily fractional removal of sclerotia. Other authors have modeled soilborne pathogen survival with the two-parameter Weibull model and with more parameter-rich regression models. For *S. rolfsii* population dynamics were described with the Weibull model (Shlevin et al. 2003). Despite its simplicity, our model summarizes in parameter *b* the variations in crop tissue composition and differences in temperature and humidity across years and experimental conditions. While the equation does not allow disentangling the contribution of each factor, it provides an integral summary of the overall process.

4.2 Population dynamics under different crop sequences

The second objective was to design and test a research approach where experimental data and model simulations are combined to explore crop sequences that minimize Southern blight incidence. Concatenating a model for the build-up of inoculum in the presence of a host and a model for the decline of inoculum after incorporation of crop debris into soil resulted in equilibrium densities of *S. rolfsii* sclerotia in soil that differed among crop successions. The differences were more related to the frequency of the sweet pepper host than to the inclusion of various other crops in the rotation. However, the equilibrium inoculum densities were much higher if the soil was left fallow between sweet pepper crops than if any intercrop was planted.

The results from the microplot experiment did not completely agree with the simulation results. The build-up of sclerotia populations in the microplots was not only dependent on a particular crop like sweet pepper but also on the crops preceding the sweet pepper crop in that sequence. For example, the final soil sclerotia densities at the harvest time of sweet pepper grown after black oats were significantly greater than those after onion or a fallow period. This is in accordance with previous reports where pathogen dynamics were affected by the previous crop (Taylor and Rodriguez-Kabana 1999a). Nevertheless, model parameters for inoculum build-up were estimated from the combined data for different crop sequences, although $1/\alpha$ and $1/\beta$ actually varied slightly (but not significantly) depending on previous crops. In another model for *S. rolfsii* dynamics in crop rotations the parameters were specific for each crop sequence, indicating that this may also be required for our concatenated model.

The observed differences between the model outcomes and the experimental results could be explained by differential survival of sclerotia produced during a previous crop, by differential germination capacity of sclerotia in different soil environments created by the various intercrop situations, or due to differential susceptibility of the second sweet pepper crop as a result of the different soil environments. Soil structure and porosity following a poaceous green manure are usually better than after an onion crop or fallow, allowing better survival and germination of sclerotia because *S. rolfsii* is highly aerobic (Punja 1985). Finally, soil microbial communities could also explain the differences in final sclerotia populations, although no differences among microbial antagonists like *Trichoderma* spp. (Bulluck and Ristaino 2002, Liu et al. 2007), *Pseudomonas* spp. (Le 2011) or actinomycete populations were found in our experiments (data not shown).

Despite the minor inaccuracies obtained with the current concatenated models, the concatenation approach could be very useful after additional parameter calibration and validation using results from additional microplot and on-farm experiments. The novelty of the concatenation approach is that data from individual short experiments on component crops can be combined to predict the final inoculum levels in different sequences of these component crops after many years. This approach was also proposed for nematode dynamics under different crop sequences (van der Berg and Rossing 2005) and applied to *Fusarim oxysporum* f.sp. *cepae* population dynamics (Leoni et al. 2013). Results from such concatenation models can be included into models for designing crop rotations, such as ROTAT (Dogliotti et al. 2003) or more complex models that aim at the re-design of whole-farm system, such as FarmIMAGES (Casagrande et al. 2010, Dogliotti et al. 2005).

5 Conclusions

We investigated and modelled the dynamics of *S. rolfsii* sclerotia for two distinctive phases of the cropping sequence, the crop phase and the intercrop phase. We combined the models of the two phases to simulate the pathogen dynamics under different cropping sequences. The research strategy followed to generate quantitative data was quite efficient compared with long-term experiments usually needed for modelling pathogen population dynamics. The strategy could be further improved by combining the data from controlled experiments with data from farm surveys, to cover a wider range of crops and to account in the models for the variability found in different farming systems. Moreover, the data from farm surveys should be used for model validation. A final step will be to link the information on population dynamics to yield loss, thus allowing economic assessment of alternative options.

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Minas, Uruguay, and at the Latin-American Regional Workshop - International Soil Tillage Research Organization (ISTRO), July 12-14th 2010, Colonia del Sacramento, Uruguay.

References

Bahraminejad, S., Asenstorfer, R.E., Riley, I.T., Schultz, C.J. 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.). *Journal of Phytopathology* 156:1-7.

Bates, D., Maechler, M., Bolker, B. 2011. lme4: Linear mixed-effects models using S4 classes. R package version 0.999375-42. http://CRAN.R-project.org/package=lme4. Accessed June 10, 2013.

Bailey, K.L. and Lazarovits, G. 2003. Suppressing soil-borne diseases with residue management and organic amendments. *Soil and Tillage Research* 72:169 – 180.

Beute, M. K. and Rodríguez-Kabana, R. 1981. Effects of soil moisture, temperature, and field environment on survival *of Sclerotium rolfsii* in Alabama and North Carolina. *Phytopathology* 71: 1293-1296.

Bulluck, L.R. III. and Ristaino, J.B. 2002. Effect of synthetic and organic soil fertility amendments on southern blight, soil microbial communities, and yield of processing tomatoes. *Phytopathology* 92: 181 – 189.

Casagrande, M., Dogliotti, S., Groot, J.C.J., Aguerre, V., Abbas, A., Albin, A., Claassen, G.D.H., Chilibroste, P., Rossing W.A.H. 2010. Exploring options for sustainable farming systems development for vegetable family farmers in Uruguay using a modelling toolkit. In: 9th European IFSA Symposium, Building sustainable rural futures. The added value of systems approaches in times of change and uncertainty, Vienna, Austria, 04 - 07 July, 2010, pp. 463 – 469.

Colbach, N., Meynard, J-M, Duby, C., Huet, P. 1999. A dynamic model of the influence of rotation and crop management on the disease development of eyespot. Proposal of cropping systems with low disease risk. *Crop Protection* 18: 451 - 461.

de Mendiburu, F. 2012. agricolae: Statistical Procedures for Agricultural Research. R package version 1.1-3. http://CRAN.R-project.org/package=agricolae. Accessed June 10, 2013.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2003. ROTAT, a tool for systematically generating crop rotations. *European Journal of Agronomy* 19: 239-250.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2004. Systematic design and evaluation of crop rotations enhancing soil conservation, soil fertility and farm income: a case study for vegetable farms in South Uruguay. *Agricultural Systems* 80: 277 - 302.

Dogliotti, S., Van Ittersum, M.K., Rossing, W.A.H. 2005. A method for exploring sustainable development options at farm scale: a case study for vegetable farms. *Agricultural Systems* 86: 29-51.

Ferguson, L. M., Shew, B.B. 2001. Wheat straw mulch and its impacts on three soilborne pathogens of peanut in microplots. *Plant Disease* 85: 661-667.

Flores-Moctezuma, H.E., Montes-Belmont, R., Jiménez-Pérez, A., Nava-Juarez, R. 2006. Pathogenic diversity of *Sclerotium rolfsii* isolates from Mexico, and potential control of southern blight through solarization and organic amendments. *Crop Protection* 25: 195–201.

Gelman A. and Hill J. 2007. Data analysis using regression and multilevel/hierarchical models. Cambridge University Press, New York.

Gilsanz, J.C., Arboleda, J., Maeso, D., Paullier, J., Behayout, E., Lavandera, C., Sanders, D.C., Hoyt, G.D. 2004. Evaluation of limited tillage and cover crop systems to reduce N use and disease population in small acreage vegetable farms mirror image projects in Uruguay and North Carolina, USA. In: Bertschinger, L., Anderson, J.D. (eds). Proceedings of XXVI IHC, Sustainability of horticultural systems, *Acta Horticulturae* 638: 163 – 169.

Grünwald, N.J., Hu, S., van Bruggen, A.H.C. 2000. Short-term cover crop decomposition in organic and conventional soils; characterization of soil C, N, microbial and plant pathogen dynamics. *European Journal of Plant Pathology* 106: 37 - 50.

Jenkins, S.F. and Averre, C.W. 1986. Problems and progress in integrated control of Southern blight of vegetables. *Plant Disease* 70: 614 – 619.

Le, C.N. 2011. Diversity and biological control of *Sclerotium rolfsii*, causal agent of stem rot of groundnut. PhD Thesis, Wageningen University, Wageningen, The Netherlands.

Leoni, C., de Vries, M., ter Braak, C.J.F., van Bruggen, A.H.C., Rossing, W.A.H. 2013. *Fusarium oxysporum* f.sp. *cepae* dynamics: in-plant multiplication and crop sequence simulations. *European Journal of Plant Patholog* 137: 545 - 561. doi: 10.1007/s10658-013-0268-6.

Liu B., Tu, C., Hu, S., Gumpertz, M., Ristaino, J.B. 2007. Effect of organic, sustainable and conventional management strategies in grower fields on soil physical, chemical, and biological factors and the incidence of Southern blight. *Applied Soil Ecology* 37: 202-214.

Lodha, S., Sharma, S.K., Mathur B.K., Aggarwal, R.K. 2003. Integrating sub-lethal heating with *Brassica* amendments and summer irrigation for control of *Macrophomina phaseolina*. *Plant and Soil* 256: 423 – 430.

Martins, M.V.V., Silveira, S.F., Mussi-Dias, V., Duarte Vieira, H. 2010. Efeito da temperatura e umidade do substrato na viabilidade de *Sclerotium rolfsii*. *Acta Scientiarum- Agronomy* 32: 217-222.

Mol, L., Huisman, O.C., Scholte, K., Struik, P.C. 1996. Theoretical approach to the dynamics of the inoculum density of *Verticillium dahliae* in the soil: first test of a simple model. *Plant Pathology* 45: 192 – 204.

Njoroge, S.M.C., Riley, M.B., Keinath, A.P. 2008. Effect of incorporation of *Brassica* spp. residues on population densities of soilborne microorganisms and on Damping-off and Fusarium wilt of watermelon. *Plant Disease* 92: 287 – 294.

Punja, Z.K. and Grogan, R.G. 1981. Eruptive germination of sclerotia of *Sclerotium rolfsii*. *Phytopathology* 71: 1092 – 1099.

Punja, Z.K., Jenkins, S.F., Grogan, R.G. 1984. Effect of volatile compounds, nutrients, and source of sclerotia on eruptive sclerotial germination of *Sclerotium rolfsii*. *Phytopathology* 74: 1290 – 1295.

Punja, Z.K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology* 23: 97-127.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, URL http://www.R-project.org/. Accessed June 10, 2013.

Raaijmakers, J. M., Paulitz, T. C., Steinberg, C., Alabouvette, C., Moënne-Loccoz, Y. 2009. The rhizosphere: a playground and battlefield for soilborne pathogens and beneficial microorganisms. *Plant and Soil* 321: 341 – 361

Rodriguez-Kabana, R., Beute, M.K., Backman, P.A. 1980. A method for estimating numbers of viable sclerotia of *Sclerotium rolfsii* in soil. *Phytopathology* 70: 917 – 919.

Rodriguez-Kabana, R., Kokalis-Burelle, N., Robertson, D.G., King, P.S., Wells, L.W. 1994. Rotations with coastal bermudagrass, cotton, and bahiagrass for management of *Meloidogyne arenaria* and Southern blight in peanut. *Journal of Nematology* 26 (4S): 665 - 668.

Shlevin, E., Saguy, I.S., Mahrer, Y., Katan, J. 2003. Modeling the survival of two soilborne pathogens under dry structural solarization. *Phytopathology* 93: 1247 – 1257.

Smolinska, U. 2000. Survival of *Sclerotium cepivorum* sclerotia and *Fusarium oxysporum* chlamydospores in soil amended with cruciferous residues. *Journal of Phytopathology* 148: 343 – 349.

Stapleton, J.J., Summers, C.G., Mitchell, J.P., Prather, T.S. 2010. Deleterious activity of cultivated grasses (Poaceae) and residues on soilborne fungal, nematode and weed pests. *Phytoparasitica* 38: 61–69.

Taylor, C.R. and Rodriguez-Kábana, R. 1999 a. Population dynamics and crop yield effects of nematodes and white mold in peanuts, cotton and velvet beans. *Agricultural Systems* 59: 177 – 191.

Taylor, C.R. and Rodriguez-Kábana, R. 1999 b. Optimal rotation of peanuts and cotton to manage soil-borne organisms. *Agricultural Systems* 61: 57 – 68.

ter Braak, C.J.F. and Vrugt, J.A. 2008. Differential evolution Markov Chain with snooker update rand fewer chains. *Statistics and Computing* 18: 435 – 446.

Tixier, P., Risède, J.M., Dorel, M., Malézieux, E. 2006. Modelling population dynamics of banana plant-parasitic nematodes: A contribution to the design of sustainable cropping systems. *Ecological Modelling* 198: 321–331.

van Bruggen, A.H.C. and Semenov, A.M. 1999. A new approach to the search for indicators of root disease suppression. *Australasian Plant Pathology* 28: 4-10.

van den Berg, W. and Rossing W.A.H. 2005. Generalized linear dynamics of a plant-parasitic nematode population and the economic evaluation of crop rotations. *Journal of Nematology* 37: 55-65.

Wu, B. M., Subbarao, K. V., Liu, Y. B. 2008. Comparative survival of sclerotia of *Sclerotinia minor* and *S. sclerotiorum*. *Phytopathology* 98: 659-665.

Xu, Z., Gleason, M.L., Mueller, D.S., Esker, P.D., Bradley, C.A., Buck, J.W., Benson, D.M., Dixon, P.M., Monteiro, J.E.B.A. 2008. Overwintering of *Sclerotium rolfsii* and *S. rolfsii* var. *delphinii* in different latitudes of the United States. *Plant Disease* 92: 719 – 724.

Supplementary data

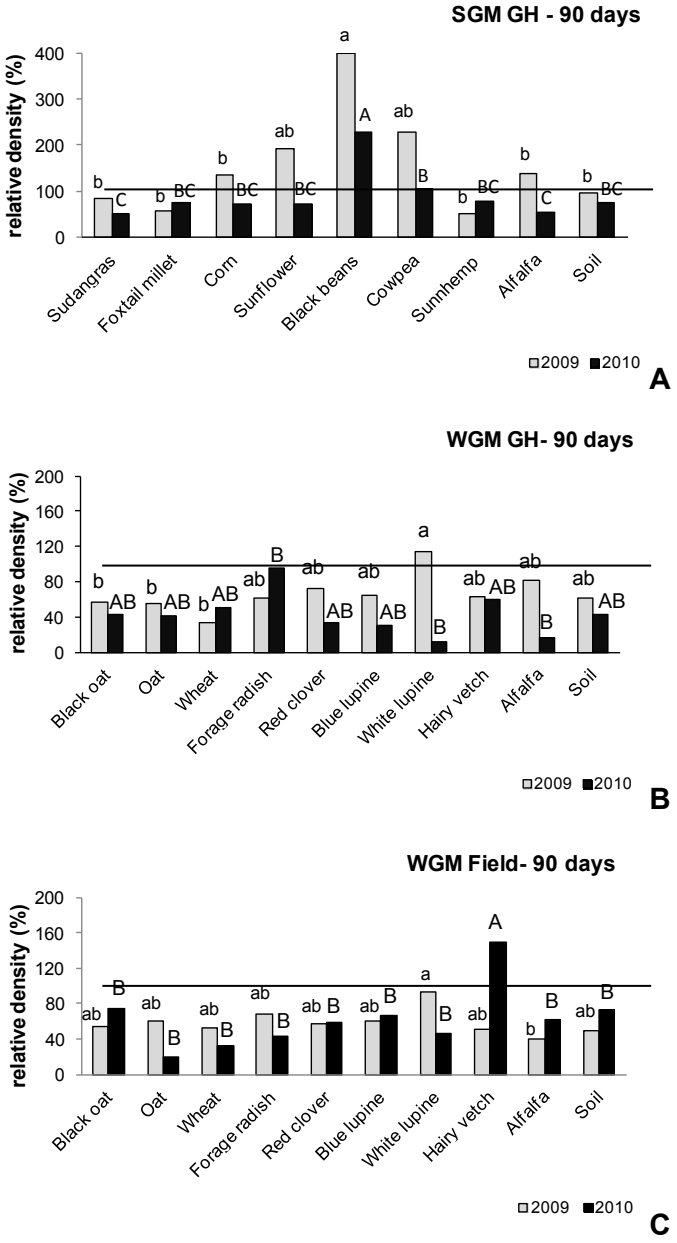

Figure S1. Relative density of *Sclerotium rolfsii* sclerotia 90 days after burying 25 sclerotia in un-amended soil (soil) or soil amended with summer green manure (SGM) or winter green manure (WGM) in nylon sock bags inside a plastic pot placed in a greenhouse (A,B) or in the field (C), in 2009 and 2010. The experiments with SGM started in April or March, and those with WGM in October. Viable sclerotia on day 0 = 100 % relative density. Bars indicated with the same letter do not differ according to Duncan test (alpha = 0.05), upper case letters for 2009 and lower case letters for 2010.

Table S1. Green manures crops assayed in the pot experiments: sowing rates, equivalent amount of incorporated dry matter per ha, Carbon-Nitrogen ratios (C/N) and percentages of dry matter (DM).

	Sowing rate	Incorporated GM	2009		2010	
	(kg.ha^{-1})	(Mkg DM.ha^{-1})	C/N[a]	DM (%)	C/N	DM (%)
Summer green manure (SGM)[b]						
Sudangrass *(Sorghum × drummondi)*	60	10.5	43.2	26.7	64.7	13.1
Foxtail Millet (*Setaria italica*)	30	6.0	24.2	45.1	46.5	17.0
Corn (*Zea mays*)	45	10.0	22.9	21.9	27.8	7.8
Sunflower (*Heliantus annus*)	60	10.0	25.8	23.8	68.0	25.0
Black beans *(Phaseolus vulgaris*)	40	4.0	18.8	24.1	36.1	21.2
Cowpea (*Vigna unguiculata*)	40	4.5	27.2	14.9	34.1	7.4
Sunn hemp (*Crotalaria juncea*)	60	6.0	13.1	23.0	31.5	14.8
Alfalfa (*Medicago sativa*) [c]	18	6.5	na [e]	na	15.2	15.8
Winter green manure (WGM) [4]						
Black oats (*Avena strigosa*)	120	11.4	27.0	17.3	47.7	13.4
Oats (*Avena byzantina*)	96	13.4	23.5	13.4	34.7	15.2
Wheat (*Triticum aestivum*)	96	11.9	24.7	20.0	55.6	19.6
Forage radish (*Brassica napus*)	12	3.3	27.5	10.4	39.8	8.5
Red clover (*Trifolium pratense*)	15	4.5	13.3	11.7	26.0	10.8
Blue lupine (*Lupinus angustifolius*)	80	3.1	15.2	13.3	23.7	9.6
White lupine (*Lupinus albus*)	80	10.7	15.8	13.4	19.4	10.0
Hairy vetch (*Vicia villosa*)	37	2.5	13.0	12.7	16.0	10.3
Alfalfa (*Medicago sativa*) [d]	18	6.5	na	na	23.4	13.2

[a] Carbon and Nitrogen tissue analyses were performed at the Soil Laboratory of INIA La Estanzuela, Uruguay (http://www.inia.org.uy/online/site/92326I1.php)

[b] SGM of 2009 were sown on December 17th 2008 and harvested and soil incorporated on April 1st 2009. SGM of 2010 were sown on December 18th 2009 and harvested and soil incorporated on March 29th 2010.

[c] Alfalfa was sown on April 2007, and harvested and soil incorporated on the same dates as the SGM and WGM.

[d] WGM of 2009 were sown on May 19th 2009 and harvested and soil incorporated on October 26th 2009. WGM of 2010 were sown on May 11th 2010 and harvested and soil incorporated on October 20th 2010.

[e] No data available

Table S2. Average, maximum and minimum air temperature (ºC) and relative humidity (HR %), registered in the greenhouse with a data logger located at the height of the pots.

	April		May		June		October		November		December		January	
	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)
							2009-2010							
Average	19.8	60.1	16.9	62.4	13.3	67.4	18.5	59.6	21.5	66.2	22.3	64.5	25.6	59.4
Max	38.1	96.2	40.0	94.5	35.1	96.4	40.4	100	38.7	99.1	37.7	98.3	40.0	97.1
Min	5.7	20.7	3.2	20.7	-0.8	20.7	1.4	20.7	7.3	20.7	8.4	20.7	10.8	20.7
							2010-2011							
Average	18.6	70.6	17.7	79.2	13.7	69.4	18.7	60.8	20.0	60.3	27.2	47.2	26.6	54.7
Max	33.9	97.7	29.7	100	29.3	99.7	33.3	100	41.2	100	43.2	90.8	40.5	92.5
Min	7.9	20.7	6.9	21.4	2.5	20.7	6.0	22.6	6.7	20.7	12.9	20.7	12.3	20.7

Table S3. Average, maximum and minimum air temperature (Temp., ºC), relative humidity (RH, %), effective precipitation (EP, mm) and evapotranspiration (Evap., Class A pan evaporation, mm) registered in the field with an automatic weather station located at INIA Las Brujas Research Station (http://www.inia.org.uy/online/site/692646I1.php).

	October				November				December				January			
	Temp	RH	Rain	Evap	Temp	RH	Rain	Evap	Temp	RH	Rain	Evap	Temp	RH	Rain	Evap
	˚C	%	mm	mm	˚C	%	mm	mm	˚C	%	mm	mm	˚C	%	mm	mm
								2009-2010								
Average	15.3	72	158	114	19.6	76	133	131	20.8	73	62	163	23.9	67	112	239
Max	34.3	100			32.0	100			35.2	100			35.6	100		
Min	2.2	23			6.3	21			7.8	25			10.2	21		
								2010-2011								
Average	15.5	73	48	134	18.2	69	18	166	23.3	55	3	269	24.6	73	39	278
Max	28.3	100			34.1	100			38.1	99			39.1	100		
Min	5.0	25			5.8	21			7.5	21			5.0	24		
AVERAGE (from INIA Las Brujas database 1971 - 2000)																
Average	15.9	75	96	149	18.4	72	91	181	21.3	69	71	235	23.0	70	86	245

Table S4. Average air temperature (Temp, °C), mean air relative humidity (RH, %), effective precipitation (EP, mm) and evapotranspiration (Evap., Class A evaporation pan, mm) during the summer - fall 2010 and 2011, registered in the field with an automatic weather station located at INIA Las Brujas Research Station (http://www.inia.org.uy/online/site/692646I1.php)

	February				March				April				May			
	Temp	RH	EP	Evap	Temp	RH	EP	Evap	Temp	RH	EP	Evap	Temp	RH	EP	Evap
	˚C	%	mm	mm	˚C	%	mm	mm	˚C	%	mm	mm	˚C	%	mm	mm
								2010								
Average	22.5	91	173	169	20.7	83	38	179	15.8	89	99	115	14.6	85	101	62
Max	26.3	100			23.8	97			21.2	100			19.6	100		
Min	16.9	76			16.7	46			11.0	77			9.1	61		
								2011								
Average	22.2	65	48	205	20.6	66	47	183	17.2	72	80	102	12.7	75	44	65
Max	26.3	82			24.8	87			21.8	86			16.3	86		
Min	20.0	40			15.7	56			14.1	53			9.2	64		
AVERAGE (from INIA Las Brujas database 1971 - 2000)																
Average	22.1	74	94	179	20.4	76	86	157	17.0	79	86	98	13.5	81	74	68

Chapter 4

Fusarium oxysporum f.sp. *cepae* dynamics: in-plant multiplication and crop sequence simulations

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Abstract

To reduce Fusarium Basal Rot caused by *Fusarium oxysporum* f.sp. *cepae* (*Foc*) through crop rotation, plant species should be selected based on *Foc* multiplication in their roots. *Foc* multiplication rates in 13 plant species were tested in a greenhouse. All plant species enabled *Foc* multiplication. The lowest *Foc* levels (cfu g^{-1} dry root) were found for wheat, sunflower, cowpea and millet, the highest for black bean. The highest *Foc* levels per plant were calculated for sudangrass. These data were used to calibrate the model $Pf = Pi / (\alpha + \beta Pi)$ relating final (*Pf*) and initial (*Pi*) *Foc* levels in the soil. The rate of population increase at low *Pi* ($1/\alpha$) was highest for onion and black oat and smallest for sunflower. The pathogen carrying capacity ($1/\beta$) was highest for black oat and black bean, and lowest for wheat, cowpea and foxtail millet. *Foc* soil population dynamics was simulated for crop sequences by concatenating *Pi-Pf* values, considering instantaneous or gradual pathogen release after harvest. Different soil *Foc* populations were attained after reaching steady states. *Foc* populations in the sequence onion –foxtail millet - wheat – cowpea were 67% lower than in the sequence onion – sudangrass - black oat - black beans. In this work, by combining detailed greenhouse experiments with modelling, we were able to screen crops for their ability to increase *Foc* population and to explore potential crop sequences that may limit pathogen build-up.

Keywords:
Fusarium oxysporum f.sp. *cepae* , hosts, reservoir-hosts, population dynamic models, simulation

1 Introduction

Rapid changes in agricultural production due to intensification and specialization have resulted in soil degradation and the associated reduction of soil physical, chemical and biological fertility, as well as an increase in pest problems (Cassman 1999, Hochman et al. 2013, Janvier et al. 2007, van Bruggen and Semenov 1999). In particular, soilborne pathogens may cause extensive crop losses by root necrosis and death limiting water and nutrient uptake and transfer towards the upper plant parts, or by reducing the quality and mass of below-ground crop products (Lucas 2006). Soilborne pathogens are ubiquitous and can persist in the soil for many years either by their surviving structures (e.g. sclerotia, chlamydospores, oospores, rhizomorphs), as saprophytes on crop debris or as endophytes on non-host plants (Lucas 2006, Grünwald et al. 2000). These attributes made soilborne pathogen control a difficult task.

Soilborne disease control has relied on seed dressing with chemical or biological fungicides, chemical or biological soil disinfestation, the use of resistant varieties, soil organic amendments to enhance antagonists, and crop rotation (Stirling 2008, van Bruggen and Termorshuizen 2003). Although not practiced extensively, crop rotation can promote a decline in pathogen populations and disease expression by improving soil structure and creating an unfavourable environment for pathogen development, by changing the nutritional status of the crop or indirectly, by increasing host plant resistance. Crop rotation can also influence pathogen dynamics by releasing toxic compounds from crop residues or root exudates, and by promoting antagonist populations exerting antibiosis, parasitism or predation of soilborne pathogens (Leoni et al., 2013a).

Management of soilborne pathogens through crop rotation involves the selection of the appropriate crops in the rotation. To carry out the selection, quantitative information on soilborne pathogens dynamics in the presence of different crops and intercrop activities (e,g. green manures, fallow) is needed (Grünwald et al. 2000) and several population dynamics models describing soilborne pathogens dynamics have been proposed (Mol et al. 1996, Taylor and Rodriguez-Kábana 1999, Termorshuizen and Rouse 1993, Tixier et al. 2006, Thrall et al. 1997, van den Berg and Rossing 2005; van den Berg et al. 2006). These models can be used for describing, explaining and/or exploring soilborne pathogen population dynamics, and furthermore can support the design of crop rotations suppressive to soilborne diseases (Leoni et al. 2013a). Quantitative information to feed the models can be retrieved from literature for several soilborne pathogens, however the effects of the local agro-

environment, the intercrop period and intercrop management are not always well quantified (Lucas 2006).

Fusarium oxysporum f.sp. *cepae* (*Foc*) is a soilborne pathogen of *Allium* species (onion, garlic, leeks) as well as *Asparagus officinalis* (Brayford 1996). On onions, the pathogen causes Fusarium Basal Rot, and is a significant problem in areas with temperatures above 25˚C during the growing season. Onion Fusarium Basal Rot causes yield losses up to 50% in the field, and additional losses accrue during storage due to disease progress and mixed infections with other pathogens (McDonald et al. 2004). Like other *Fusarium oxysporum* species, *Foc* survives as macro- and micro-conidia in crop debris, as chlamydospores in the soil or as mycelium and conidia in colonized roots of non-*Allium* plant species without causing disease (Abawi. and Lorbeer 1972, Brayford 1996).

Like for other soilborne pathogens (Hiddink et al. 2005, Stirling 2008), several strategies need to be combined for the management of Fusarium Basal Rot. To reduce Fusarium Basal Rot incidence and severity by crop rotation, 4 to 5 years without onions are recommended (Agamennoni et al. 2008, Özer et al. 2002, Özer and Köycü 2004). However, failures and inconsistency in disease control are common, possibly due to the ability of other crops to harbour the pathogen without displaying disease symptoms. To improve the chances of success through crop rotation, plant species need to be selected that limit the multiplication of *Foc.* In other words, the crop sequence should include "non-hosts" rather than "reservoir-hosts" for *Foc*, following the terminology proposed by Dhingra and Coelho-Neto (2001) for the bean pathogen *F.oxysporum* f. sp. *phaseoli*.

In this study we investigate through greenhouse experiments the ability of *Foc* to multiply in the roots of different plant species. Then we explore in simple crop rotations how *Foc* multiplication in reservoir-hosts can affect inoculum dynamics in soil by entering the experimental data into a population dynamics model. In further studies the outcomes of this population dynamic model will be compared with data obtained from field experiments and on-farm observations.

2 Materials and Methods

2.1 *Foc* benomyl resistant strain and inoculum production

Fusarium oxysporum f.sp. *cepae* (*Foc)* cannot be distinguished visually from other *formae*

speciales of *F. oxysporum*. We developed a benomyl resistant strain to be able to trace *Foc* in the experiments. Wild isolates from a Uruguayan *Foc* collection (Galván et al. 2008) were exposed during 1 or 2 minutes to UV radiation (UV-lamp: Osram deluxe, Table-S, 11 Watt, 230 Volt~50 Hz) to induce mutations, according to the procedure described by Postma and Luttikholt (1993). After four consecutive transfers onto malt-agar medium (MA) amended with benomyl at 50 mg l^{-1}, the isolates that were able to growth on benomyl-MA media were evaluated for their morphology, growth rate, "in vitro" competitive ability and "in vitro" pathogenicity on onions seedlings (Altier and Groth 2005). Finally, the isolate *Foc* UR17-8B8 was selected, and stored on silica gel at 4˚C till use.

Foc UR17-8B8 inoculum was produced on malt broth (Oxoid, UK). First, silica gel crystals with *Foc* UR17-8B8 were transferred onto MA amended with benomyl at 10 mg l^{1} and incubated for seven days at 25˚C. Plugs from the edge of these 7-day old colonies were transferred into malt broth and incubated for five days at 25˚C under continuous agitation. Finally, the broth with the fungal growth was blended, filtered through four layers of gauze to remove the mycelium, and adjusted using a hemacytometer to a final concentration of 10^5 or 10^7 conidia ml^{-1} for the different experiments.

2.2 Evaluation of the ability of different plant species to host *Foc*

Two pot experiments were established in a greenhouse at INIA Las Brujas Research Station (34° 40' S., 56° 20' W., 32 m over sea level), Rincón del Colorado, Canelones, Uruguay, to provide information about multiplication and/or survival of *Foc* on major plant species used in vegetable crop rotations in Uruguay.

From February to June 2009, an explorative experiment was carried out to assess if *Foc* was able to colonize the roots of different plant species. Following Dinghra and Coelho-Neto's (2001) experimental design, seeds of two onion varieties and 11 *Foc* non-hosts (oat, black oat, wheat, forage radish, red clover, tomato, soybean, corn, sweet corn and sudangrass) were sown in autoclaved sand. About three weeks after sowing, the plantlets were uprooted and the root system pruned to 3 cm. The pruned roots were dip inoculated with a *Foc* UR-17-8 B8 conidial suspension with 5 x 10^5 conidia ml^{-1}. As a control treatment, roots of each host were dipped in sterile water. After inoculation or water dip, the plantlets were transferred individually into 3 l pots filled with a mix of one third of sterile sand and two thirds (v/v) of natural soil (Typic Hapludert). Pots were kept in the greenhouse, irrigated and fertilized (10% N, 4% P_2O_5, 7% K_2O, 0.2% Mg w/v, plus micro-nutrients) as needed to allow optimal plant

growth and development. Temperature and relative humidity of the greenhouse was recorded with a data logger located at the height of the pots. After three to four months when the plants reached the flowering stage, they were uprooted; the root system was washed in tap water and collected over two sieves (5 mm and 1 mm mesh). The root system of each plant was kept in a plastic pot with water at 4°C until analysis within 1 to 3 weeks. After visual examination for disease symptoms, one representative sample of each root system was used for confirming *Foc* colonization, following the procedure described below for *Foc* quantification in the roots.

In 2011, the second experiment was installed. In March, surface disinfected seeds of 13 *Foc* non-hosts and one Fusarium Basal Rot susceptible onion variety (Table 3) were sown in sterile (autoclaved) peat moss. About three weeks after sowing, the plantlets at the development stage described in the first experiment were carefully uprooted to minimize root damage and transplanted into 3 l plastic pots filled with inoculated and non-inoculated two thirds soil - one third sand (v/v) mixture. With the aid of a cement mixer, the soil-sand mixture was inoculated with a conidial suspension of *Foc* UR 17-8 B8 produced on malt broth with 6 × 10^5 conidia ml^{-1}, and for the non-inoculated treatments we used an autoclaved conidial suspension. The plastic pots with one plant each were placed in the greenhouse under ambient conditions to allow *Foc* natural root infection and colonization. Pots were watered and fertilized (10% N, 4% P_2O_5, 7% K_2O, 0.2% Mg w/v, plus micro-nutrients) as needed for plant growth and development. Temperature and relative humidity of the greenhouse was recorded with a data logger located at the height of the pots .The experimental design was a split-plot design with four blocks with inoculation level (inoculated vs. non inoculated) as main plot and plant species as sub-plot. The experimental unit consisted of a 3 l black plastic pot with one plant per pot, resulting in a total of 112 pots.

At flowering, three to four months after transplanting, plants were uprooted and the roots were washed, collected, and kept at 4°C until analysis within 1 to 3 weeks, as described in the next paragraph. After assessing presence of diseases symptoms, 1/4 of the root system was used for *Foc* quantification in the roots and another 1/4 for root length estimation. A soil sample from each bucket of inoculated and non-inoculated soil at transplanting, and from each pot at harvest was collected for quantification of *Foc* populations in soil through soil dilution plating on Komada's-agar medium amended with benomyl (10 mg l^{-1}).

Quantification of *Foc* UR-17-8 B8-population density in roots was performed by counting the number of *Foc* colony forming units (cfu) after growth on selective media. The washed roots

were cut into small pieces (less than 5 mm) and put into sterile water (200 – 300 ml). An aliquot of 15 ml of the suspension was mixed with 100 ml of molten Komada+benomyl (10 mg l^{-1}), and immediately plated onto 9 cm diameter Petri plates (7 plates). The plates were incubated at 25˚C with a 12 h light/dark cycle for 7 to 10 days and then scored for the total number of *Foc* UR-17-8 B8 colonies across the seven plates. Three aliquots of 15 ml of the root suspension were used for determining root dry weight, by drying the suspension at 105 °C for 48 hrs.

Quantification of root length was done by scanning a quarter of the root system with a flatbed scanner (HP Scanjet 2800 (tma), 4800 optical dpi, 48-bit colour). The acquired images were analyzed for root length (L, mm) with the 'Assess' image analysis software for plant disease quantification (Lamari 2002). Total L was estimated by multiplying measured sample L by four, and volumetric root length density (L_V) in cm of roots per cm^3 of soil was calculated assuming a soil root exploration of 3000 cm^3 (3 l pots). The scanned roots were dried in an oven for 48 h at 105 °C and the total dry weight (W) of each plant was calculated.

2.3 Onion root decomposition

Decomposition rate of Fusarium Basal Rot diseased onion roots was estimated in a pot experiment established in 2011 in a greenhouse located at INIA Las Brujas Research Station, Uruguay. At onion harvest (December 27, 2010), soil and roots were collected from an onion crop that had been planted in a 1.5 m x 2.0 m field plot artificially infested with a conidial suspension of *Foc* UR17-8B8 (3×10^7 conidia ml^{-1}, 11 ml per l of soil,15 cm deep). Fusarium Basal Rot disease incidence was 100% and *Foc* root colonization was 1.1 x 10^4 cfu g^{-1} dry root. Soil was collected from the centre of the raised beds in the microplot and sieved through a 1 cm mesh. Roots were cut from the harvested bulbs, shaken vigorously to remove organic residues and soil clumps, and kept in a plastic bag at 5˚C till the following day. Nearly two grams of roots were placed into 8 by 6 cm plastic litter bags (aphid net, 50 mesh) and buried in 500 ml plastic pots filled with the sieved non-inoculated soil used in the second *Foc* infection experiment, with only one bag per pot. Pots were placed in the greenhouse and watered twice a month by replacing the evaporated water to approximately field capacity. Temperature and relative humidity of the greenhouse was recorded with a data logger located at the height of the pots. The experimental design was a completely randomized block with 4 repetitions for each of 13 sampling dates. On days 1, 7, 11, 14, 21, 28, 44, 71, 84, 98, 133, 177 and 213, four bags were dug up, and root fresh and dry weights were measured. Dry weight was determined after drying the roots at 105 °C for 72 hr.

The experimental data were used to estimate the parameters of a model that describes root decomposition (Silver and Miya, 2001):

$$y = y_0 * e^{(-bt)} \quad (1)$$

with y_0 = initial amount of roots (dry weight, in g), b = relative rate of decomposition of onion roots (day^{-1}) and t = time (day).

2.4 Population model

A simple population model that relates the inoculum level at the end of the cropping season (final level, *Pf*) to the inoculum level at the start (initial level, *Pi*), was used (van den Berg and Rossing, 2005):

$$Pf = Pi/(\propto + \beta Pi) \quad (2)$$

where 1/α represents the slope of the curve at the origin or the rate of population increase at low *Pi*, and 1/β the horizontal asymptote or the carrying capacity of the crop for the pathogen. The 2011 experimental data on multiplication of *Foc* in different plant species were used to estimate the parameters of equation (2). *Pi* was taken to be the *Foc* population density in the soil (cfu g^{-1} dry soil) at the start of the experiment. *Pf* was defined as the sum of the final *Foc* population density in the soil (cfu g^{-1} dry soil) and the final *Foc* population in the roots (cfu g^{-1} dry root), representing the potential *Foc* population density after complete decomposition of the infected tissues.

2.5 Simulation of *Foc* population dynamics

Using the calibrated models, we simulated *Foc* soil dynamics in repetitive simple crop sequences. The simulations were carried out for a range of initial values (*Pi*) from 5 x 10^1 to 5 x 10^4 cfu g^{-1} dry soil commonly encountered in onion fields (Abawi and Lorbeer 1972). The simulations were applied to sequences of onion – fallow (O-F), onion – summer green manure (O-SGM), onion followed by three summer and winter green manures (O-SGM-WGM-SGM), onion followed by tomato and then two green manures (O-T-WGM-SGM) or onion followed by two green manures and then tomato (O-WGM-SGM-T).

First *Foc* soil dynamics was simulated concatenating equation (2). The simulation starts with

the given *Foc* densities for the first crop ($Pi_{(1)}$), and then initial inoculum level of crop *n* ($Pi_{(n)}$) was equal to the final inoculum level of crop *n* ($Pf_{(n-1)}$). For these simulations, the following assumptions were made: (i) when the crop ends, all infected roots are immediately decomposed and release all *Foc* infective units, which are ready for starting a new infection process; (ii) *Foc* multiplication in plant roots is the result of the infections that occurred at transplant/seeding, no later infections are considered; (iii) *Foc* population remains constant during the intercrop periods (fallow, soil preparation or green manure decomposition); and (iv) the potential decline of *Foc* population due to predators, antagonists or toxins is not considered.

Second, *Foc* soil dynamics was simulated considering that *Foc* release is proportional to root decomposition, and the root decomposition and pathogen release processes occur simultaneously (Figure 1). The three last assumptions of the previous simulations also apply here. $Pi_{(n)}$ values were calculated according to the following equation:

$$Pi_{(n)} = \sum_{j=1}^{j=j} Pf_{(n-j)} * FDR_{(n-j)} \quad (3),$$

where $Pf_{(n-j)}$ is calculated applying equation 2 where *j* indicates the number of preceding crops that contribute with *Foc* infective units, and FDR is the fraction of decomposed roots calculated as:

$$FDR_{(n)} = y_{(n)} - y_{(n)}e^{(-bt)} \quad (4),$$

where $y_{(n)}$ is the relative amount of roots at time n, *t* is the time in days and *b* values are 0.0017 day^{-1} for Fusarium Basal Rot diseased onion roots (this work), 0.0038 day^{-1} for roots of gramineous plants and 0.0012 day^{-1} for roots of broad leaf plants (Silver and Miya 2001). According to these *b* values, the mean time for root decomposition (1/*b*) for onions is approximately 19 months, 12 months for gramineous plants and 24 months for broad leaf species.

2.6 Data analysis

All statistical analyses were performed with R software version 2.12.2 (R Development Core Team 2011). *Foc* root population density (*Foc*, cfu g^{-1} dry soil), plant root length (L, cm), plant root length density (L_V, cm cm^{-3}) and plant root dry weight (W, g) were analyzed by split-plot ANOVA.

Plant species were grouped according to their ability to allow *Foc* multiplication by hierarchical agglomerative cluster analysis with the packages “stats” and “pvclust” (Suzuki and Shimodaira 2011). The variables analysed were: *Foc* cfu g^{-1} dry soil, *Foc* cfu $plant^{-1}$ and *Foc* cfu ha^{-1}. Clusters were defined when the approximately unbiased *P*-values (AU *P*-value) were > 0.95, and AU *P*-values were calculated using a multiscale bootstrap resampling technique.

Parameters α and β of equation (2) for each plant species were estimated by Bayesian non-linear regression with un-informative prior distributions, using (0.1, 10) as acceptable interval for $1/\alpha$ and (10^2, 10^5) for $1/\beta$, and the DE-MC-ZS sampler algorithm (ter Braak and Vrugt 2008). The median of the posterior probability distribution for α and β was selected as the best estimate, since it was more stable than the mean.

The relative rate of onion root decomposition (*b*) of equation (2) was estimated by non-linear regression with the packages "lattice" (Sarkar 2008) and "car" (Fox and Weisberg 2011).

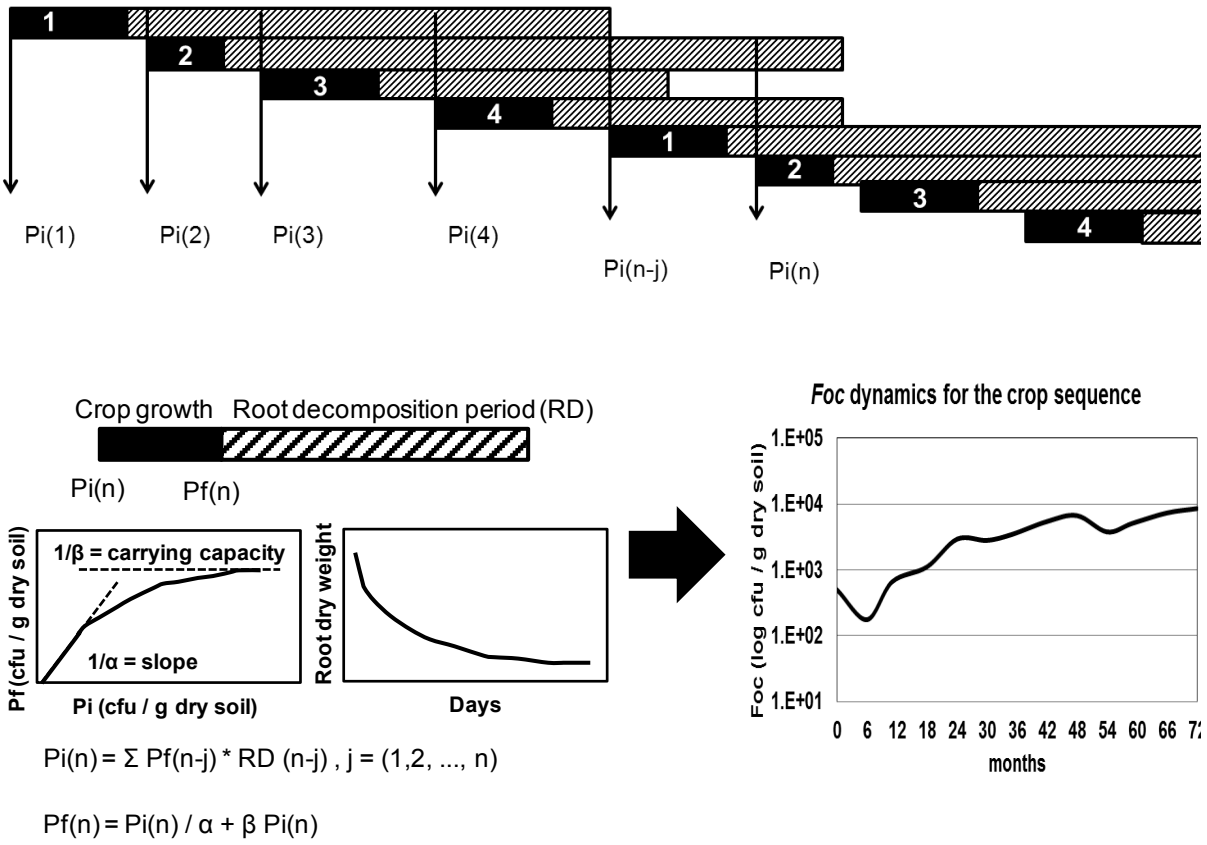

Figure 1. Schematic representation of a crop sequence and the processes involved in soil *Foc* dynamics: pathogen multiplication during crop growth and pathogen release after root decomposition.

3 Results

3.1 Evaluation of the ability of different plant species to host *Foc*

Both in 2009 and 2011 during the first months of the experiment, air temperature and relative humidity were within the range needed for the pathogen to colonize plant roots, between 24 to 27 ˚C and 60 to 70% relative humidity (Abawi and Lorbeer 1972; Özer and Köycü 2004) (Table 1). All plant species were colonized by *Foc* and facilitated multiplication of the pathogen, however only onions developed disease symptoms confirming the *formae speciales* of the pathogen *Foc* UR17-8B8.

Foc inoculum level did not influence root dry weight (W), root length (L) or root length density (L_V) of any of the plant species tested, and plant species was the only significant treatment effect for these variables ($P < 0.001$) (Table 2). Among the plant species tested, onion had the lowest root weight and length density (W = 0.36 ± 0.03 g and L_V = 1.31 ± 0.07 cm cm^{-3}). In contrast, grasses had an average value of W = 3.21 ± 0.81 g and L_V = 10.6 ± 1.35 cm cm^{-3}, and broad leaf species had intermediate values with an average of W = 1.30 ± 0.26 g and L_V = 4.32 ± 1.34 cm cm^{-3} (Table 3).

In 2009, when the roots of plants were clipped, only the inoculation level was significant (F value = 1189.44, $P > F = 0.018$), nevertheless, the lowest *Foc* levels were found in red clover, wheat and soybean, whereas the highest were in tomato, sudangrass, and sweet corn.

In 2011, when roots were not damaged, the inoculation level, the plant species and their interaction were significant (Table 2). The lowest *Foc* levels per g of dry weight of root were found in wheat, sunflower, cowpea and millet, whereas the highest *Foc* density developed in black bean (Table 3). Cluster analysis for *Foc* colonization identified three groups of species: A) black beans, B) onion, oat, black oat, white lupine, blue lupine, tomato, sudangrass, corn and sweet corn; and C) wheat, millet, sunflower and cowpea. The group means were 4.3 x 10^4, 1.1 x 10^4 and 4.0 x 10^2 *Foc* cfu g^{-1} dry root for A, B and C respectively (Table 3).

Combining *Foc* levels per g root dry weight with plant specific root weight and root density resulted in estimates of total inoculum production and potential release into the soil (Table 3). Sudangrass was the species that allowed the highest *Foc* multiplication per plant (7.49 x 10^4 cfu $plant^{-1}$), followed by the group of oat, black oat, white lupine, black bean, corn, sweet corn and tomato with a group mean of 3.11 x 10^4 cfu $plant^{-1}$. The group with the highest

production of *Foc* per ha comprised black oat and sudangrass, with a group mean of 2.2 x 10^{11} cfu ha^{-1} (Table 3).

Table 1. Average, maximum and minimum air temperature (˚C) and relative humidity (RH, %), registered in the greenhouse during the experiments conducted for the evaluation of the ability of different plant species to host *Foc.* The information was recorded with a data logger located at the height of the pots.

	February		March		April		May		June		July	
	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)	Temp (˚C)	RH (%)
						2009						
Average	24.7	58.3	22.4	67.5	19.8	60.1	16.9	62.4	13.3	67.4	11.2	70.5
Max.	41.0	93.1	37.8	95.0	38.1	96.2	40.0	94.5	35.1	96.4	31.6	97.7
Min.	11.8	20.7	11.1	20.7	5.7	20.7	3.2	20.7	-0.8	20.7	-3.1	20.7
						2011						
Average	24.8	58.6	22.9	58.1	19.5	63.3	18.9	57.3	15.8	65.5	13.5	66.8
Max.	39.8	96.2	39.9	92.9	36.7	96.7	35.3	98.1	32.9	98.9	35.5	98.7
Min.	12.3	20.7	8.0	20.7	5.7	20.7	12.5	20.7	2.6	20.7	-1.7	20.7

Table 2. Analysis of variance for the variables *Fusarium oxysporum* f.sp. *cepae* root colonization (*Foc,* cfu g^{-1} dry root), root dry weight (W, g), root length (L, mm) and root length density ($L_{V,}$ cm cm^{-3}) for the greenhouse experiment conducted in 2011 to evaluate the ability of different plant species to host *Foc*, showing *F* values and levels of significance for each source of variation and each dependent variable, respectively. The experiment was analyzed as a split plot design with inoculum as main plot and plant species as sub-plot.

		Dependent variables							
		Foc		**W**		**L**		**L_V**	
Source of variation	**df[a]**	**F values**	***P >F***	**F values**	***P >F***	**F values**	***P >F***	**F values**	***P >F***
Inoculum [b]	1	574.29	0.027	3.80	0.302	1.42	0.445	1.42	0.445
Plant species	13	7.86	<0.001	33.02	<0.001	22.54	<0.001	22.54	<0.001
Inoculum x plant species	13	7.62	<0.001	1.64	0.100	1.01	0.454	1.01	0.454

[a] degrees of freedom

[b] Plantlets were transplanted into soil-sand mixture non-inoculated or inoculated with a conidial suspension of *Foc* UR 17-8 B8 with 6 × 10^5 conidia ml^{-1}.

Table 3. Plant species root dry weight (W), root length density (L_V), *Fusarium oxysporum* f.sp. *cepae* root colonization and potential release of the pathogen into the soil (total *Foc* production), for the greenhouse experiment conducted in 2011 to evaluate the ability of different plant species to host *Foc*.

Plant species [a]	Root dry weight (W) (g plant^{-1})	Root length density (L_V) (cm cm^{-3})	*Foc* root colonization (cfu g^{-1}dry root)	Total *Foc* production (cfu plant^{-1}) [b]	(cfu ha^{-1}) [c]
Onion INIA Dulce (*Allium cepa* L.)	0.36 [d]	1.31 [d]	2.09 x 10^4 [d] B [e]	7.57 x 10^3 C [e]	1.89 x 10^9 C [e]
Oat (*Avena sativa* L.)	2.30	10.18	1.00 x 10^4 B	2.32 x 10^4 B	5.56 x 10^{10} B
Black oat (*Avena strigosa* Schreb.)	2.35	13.39	1.96 x 10^4 B	4.67 x 10^4 B	2.38 x 10^{11} A
Wheat (*Triticum aestivum* L.)	2.39	12.43	1.09 x 10^3 C	2.41 x 10^3 D	5.18 x 10^9 C
Blue lupine (*Lupinus angustifolius* L.)	1.11	1.43	7.08 x 10^3 B	8.12 x 10^3 C	3.25 x 10^9 C
White lupine (*Lupinus albus* L.)	0.92	2.26	9.15 x 10^3 B	1.20 x 10^4 B	2.03 x 10^9 C
Tomato (*Solanum lycopersicum* L.)	2.58	10.66	1.27 x 10^4 B	2.90 x 10^4 B	9.58 x 10^8 C
Sunflower (*Helianthus annuus* L.)	1.24	3.68	1.34 x 10^3 C	1.58 x 10^3 D	4.59 x 10^8 C
Black bean (*Phaseolus vulgaris* L.)	0.96	4.31	4.33 x 10^4 A	4.26 x 10^4 B	3.83 x 10^{10} B
Cowpea (*Vigna unguiculata* L. Walp.)	0.98	3.60	1.63 x 10^3 C	1.72 x 10^3 D	2.99 x 10^8 C
Sweet corn (*Zea mays* L.)	2.98	9.99	6.89 x 10^3 B	2.00 x 10^4 B	2.00 x 10^9 C
Corn (*Zea mays* L.)	4.24	11.77	1.12 x 10^4 B	4.38 x 10^4 B	5.70 x 10^9 C
Foxtail millet (*Setaria italica* (L.) P. Beauvois)	0.73	3.12	3.03 x 10^3 C	2.47 x 10^3 D	2.18 x 10^{10} B
Sudan grass (*Sorghum × drummondi* (Steud.) Millsp. & Chase)	7.46	13.32	9.64 x 10^3 B	7.49 x 10^4 A	2.02 x 10^{11} A
Non-inoculated plants			---------- C [f]	---------- D	---------- C
Mean of Group A			4.33 x 10^4	7.49 x 10^4	2.20 x 10^{11}
Mean of Group B			1.06 x 10^4	3.11 x 10^4	3.86 x 10^{10}
Mean of Group C			4.00 x 10^2	7.85 x 10^3	9.91 x 10^8
Mean of Group D				4.71 x 10^2	

[a] The seedlings were transplanted into inoculated and non-inoculated soil, and W, L_V and *Foc* colonization were evaluated at the flowering stage. [b] cfu plant^{-1}= *Foc* root colonization (cfu g^{-1} dry root) x plant root dry weigh (g). [c] *Foc* cfu ha^{-1}= cfu plant^{-1}x planting density (plants ha^{-1}). Values for planting density are typical for Uruguay. [d] Means of four repetitions. [e] Letters in the column define the groups according to agglomerative hierarchical cluster analysis using Euclidean distances and Ward's, cluster method. Groups were defined when AU p-values ≥ 0.95) . [f] All non-inoculated treatments are presented together. *Foc* root colonization due to natural infection was zero for onion, oat, blue lupine, black beans, cowpea, foxtail millet and sudangrass. *Foc* root colonization ± standard error in cfu g dry root^{-1} were 38 ± 38 for black oat, 7 ± 7 for wheat, 10 ± 10 for white lupine, 4 ± 4 for tomato, 18 ± 18 for sunflower, 7 ± 4 for sweet corn and 2 ± 2 for corn.

3.2 Onion root decomposition

Dry weight of onion root biomass in the litter bags was well described by the negative exponential model, with a relative decomposition rate b = - 0.0017 day^{-1} ± 0.0004 s.e. (P < 0.001) (Figure 2).

During the experiment, soil moisture in the pots was maintained near field capacity, while mean air temperatures and relative air humidity in the greenhouse ranged from 26.6°C and 55% in January, to 10.8°C and 69% in August. Mean field climatological records for Southern Uruguay for the period January – August were: precipitation between 75 to 78 mm per month, mean air temperatures and air relative humidity going from 23°C and 75% to 10°C and 78% respectively (INIA 2012).

3.3 Population model

Median values for $1/\alpha$, the rate of population increase at low *Pi*, were largest for onion (7.98) and black oat (7.89) and smallest for sunflower (1.15). Values for the population maximum $1/\beta$, the horizontal asymptote of the model and indicative of the crop carrying capacity for the pathogen, were largest for black oat (6.44×10^4 cfu g^{-1} dry soil) and black bean (5.54×10^4 cfu g^{-1} dry soil), and smallest for wheat (1.20×10^3 cfu g^{-1} dry soil), cowpea (2.48×10^3 cfu g^{-1} dry soil) and foxtail millet (3.99×10^3 cfu g^{-1} dry soil) (Table 4).

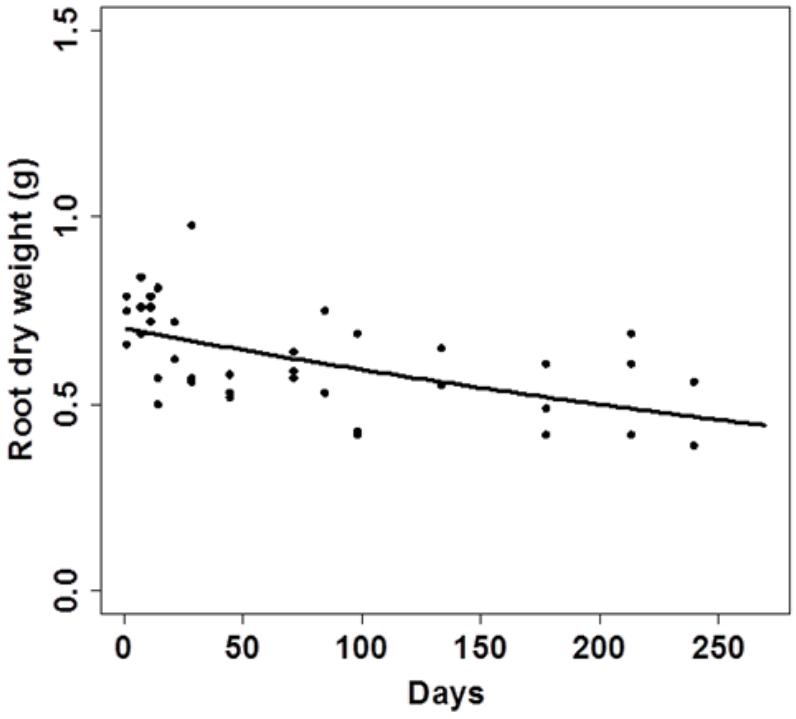

Figure 2. Decrease of onion root dry weight in litter bags over time in a greenhouse pot experiment. Roots were put in plastic litter bags (50 mesh) on day 0, and dug up during seven months. The line represents the fitted negative exponential model $y_t = y_0\, e^{-bt}$, with y_0= 0.703 g ± 0.027 s.e. and b = 0.0017 days-1 ± 0.0004 s.e.

Table 4. Median of the estimated parameters *1/α* and *1/β* (cfu g^{-1} dry soil) of the population model *Pf = Pi/(α + β Pi)* for fourteen plant species. Parameters were estimated by Bayesian non-linear regression with un-informative prior distributions.

Crop	1/α	1/β	AP [a]
Onion	7.98	3.22×10^4	0.27
Oat	5.10	1.92×10^4	0.26
Black oat	7.89	6.44×10^4	0.29
Wheat	6.05	1.20×10^3	0.21
Blue lupine	3.66	1.24×10^4	0.20
White lupine	3.19	2.52×10^4	0.25
Tomato	6.23	4.83×10^4	0.33
Sunflower	1.15	3.16×10^4	0.19
Black bean	6.29	5.54×10^4	0.32
Cowpea	4.01	2.48×10^3	0.13
Sweet corn	4.53	4.31×10^4	0.31
Corn	5.86	4.79×10^4	0.34
Foxtail millet	5.39	3.99×10^3	0.21
Sudangrass	5.68	4.52×10^4	0.34

[a] Acceptance Probability. AP reference rates are 0.4 for *d*=1 and 0.28 for *d*=5, where *d*= number of estimated parameters of the posterior distribution (ter Braak and Vrugt, 2008)

3.4 Simulation of *Foc* population dynamics

Two sets of simulation runs were carried out, differing in the way that root decomposition and associated release of the pathogen infective units was considered. For the first runs, instantaneous and complete release of *Foc* was assumed at the end of the cropping season. For the second set of runs, *Foc* release was assumed to be proportional to root decomposition.

The first simulations of the onion-summer green manure sequences showed that *Foc* soil densities reached the carrying capacity of the individual crops after 3 cycles, irrespective of initial inoculum level or summer green manure species. For the onion – fallow sequence, the pathogen population reached the carrying capacity after 4 years when the simulation started at the lowest inoculum level (5×10^2 cfu g^{-1} dry soil) (Figure 3).

Among the summer green manures, cowpea and foxtail millet resulted in important decreases in the pathogen population (Figure 3), reducing population densities from 1.2 x 10^4 to 2.4 x 10^3 cfu g^{-1} dry soil in the case of cowpea, and from 1.6 x 10^4 to 3.8 x 10^3 cfu g^{-1} dry soil in the case of foxtail millet. For sunflower, the reduction in inoculum was less obvious, from 2.5 x 10^4 to 1.5 x 10^4 cfu g^{-1} dry soil.

For the 2-year sequences the steady states were reached after two cycles (4 years), and soil *Foc* population densities were different for the different crop sequences. When foxtail millet, wheat and cowpea were selected as GM following an onion crop, the *Foc* soil population at the start of a new onion crop was 67% lower than in the sequence with sudangrass, black oat and black beans (Figure 4A and 4B, Table 5), with final values of 1.6 x 10^3 and 4.9 x 10^3 cfu g^{-1} dry soil, respectively.

When tomato (T) was included in the sequence and the GM were wheat (W) and foxtail millet (FM), the *Pi* for a new onion crop was 2.5 x 10^3 cfu g^{-1} dry soil for the sequence O-T–W–FM and 6.2 x 10^3 cfu g^{-1} dry soil for O-W-FM-T. But if black oat and sudangrass were used as GM, the final *Foc* values were one order of magnitude greater (Figure 4, Table 5).

When the effect of root decomposition was considered, and therefore the gradual release of the pathogen, the time needed to reach the equilibrium increased to 6 years (Figure 5) for the Onion – GM sequences. For the monoculture (onion - fallow), the steady state was around 1.1 x 10^4 cfu g^{-1} soil. When including the GM in the sequence, the equilibrium values at the time of starting a new onion crop were above those attained with the monoculture for the sequences onion – sweet corn and onion – sudangrass, with values of 2.1 and 2.4 x 10^4 cfu g^{-1} soil respectively (Figure 5, Table 4). On the other hand, smaller *Pi* values were obtained with the sequence onion – cowpea, with values of 9.4 x 10^3 cfu g^{-1} soil (Figure 5, Table 5).

For the 2-year sequences the steady states were reached after three cycles (6 years), except for the simulations that started at very low inoculum levels (Figure 6). Again, the sequences that included foxtail millet and wheat as GM, resulted in pathogen populations at least three times smaller than the ones with sudangrass and black oat (Table 5).

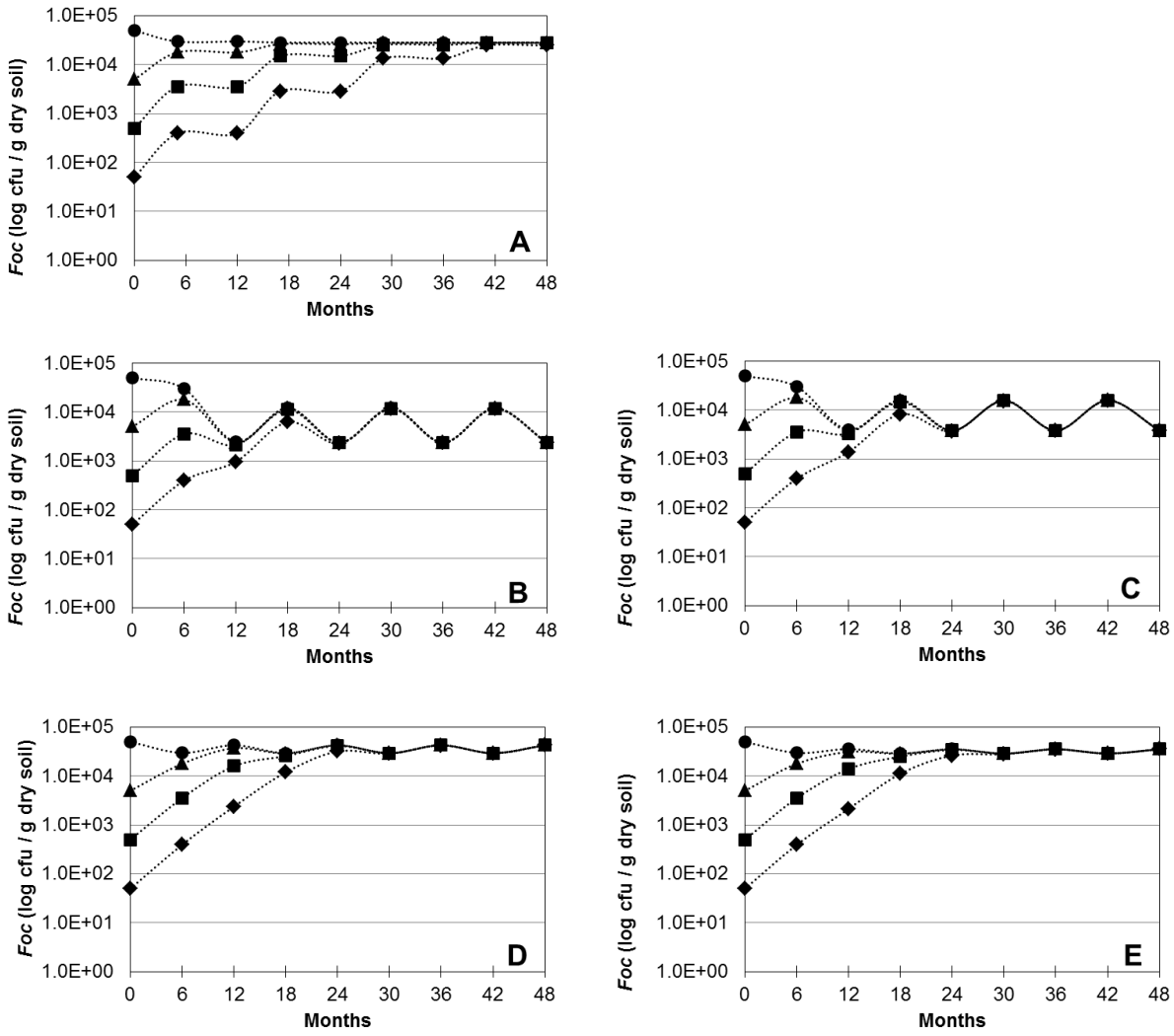

Figure 3. Dynamics of *Fusarium oxysporum* f.sp. *cepae* soil populations (log cfu g^{-1} dry soil) for different onion – summer green manure crop sequences, simulated over four years assuming complete *Foc* release at the end of the cropping season ($Pi_{(n)} = Pf_{(n-1)}$,). Sequence (A) onion – fallow, (B) onion – cowpea, (C) onion – foxtail millet, (D) onion – black bean, and (E) onion – sudangrass. Symbols are estimated *Pi* values for each crop: diamonds – $Pi_{(0)} = 5 \times 10^1$ cfu g^{-1} dry soil , squares – $Pi_{(0)} = 5 \times 10^2$ cfu g^{-1} dry soil , triangles – $Pi_{(0)} = 5 \times 10^3$ cfu g^{-1} dry soil, and dots – $Pi_{(0)} = 5 \times 10^4$ cfu g^{-1} dry soil.

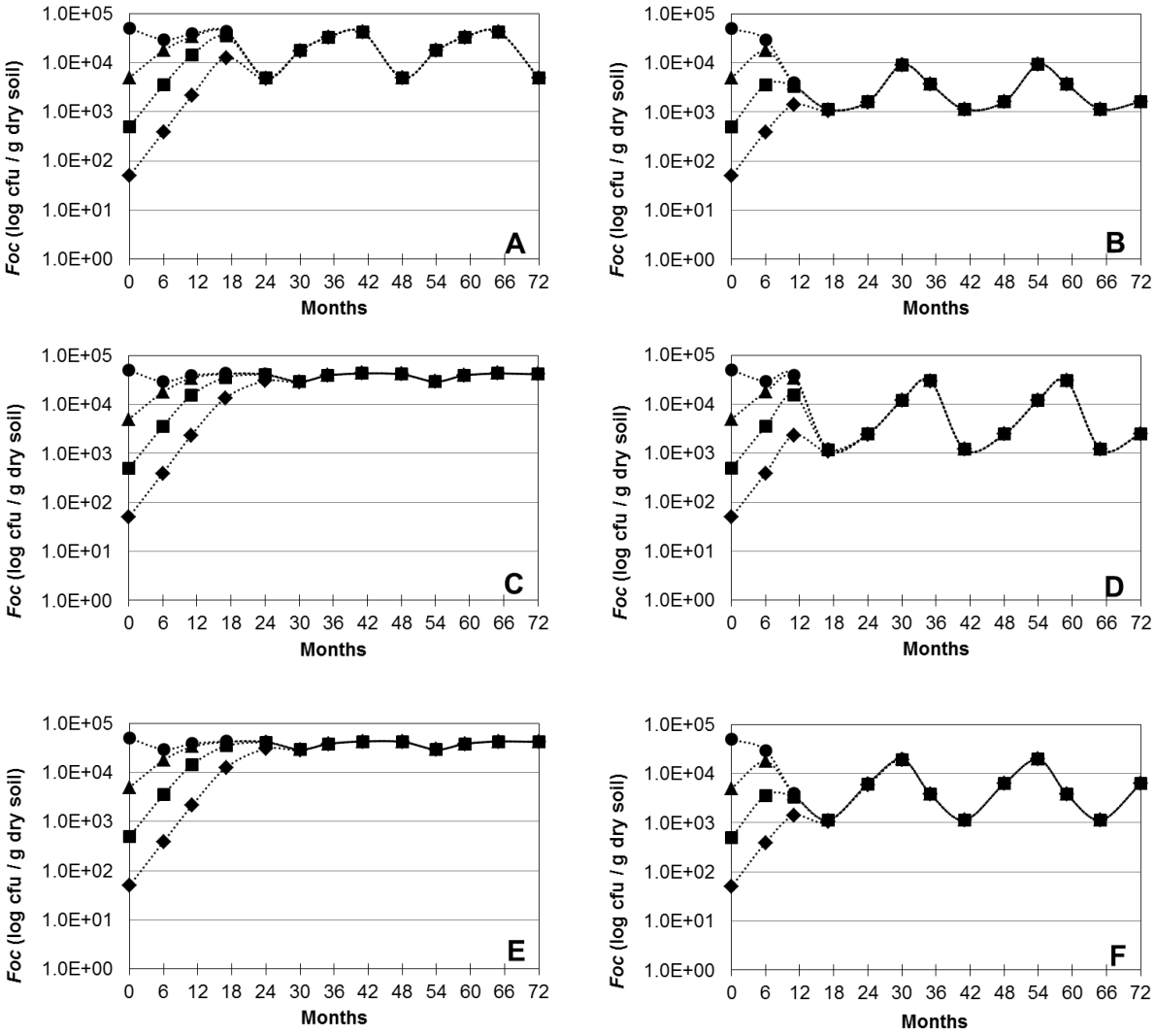

Figure 4. Dynamics of *Fusarium oxysporum* f.sp. *cepae* soil populations (log cfu g^{-1} dry soil) for different crop sequences, simulated over six years assuming complete *Foc* release at the end of the cropping season ($Pi_{(n)} = Pf_{(n-1)}$,). Sequence (Aa) onion – sudangrass – black oat – black beans, (B) onion – foxtail millet – wheat – cowpea, (C) onion – tomato – black oat – sudangrass, (D) onion – tomato – wheat – foxtail millet, (E) onion – sudangrass – black oat – tomato, and (F) onion – foxtail millet – wheat – tomato. Symbols are estimated *Pi* values for each crop: diamonds – $Pi_{(0)} = 5 \times 10^1$ cfu g^{-1} dry soil , squares – $Pi_{(0)} = 5 \times 10^2$ cfu g^{-1} dry soil, triangles – $Pi_{(0)} = 5 \times 10^3$ cfu g^{-1} dry soil, and dots – $Pi_{(0)} = 5 \times 10^4$ cfu g^{-1} dry soil.

Table 5. Simulated *Pi* values (cfu g^{-1} dry soil) at the start of a new onion crop when steady states were reached, for different crop sequences.

Crop sequence	simulation 1 [a]	simulation 2 [b]
Onion – Fallow	2.8×10^4	1.1×10^4
Onion - Foxtail Millet	3.8×10^3	1.0×10^4
Onion – Sudangrass	3.5×10^4	2.3×10^4
Onion – Sweet Corn	3.2×10^4	2.1×10^4
Onion – Cowpea	2.4×10^3	9.4×10^3
Onion – Black bean	4.3×10^4	2.1×10^4
Onion – Sunflower	1.5×10^4	1.1×10^4
Onion – Foxtail millet – Wheat – Cowpea	1.6×10^3	8.4×10^3
Onion – Sudangrass – Black oat – Black beans	4.9×10^3	2.9×10^4
Onion – Tomato – Wheat – Foxtail millet	2.5×10^3	1.0×10^4
Onion – Tomato – Black oat – Sudangrass	4.2×10^4	3.7×10^4
Onion – Foxtail millet – Wheat – Tomato	6.2×10^3	7.8×10^3
Onion – Sudangrass – Black oat – Tomato	4.2×10^4	2.5×10^4

[a] Simulation 1: complete *Foc* release at the end of the cropping season and $Pi_{(n)} = Pf_{(n-1)}$. [b] Simulation 2: *Foc* release proportional to root decomposition and $Pi_{(n)} = \sum_{j=1}^{j=j} Pf_{(n-j)} * FDR_{(n-j)}$

4 Discussion

4.1 Evaluation of the ability of different plant species to host *Foc*

Foc colonized and multiplied in the root systems of all plant species tested, to varying extent, without inducing disease symptoms or growth retardation as previously reported for *Oxalis corniculata* (Abawi and Lorbeer 1972, Brayford 1996). The ability of different plant species to allow multiplication of particular *formae speciales* of *F. oxysporum,* while remaining symptomless, is well documented for the *Fusarium oxysporum* group (Banihashemi and de Zeeuw 1975, Bishop and Cooper 1983, Dhingra and Coelho Netto 2001, Gordon et al. 1989, Helbig and Carroll 1984, Katan 1971, Leslie et al. 1990, Leslie and Summerell 2006, Oritsejafor and Adeniji 1990).

Following Dhingra and Cohelo-Neto (2001) we conclude that all the plant species tested are "reservoir-hosts" for *Foc,* even though the extent of *Foc* multiplication differed for each plant species (Tables 2 and 3). Multiplication of soilborne pathogens on "reservoir-hosts" depends not only on their capacity to colonize the cortex of roots but also on their ability to benefit

from particular root exudates (Edel et al. 1997, Hartmann et al. 2009, Mazzola 2007). For example, corn is a good multiplier for *F. oxysporum* f.sp. *elaedis* (Oritsejafor and Adeniji 1990), an intermediate multiplier for *Foc* (Table 3) and a poor multiplier for *F. oxysporum* f.sp. *phaseoli* (Dhingra and Coelho Netto 2001)

4.2 Population model

To explore the potential contribution of different plant species to *Foc* inoculum build-up in the soil, we successfully used a population model (equation 2) that had been used to describe the nematode dynamics for a sequence of different crops (van den Berg and Rossing, 2005). Parameters α and β were estimated with measured *Foc* populations in plant roots and soil from the experiment in 2011. The inoculation method used in that experiment (transplant intact plantlets into inoculated soil) provided more useful information than the preliminary experiment (with pruned roots dipped in a *Foc* suspension) about the ability of the pathogen to colonize the roots of different plant species, because the natural barriers of the rhizosphere and rhizoplane were present. Therefore, the generated data were suitable for fitting the population model proposed by van den Berg and Rossing (2005) (equation. 2).

The population model provides an indication of the maximum expected *Foc* soil densities after one particular crop. In the case of onions, the estimated carrying capacity ($1/\beta$ = 3.22 x 10^4 cfu g^{-1} dry soil) was similar to *Foc* densities found by Abawi and Lorbeer (1972) in composite soil samples from inoculated soil (5 x 10^4 cfu g^{-1} dry soil). However, the same authors reported that in commercial onion farms, *Foc* densities ranged from 3.0 x 10^2 to 6.5 x 10^3 cfu g^{-1} dry soil.

Indeed, model predictions of the final soil inoculum levels (*Pf*) could have overestimated the actual inoculum levels, because pathogen mortality was not considered. It was assumed that 100% *Foc* propagules survived after decay of the roots and subsequent release of the pathogen from the infected tissue. It is known that during root decomposition biotoxic compounds can be released which potentially affect *Foc* survival, either lethally or sub-lethally by weakening the chlamydospores. Various biotoxic compounds have been reported to be released by decaying plants. Sudangrass releases cyanoglucoside and isothiocyanates (Abawi and Widmer 2000), oats release saponins (avenacoside) flavonoids, phytoalexins and avenanthramides (hydroxycinnamic acid amide) (Bahraminejad et al. 2008), and various *Brassica* species release isothiocyanates after hydrolysis of aliphatic and aromatic glucosinolates (Matthiessen and Kirkegaard 2006).

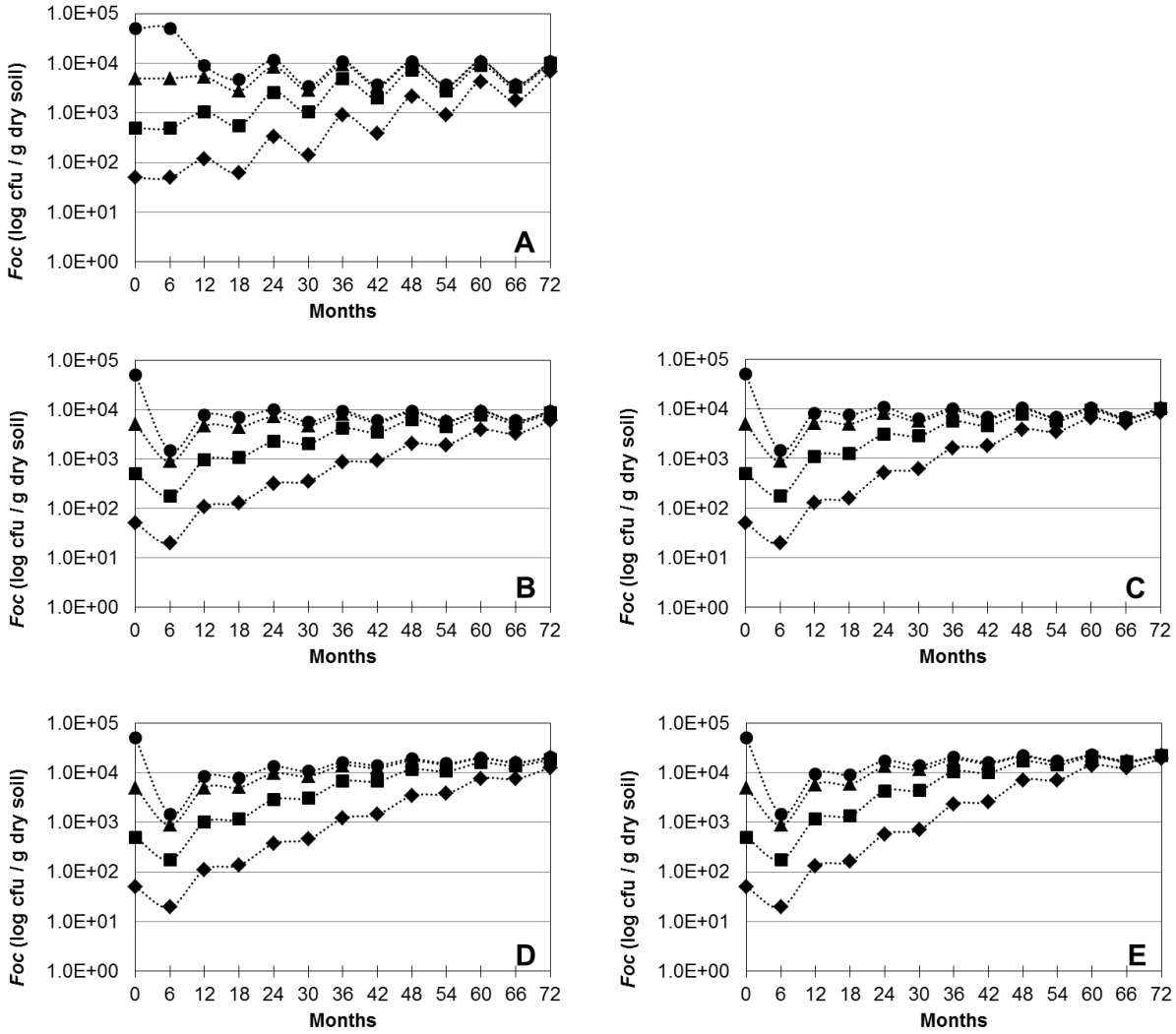

Figure 5. Dynamics of *Fusarium oxysporum* f.sp. *cepae* soil population (log cfu g^{-1} dry soil) for different onion-fallow or onion – green manure sequences simulated over six years assuming *Foc* release proportional to root decomposition ($Pi_{(n)} = \sum_{j=1}^{j=j} Pf_{(n-j)} * FDR_{(n-j)}$). Sequence (A) onion – fallow, (B) onion – cowpea, (C) onion – foxtail millet, (D) onion – black bean, and (E) onion – sudangrass. Symbols are estimated *Pi* values for each crop: diamonds – $Pi_{(0)}$ = 5 x 10^1 cfu g^{-1} dry soil , squares – $Pi_{(0)}$ = 5 x 10^2 cfu g^{-1} dry soil , triangles – $Pi_{(0)}$ = 5 x 10^3 cfu g^{-1} dry soil, and dots – $Pi_{(0)}$ = 5 x 10^4 cfu g^{-1} dry soil.

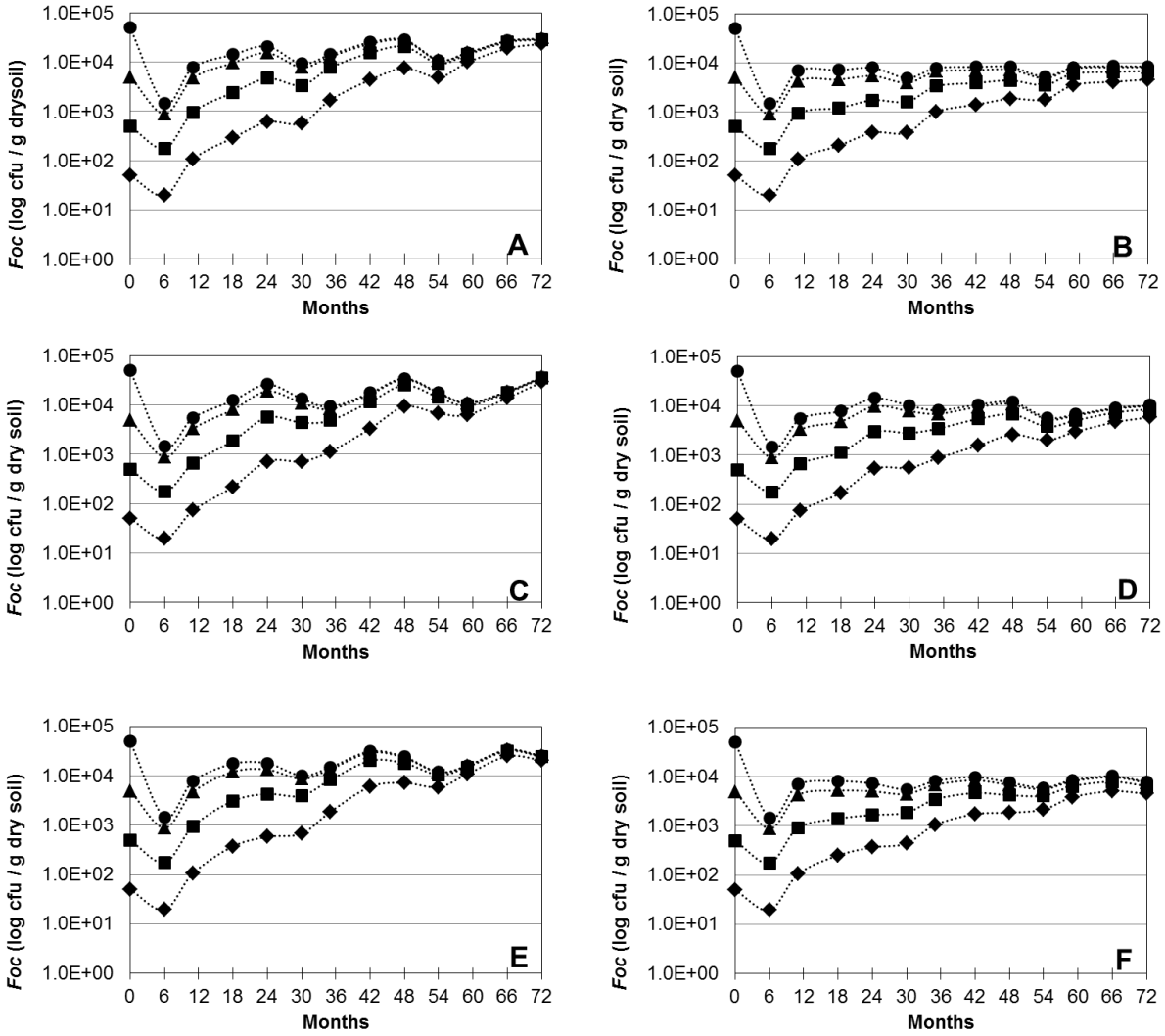

Figure 6. Dynamics of *Fusarium oxysporum* f.sp. *cepae* soil population (log cfu g^{-1} dry soil) for different 2-years crop sequences simulated over six years assuming *Foc* release proportional to root decomposition ($Pi_{(n)} = \sum_{j=1}^{j=j} Pf_{(n-j)} * FDR_{(n-j)}$). Sequence (A) onion – sudangrass – black oat – black beans, (B) onion – foxtail millet – wheat – cowpea, (C) onion – tomato – black oat – sudangrass, (D) onion – tomato – wheat – foxtail millet, (E) onion – sudangrass – black oat – tomato, and (F) onion – foxtail millet – wheat – tomato. Symbols are estimated *Pi* values for each crop: diamonds – $Pi_{(0)}$ = 5 x 10^1 cfu g^{-1} dry soil , squares – $Pi_{(0)}$ = 5 x 10^2 cfu g^{-1} dry soil , triangles – $Pi_{(0)}$ = 5 x 10^3 cfu g^{-1} dry soil, and dots – $Pi_{(0)}$ = 5 x 10^4 cfu g^{-1} dry soil.

In addition, soil microbial and microfaunal communities, as affected by plant species, likely contribute to a decline in real *Foc* propagules before they can infect a new host (Hartmann et al. 2009). *Foc* survival can be affected by the release of biotoxic compounds (Abawi and

Widmer 2000, Smolińska 2000) and the promotion of antagonistic communities like fluorescent pseudomonads, spore-forming bacteria, *Trichoderma* spp., and earthworms (Elmer 2009, Smolińska 2000, Vinale et al. 2008). Thus, a "non-host" as well as a "reservoir-host" crop could not only affect the inoculum density of *Foc* but also its inoculum potential.

4.3 *Foc* population dynamics

Using the calibrated model, we explored *Foc* soil population dynamics in repetitive simple crop sequences by concatenating *Pi-Pf* values, either considering instantaneous (equation 2) or gradual (equation 3) pathogen release at the end of the cropping season. In both cases, different soil *Foc* population values were attained after reaching the steady states (Table 5; Figures 3 to 6).

The wide range of plant species tested here differed in their ability to facilitate multiplication of *Foc* and therefore in their contribution to inoculum build-up and potential risk for disease development and yield losses (Goud et al. 2011, Lucas 2006). Thus crops cannot be grouped just according to standard agronomic criteria, which distinguish grasses from legumes, or from Brassicaceae. A more refined grouping is needed in designing Fusarium Basal Rot-suppressive crop rotations as demonstrated by our simulations. Similar results were found for other soilborne pathogens. *Fusarium oxysorum* f.sp. *phaseoli* multiplied more in *Crotolaria spectabilis* than in *C. juncea*, and equally in *C. juncea* and *Sorghum bicolor* (Dhingra and Coelho Netto 2001). Disease severity of *Rhizoctonia solani* on sugar beet was more severe after maize than after wheat or oat, while the last two crops were considered "non-hosts" for the pathogen (Buhre et al., 2009). The risk of wheat take-all (*Gaeumannomyces graminis* var. *tritici*), sharp eyespot (*Rhizoctonia cerealis*) and eyespot (*Pseudocercosporella herpotrichoides*) increased after wheat and barley, decreased after the non-host crops alfalfa, peas and sunflower, and had an intermediate effect after maize (Colbach et al. 1994).

When pathogen dynamics were simulated accounting for root decomposition and the associated *Foc* release using our simple model (equation 1), the results were more realistic compared to the model with instantaneous release of all propagules. The relative onion root decomposition rate (parameter *b*) was estimated in this work, while the average relative rates used for grasses (1.38 ± 0.39 $year^{-1}$) and broad leaf species (0.44 ± 0.05 $year^{-1}$) were taken from the literature (Silver and Miya, 2001). This simplification treated all plant species of the two groups in the same way, while not all roots have the same decomposition rate. Root

decomposition is controlled mainly by their chemical composition (for example the C:N ratio, calcium and lignin content), followed by climate and environmental conditions, including the effects of root pathogens and the non-pathogenic soil community (Coleman 2008, Silver and Miya 2001). Recently, Gan et al. (2011) reported large variations among root C:N ratios for oilseed, pulse crops and wheat. If specific relative root decomposition parameters (*b*) are available for the different plant species, pathogen dynamics could be estimated more precisely.

Besides the specific crops used in a rotation, the order of those crops can influence disease development and yield (Abawi and Widmer 2000, van den Berg and Rossing 2005). However, from our simulations it appeared that the sequence of the crops was less important than the actual crops included in the rotation. This may be due to the fact that a potential decline in pathogen population was not taken into account. A more realistic model that simulates growth and death of roots and microbial communities, including pathogens and predators, may result in more realistic data about pathogen dynamics (Termorshuizen and Rouse 1993, Zelenev et al. 2006.). However, more complex models miss the simplicity and elegance of analytical models.

Regardless of the simplicity and discussed limitations of the population model described here, the simulated *Foc* population dynamics could be used to explore and design crop rotations suppressive to Fusarium Basal Rot. For example, sustainable cropping sequences with *Allium* species as the main crop could be designed using the ROTAT program (Dogliotti et al. 2003). ROTAT was developed to create all feasible crop rotations based on a list of crops and well-defined quantitative agronomic rules. These agronomic rules are filters included to eliminate undesirable crop sequences, and are built on previous research or on expert knowledge. One particular filter sets the “maximum cropping frequency of a crop or groups of related crops”, and aims to minimize the inoculum build-up of key soilborne pathogens. The outcomes from simulations as carried out in this work can be used to improve the filters in the ROTAT program in order to reduce the uncertainty about crop yields due to soilborne pathogens. The results from simulations in the ROTAT program could then be validated with pathogen population data from farm fields, with different crop rotations, management practices and environmental conditions.

5 Conclusions

The approach followed in this work, combining detailed greenhouse experiments and extrapolating the data to feed simple pathogen population models, allowed us to screen crops for their ability to increase the population of *Foc* and to explore potential crop sequences that may limit pathogen build-up. This approach will be also relevant for the study of other soilborne pathogens with different survival strategies, like *Sclerotium rolfsii* (Leoni et al. 2013b). However, the generated information will need to be contrasted and validated with pathogen population data from field experiments and from commercial farm fields to further design crop rotations that minimize epidemic development of soilborne pathogens (Chapter 5). The combination of novel analytical tools (meta-analysis, modeling, simulation) and on-farm observations, will contribute to select optimal crop rotations without the extensive requirements of time, space and economic resources demanded by classical experimental research (Ratnadass et al. 2012, van den Berg and Rossing 2005).

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References

Abawi, G.S. and Lorbeer, J.W. 1972. Several aspects of the ecology of *Fusarium* oxysporum f. sp. *cepae*. *Phytophatology* 62*:* 870-876.

Abawi, G. S. and Widmer, T. L. 2000. Impact of soil health management practices on soil-borne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology* 15: 37-47.

Agamennoni, R., Prioletta, S., Baffoni, P., Vanzolini, J. I., Caracotche, V., Reinoso, O. 2008. Efecto de

las rotaciones con pasturas y cultivos agrícolas. II. incidencia de podredumbre basal (*Fusarium oxysporum* f.sp. *cepae*) en la cebolla en el sur de Buenos Aires. In: *XXXI Congreso Argentino de Horticultura Resúmenes de trabajos*.; Mar del Plata, Buenos Aires, Argentina.

Altier, N.A. and Groth, J.V. 2005. Characterization of aggressiveness and vegetative compatibility diversity of *Fusarium oxysporum* associated with crown and root rot birdsfoot trefoil. *Lotus Newsletter* 35: 59-74.

Bahraminejad, S., Asenstorfer, R. E., Riley, I. T., Schultz, C. J. 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.). *Journal of Phytopathology* 156: 1-7.

Banihashemi, Z., and de Zeeuw, D.J. 1975. The behaviour of *Fusarium oxysporum* f.sp. *melonis* in the presence and absence of host plants. *Phytopathology* 65: 1212 - 1217.

Bishop, C. and Cooper, R. M. 1983. An ultrastructural study of vascular colonization in three vascular wilt diseases. *Physiolgical Plant Pathology* 22: 15-27.

Brayford, D. 1996. IMI Descriptions of Fungi and Bacteria No. 1263. *Fusarium oxysporum* f. sp. *cepae*. *Mycopathologia* 133: 39 - 40.

Buhre, C., Kluth, C., Bürcky, K., Märländer, B., Varrelmann, M. 2009. Integrated control of root and crown rot in sugar beet: combined effects of cultivar, crop rotation, and soil tillage. *Plant Disease* 93: 155-161.

Cassman, K.G. 1999. Ecological intensification of cereal production systems: yield potential, soil quality, and precision agriculture. *Proceedings of The National Academy of Sciences of the United States of America* 96: 5952 – 5959 (Colloquium paper).

Colbach, N., Lucas, P., Cavelier, N. 1994. Influence des successions culturales sur les maladies du pied et des racines du blé d'hiver. *Agronomie* 14: 525-540.

Coleman, D.C., 2008. From peds to paradoxes: linkages between soil biota and their influences on ecological processes. *Soil Biolgy and Biochemistry* 40: 271 – 289.

Dhingra, O.D. and Coelho Netto, R.A. 2001. Reservoir and non-reservoir hosts of bean-wilt pathogen, *Fusarium oxysporum* f.sp. *phaseoli*. *Journal of Phytopathology* 149: 463 – 467.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2003. ROTAT, a tool for systematically generating crop rotations. *European Journal of Agronomy* 19: 239-250.

Edel, V., Steinberg, C., Gautheron, N., Alabouvette, C. 1997. Populations of non-pathogenic *Fusarium oxysporum* associated with roots of four plant species compared to soilborne populations. *Phytopathology* 87: 693 - 697.

Elmer, W. H. 2009. Influence of earthworm activity on soil microbes and soilborne diseases of vegetables. *Plant Disease* 93: 175-179.

Fox, J, and Weisberg, S. 2011. An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion. Accesed 25 June 2012.

Galván, G. A., Koning-Boucoiran, C.F.S, Koopman, W.J.M., Burger-Meijer, K., González, P.H., Waalwijk, C., Kik, C., Scholten, O.E. 2008. Genetic variation among Fusarium isolates from onion, and resistance to Fusarium basal rot in related *Allium* species. *European Journal of Plant*

Pathology 121: 499 – 512.

Gan, Y.T., Liang, B.C., Liu, L.P., Wang, X.Y., McDonald, C.L. 2011. C:N ratios and carbon distribution profile across rooting zones in oilseed and pulse crops. *Crop and Pasture Science* 62: 496 – 503.

Gordon, T.R., Okamoto, D., Jacobson, D.J. 1989. Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f.sp. *melonis* and other species of *Fusarium*. *Phytopathology* 79: 1095 - 1100.

Goud, J.C., Termorshuizen, A.J., van Bruggen, A.H.C. 2011. Verticillium wilt in nursery trees: damage thresholds, spatial and temporal aspects. *European Journal of Plant Pathology* 131: 451–465.

Grünwald, N.J., Hu, S., van Bruggen, A.H.C. 2000. Short-term cover crop decomposition in organic and conventional soils; Characterization of soil C, N, microbial and plant pathogen dynamics. *European Journal of Plant Pathology* 106: 37-50.

Hartmann, A., Schmid, M., van Tuinen, D., Berg, G. 2009. Plant-driven selection of microbes. *Plant and Soil* 321: 235-257.

Helbig, JB. and Carroll, R.B. 1984. Dicotyledonous weeds as a source of *Fusarium oxysporum* pathogenic on soybean. *Plant Disease* 68: 694 - 696.

Hiddink, G.A., van Bruggen, A.H.C., Termorshuizen, A.J., Raaijmakers, J.M., Semenov, A.V. 2005. Effect of organic management of soils on suppressiveness to *Gaeumannomyces graminis* var. *tritici* and its antagonist, *Pseudomonas fluorescens*. *European Journal of Plant Pathology* 113: 417-435.

Hochman, Z., Carberry, P.S., Robertson, M.J., Gaydon, D.S., Bell, L.W., McIntosh, P.C. 2013. Prospects for ecological intensification of Australian agriculture. *European Journal of Agronomy* 44: 109-123.

INIA. 2012. Banco de Datos Agroclimático - GRAS (GRupo Agroclima y Sistemas de información). http://www.inia.org.uy/online/site/gras.php. Accesed 15 August 2012.

Janvier, C., Villenueve, F., Alabouvette, C. Edel-Hermann, V., Mateille, T., Steinberg, C. 2007. Soil health through soil disease suppression: which strategy from descriptors to indicators?. *Soil Biology and Biochemistry,* 39: 1-23.

Katan, J. 1971. Symptomless carriers of the tomato Fusarium wilt pathogen. *Phytopathology* 61: 1213 - 1217.

Lamari, L. 2002. *ASSESS – Image Analysis Software for Plant Disease* Quantification. St. Paul, MN, American Phytopathological Society.

Leoni, C., Rossing, W.A.H., van Bruggen, A.H.C. 2013a. Crop Rotation. In: Finckh, M., Tamm, L., van Bruggen, A.H.C. (Eds) *Plant Disease Management in Organic Agriculture.* St. Paul, MN, American Phytopathological Society (in press*).*

Leoni, C., ter Braak, C.J.F., Gilsanz, J.C, Dogliotti, S., Rossing, W.A.H., van Bruggen, A.H.C. 2013b. *Sclerotium rolfsii* dynamics in soil as affected by crop sequences. *Applied soil Ecology* (accepted).

Leslie, J.F., Pearson, C.A.S., Nelson, P.E., Toussoun, T.A. 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the central and eastern United States. *Phytopathology,* 80: 343 – 350.

Leslie, J.F. and Summerell, B.A. 2006. *The Fusarium Laboratory Manual*. Iowa, Blackwell Publishing.

Lucas, P. 2006. Diseases caused by soil-borne pathogens. In: Cooke, B.M., Gareth Jones, D. and Kaye, B. (Eds.) *The Epidemiology of Plant Diseases*, 2nd ed. (pp. 373 – 386). The Netherlands, Springer.

Matthiessen, J. N. and Kirkegaard, J. A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Critical Reviews in Plant Science* 25: 235-265.

Mazzola, M. 2007. Manipulation of rhizosphere bacterial communities to induce suppressive soils. *Journal of Nematology* 39: 213-220.

Mol, L., Huisman, O.C., Scholte, K., Struik, P.C. 1996. Theoretical approach to the dynamics of the inoculum density of *Verticillium dahliae* in the soil: first test of a simple model. *Plant Pathology* 45: 192 – 204.

McDonald M., Jaime M., Hovius M. 2004. Management of diseases of onions and garlic. In: Naqvi, S.A.M.H. (Ed.) *Diseases of fruits and vegetables, Vol II*. (pp. 149-200). The Netherlands, Springer.

Oritsejafor, J.J. and Adeniji, M.O. 1990. Influence of host and non-host rhizospheres and organic amendments on survival of *Fusarium oxysporum* f.sp. *elaeidis*. *Mycological Research* 94: 57 - 63.

Özer N., Köycü, N.D., Mirik, M., Soran, H., Boyraz, D. 2002. Effect of some organic amendments on Onion bulb rot. *Phytoparasitica* 30: 429-433.

Özer N., and Köycü D. 2004. Seed-borne fungal diseases of onion, and their control. *Fruit and Vegetable Diseases* 1: 281-306.

Postma, J. and Luttikholt, A.J.G. 1993. Benomyl-resistant Fusarium isolates in ecological studies on the biological control of fusarium wilt in carnation. *Netherlands Journal of Plant Pathology* 99: 175-188.

R Development Core Team. 2011. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org/.

Ratnadass, A., Fernandes, P., Avelino, J. Habib, R. 2012. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. *Agronomy for Sustainable Development* 32: 273 – 303.

Sarkar, D. 2008. *Lattice: Multivariate Data Visualization with R*. New York, Springer.

Silver, W.L. and Miya, R.K. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. *Oecologia* 129: 407 – 419.

Smolińska, U. 2000. Survival of *Sclerotium cepivorum* sclerotia and *Fusarium oxysporum* chlamydospores in soil amended with cruciferous residues. *Journal of Phytopathology* 148: 343 – 349.

Stirling, G.R. 2008. The impact of farming systems on soil biology and soilborne diseases:examples from the Australian sugar and vegetable industries – the case for better integration of sugarcane and vegetable production and implications for future research. *Australasian Plant Pathology* 37:

1–18.

Suzuki, R. and Shimodaira, H. 2011. Pvclust: Hierarchical Clustering with P-Values via Multiscale Bootstrap Resampling. R package version 1.2-2. http://CRAN.R-project.org/package=pvclust. Accesed 25 June 2012.

Taylor, C.R. and Rodriguez-Kábana, R. 1999. Optimal rotation of peanuts and cotton to manage soil-borne organisms. *Agricultural Systems* 61: 57 – 68.

ter Braak, C.J.F. and Vrugt, J.A. 2008. Differential evolution Markov Chain with snooker update rand fewer chains. *Statistics and Computing* 18: 435 – 446.

Termorshuizen, A.J. and Rouse, D.I. 1993. Towards a mechanistic model for the *Verticillium dahliae* - potato system. *European Journal of Plant Pathology* 99 (Suppl. 3): 201-218.

Tixier, P., Risède, J.M., Dorel, M., Malézieux, E. 2006. Modelling population dynamics of banana plant-parasitic nematodes: a contribution to the design of sustainable cropping systems. *Ecological Modelling* 198: 321 – 331.

Thrall, P.H., Bever, J.D., Mihail, J.D., Alexander, H.M. 1997. The population dynamics of annual plants and soil-borne fungal pathogens. *Journal of Ecology* 85: 313 – 328.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., Lorito, M. 2008. Trichoderma–plant–pathogen interactions. *Soil Biololgy and Biochemistry* 40: 1–10.

van Bruggen, A.H.C. and Semenov, A.M. 1999. A new approach to the search for indicators of root disease suppression. *Australasian Plant Pathology* 28: 4-10.

van Bruggen, A.H.C. and Termorshuizen, A.J. 2003. Integrated approaches to root disease management in organic farming systems. *Australasian Plant Pathology* 32: 141-156.

van den Berg, W. and Rossing W.A.H. 2005. Generalized linear dynamics of a plant-parasitic nematode population and the economic evaluation of crop rotations. *Journal of Nematology* 37: 55-65.

van den Berg, W., Rossing W.A.H., Grasman, J. 2006. Contest and scramble competition and the carry-over effect in *Globodera* spp. in potato-based crop rotation using an extended Ricker model. *Journal of Nematology* 38: 210 – 220.

Zelenev, V.V, van Bruggen, A.H.C., Leffelaar, P.A., Bloem, J., Semenov, A.M. 2006. Oscillating dynamics of bacterial populations and their predators in response to fresh organic matter added to soil: the simulation model 'BACWAVE-WEB'. *Soil Biololgy and Biochemistry* 38: 1690-1711.

Chapter 5

Fusarium dynamics in soil as affected by crop sequences: combining controlled experiments, simulation models and field measurements

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Abstract

The design of a crop sequence is complex due to the many interacting objectives and processes. Models can support the design of crop sequences and help to reveal synergies and trade-offs among farmer objectives, on-farm resources, and socio-economic constraints. In this study our objective was to generate information on *Fusarium oxysporum f.sp. cepae (Foc)* dynamics for different crops and intercrops, and develop a research approach where experimental data and a simulation model are combined to explore crop sequences that minimize *Foc* inoculum build-up.

Data from microplots as well as farm fields showed that populations of *Fusarium* propagules in soil were strongly influenced by the cropping history. In onion (*Allium cepae*) crops, *Fusarium* populations increased from transplanting to harvest when another onion crop had been grown in the same field the previous winter whereas *Fusarium* populations decreased when a winter green manure had been planted. Changes in initial (*Pi*) and final (*Pf*) inoculum densities in one cropping season could be seen as the result of of multiplication of fungal propagules in and release from roots of the previous and pre-previous crops.

Foc dynamics in soil under different crop sequences were simulated with a model that described two processes: build-up of *Foc* within a crop and release of the pathogen from decomposed roots after crop harvest. The model was calibrated with data from greenhouse pot experiments and validated with data collected in microplots and on farm fields. The proposed model was able to describe an increase or decrease in pathogen populations in soil, despite some discrepancies in pathogen population trends (*Pi-Pf*) and overestimations of simulated pathogen populations. Nevertheless, model outcomes point at the importance of the cropping history on *Fusarium* soil dynamics.

Keywords:
Fusarium oxysporum f.sp. *cepae*, population dynamics, simulation model, crop rotation

1 Introduction

Sustainable farming systems are usually associated with relatively long crop rotations, spanning a period of five to eight years. An adequate crop rotation not only provides a regular supply of nutrients, maintains a good soil structure that enhances the water-holding capacity and allows for extensive root formation, reduces soil erosion and regulates disease, pest and weed outbreaks, but also spreads the workload evenly over the seasons and provides food and income security to farmers. Further considerations in designing crop rotations are the farmers' long-term objectives and ambitions at the whole farm level, on-farm resources and climate, and shorter-term socio-economic conditions. Models can support the design of crop sequences and help to reveal synergies and trade-offs among objectives (Casagrande et al. 2010, Dogliotti et al. 2003, Dogliotti et al. 2013). In this paper we focus on minimizing build-up and survival of soilborne pathogens.

Crop rotation experiments are resource demanding due to the amount of time and labour involved, and usually only involve few experimental factors. More resource-effective methods would allow larger numbers of experimental factors to be investigated, and adjustment of factors, such as cultivars, crop types or nutrient management techniques, to current technologies. Alternatively, soilborne pathogen dynamics in a crop rotation can be described by interlinked population models where different equations describe the increase and decline of the pathogen population during and after a crop and intercrop activity, respectively (Bailey and Gilligan 2004, Colbach et al. 1999, Leoni et al. 2013a, Leoni et al. 2013b, Mol et al. 1996, Tixier et al. 2006, van den Berg and Rossing 2005, van den Berg et al. 2006). In order to calibrate and evaluate the models, quantitative information on soilborne pathogen dynamics in the presence of different crops and intercrop activities (e,g. green manures, fallow) is needed.

Two important pathogens in vegetable production are *Sclerotium rolfsii* (Leoni et al. 2013b), and *Fusarium oxysporum* f.sp. *cepae, Foc* (Leoni et al., 2013a). In this paper, *Foc* population dynamics are investigated. *Foc* is a soilborne pathogen of *Allium* species (onion, garlic, leeks). On onions, the pathogen causes Fusarium Basal Rot, and is a significant problem in areas with temperatures above 25˚C during the growing season. Onion Fusarium Basal Rot causes yield losses up to 50% in the field, and additional losses occur during storage due to disease progress and mixed infections with other pathogens (McDonald et al. 2004). Like for other soilborne pathogens several strategies need to be combined for the management of this disease. Among them, crop rotations without onions for 4 to 5 years have been

recommended (Agamennoni et al. 2008, Özer et al. 2002, Özer and Köycü 2004). However, failures in disease control are common due to the ability of several crops to harbour the pathogen without displaying disease symptoms (Leoni et al. 2013a). By selecting crops that limit the multiplication of *Foc* inoculum, the chances of success of Fusarium Basal Rot management through crop rotation could be improved.

In this study our objective was to generate information on *Foc* dynamics for different crops and intercrops, and design and test a research approach where experimental data and model simulations are combined to explore crop sequences that minimize *Foc* inoculum build-up.

2 Material and methods

2.1 Soil microbial population in farm fields

During the winter cropping seasons of 2009, 2010 and 2011, in total 35 onion (*Allium cepae* L.), eight white oat (*Avena sativa* L.) and four fallow fields at seven farms were surveyed to study the effect of crop sequences on microbial population dynamics in the soil. The farms were located in Southern Uruguay, where most of the vegetable farms of the country are found. The farms, which had onion as one of the main cash crops, were selected to represent a large range of variation in resource availability, soil quality and distance to the market. A description of environmental data for the region is presented in the supplementary material (Table S1).

For quantification of total *Fusarium oxysporum* (*Fox)*, actinomycete, fluorescent *Pseudomonas* and *Trichoderma* spp populations, soil samples were collected at transplanting and harvest of onion crops in 2009, 2010 and 2011. Soil samples were composites of 15-20 soil cores (2 cm diameter, 10 cm depth) collected within the rows of the crop, and after sieving to remove roots, 1 g of soil was used for preparing 10-fold serial dilutions with 0.1% sterile water agar. For each microorganism a particular dilution and semi-selective medium was used, as detailed in Table 1. *Fox* populations were estimated by the most probable number (mpn) method, with 6 dilutions in three replications. To determine whether the initial (*Pi*) and final (*Pf*) soil microbial populations before and after an onion crop were different a paired t-test was used. To determine whether the increase or decrease in *Fox* and *Trichoderma* spp. populations during onion was related to the previous crop (onion or green manure) the Chi-squared test for 2 x 2 contingency tables was used. Both tests

Table 1. Organisms culture, media, dilution factors and incubation conditions for microorganisms isolated from soil samples on farms in Uruguay.

Organism cultured	Media	Dilution factors [1]	Temperature, incubation and light conditions	Reference
Foc[2]	Komada with benomyl (10 mg l^{-1})	10^{-2} - 10^{-7} (for mpn [3])	25 C 7 - 10 days 12hs light	Leoni et al., 2013a
Fox[4]	Komada	10^{-2} , 10^{-3} , 10^{-2} - 10^{-7} (for mpn)	25 C 7 - 10 days 12hs light	Leslie and Summerell, 2006
Actinomycetes	Casein-starch	10^{-3} , 10^{-4}	25 C 5-7 days Dark	Leoni and Ghini, 2002
Fluorescent *Pseudomonas*	King B with cyclohexymide (100 mg l^{-1}), ampiciline (40 mg l^{-1}), chloram-phenicol (13 mg l^{-1})	10^{-4} , 10^{-5}	25 C 2-3 days Dark	Hiddink et al., 2005.
Trichoderma spp.	THSM	10^{-2} , 10^{-3}	25 C 5-7 days Dark	Williams et al, 2003.

[1] Dilutions were 10 fold serial dilutions on 0.1% sterile water agar.

[2] *Fusarium oxysporum* f.sp. *cepae*

[3] most probable number method

[4] Total *Fusarium oxysporum*

were done with R software version 2.15.0 (R Development Core Team 2012).

2.2 Soil microbial populations in microplots

To study the effect of crop sequences on *Fusarium oxysporum* f.sp. *cepae* (*Foc*) dynamics in the soil, a microplot experiment was installed in April 2009 at the INIA Las Brujas Research Station (34° 40' S., 56° 20' W., 32 m above sea level). The soil in the experiment was a Typic-Vertic Argiudolls, with a clay loam texture (34% sand, 28% silt, 38% clay) and pH 6.2, with 1.76% organic carbon, 0.16% total N, 36 µg /g P_2O_5, and 1.14 meq/100g K_2O. Each 2.5 m x 2.0 m microplot was delimited by a 50 cm high steel frame, inserted 30 cm into the

ground. Rows between plots were 2 m wide and covered by permanent grass strips. A total of 21 microplots were arranged in three blocks and installed in a field which had been under grass-clover pasture during the previous 10 years.

To generate different soil *Foc* populations in the microplots, in July 2009 in each of the three blocks four plots were inoculated with the benomyl-resistant *Foc* isolate *Foc* UR-17-8 B8 (Leoni et al., 2013a), and three plots remained un-inoculated. Among the inoculated plots, three were inoculated with a conidial suspension of 3 x 10^6 conidia ml^{-1}, and one with a conidial suspension of 3 x 10^7 conidia ml^{-1}. The day after inoculation, a winter onion crop was planted in each plot (110 plantlets per plot). In December 2009, the onion crop was harvested and followed by one of the three activities: fallow, a summer green manure crop of sudangrass (*Sorghum* × *drummondi* (Steud.) Millsp. & Chase) or a summer cash crop of sweet pepper (*Capsicum annuum* L.). This resulted in three cropping sequences: onion monocrop, Onion – Sudangrass and Onion – Sweet pepper. All plots were managed according to integrated crop management practices proposed for the region. Air temperature, relative humidity, precipitation and soil evapotranspiration (Class A pan evaporation) were registered with an automatic meteorological station (data available at http://www.inia.org.uy/online/site/692646I1.php). A summary of the data is presented in the supplementary material (Table S1).

For quantification of total *Fusarium oxysporum* (*Fox)*, actinomycete, fluorescent *Pseudomonas* and *Trichoderma* spp populations, soil samples were collected at transplanting and harvest of the onion crops in 2010 and 2011. Soil samples were composites of 15 soil cores (2 cm diameter, 10 cm depth) collected within the crop rows, and after sieving to remove roots, 1 g of soil was used for preparing 10-fold serial dilutions with 0.1% sterile water agar. For each microorganism a particular dilution and semi-selective medium was used, as detailed in Table 1. *Foc* and *Fox* populations were estimated by the most probable number (mpn) method, with six dilutions and three replicates. To determine whether the means of initial (*Pi*) and final (*Pf*) soil microbial populations were different; a paired t-test was used with R software version 2.15.0 (R Development Core Team, 2012).

2.3 Simulated soilborne *Fusarium* population dynamics in crop sequences

*Foc s*oil population dynamics in crop sequences was simulated using the model proposed by Leoni et al. (2013a), by concatenating initial (*Pi*) and final (*Pf*) soil *Fusarium* population densities in subsequent crops and considering gradual pathogen release from plant roots

after harvest. In the model, the following assumptions were made: (i) *Foc* multiplication in plant roots is the result of infection by all inoculum available at transplanting/seeding, thus depleting plant available soil inoculum; (ii) when the crop is harvested, all infected roots start to decompose and to release *Foc* infective units, which accumulate until the new crop is transplanted/seeded; (iii) the potential decline in *Foc* population due to predators, antagonists or toxins is not considered.

Pf values were calculated with equation (1) which relates soil population densities at the start (*Pi*) and at the end (*Pf*) of the activity *n* –here the current crop-, expressed in colony forming units in one gram of dry soil including roots (cfu g^{-1} dry soil):

$$Pf_{(n)} = Pi_{(n)}/(\alpha_{(n)} + \beta_{(n)}\ Pi_{(n)}) \quad (1)$$

where *1/α* represents the slope at the origin and *1/β* the horizontal asymptote (van den Berg and Rossing, 2005).

At harvest of a crop, *Pf* constitutes the size of a pool of inoculum in the roots of crop *n*, which is released over time according to

$$Pf_{(n,t)} = Pf_{(n,0)}(1 - e^{-bt}) \quad (2)$$

where t is time since harvest of crop *n* and *b* represents the relative decay rate (day^{-1}), thus 1/*b* (day) represents the average lifetime of the roots. Inoculum density at the start of a crop *Pi* depends on the amount of inoculum released into the soil by decomposition of roots of previous crops, taking into account that inoculum is lost from the soil pool as it is assumed to infect each starting crop.

The model parameters *α, β* and *b* were estimated with data generated in the pot experiments reported in Leoni et al. (2013a). Briefly, the relative decomposition rate (*b,* day^{-1}) of onion roots was estimated with data from a pot experiment with roots placed in plastic litter bags and retrieved in the course of seven months. Parameters *1/α* and *1/β* for the different crops were estimated with data generated in a pot experiment where several plant species were planted in soil inoculated with the benomyl -resistant *Foc* isolate UR 17-8B8. Measured soil *Foc* population at transplanting was considered as *Pi*, and *Pf* was defined as the sum of soil and root *Foc* populations. The details are as follows.

Parameters in equations (1) and (2) were estimated using R software version 2.15.0 (R Development Core Team, 2012). Parameters *α* and *β* in equation (1) were estimated for each crop by Bayesian non-linear least-squares regression after log transformation of the data, assuming uniform (flat) prior distributions U (0.1, 100) for *1/α* and U (10, 5 x 10^4) for *1/β* using the DE-MC_{ZS} algorithm (ter Braak and Vrugt, 2008) with three parallel Markov chains and 33333 generations. The upper bound of the uniform distribution for *1/β* was defined based on the threshold described by Abawi and Lorbeer (1972) for *Foc* to start an infection in onions on peat soils. The posterior distribution was represented by 1000 samples (draws) obtained by thinning the second half of each chain. The median of these samples for *1/α* and *1/β* was selected as the best estimate, since it was more stable than the mean, but we report the mean and its standard error as well. Parameter *b* in equation (2) was estimated by non-linear least-squares regression with the packages "lattice" (Sarkar, 2008) and "car" (Fox and Weisberg, 2011).

Model simulations were run with fixed parameters (deterministic simulation). The mean of *b* and the median of *1/α* and *1/β* were used (Figure 1, Table 2). To simplify calculations, root decomposition and associated *Foc* release was assumed to cease after 2 times 1/*b* days.

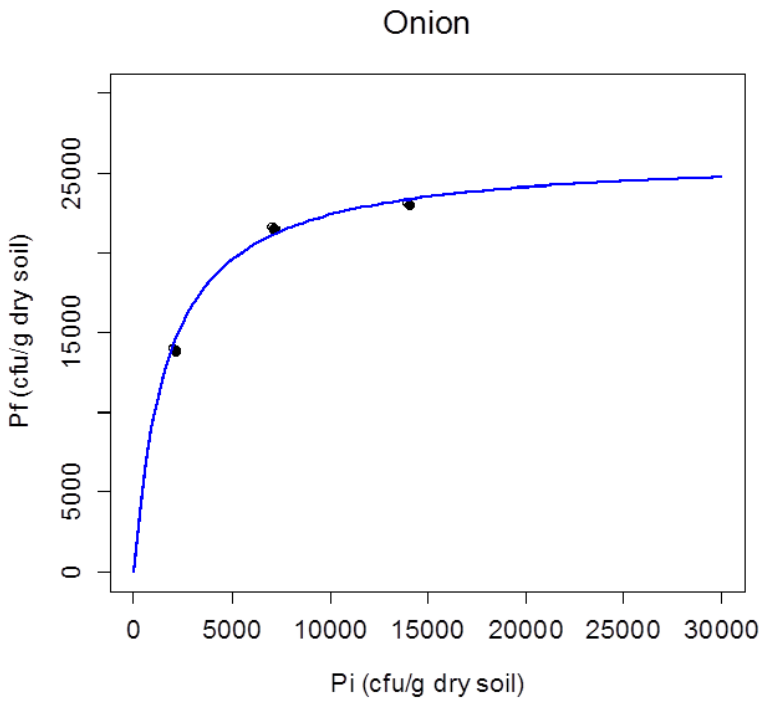

Figure 1. Relation between final (*Pf*) and initial (*Pi*) *Fusarium oxysporum* f.sp. *cepae* soil populations (cfu g^{-1} dry soil, including roots) for onion. Dots are measured values in a greenhouse pot experiment (Leoni et al, 2013a), and the line represents the fitted model $Pf = Pi / (\alpha + \beta Pi)$, where *1/α* is the slope at the origin and *1/β* the horizontal asymptote. *Pi* was measured soil *Foc* population at transplanting and *Pf* was defined as the sum of measured soil and root *Foc* population at harvest.

Table 2. Parameters used for simulation of the population dynamics of *Fusarium oxysporum* f.sp. *cepae*.

Crop	*1/α* [1]			*1/β* [1] (cfu g^{-1}dry soil)			AP [2]	b [3] (day^{-1})	
	median	mean	s.e	median	mean	s.e		mean	s.e
Onion (*Allium cepa* L.)	15.58	21.3	16.22	2.62×10^4	2.58×10^4	3.31×10^3	0.28	0.0017	0.00040
White oat (*Avena sativa* L.)	55.39	54.16	27.11	1.20×10^4	1.28×10^4	5.20×10^3	0.31	0.0038	0.00107
Wheat (*Triticum aestivum* L.)	57.59	56.29	26.78	1.12×10^3	1.13×10^3	3.18×10^2	0.33	0.0038	0.00107
Corn (*Zea mays* L.)	46.76	47.88	28.96	1.44×10^4	1.69×10^4	9.96×10^3	0.29	0.0038	0.00107
Sweet corn (*Zea mays* L.)	30.55	38.08	30.57	9.88×10^3	1.37×10^4	1.07×10^4	0.22	0.0038	0.00107
Tomato (*Solanum lycopersicum* L.)	51.37	51.41	27.92	1.64×10^4	1.81×10^4	9.10×10^3	0.30	0.0012	0.00014
Sudangrass *(Sorghum × drummondi* (Steud.) Millsp. & Chase)	42.37	44.91	29.31	1.34×10^4	1.63×10^4	1.04×10^4	0.28	0.0038	0.00107

[1] Parameters of the equation Pf = Pi/(α + β Pi) describing soil population dynamics of *Foc* . Parameters were estimated with data from a greenhouse pot experiment (Leoni et al, 2013a), with lower and upper bounds for *1/α* : 0.1 and 100, and for *1/β* : 10 and 5×10^4 .

[2] Acceptance Probability. AP reference rates are 0.4 for d=1 and 0.28 for d=5, where d= number of estimated parameters of the posterior distribution (Ter Braak and Vrugt, 2008)

[3] Parameter of the equation $y_t = y_0 \exp(-bt)$ describing root decomposition. The value for onion roots was determined in Leoni et al. (2013a*)*; *b* values for gramineous crops and tomato were taken from Silver and Miya (2001).

Foc or *Fox* soil populations measured in soil samples collected either on farm fields or in microplots from a total of sever crop sequences were used as independent datasets to assess model performance. The simulated crop sequences were: onion monocrop, onion - white oat, onion – wheat, onion – sudangrass, onion – white oat – tomato, onion - white oat - corn and onion – sweet corn - white oat. In all sequences onion, white oat and wheat (*Triticum aestivum* L.) are winter crops, whereas sudangrass, tomato (*Solanum lycopersicum* L.) corn (*Zea mays* L.) and sweet corn (*Zea mays* L.) are summer crops. Each winter or summer crop was followed by a fallow period with variable length. Crop and fallow lengths are specified in Table 6.

3 Results

3.1 Soil microbial population dynamics in farm fields

In the 35 onion fields surveyed the average total *Fusarium oxysporum* (*Fox*) populations at transplanting and harvest were 2.4×10^3 and 1.6×10^3 cfu g^{-1} dry soil, respectively (Table 3).

Table 3. Soil *Fusarium oxysporum (Fox)*, actinomycete, fluorescent *Pseudomonas* and *Trichoderma spp.* populations (cfu g^{-1} dry soil) in commercial onion fields at transplanting (*Pi*) and harvest (*Pf*), analysed according to cropping sequence.

	All onion fields [1]		Onion fields with onions the previous winter		Onion fields with winter green manure the previous winter	
	Pi	*Pf*	*Pi*	*Pf*	*Pi*	*Pf*
Fusarium oxysporum						
Mean	$2.4x10^3$	$1.6x10^3$	$4.1x10^2$	$2.4x10^3$	$3.8x10^3$	$7.8x10^2$
s.e.	$7.1x10^2$	$3.3x10^2$	$1.6x10^2$	$6.4x10^2$	$1.2x10^3$	$1.7x10^2$
n	35	35	13	13	20	20
p [2]	0.357		0.005		0.020	
Actinomycetes						
Mean	$1.5x10^6$	$4.3x10^6$	$1.1x10^6$	$2.0x10^6$	$1.9x10^6$	$6.2x10^6$
s.e.	$2.7x10^5$	$8.7x10^5$	$2.7x10^5$	$4.4x10^5$	$4.6x10^5$	$1.4x10^6$
n	34	35	13	13	18	19
p [2]	<0.001		0.032		0.002	
Fluorescent *Pseudomonas*						
Mean	$6.8x10^5$	$4.2x10^4$	$3.4x10^5$	$2.9x10^4$	$5.6x10^5$	$1.9x10^4$
s.e.	$2.9x10^5$	$2.1x10^4$	$1.9x10^5$	$1.1x10^4$	$1.1x10^5$	$9.9x10^3$
n	32	24	13	8	16	12
p [2]	0.075		0.226		0.010	
Trichoderma spp.						
Mean	$6.6x10^3$	$5.2x10^3$	$5.6x10^3$	$4.2x10^3$	$8.2x10^3$	$6.0x10^3$
s.e.	$1.4x10^3$	$6.7x10^2$	$1.1x10^3$	$1.0x10^3$	$2.6x10^3$	$9.7x10^2$
n	35	35	13	13	20	20
p [2]	0.167		0.263		0.248	

[1] 14 onion fields were evaluated in 2009, 10 in 2010 and 11 in 2011.

[2] *p* values of the paired t-test- (*Pi* and *Pf*).

Fox populations increased from transplant to harvest in 17 fields; on 71% of these fields an onion crop had been grown the previous winter. Of the 18 fields in which *Fox* decreased 89% had had a winter green manure species the previous winter (p= 0.002, Chi squared test with one degree of freedom) (Table 4, Figure 2).

Among the beneficial soil microbial populations different trends were observed for actinomycetes, fluorescent *Pseudomonas* and *Trichoderma* spp. (Table 3). Actinomycete soil populations significantly increased (p <0.001) from transplanting to harvest, with average population densities of 1.5 x 10^6 and 4.3 x 10^6 cfu g^{-1} dry soil, respectively. Fluorescent *Pseudomonas* soil populations significantly decreased (p = 0.075) from 6.8 x 10^5 to 4.2 x 10^4

cfu g^{-1} dry soil. *Trichoderma* spp. populations did not vary significantly between transplant and harvest (p = 0.167). However, it was observed that in 60% of the fields *Trichoderma* spp. populations increased when a winter green manure crop had been planted the previous winter (Table 4).

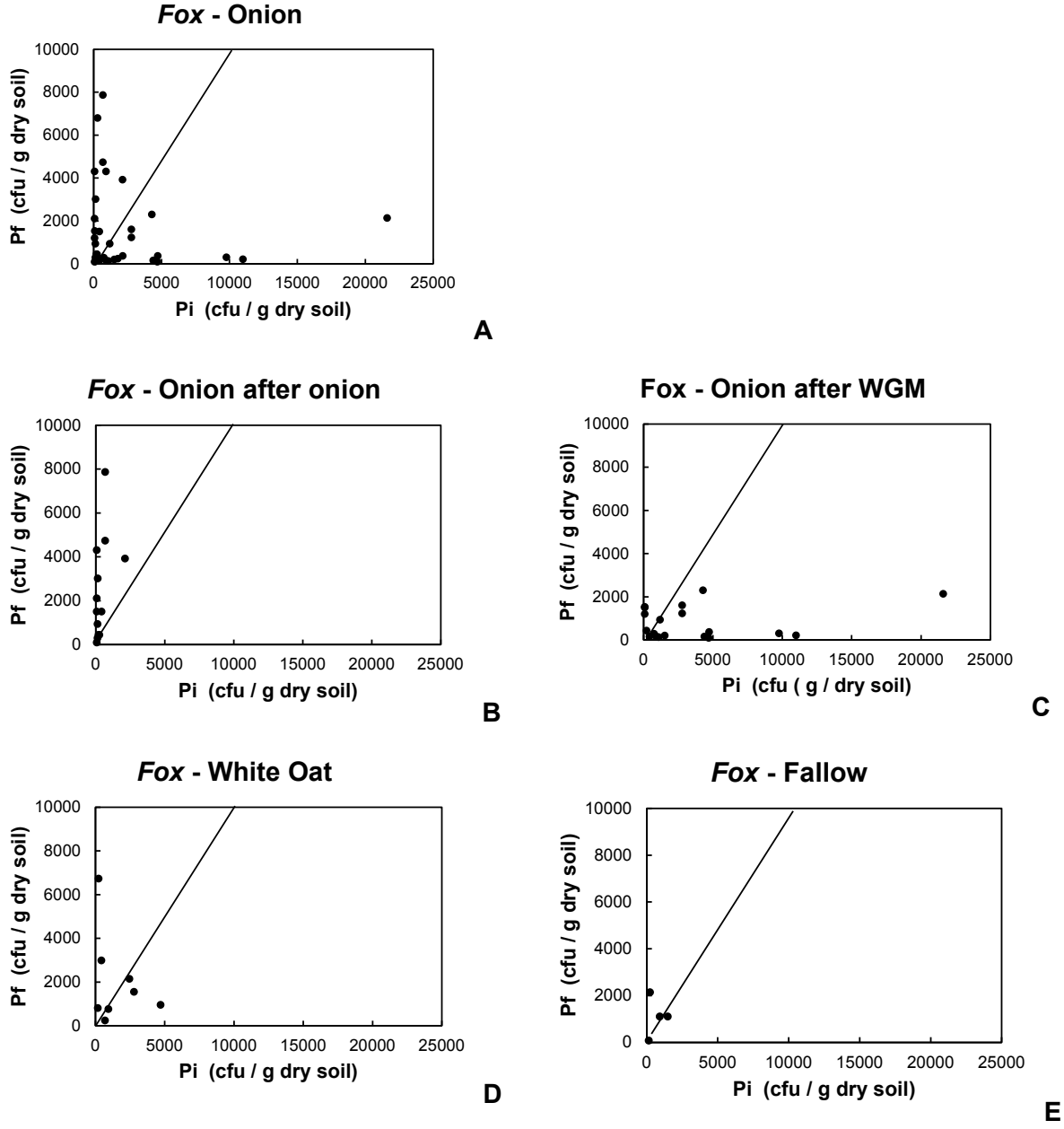

Figure 2. Relation between final (*Pf*) and initial (*Pi*) *Fusarium oxysporum* soil populations (cfu g^{-1} dry soil). A: all onion crops, B: onion in winter after onion in previous winter, C: onion in winter after winter green manure in previous winter, D: white oat and E: fallow. Dots are values measured between 2009 and 2011 in farm fields. The line represents de 1:1 relationship.

Table 4. Changes in soil *Fusarium oxysporum* and *Trichoderma spp.* populations in commercial onion fields between transplanting (*Pi*) and harvest (*Pf*), analysed according to cropping sequence.

	All onion fields[1]	Onion fields with onions the previous winter	Onion fields with winter green manure the previous winter
Fusarium oxysporum			
Pf>Pi	17	12	4
Pf<Pi	18	1	16
Total	35	13	20
Trichoderma spp.			
Pf>Pi	19	7	11
Pf<Pi	16	7	6
Total	35	14	17

[1] 14 onion fields were evaluated in 2009, 10 fields in 2010 and 11 fields in 2011.

3.2 Soil microbial population dynamics in microplots

In the microplots, average *Fusarium oxysporum f.sp. cepae (Foc)* and total *Fusarium oxysporum* (*Fox*) populations decreased between transplanting and harvest when all three crop sequences were considered jointly (Table 5). This trend was opposite to the one observed at the farms for *Fox* populations in the soil after two consecutive onion crops. However, for *Foc* soil populations an increase was observed in nine plots (50%) in 2010 and eight (44%) in 2010. For *Fox* this happened in four plots (22%) in 2010 and 7 (39%) plots in 2011 (Figure 3).

Beneficial soil microbial populations showed similar trends as in farm fields. Soil populations of actinomycetes increased and fluorescent *Pseudomonas* and *Trichoderma* spp. decreased from transplanting to harvest (Table 5).

3.3 Simulated soil *Fusarium* population dynamics in crop sequences

For the different simulated sequences, dynamic equilibrium was reached after four cycles irrespective of the initial inoculum density. The exception was the onion monocrop sequence that needed eight cycles (Figure 4, Figure 5). At equilibrium, the highest *Pi* values when starting a new onion crop were reached by the onion monocrop sequence, with values of 1.3 x 10^4 cfu g^{-1} dry soil. The other sequence with one onion crop per year (onion-sudangrass) resulted in a 58% lower *Pi* for onion than the onion monocrop sequence. Among the sequences with one onion crop in two years, the lowest *Pi* values were reached with the sequence onion – white oat – corn with 4.6 x 10^3 cfu g^{-1} dry soil (Table 6). Simulated soil

inoculum levels were generally higher than the measured soil *Fusarium* populations, both in farm fields and microplots (Figure 4, Figure 5).

Table 5. Soil *Fusarium oxysporum f.sp. cepae (Foc),* total *Fusarium oxysporum* (*Fox),* Actinomycetes, fluorescent *Pseudomonas* and *Trichoderma spp.* populations (cfu g^{-1} dry soil) in the microplots at transplanting (*Pi*) and harvest (*Pf*) of onions. Soil microbial populations are combined data from 2010 and 2011[1].

	All Onion crops		Onion after Fallow		Onion after Sudangrass		Onion after Sweet pepper	
	Pi	Pf	Pi	Pf	Pi	Pf	Pi	Pf
Fusarium oxysporum f.sp. cepae (Foc)								
Mean	$9.8x10^2$	$2.9x10^2$	$1.6x10^3$	$4.3x10^2$	$6.3x10^2$	$1.4x10^2$	$4.2x10^2$	$2.4x10^2$
s.e.	$2.8x10^2$	$5.5x10^1$	$5.9x10^2$	$1.1x10^2$	$4.1x10^2$	$3.5x10^1$	$1.5x10^2$	$9.4x10^1$
n	42	42	18	18	12	12	12	12
p [2]	0.020		0.066		0.253		0.327	
Fusarium oxysporum (*Fox)*								
Mean	$4.2x10^3$	$2.5x10^3$	$6.4x10^3$	$3.7x10^3$	$4.4x10^3$	$2.2x10^3$	$4.1x10^3$	$1.6x10^3$
s.e.	$8.0x10^2$	$4.3x10^2$	$1.6x10^3$	$9.0x10^2$	$1.3x10^3$	$6.6x10^2$	$1.2x10^3$	$4.8x10^2$
n	42	42	18	18	12	12	12	12
p [2]	0.042		0.175		0.407		0.186	
Actinomycetes								
Mean	$1.3x10^6$	$3.2x10^6$	$1.2x10^6$	$3.0x10^6$	$1.6x10^6$	$3.5x10^6$	$1.1x10^6$	$3.2x10^6$
s.e.	$1.0x10^5$	$1.3x10^5$	$1.5x10^5$	$1.7x10^5$	$2.4x10^5$	$3.2x10^5$	$1.4x10^5$	$2.4x10^5$
n	42	42	18	18	12	12	12	12
p [2]	<0.001		<0.001		<0.001		<0.001	
Fluorescent *Pseudomonas*								
Mean	$3.3x10^5$	$3.1x10^4$	$3.3x10^5$	$5.1x10^4$	$4.1x10^5$	$1.6x10^4$	$2.3x10^5$	$2.3x10^4$
s.e.	$8.0x10^4$	$9.4x10^3$	$8.5x10^4$	$2.3x10^4$	$2.3x10^5$	$7.0x10^3$	$6.5x10^4$	$1.0x10^4$
n	33	31	12	12	11	11	10	8
p [2]	<0.001		0.016		0.017		0.047	
Trichoderma spp.								
Mean	$4.3x10^3$	$2.8x10^3$	$4.2x10^3$	$3.2x10^3$	$5.0x10^3$	$2.6x10^3$	$3.8x10^3$	$2.3x10^3$
s.e.	$3.5x10^2$	$2.0x10^2$	$6.8x10^2$	$3.3x10^2$	$5.8x10^2$	$4.1x10^2$	$5.2x10^2$	$3.1x10^2$
n	42	42	18	18	12	12	12	12
p [2]	<0.001		0.157		0.008		0.021	

[1] Soil microbial populations were evaluated in 2010 and 2011. All microplots were planted with onions the previous year.

[2] *p* values of the paired t-test (*Pi* and *Pf*).

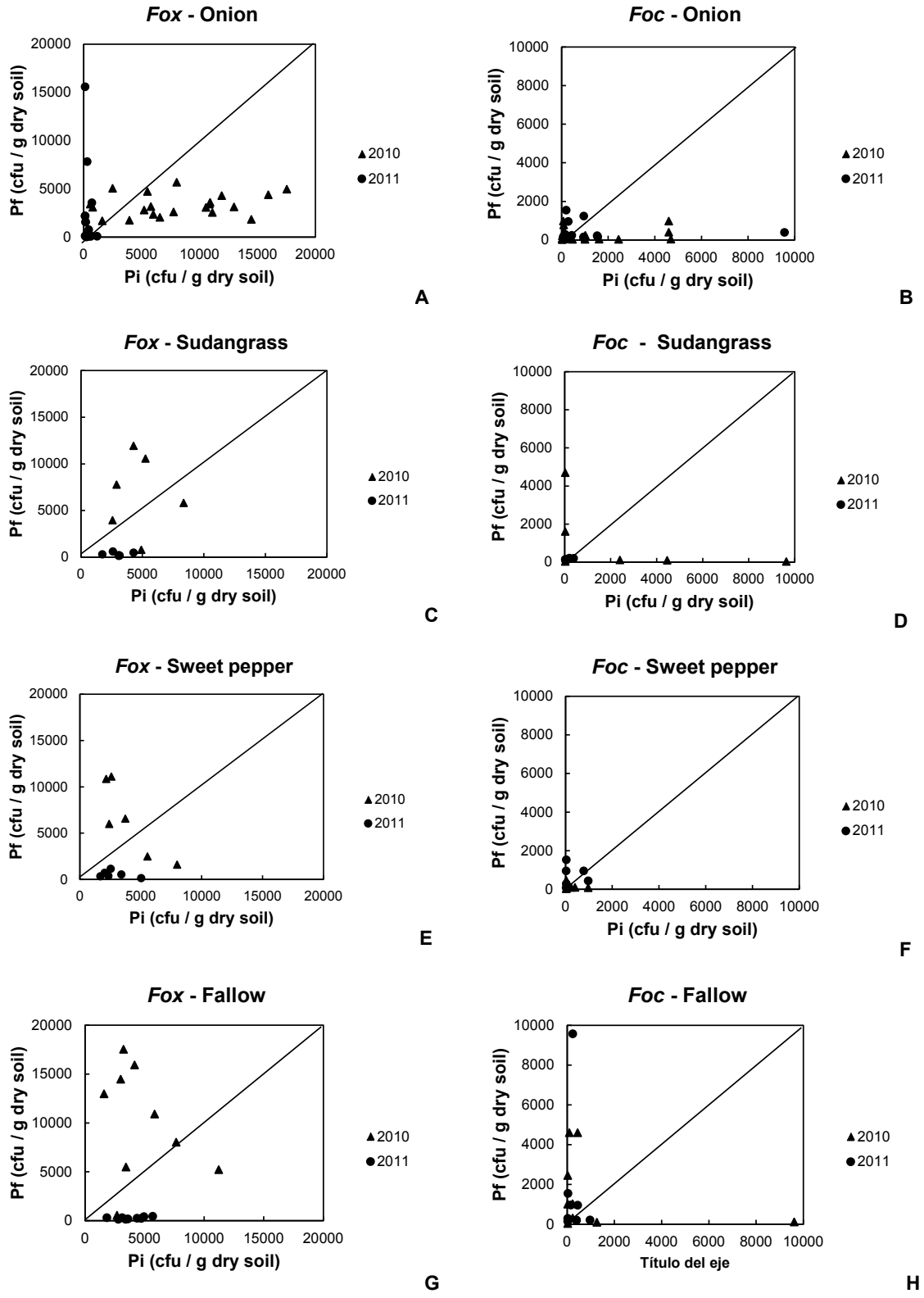

Figure 3. Relation between final (*Pf*) and initial (*Pi*) *Fusarium oxysporum* and *Fusarium oxysporum* f.sp. *cepae* soil populations (cfu g^{-1} dry soil) for onion, sudangrass, sweet pepper and fallow, measured in microplots between 2010 – 2011. The line represents de 1:1 relationship.

Table 6. Simulated *Pi* values (cfu g^{-1} dry soil) of *Fusarium oxysporum* f.sp. *cepae* for different crop sequences at steady state.

Crop sequence[1]	Component length[2]	*Pi* O	*Pi* F	*Pi* Crop	*Pi* F	*Pi* Crop	*Pi* F
O-F	6-6	$1.3x10^4$	$6.3x10^3$	---	---	---	---
O-F-Sg-F	6-1-3-2	$5.5x10^3$	$1.1x10^4$	$2.3x10^3$	$5.4x10^3$	---	---
O-F-WO-F	6-4-7-7	$9.6x10^3$	$5.6x10^3$	$6.3x10^3$	$5.5x10^3$	---	---
O-F-Wt-F	6-4-7-7	$9.2x10^3$	$5.3x10^3$	$6.2x10^3$	$5.4x10^3$	---	---
O-F-WO-F-T-F	6-3-5-3-5-2	$7.1x10^3$	$3.2x10^3$	$5.6x10^3$	$5.8x10^3$	$6.2x10^3$	$7.6x10^3$
O-F-WO-F-C-F	6-3-5-3-5-2	$4.6x10^3$	$9.2x10^3$	$5.6x10^3$	$5.4x10^3$	$5.8x10^3$	$6.0x10^3$
O-F-SC-F-WO-F	6-1-3-2-6-6	$1.1x10^4$	$6.1x10^3$	$1.9x10^3$	$3.8x10^3$	$3.9x10^3$	$7.7x10^3$

[1] O: onion, Sg: sudangrass, WO: white oat, Wt: wheat, T: tomato, C: corn, SC: sweet corn. Each crop was followed by an intercrop activity, denoted as F, which could be fallow, soil preparation or green manure incorporation and decomposition, and was parameterized as fallow.

[2]Length in months for each crop or intercrop period

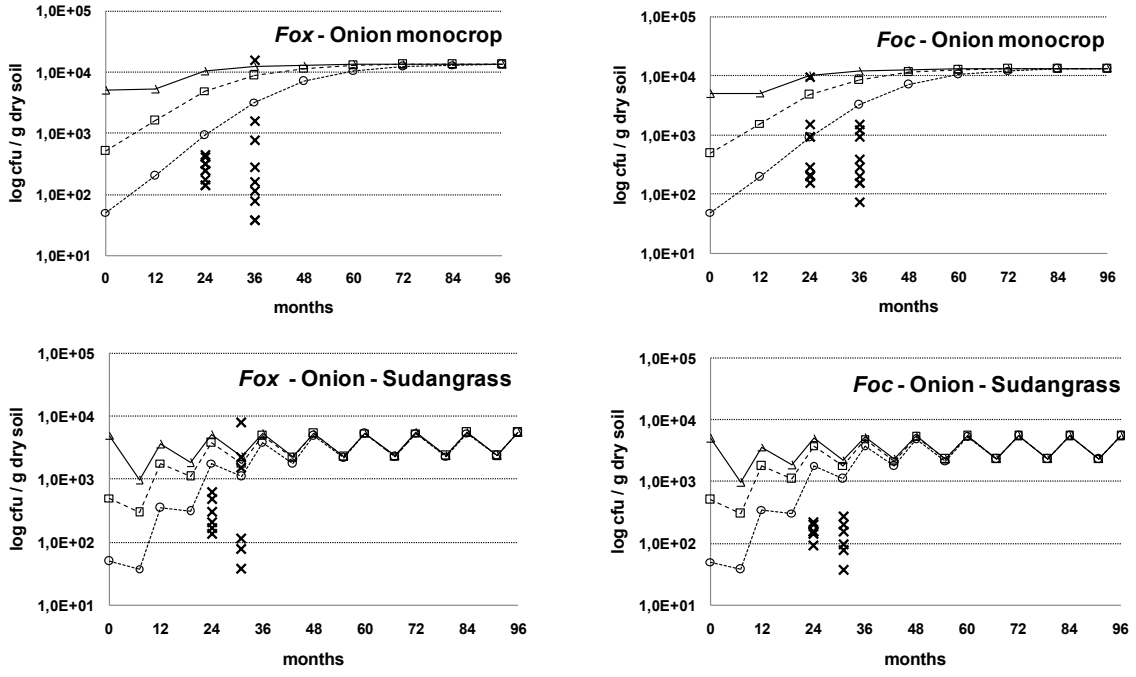

Figure 4. Simulated dynamics of soil inoculum of *Fusarium oxysporum f.sp. cepae* (cfu g^{-1} dry soil) in different crop sequences and measured values (mpn) of *Fox* and *Foc* in microplots at transplant and harvest of onion in 2011 (isolated crosses). All graphs start at month 0 – initial of first onion crop- and end at month 96, indicating the *Pi* value for starting a new onion crop. Simulations were run for three initial *Pi* values: 5 x 10^1 cfu g^{-1}dry soil, 5 x 10^2 cfu g^{-1}dry soil, and 5 x 10^3 cfu g^{-1}dry soil.

4 Discussion

4.1 Soil microbial population dynamics in farm fields and microplots

Our first objective was to study *Fusarium* soil dynamics under different crop sequences. Changes in *Fusarium oxysporum* populations from transplanting or seeding to harvest either increased or decreased. The observed *Pf* and *Pi* values obtained from the farm fields or microplots for different land uses (crops, fallow) when plotted against each other presented no clear deterministic relationship (Figure 2 and Figure 3), but resembled points on the phase-plane curves reported for the population dynamics of different phases in disease development (like latent and symptomatic populations) of a vector-transmitted disease with an attractor (Chiyaka et al. 2012). This similarity suggests that pathogen dynamics could be the result of different phases in a dynamic process: trapping of fungal propagules by the crop and release after in-root multiplication. In a crop sequence in the field, both phases occur at the same time, therefore *Pi* and *Pf* can be seen as different points in a process where production and release of fungal propagules occur, possibly resulting in oscillatory propagule dynamics which has been demonstrated for many micro-organisms in soil (He et al., 2010 and 2012; Semenov et al., 2010; Zelenev et al., 2006).

If such complex pathogen dynamics occurred, saturation curves could not be fit to the measured *Pi* and *Pf* in different fields (Figure 1). However, saturation curves could be fit to *Pi* and *Pf* data from pot experiments where pathogen populations in roots were included in the total population density at the end of the experiments (*Pf* values). Therefore, these saturation curves were used in the model for long-term behaviour of inoculum densities in soil.

Our field results demonstrate that the previous crop affected pathogen dynamics between transplanting and harvest. In most of the onion crops in farm fields and in microplots in 2011, *Fox* populations increased from transplanting to harvest when another onion crop was planted in the same field the previous winter (Figure 2B and Figure 3A). On the other hand, *Fox* populations decreased when a winter green manure – mostly white oat – was planted the previous winter (Figure 2C). Thus *Fusarium* values **in soil** reflect released pathogen propagules **from roots** from the previous and pre-previous crops. In other words, there is a strong delay in soil *Foc* populations in response to cropping history, thus *Pi* and *Pf* values are the result of the cropping history and not the result of the current land use.

The effect of different crops on microbial populations has been extensively reported. Plants and their management are the main driving forces for soil biological processes and strongly

affect soil biological composition, diversity and health (van Bruggen et al. 2006). For a given soil, crop rotation is the main “driver” in selecting soil functional groups, and the differences in quality and quantity of root exudates and dead plant materials from successive plants lead to the maintenance of different biological communities in soil (Garbeva et al. 2004, Hartmann et al. 2009).

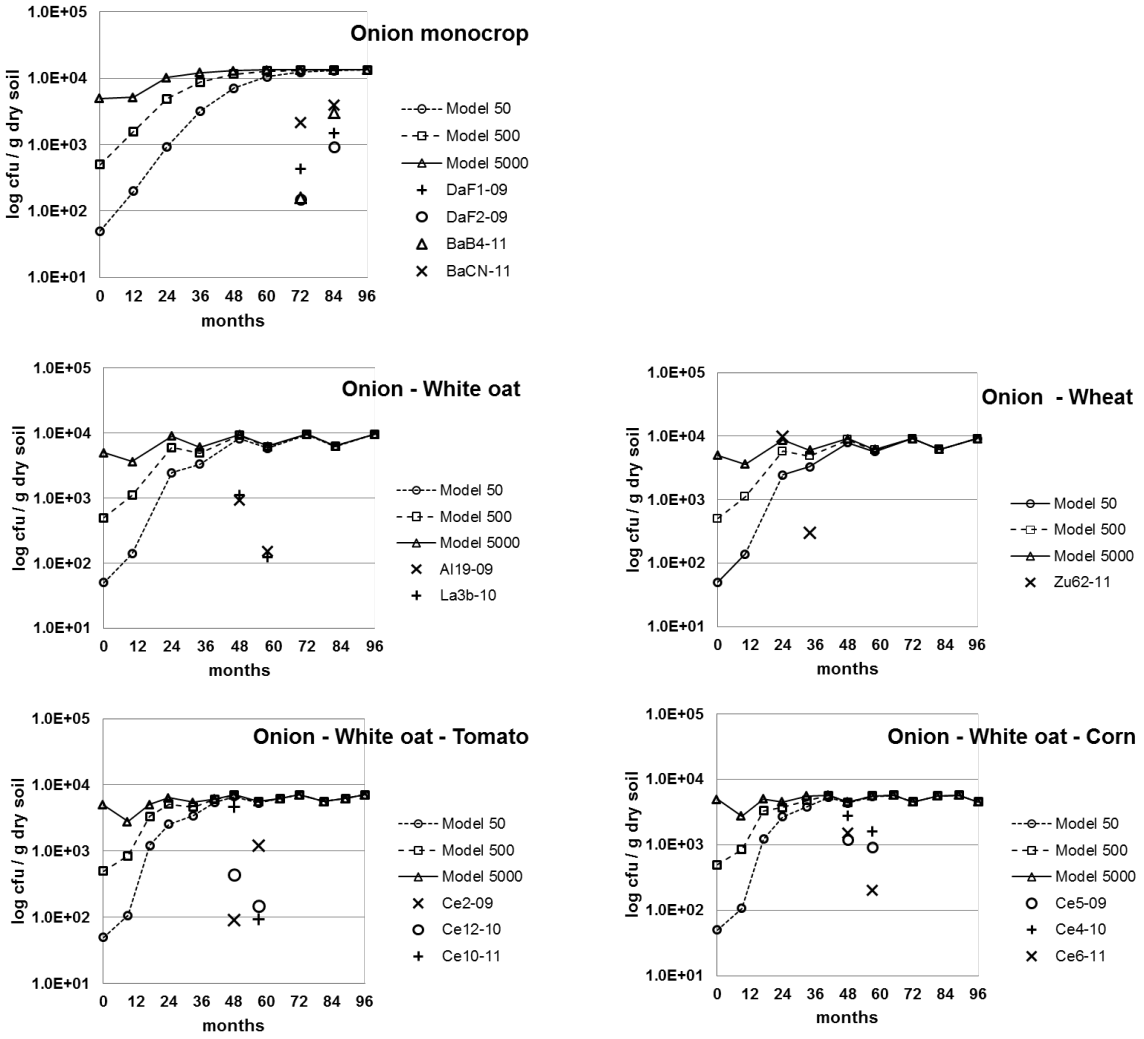

Figure 5. Simulated dynamics of soil inoculum of *Fusarium oxysporum f.sp. cepae* (cfu g^{-1} dry soil) in different crop sequences and measured values (mpn) of *Fox* in farm fields at transplant and harvest of onion (isolated symbols). All graphs start at month 0 – initial of first onion crop- and end at month 96, indicating the *Pi* value for starting a new onion crop. Simulations were run for three initial *Pi* values: 5 x 10^1 cfu g^{-1}dry soil, 5 x 10^2 cfu g^{-1}dry soil, and 5 x 10^3 cfu g^{-1}dry soil. Symbols for measured data are different for each farm field; the first part of the legend indicates the farm field (e.g. DaF1, DaF2, Bab4, etc.) and the second part the year of the measurements (-09 for 2009, -10 for 2010 and -11 for 2011).

4.2 Simulated Fusarium population dynamics in crop sequences

The second objective of this study was to design and test a research approach where experimental data and model simulations are combined to explore crop sequences that minimize *Foc* inoculum build-up. *Fusarium* dynamics was simulated with a model that described two processes: build-up of *Fusarium* within a crop and release by decomposition of the residues after crop harvest. The model was calibrated with greenhouse pot experiments and validated with data collected in microplots and on farm fields.

Model results supported the observations on the effect of cropping history on soil pathogen dynamics. Average duration of *Foc* inoculum retention in organic matter calculated as the inverse of the root decomposition parameter *b,* depended on crop species and ranged from 0.7 year (most crops) to 1.6 years for onion and 2.3 years for tomato (Table 2). This delay explains why after the same crop but with different cropping histories inoculum levels were found to both increase and decrease compared to levels at the start of the crop. In the case of onion the two simulations where *Pf* was higher than *Pi* were O-Sg and O-WO-C, due to the gramineous summer green crop immediately before onions (Table 6). Both sudangrass and corn enable multiplication of *Foc* in their root system (Leoni et al., 2013a), and according to the root decomposition parameter *b*, most of the propagules will have been released at the end of the onion crop following the summer crop. The opposite trend was observed when tomato was the previous summer crop. Tomato also allows multiplication of *Foc* (Leoni et al., 2013a), but root decomposition is slower than in gramineous plants (Tabe 2), and therefore most of the propagules will not be available at the end of the onion crop following a tomato crop.

When model results at equilibrium were compared with soil *Fusarium* populations in farm fields, the observed *Pi-Pf* trends within one cropping season were similar to the simulated trends for the sequences O-WO and O-Wt, and for two out of three observations for the sequence O-WO-T. In the case of onion monocrops, observed *Fox* dynamics in farm fields showed an increase in *Fox* populations during the onion crops, while the simulated populations had already stabilized (Figure 4).

The model generally overestimated *Fusarium* soil densities. This can be explained by the way model parameters were estimated and by the model definition and assumptions. Model parameters for equation 1 were estimated with experimental data from pot experiments, where a high inoculum level was used to investigate *Foc* multiplication in plants (Leoni et al. 2013a). In addition, the boundary for estimating *1/β* was quite high, with a value of 5×10^4

cfu g^{-1} dry soil, based on the threshold reported for starting an onion infection in peat soils (Abawi and Lorbeer 1972). However *Fox* populations in our soils were below 1 x 10^4 cfu g^{-1} dry soil and generally one and two orders of magnitude lower, than those reported by Abawi and Lorbeer (1972). Thus in future simulations, parameter *1/β* for the model can be best estimated by using the *Fusarium* soil population found in Uruguayan commercial farm fields as the prior distribution. In addition, model parameters were estimated from the multiplication of *Foc* in individually grown plants in pots, expressed as cfu g^{-1} dry root. In the model we assumed that these estimates were equivalent to cfu g^{-1} dry soil under field conditions. However, in the pots high root densities were attained which may not have been representative of root densities in the field. By including root and plant density in the calculation of the model parameters, the model will likely result in lower simulated pathogen populations and be more in agreement with field observations.

The second reason for overestimation of field measurements by the model was probably our simplification of the abiotic and biotic interactions in the soil by assuming that *Fusarium* survival and reproduction were not reduced by adverse environmental conditions in soil or other microbial populations. This simplification ignored, for example, that during root decomposition chemical compounds toxic to *Fusarium* conidia and chlamydospores could be released (Abawi and Widmer 2000, Bahraminejad 2008, Matthiessen and Kirkegaard 2006, Smolińska 2000). Also, the potential effect of different crops on soil microbial communities with potentially antagonistic effects on *Fusarium* propagules, like fluorescent *Pseudomonads* or *Trichoderma* (Hartmann et al. 2009, Smolińska 2000, Vinale et al. 2008) was ignored. Incorporation of such effects awaits advances in our knowledge about the ecology of the pathogen.

In addition to model structure and model parameter values, the observations in fields and microplots reflected large uncertainty. For instance, starting from similar and controlled initial conditions in the microplots, *Fox* as well as *Foc* values at the end of the last onion crop spanned one to two orders of magnitude. Evaluation of any model should take this uncertainty in field data into account. Irrespective of the 'truthfulness' of the model, the heuristic usefulness of the model is in showing the importance of cropping history in determining soil inoculum levels of *Foc*.

5 Conclusions

We demonstrated that there was not a simple linear or non-linear relationship between final and initial population densities (*Pf* versus *Pi*) of a plant pathogen in soil, but resembled points on typical phase-plane curves. A model concatenating pathogen population build-up in roots and decline in soil, based on results from pot experiments, was able to describe an increase or decrease in pathogen populations in real soil, despite some discrepancies in pathogen population trends (*Pi-Pf*) and general overestimations of simulated pathogen populations. Nevertheless, model outcomes pointed at the importance of cropping history for *Fusarium* dynamics in soil. In view of the considerable uncertainty in soil inoculum data for both model calibration and model evaluation, uncertainty could be taken into account in future studies as suggested in other studies (Leoni et al. 2013b; Chapter 3). To validate more complex and realistic crop sequences, pathogen populations and root densities would need to be monitored for several consecutive years in several fields with different crop rotations, taking crop history into account.

6 Acknowledgments

We thank Beatriz Dini, Jonathan Machi and Alfredo Fernandez (INIA - Las Brujas Research Station) for their assistance in the greenhouse and microplot experiments, and José Pedro Dieste and Sebastián Peluffo for their assistance in sampling soil from farm fields. We are also very grateful to the farmers who allowed us to work in their farms and shared their experiences with us. The first author thanks the Alban Programme of the European Union (Fellowship E07D401358UY), the Erasmus Mundus – Monesia scholarship program, and The National Agricultural Research Institute of Uruguay (INIA) for their financial support.

References

Abawi, G.S. and Lorbeer, J.W. 1972. Several aspects of the ecology of *Fusarium oxysporum* f. sp. *cepae*. *Phytophatology* 62: 870-876.

Abawi, G. S. and Widmer, T. L. 2000. Impact of soil health management practices on soil-borne pathogens, nematodes and root diseases of vegetable crops. *Applied Soil Ecology* 15 (1), 37-47.

Agamennoni, R., Prioletta, S., Baffoni, P., Vanzolini, J. I., Caracotche, V., Reinoso, O. 2008. Efecto de las rotaciones con pasturas y cultivos agrícolas. II. incidencia de podredumbre basal (*Fusarium*

oxysporum f.sp. *cepae*) en la cebolla en el sur de Buenos Aires. In: XXXI Congreso Argentino de Horticultura Resúmenes de trabajos.; Mar del Plata, Buenos Aires, Argentina.

Bahraminejad, S., Asenstorfer, R. E., Riley, I. T., Schultz, C. J. 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.). *Journal of Phytopathology* 156 (1),1-7.

Bailey, D.J. and Gilligan, C.A. 2004. Modeling and analysis of disease-induced host growth in the epidemiology of take-all. *Phytopathology* 94: 535 – 540.

Casagrande, M., Dogliotti, S., Groot, J.C.J., Aguerre, V., Abbas, A., Albin, A., Claassen, G.D.H., Chilibroste, P., Rossing W.A.H. 2010. Exploring options for sustainable farming systems development for vegetable family farmers in Uruguay using a modelling toolkit. In: 9th European IFSA Symposium, Building sustainable rural futures. The added value of systems approaches in times of change and uncertainty, Vienna, Austria, 04 - 07 July, 2010, pp. 463 – 469.

Chiyaka, C., Singer, B.H., Halbert, S.E., Morris, J.G.Jr., van Bruggen, A.H.C. 2012. Modeling huanglongbing transmission within a citrus tree. *Proceedings of the National Academy of Sciences* 109: 12213 – 12218.

Colbach, N., Meynard, J-M, Duby, C., Huet, P. 1999. A dynamic model of the influence of rotation and crop management on the disease development of eyespot. Proposal of cropping systems with low disease risk. *Crop Protection* 18, 451 - 461.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2003. ROTAT, a tool for systematically generating crop rotations. *European Journal of Agronomy* 19: 239-250.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2004. Systematic design and evaluation of crop rotations enhancing soil conservation, soil fertility and farm income: a case study for vegetable farms in South Uruguay. *Agricultural Systems* 80, 277 - 302.

Dogliotti, S., Garcia, M.C., Peluffo, S., Dieste, J.P., Pedemonte, A.J., Bacigalipe, G.F., Scarlato, M., Alliaume, F., Alvarez, J., Chiappe, M., Rossing, W.A.H. 2013. Co-innovation of family farm systems: A systems approach to sustainable agriculture. *Agricultural Systems.* http://dx.doi.org./10.1016/j.agsy.2013.02.009.

Fox, J., and Weisberg, S. 2011. An {R} Companion to Applied Regression, Second Edition. Thousand Oaks CA: Sage. http://socserv.socsci.mcmaster.ca/jfox/Books/Companion. Accesed 25 June 2012.

Garbeva, P., van Veen, J.A., van Elsas, J.D. 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease supressiveness. *Annual Review of Plant Pathology* 42: 243 – 270.

Hartmann, A., Schmid, M., van Tuinen, D., Berg, G. 2009. Plant-driven selection of microbes. *Plant and Soil* 321: 235-257. doi: 10.1007/s11104-008-9814-y.

He, M., Ma, W., Tian, G., Blok, W., Khodzaeva, A., Zelenev, V.V., Semenov, A.M., van Bruggen, A.H.C. 2010. Daily changes of infections by *Pythium ultimum* after a nutrient impulse in organic versus conventional soils. *Phytopathology* 100: 593-600.

He, M., Tian, G., Semenov, A.M. van Bruggen, A.H.C. 2012. Short-term fluctuations of sugar-beet damping-off by *Pythium ultimum* in relation to changes in bacterial communities after organic

amendments to two soils. *Phytopathology* 102: 413-420.

Hiddink, G.A., van Bruggen, A.H.C., Termorshuizen, A.J., Raaijmakers, J.M., Semenov, A.V. 2005. Effect of organic management of soils on suppressiveness to *Gaeumannomuces graminis* var. *tritici* and its antagonist, *Pseudomonas fluorescens*. *European Journal of Plant Pathology* 113: 417 – 435.

Leoni, C. and Ghini, R. 2002. Efeito do lodo de esgoto na indução de supressividade *in vitro* a *Phytophthora nicotianae*. *Fitopatologia Brasileira* 28: 67-75.

Leoni, C., de Vries, M., ter Braak, C.J.F., van Bruggen, A.H.C, Rossing, W.A.H. 2013a. *Fusarium oxysporum* f.sp. *cepae* dynamics: in-plant multiplication and crop sequence simulations. *European Journal of Plant Pathology* 137: 545-561.

Leoni, C., ter Braak, C.J.F., Gilsanz, J.C, Dogliotti, S., Rossing, W.A.H., van Bruggen, A.H.C. 2013b. *Sclerotium rolfsii* dynamics in soil as affected by crop sequences. *Applied soil Ecology* (accepted).

Leslie, J.F. and Summerell, B. A. 2006. The Fusarium Laboratory Manual. Blackwell Publishing. 388p. ISBN: 978-0-8138-1919-8.

Matthiessen, J. N. and Kirkegaard, J. A. 2006. Biofumigation and enhanced biodegradation: opportunity and challenge in soilborne pest and disease management. *Critical Reviews in Plant Science* 25: 235-265.

McDonald M., Jaime M., Hovius M. 2004. Management of diseases of onions and garlic. In: Naqvi, S.A.M.H. (Ed.) Diseases of fruits and vegetables, Vol II. (pp. 149-200). The Netherlands, Springer.

Mol, L., Huisman, O.C., Scholte, K., Struik, P.C. 1996. Theoretical approach to the dynamics of the inoculum density of Verticillium dahliae in the soil: first test of a simple model. *Plant Pathology* 45: 192 – 204.

Özer N., N.D. Kycti, N.D., Mirik, M., Soran, H., Boyraz, D. 2002. Effect of some organic amendments on onion bulb rot. *Phytoparasitica* 30: 429-433.

Özer N. and Köycü D. 2004. Seed-borne fungal diseases of onion, and their control. *Fruit and Vegetable Diseases* 1: 281-306.

R Development Core Team. 2012. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0, http://www.R-project.org/.

Semenov, A.V., Franz, E., van Bruggen, A.H.C. 2010. COLIWAVE: a simulation model for survival of *E. coli* O157:H7 in dairy manure and manure-amended soil. *Ecological Modelling* 221: 599-609.

Silver, W.L. and Miya, R.K. 2001. Global patterns in root decomposition: comparisons of climate and litter quality effects. *Oecologia 129:* 407 – 419.

Sarkar, D. 2008. *Lattice: Multivariate Data Visualization with R*. New York, Springer.

Smolińska, U. 2000. Survival of *Sclerotium cepivorum* sclerotia and *Fusarium oxysporum* chlamydospores in soil amended with cruciferous residues. *Journal of Phytopathology* 148: 343 – 349.

ter Braak, C.J.F. and Vrugt, J.A. 2008. Differential evolution Markov Chain with snooker update rand

fewer chains. *Statistics and Computing 18:* 435 – 446.

Tixier, P., Risède, J.M., Dorel, M., Malézieux, E. 2006. Modelling population dynamics of banana plant-parasitic nematodes: A contribution to the design of sustainable cropping systems. *Ecological Modelling* 198: 321 – 331.

van Bruggen, A.H.C., Semenov, A.M., van Diepeningen, A.D., de Vos, O.J., Blok, W.J. 2006. Relation between soil health, wave-like fluctuations in microbial populations, and soil-borne plant disease management. *European Journal of Plant Pathology* 115:105–122.

van den Berg, W. and Rossing W.A.H. 2005. Generalized linear dynamics of a plant-parasitic nematode population and the economic evaluation of crop rotations. *Journal of Nematology 37*: 55-65.

van den Berg, W., Rossing, W.A.H., Grasman, J. 2006. Contest and scramble competition and the carry-over effect in *Globodera* spp. in potato-based crop rotations using an extended Ricker model. *Journal of Nematology* 38: 210 – 220.

Vinale, F., Sivasithamparam, K., Ghisalberti, E. L., Marra, R., Woo, S. L., Lorito, M. 2008. Trichoderma–plant–pathogen interactions. *Soil Biololgy and Biochemistry* 40: 1–10.

Williams, J., Clarkson, J.M., Mills, P.R., Cooper, R.M. 2003. A selective medium for quantitative reisolation of *Trichoderma harzianum* from *Agaricus bisporus* compost. *Applied and Environmental Microbiology* 69: 4190–4191.

Zelenev, V.V., van Bruggen, A.H.C., Leffelaar, P.A., Bloem, J., Semenov, A.M. 2006. Oscillating dynamics of bacterial populations and their predators in response to fresh organic matter added to soil: the simulation model 'BACWAVE-WEB'. *Soil Biology and Biochemistry* 38: 1690-1711.

Supplementary data

Table S1. Average air temperature (Temp, °C), mean air relative humidity (RH, %), effective precipitation (EP, mm) and evapotranspiration (Evap., Class A evaporation pan, mm) during 2009, 2010 and 2011, registered in the field with an automatic weather station located at INIA Las Brujas Research Station (http://www.inia.org.uy/online/site/692646I1.php), and average values for a 30-year period (1980-2009).

	January				**February**				**March**			
	Temp	RH	EP	Evap	Temp	RH	EP	Evap	Temp	RH	EP	Evap
	˚C	%	mm	mm	˚C	%	mm	mm	˚C	%	mm	mm
						2009						
Average	23	73	49	288	22	83	118	180	21	90	116	140
Max	27	100			27	100			24	100		
Min	18	42			18	69			17	73		
						2010						
Average	24	84	102	239	22	91	173	169	21	83	38	179
Max	26	99			26	100			24	97		
Min	18	69			17	76			17	46		
						2011						
Average	24	62	38	286	22	65	48	205	21	66	47	183
Max	28	77			26	82			25	87		
Min	21	48			20	40			16	56		
AVERAGE (from 1980 to 2009)												
Average	17	75	113		14	78	95		11	79	100	

	April				**May**				**June**			
	Temp	RH	EP	Evap	Temp	RH	EP	Evap	Temp	RH	EP	Evap
	˚C	%	mm	mm	˚C	%	mm	mm	˚C	%	mm	mm
						2009						
Average	17	86	7	119	14	91	57	105	9	94	86	64
Max	21	97			22	100			16	100		
Min	13	73			8	74			5	86		
						2010						
Average	16	89	99	115	15	85	101	62	11	81	74	52
Max	21	100			20	100			16	100		
Min	11	77			9	61			7	57		
						2011						
Average	17	72	80	102	13	75	44	65	10	79	110	44
Max	22	86			16	86			17	88		
Min	14	53			9	64			5	63		
AVERAGE (from 1980 to 2009)												
Average	17	75	113		14	78	95		11	79	100	

	July				August				September			
	Temp	RH	EP	Evap	Temp	RH	EP	Evap	Temp	RH	EP	Evap
	˚C	%	mm	mm	˚C	%	mm	mm	˚C	%	mm	mm
						2009						
Average	8	91	104	97	13	89	38	124	12	91	77	79
Max	14	100			24	99			15	100		
Min	4	81			6	73			8	77		
						2010						
Average	10	83	128	46	11	78	94	61	14	77	96	93
Max	21	96			18	93			19	93		
Min	4	64			5	60			10	65		
						2011						
Average	9	75	103	56	10	76	87	63	13	66	21	113
Max	18	89			16	92			19	87		
Min	4	63			5	61			7	52		
AVERAGE (from 1980 to 2009)												
Average	10	78	81		12	75	87		13	73	88	

	October				November				December			
	Temp	RH	EP	Evap	Temp	RH	EP	Evap	Temp	RH	EP	Evap
	˚C	%	mm	mm	˚C	%	mm	mm	˚C	%	mm	mm
						2009						
Average	15	85	133	115	19	89	125	141	20	88	55	173
Max	24	100			24	100			24	99		
Min	9	75			15	71			15	74		
						2010						
Average	15	71	48	141	18	68	21	181	23	55	29	282
Max	20	91			23	80			27	76		
Min	11	55			12	52			14	38		
						2011						
Average	15	72	70	133	20	63	114	212	20	67	64	216
Max	19	85			19	85			27	84		
Min	11	52			16	50			16	50		
AVERAGE (from 1980 - 2009)												
Average	16	71	117		19	69	105		21	66	79	

Chapter 6

General discussion

1 Introduction

Crop rotation has been used for the management of soilborne diseases for centuries, but has not often been planned based on scientific knowledge. Crops in the rotation should be selected in such a way that the probabilities for pathogens to infect and colonize the host are minimized. If a non-host is included in the rotation scheme, the pathogen cannot infect the non-host plant and therefore its reproduction and survival are affected, resulting in a decline in the pathogen population. Depending on the pathogen's survival ability, the period without hosts may need to range from one to more than 10 years; not an easy task for the management of pathogens with multiple hosts (Figure 1).

Crop rotations are at the core of farming systems, and should accomplish several objectives, not only soilborne disease management. Models can support the design of crop sequences and help to reveal synergies and trade-offs among objectives (Casagrande et al. 2010, Dogliotti et al. 2003, Dogliotti et al. 2004). Additionally, models can contribute to the re-design of farming systems by exploring several crop sequences followed by the discussion of the most feasible options with farmers and advisors, in order to find "tailor-made" solutions (Dogliotti et al. 2013). Despite their importance, pathogen dynamics are rarely taken into account in cropping system models, or at best are introduced as forcing functions influencing the state of the system without being affected by the system (Tixier et al. 2006).

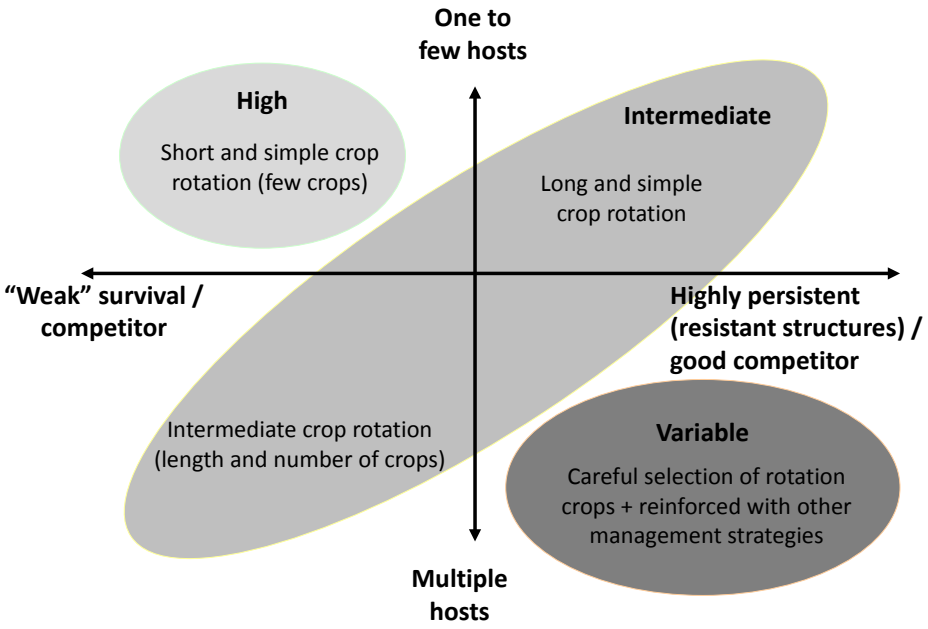

Figure 1. Effectiveness of crop rotation for disease management depending on frequency of hosts and survival characteristics of the pathogen (taken from Chapter 2).

Management of soilborne pathogens through crop rotation involves the selection of the appropriate crops and their sequence in the rotation. To carry out the selection, quantitative information on soilborne pathogen dynamics in the presence of different crops and intercrop activities (e,g. green manures, fallow) is needed (Grünwald et al. 2000). Several population dynamics models describing soilborne pathogen dynamics have been proposed (Mol et al. 1996, Taylor and Rodriguez-Kábana 1999 b, Termorshuizen and Rouse 1993, Tixier et al. 2006, Thrall et al. 1997, van den Berg and Rossing 2005, van den Berg et al. 2006, and in Chapters 3, 4, and 5 of this thesis). Quantitative information to feed the models can be retrieved from literature for several soilborne pathogens, however the effects of the local agro-environment, the intercrop period and intercrop management are not always well quantified (Lucas, 2006).

The general aim of this thesis was to explore a research approach where experimental data and model simulations were combined to explore crop sequences that minimize soilborne pathogen inoculum build up, to subsequently include this information into models for designing sustainable crop rotations. More specifically, the aims were to provide a quantitative understanding of population dynamics of *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *cepae* in vegetable production systems and to apply insight in population dynamics of these soilborne pathogens for designing sustainable cropping systems.

2 Main findings

2.1 Soil population dynamics of *Sclerotium rolfsii*

The dynamics of *S. rolfsii* sclerotia was studied in two different processes: survival in crop debris amended and un-amended soil and population changes under different crop sequences. Survival of sclerotia was studied through pot experiments during two years, whereas the effect of three cropping sequences (sweet pepper – fallow, sweet pepper – black oat and sweet pepper – onion) on sclerotia dynamics was analyzed in microplot experiments (Chapter 3).

Sclerotia survival in soil after winter green manure (WGM) incorporation and decomposition during summer was generally lower than after summer green manure (SGM) incorporation and decomposition during winter, consistent with decreased survival of *S. rolfsii* at temperatures above 20°C (Beute and Rodríguez-Kábana 1981). But the most important result was that certain crops allowed multiplication of sclerotia while others resulted in a

reduction of the sclerotia densities (Chapter 3). In particular, the incorporation of the residues of various legume crops (black beans, cowpea, hairy vetch and lupines) resulted in the highest sclerotia densities, suggesting either saprophytic growth or sclerotia germination and colonization, resulting in formation of new sclerotia (Beute and Rodriguez-Kabana 1981, Flores-Moctezuma et al. 2006, Punja and Grogan 1981, Punja et al. 1984). On the other hand, various grasses (sudangrass, foxtail millet, oats and wheat) as well as sunhemp resulted in a reduction of sclerotia in the soil, probably due to release of toxic chemical compounds during plant tissue decomposition (Bahraminejad et al. 2008, Stapleton et al. 2010).

In the microplots, the summer crop sweet pepper was the crop that supported the greatest multiplication of the pathogen, whereas the winter crops black oat and onion hardly allowed multiplication of the pathogen. The differences in sclerotia multiplication were mainly due to pathogen requirements of moderately high temperatures and high soil humidity to multiply and cause disease (Jenkins and Averre 1986, Punja 1985). The build-up of sclerotia populations in the microplots was also dependent on the crop sequence, resulting in greater multiplication by sweet pepper after black oat than after onion or fallow (Chapter 3), in accordance with previous reports (Taylor and Rodriguez-Kabana 1999a).

2.2 Soil population dynamics of *Fusarium oxysporum* f.sp. *cepae*

The dynamics of *Fusarium oxysporum* f.sp. *cepae* (*Foc*) was studied at two different levels: multiplication in single plants and survival or growth in different crop sequences. *Foc* multiplication in different plant species was investigated through pot experiments (Chapter 4), whereas the effect of cropping sequences on *Foc* dynamics in soil was analysed in microplot experiments and in farm fields located in Southern Uruguay (Chapter 5).

Foc colonized and multiplied in the root systems of the 13 non *Allium* plant species tested without inducing disease symptoms or growth retardation, as demonstrated for other *formae speciales* of *Fusarium oxysporum* (Banihashemi and de Zeeuw 1975, Dhingra and Coelho Netto 2001, Gordon et al. 1989, Helbig and Carroll 1984, Katan 1971, Leslie et al. 1990, Oritsejafor and Adeniji 1990). Following Dhingra and Cohelo-Neto (2001) we conclude that all the plant species tested are “reservoir-hosts” for *Foc*, even though the extent of *Foc* multiplication differed for each plant species. The lowest *Foc* levels per g of dry weight of root were found in wheat, sunflower, cowpea and millet; intermediate levels in onion, oat, black oat, white lupine, blue lupine, tomato, sudangrass, corn and sweet corn; whereas the highest

Foc level was found in black bean (Chapter 4).

Fusarium pathogen dynamics was strongly affected by the cropping history in a particular field. *Fox* populations in onions (a winter crop) increased from transplant to harvest when another onion crop was planted in the same field the previous winter in most of the onion crops in farm fields and in microplots in 2011 (Chapter 5). Crop sequence is the main "driver" in selecting different biological communities in soil due to differences in quality and quantity of root exudates and dead plant materials from successive plants (Abdel-Monaim and Abo-Elyousr 2013, Garbeva et al. 2004, Hartmann et al. 2009).

2.3 Simulation of population dynamics in crop sequences

Pathogen dynamics in crop sequences was simulated for the two soilborne pathogens *S. rolfsii* and *Foc,* by concatenating models describing build-up in host crops and subsequent decline (*S. rolfsii*) or release (*Foc*). For *S. rolfsii* the model was calibrated using quantitative data generated in pot and microplot experiments (Chapter 3), whereas for *Foc* the data for calibration came from pot experiments (Chapter 4) and the model was validated with data collected in microplots and on farm fields (Chapter 5).

The model for *S. rolfsii* described the build-up of the pathogen within a crop (equation 1) followed by the decline of inoculum after incorporation of crop debris into soil (equation 2) (Figure 2A).

$$Pf_{(n)} = Pi_{(n)}/(\alpha_{(n)} + \beta_{(n)}\ Pi_{(n)}) \quad (1)$$

where *Pi* and *Pf* are soil population densities at the start and at the end of the activity n –here the current crop-, *1/α* represents the slope at the origin and *1/β* the horizontal asymptote (van den Berg and Rossing 2005).

$$Sf_{(n)} = Si_{(n)}\ e^{(-bd)} \quad (2)$$

where *Si* and *Sf* are soil sclerotia populations at the start and at the end of the intercrop period after crop n , *b* the relative decay rate of the sclerotia after soil incorporation of crop n and *d* the length of the intercrop period (Chapter 3).

The model for *Fusarium* described the build-up of the pathogen within a crop (equation 1)

followed by gradual pathogen release from plant roots after harvest (equation 3) (Figure 2B).

$$Pf_{(n,t)} = Pf_{(n,0)}(1 - e^{-bt}) \quad (3)$$

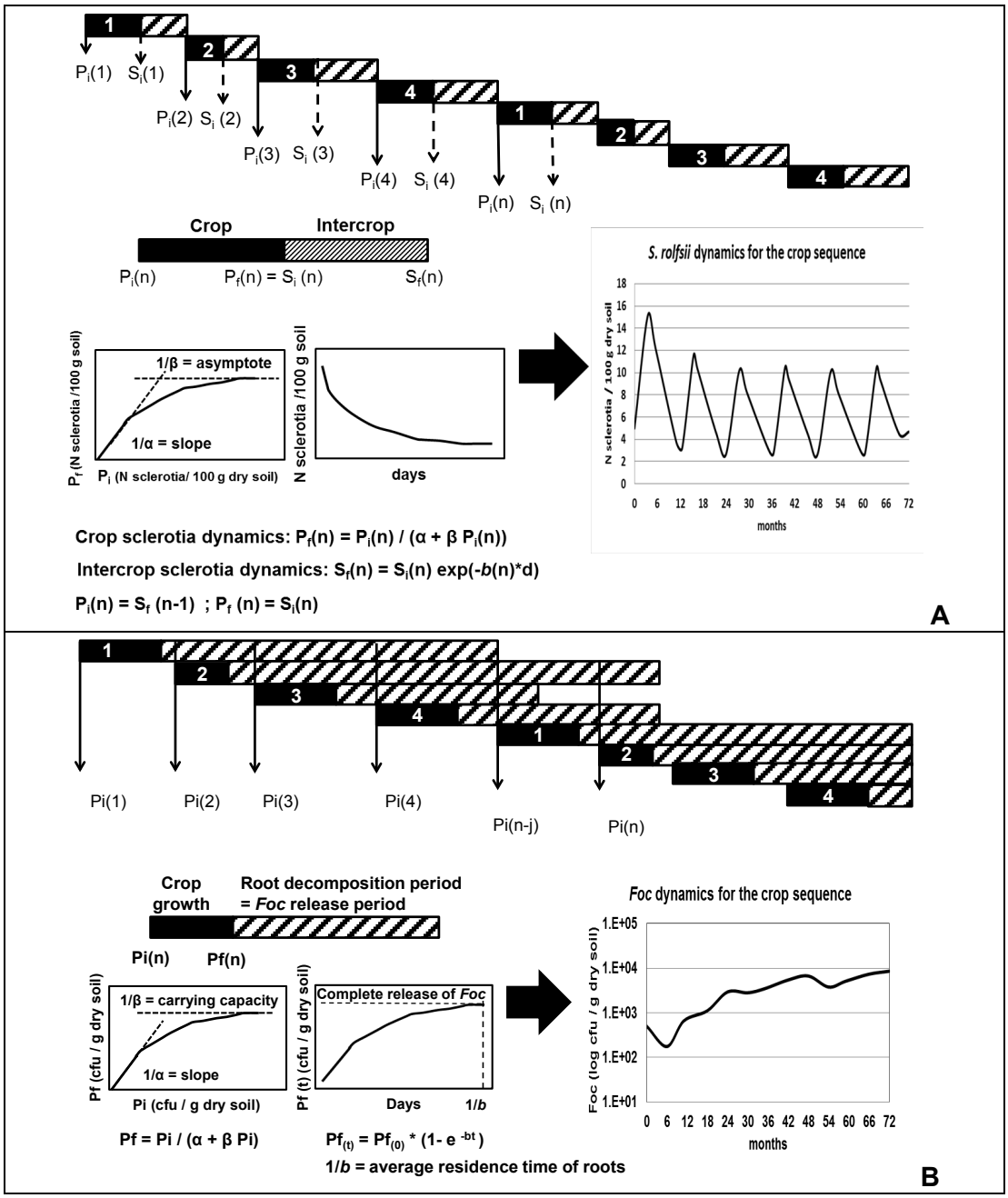

Figure 2. Schematic representation of a crop sequence and the processes involved in soil pathogen dynamics. (A) *Sclerotium rolfsii*: pathogen multiplication during crop growth and sclerotia decline after incorporation of crop debris into soil. (B) *Fusarium oxysporum:* pathogen multiplication during crop growth and pathogen release after root decomposition (modified from Chapter 3 and Chapter 4).

where *Pf* constitutes inoculum in the roots, n the corresponding crop, *t* is time since harvest of crop n and *b* represents the relative decay rate of the roots of crop n (day^{-1}). Inoculum density at the start of a crop *Pi* depends on the amount of inoculum released into the soil by decomposition of roots of previous crops, taking into account that inoculum is lost from the soil pool as it is assumed to infect each starting crop (Chapter 5).

Regardless of the simplicity and limitations of the population models described (Chapter 3, Chapter 5) differences among crop sequences and alternating cycles of increasing and decreasing soil pathogen populations were detected. Also differences at equilibrium populations were related to host frequency and to cropping history, as pointed out by several authors (Abdel-Monaim and Abo-Elyousr 2013, Buhre et al. 2009, Colbach et al. 1999, Cunfer et al. 2006, Taylor and Rodriguez-Kabana 1999a, Taylor and Rodriguez-Kabana 1999b). For *S. rolfsii*, the equilibrium inoculum densities were much higher for the sweet pepper monocrop (sweet pepper followed by a winter fallow) than for the sequences sweet pepper - winter crop (Chapter 3). For *Fusarium,* cropping history was particularly important for the dynamics of the pathogen in the soil. As an example, *Foc* soil populations during onion crops increased from transplanting to harvest when the previous summer crop was either sudangrass or corn, whereas *Foc* soil populations decreased when the previous summer crop was tomato (Chapter 5).

Both *S. rolfsii* and *Foc* colonize and multiply in several plant species, and can survive for long periods in soil due to their resistant survival structures (*S. rolfsii* by sclerotia and *Fusarium* by chlamydospores), However, the population dynamics in soil of each pathogen is different. This difference could be explained with the conceptual model presented in Chapter 1. After multiplication of the pathogen in the crop the infected plant tissue carrying the pathogen decays and releases the infective pathogen units into the soil (Figure 2 in Chapter 1). For *S. rolfsii*, the pathogen colonizes the plant near the soil line and produces sclerotia on diseased tissues. Diseased tissues decompose rapidly due to the action of oxalic acid and pectinolytic and cellulolytic enzymes, and therefore the release of the sclerotia into the soil is also a relative quick process (Le 2011, Punja 1985). In the case of *Foc,* the pathogen penetrates the roots through wounds or directly, and multiplies within plant tissues (Brayford 1996, Chapter 4). Then the infective units of the pathogen, mainly chlamydospores, are released along with root decomposition, which is a relative slow process that can last between 0.7 to 2.3 years (Chapter 5). This delay influences inoculum build-up in soil, and explains the differences in the importance of cropping histories for population dynamics in the field (Chapter 5). The long-term effects of cropping history on *Fusarium* dynamics may be partially

responsible for the discrepancies between modelled and observed population densities of *Fusarium* densities in field soil.

3 Reflections on the research methodology

To support the design of crop sequences for maximizing suppression of soilborne pathogens demands quantitative data of pathogen dynamics and integration by modelling. The novelty of the present work is that data from individual short experiments on component crops or intercrop activities can be combined in simple population models to predict the final inoculum levels in different sequences of these component crops after many years. The models described in this thesis can be used to explore and design crop rotations suppressive to soilborne pathogens.

The implementation of simple and short (3 months to 2 years) experiments conducted under controlled conditions (greenhouse pot experiments, microplots) allowed the parameterization of several component crop and intercrop activities (Chapter3, Chapter 4). This strategy is more efficient in time and space than most of the quantitative approaches found in literature for modelling soilborne pathogen population dynamics, where intensive previous research on the ecology and epidemiology of the modelled pathosystems is required (Bailey and Gilligan 2004, Colbach et al. 1999, Mol et al. 2004, Taylor and Rodriguez-Kabana 1999a, Taylor and Rodriguez-Kabana 1999b, Tixier et al. 2006, van den Berg and Rossing 2005, van den Berg et al. 2006).

The modelling approach consisted of concatenating simple population models describing the soilborne pathogen dynamics for each crop or intercrop activity in one cropping season, to simulate long term rotations. The concatenating approach was also proposed for nematode dynamics under different crop sequences as well as the use of simple population models (van den Berg and Rossing 2005). One advantage of the simple models is that few parameters need to be estimated, and therefore fewer data are required. However this oversimplification can lead to inaccurate predictions (Chapter 5).

One first step to improve the model outcome is to refine our experimental data to enable linking pot experiments with field measurements. For example for *Foc* dynamics, model parameters were estimated with measurements of in-plant pathogen multiplication per gram of roots assuming equivalence with one gram of soil in the field (Chapter 4, Chapter 5). However, specific information about the contribution of the crop, not the individual plant, to

soil inoculum build-up is needed, taking planting density, root development and soil exploration by roots into account. This information will contribute to a reduction in the discrepancies between predicted and on-farm measured soil *Foc* populations. Moreover, more *Foc* data would be needed for subsequent crops in the same fields for model validation, rather than single observations in multiple fields.

A second step to improve model predictions is to consider the uncertainty of soilborne pathogen data used for parameter estimations. Uncertainty about soilborne pathogen population levels is caused by pathogen distribution in the soil - generally in foci -, to sampling procedures and to quantification methods. To represent the uncertainties associated with the data, stochastic simulations provide a powerful approach (Rossing et al. 1994). Stochastic simulations are run while considering the distribution of the parameter values instead of using fixed parameter values (e.g. based on means) as in deterministic simulations. Stochastic approaches have been proposed and used in modelling pathogen and pest dynamics, as well as for other biological sciences, along with social and economic sciences (Fabre et al. 2006, Savage 2012).

Another strategy is to combine information from different experimental sources by Bayesian calibration methods, where information about the probability distribution (prior distribution) of the parameters to be estimated is required (Ellison 2004, Fabre et al. 2006). In subsequent cycles of model adjustment, the experimental data generated in simple experiments can be used as prior information for parameter calibration, and the resulting posterior distribution constitutes the input for the stochastic model. New data to validate the models could be obtained in other controlled experiments (pot or field experiments) or in farm surveys. In the latter case, the variability found among different farming systems and environments can be taken into account.

4 Implications for future research

In this thesis an approach to explore crop sequences that minimize soilborne pathogen inoculum build up was presented, and was developed based on two SBP with different survival strategies. The outcomes from the simulations as carried out in this work can be included in models for designing crop rotations (Bachinger and Zander 2007, Dogliotti et al. 2003, Schönhart et al. 2012) or more complex models that aim at the re-design of whole-farm system where the trade-offs of the different crop and intercrop activities with objectives other than disease suppression are analysed (Casagrande et al. 2010, Dogliotti et al. 2013). For example, ROTAT (Dogliotti et al. 2003) was developed to create all feasible crop

rotations based on a list of crops and well-defined quantitative agronomic rules. These agronomic rules are filters included to eliminate undesirable crop sequences, and are built on expert knowledge or on previous research, either classical experimentation or simulations. The outcome of the models presented in this thesis could contribute to define particular filters in order to minimize the inoculum build-up of key soilborne pathogens. For example, the "maximum cropping frequency of a crop or groups of related crops" or the "order of the crops in the sequence" can be established more precisely for the pathosystems studied (*Foc*- onion and *S. rolfsii* – sweet pepper). However, to further contribute to the design of sustainable cropping sequences, the information on soilborne pathogen population dynamics should be linked with information on disease incidence and severity and associated yield losses. This will allow for economic assessment of alternative options when this information is included in multi-objective models like FarmIMAGES (Dogliotti et al. 2005).

The development of sustainable farming systems requires cropping options that increase farm productivity by taking advantage of the benefits of biological regulation in the agro-ecosystems, reducing the dependency on non-renewable and external inputs and optimizing the use of water and energy compared to sub-optimal crop rotations or monocultures (Altieri et al. 2012, Malézieux 2012, United Nations 2013). Crop rotations consisting of single crop sequences can contribute to this general objective as demonstrated (Chapter 2), but also the use of mixed crops and intercrops need to be evaluated. Recent research has highlighted the importance of these alternatives for the management of pathogens (Abdel-Monaim and Abo-Elyousr 2013, Boudreau 2013, Hiddink et al. 2009, Ratnadass et al. 2012). Classical experimentation with mixed crops and intercrops is complex and expensive, however the research strategy proposed in this thesis where simple experiments (pot, microplot) and modelling are combined, could contribute to identify promising combinations of crops and their effect on soilborne pathogen dynamics.

In this thesis we studied the population dynamics of two soilborne pathogens (*Sclerotium rofsii* and *Fusarium oxysporum f.sp. cepae)* in crop sequences. Supporting observations were made on soil populations of known antagonists like *Trichoderma* spp., actinomycetes and fluorescent *Pseudomonas* (Chapter 5). However no clear correlations were found between these microbial groups and *Fox*, possibly because these groups were not the relevant ones. It is likely that infection by *Foc* and subsequent population build-up is affected by non-pathogenic *Fox* populations (Lemanceau et al., 1993), but it is currently difficult to distinguish between *Foc* and *Fox* populations in natural field soil. The study of the effect of different crop sequences on specific soil microbial communities may contribute to our

understanding of the processes involved in pathogen dynamics and disease expression. However, quantification of soil microbial populations is a difficult task. Quantification through plating soil dilutions on selective or semi-selective media demands too much time and labour and is selective towards profusely sporulating species; besides only cultivable microorganisms can be evaluated. Nowadays several molecular techniques are available to overcome these limitations. Soil microbial community structure, for example, can be analysed through Denaturing Gradient Gel Electrophoresis (DGGE) (Garbeva et al. 2003, Muyzer and Smalla 1998, Wakelin et al. 2008) or pyrosequencing (Roesch et al. 2007) and quantification of target microorganisms can be performed by real-time polymerase chain reaction (q-PCR) (Okubara et al. 2005). These molecular techniques will allow identification and quantification of different taxonomic and functional groups that could contribute to the elucidation of the processes involved in disease suppression or in disease development.

5 Conclusions

This thesis makes an important methodological contribution to the design of crop rotations and their effects on soil borne pathogen dynamics. The combination of data from controlled experiments, novel analytical tools (Bayesian analysis, modelling and simulation) and on-farm observations can lead to the identification of optimal crop rotations without extensive field experiments that require a lot of time, space and economic resources.

In the present work, *S. rolfsii* and *F. oxysporum* f.sp. *cepae* served as examples of two ecologically distinct soilborne pathogens to examine the potential for simple crop rotation models to predict pathogen population dynamics in farmers' fields. To further contribute to the design of sustainable vegetable crop rotations, research on other soilborne pathogens as well as on their interactions and combined effects on crop growth and development is needed.

References

Altieri, M.A., Funes-Monzote, F.R., Petersen, P. 2012. Agroecologically efficient agricultural systems for smallholder farmers: contributions to food sovereignty. *Agronomy for Sustainable Development* 32: 1-13. doi: 10.1007/s13593-011-0065-6.

Abdel-Monaim, M. F. and Abo-Elyousr, K.A.M. 2013. Effect of preceding and intercropping crops on suppression of lentil damping-off and root rot disease in New Valley e Egypt. *Crop Protection*

32: 41 -46.

Bachinger, J, and Zander, P. 2007. ROTOR, a tool for generating and evaluating crop rotations for organic farming systems. *European Journal of Agronomy* 26: 130 – 143.

Bahraminejad, S., Asenstorfer, R.E., Riley, I.T., Schultz, C.J. 2008. Analysis of the antimicrobial activity of flavonoids and saponins isolated from the shoots of oats (*Avena sativa* L.). *Journal of Phytopathology* 156:1-7.

Banihashemi, Z., and deZeeuw, D.J. 1975. The behaviour of *Fusarium oxysporum* f.sp. *melonis* in the presence and absence of host plants. *Phytopathology* 65: 1212 - 1217.

Beute, M. K. and Rodríguez-Kabana, R. 1981. Effects of soil moisture, temperature and field environment on survival of *Sclerotium rolfsii* in Alabama and North Carolina. *Phytopathology* 71: 1293-1296.

Boudreau, M.A. 2013. Diseases in Intercropping Systems. *Annual Review of Phytopathology* 51:499–519

Brayford, D. 1996. IMI Descriptions of Fungi and Bacteria No. 1263. *Fusarium oxysporum* f. sp. *cepae*. *Mycopathologia* 133: 39 - 40.

Buhre, C., Kluth, C., Bürcky, K., Märländer, B., Varrelmann, M. 2009. Integrated control of root and crown rot in sugar beet: Combined effects of cultivar, crop rotation, and soil tillage. *Plant Disease* 93:155-161.

Casagrande, M., Dogliotti, S., Groot, J.C.J., Aguerre, V., Abbas, A., Albin, A., Claassen, G.D.H., Chilibroste, P., Rossing W.A.H. 2010. Exploring options for sustainable farming systems development for vegetable family farmers in Uruguay using a modelling toolkit. In: 9th European IFSA Symposium, Building sustainable rural futures. The added value of systems approaches in times of change and uncertainty, Vienna, Austria, 04 - 07 July, 2010, pp. 463 – 469.

Colbach, N., Meynard, J-M, Duby, C., Huet, P. 1999. A dynamic model of the influence of rotation and crop management on the disease development of eyespot. Proposal of cropping systems with low disease risk. *Crop Protection* 18: 451 - 461.

Cunfer, B.M., Buntin, G.D., Phillips, D.V. 2006. Effect of crop rotation on take-all of wheat in double-cropping systems. *Plant Disease* 90:1161-1166.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2003. ROTAT, a tool for systematically generating crop rotations. *European Journal of Agronomy* 19: 239-250.

Dogliotti, S., Rossing, W.A.H., van Ittersum, M.K. 2004. Systematic design and evaluation of crop rotations enhancing soil conservation, soil fertility and farm income: a case study for vegetable farms in South Uruguay. *Agricultural Systems* 80: 277 - 302.

Dogliotti, S.; Van Ittersum, M.K.; Rossing, W.A.H. 2005. A method for exploring sustainable development options at the farm scale: a case study for vegetable farms in South Uruguay. *Agricultural Systems* 86: 29 – 51.

Dogliotti, S., Garcia, M.C., Peluffo, S., Dieste, J.P., Pedemonte, A.J., Bacigalupe, G.F., Scarlato, M., Alliaume, F., Alvarez, J., Chiappe, M., Rossing, W.A.H. 2013. Co-innovation of family farm systems: A systems approach to sustainable agriculture. *Agricultural Systems*. http://dx.doi.org./10.1016/j.agsy.2013.02.009.

Ellison, A.M. 2004. Bayesian inference in ecology. *Ecology Letters* 7: 509–520.

Fabre, F., Pierre, S., Dedryver, C.A., Plantegenest, M. 2006. Barley yellow dwarf risk assessment based on Bayesian modelling of aphid population dynamics. *Ecological Modelling* 193: 457 – 466.

Flores-Moctezuma, H.E., Montes-Belmont, R., Jiménez-Pérez, A., Nava-Juarez, R. 2006. Pathogenic diversity of *Sclerotium rolfsii* isolates from Mexico, and potential control of southern blight through solarization and organic amendments. *Crop Protection* 25: 195–201.

Garbeva, P., van Veen, J.A., van Elsas, J.D. 2003. Predominant *Bacillus* spp. in Agricultural Soil under Different Management Regimes Detected via PCR-DGGE. *Microbial Ecology* 45: 302-316.

Garbeva, P., van Veen, J.A., van Elsas, J.D. 2004. Microbial diversity in soil: selection of microbial populations by plant and soil type and implications for disease supressiveness. *Annual Review of Plant Pathology* 42: 243 – 270.

Gordon, T.R., Okamoto, D., Jacobson, D.J. 1989. Colonization of muskmelon and nonsusceptible crops by *Fusarium oxysporum* f.sp. *melonis* and other species of *Fusarium*. *Phytopathology* 79: 1095 - 1100.

Grünwald, N.J., Hu, S., van Bruggen, A.H.C. 2000. Short-term cover crop decomposition in organic and conventional soils; Characterization of soil C, N, microbial and plant pathogen dynamics. *European Journal of Plant Pathology* 106: 37 - 50.

Hartmann, A., Schmid, M., van Tuinen, D., Berg, G. 2009. Plant-driven selection of microbes. *Plant and Soil* 321: 235-257. doi: 10.1007/s11104-008-9814-y.

Helbig, JB. and Carroll, R.B. 1984. Dicotyledonous weeds as a source of *Fusarium oxysporum* pathogenic on soybean. *Plant Disease* 68: 694 - 696.

Hiddink, G.A., Termorshuizen, A.J. van Bruggen, A.H.C. 2009. Mixed cropping and suppression of soilborne diseases, a review. In: Lichtfouse, E. (Ed.), Genetic Engineering, Biofertilisation, Soil Quality and Organic Farming, *Sustainable Agriculture Reviews* 4: 119-146.

Jenkins, S.F. and Averre, C.W. 1986. Problems and progress in integrated control of Southern blight of vegetables. *Plant Disease* 70: 614 – 619.

Katan, J. 1971. Symptomless carriers of the tomato Fusarium wilt pathogen. *Phytopathology* 61: 1213 - 1217.

Le, C.N. 2011. Diversity and biological control of *Sclerotium rolfsii*, causal agent of stem rot of groundnut. PhD Thesis, Wageningen University, Wageningen, The Netherlands.

Lemanceau, P., Bakker, P.H.A.M., De Kogel, W.J., Alabouvette, C., Schippers, B. 1993. Antagonistic effect of non-pathogenic *Fusarium oxysporum* Fo47 and Pseudobactin 358 upon pathogenic *Fusarium oxysporum* f. sp. *dianthi*. *Applied Environmental Microbiology* 59: 74–82.

Leoni, C., Rossing, W.A.H., van Bruggen, A.H.C. Crop Rotation. In: Finckh, M., Tamm, L., van Bruggen, A.H.C. (Eds) *Plant Disease Management in Organic Agriculture*. St. Paul, MN., American Phytopathological Society, (*in press*)

Leoni, C., de Vries, M., ter Braak, C.J.F., van Bruggen, A.H.C., Rossing, W.A.H. 2013. *Fusarium oxysporum* f.sp. *cepae* dynamics: in-plant multiplication and crop sequence simulations.

European Journal of Plant Pathology 137: 545-561. doi: 10.1007/s10658-013-0268-6.

Leslie, J.F., Pearson, C.A.S., Nelson, P.E., Toussoun, T.A. 1990. *Fusarium* spp. from corn, sorghum, and soybean fields in the Central and Eastern United States. *Phytopathology* 80: 343 – 350.

Lucas, P. 2006. Diseases caused by soil-borne pathogens. In: Cooke, B.M., Gareth Jones, D., Kaye, B. (Eds.) *The Epidemiology of Plant Diseases*, 2nd ed. (pp. 373 – 386). The Netherlands, Springer.

Malézieux, E. 2012. Designing cropping systems from nature. *Agronomy for Sustainable Development* 32:15–29. doi10.1007/s13593-011-0027-z.

Mol, L., Huisman, O.C., Scholte, K., Struik, P.C. 1996. Theoretical approach to the dynamics of the inoculum density of *Verticillium dahliae* in the soil: first test of a simple model. *Plant Pathology* 45: 192 – 204.

Muyzer, G. and Smalla, K. 1998. Application of denaturing gradient gel electrophoresis (DGGE) and temperature gradient gel electrophoresis (TGGE) in microbial ecology. *Antonie van Leeuwenhoek* 73: 127–141.

Okubara, P.A., Schroeder, K.L., Paulitz, T.C. 2005. Real-time polymerase chain reaction: applications to studies on soilborne pathogens. *Canadian Journal of Plant Pathology* 27: 300–313.

Oritsejafor, J.J. and Adeniji, M.O. 1990. Influence of host and non-host rhizospheres and organic amendments on survival of *Fusarium oxysporum* f.sp. *elaeidis*. *Mycological Research* 94: 57 - 63.

Punja, Z.K. and Grogan, R.G. 1981. Eruptive germination of sclerotia of *Sclerotium rolfsii*. *Phytopathology* 71: 1092 – 1099.

Punja, Z.K., Jenkins, S.F., Grogan, R.G. 1984. Effect of volatile compounds, nutrients, and source of sclerotia on eruptive sclerotial germination of *Sclerotium rolfsii*. *Phytopathology* 74: 1290 – 1295.

Punja, Z.K. 1985. The biology, ecology, and control of *Sclerotium rolfsii*. *Annual Review of Phytopathology* 23: 97-127.

Ratnadass, A., Fernandes, P., Avelino, J., Habib, R. 2012. Plant species diversity for sustainable management of crop pests and diseases in agroecosystems: a review. *Agronomy for Sustainable Development* 32:273–303.

Roesch, L.F.W., Fulthorpe, R.R., Riva, A., Casella, G., Hadwin, A.K.M., Kent, A.D., Daroub, S.H., Camargo, F.A.O., Farmerie, W.G., Triplett, E.W. 2007 Pyrosequencing enumerates and contrasts soil microbial diversity. *The International Society for Microbial Ecology Journal* 1: 283 – 290.

Rossing, W.A.H., Daamen, R.A. Jansen, M.J.W. 1994. Uncertainty analysis applied to supervised control of aphids and brown rust in winter wheat. Part 1. Quantification of uncertainty in cost-benefit calculations. *Agricultural Systems* 44: 419-448.

Savage, S.L. 2012. The flaw of averages. Why we underestimate risk in the face of uncertainty. John Willey & Sons., New Jersey. 392 p.

Schönhart, M., Schmid, E., Schneider, U.A. 2012. CropRota – A crop rotation model to support integrated land use assessments. *European Journal of Agronomy* 34: 263 – 277.

Stapleton, J.J., Summers, C.G., Mitchell, J.P., Prather, T.S. 2010. Deleterious activity of cultivated grasses (*Poaceae*) and residues on soilborne fungal, nematode and weed pests. *Phytoparasitica* 38: 61–69.

Taylor, C.R. and Rodriguez-Kábana, R. 1999 a. Population dynamics and crop yield effects of nematodes and white mold in peanuts, cotton and velvet beans. *Agricultural Systems* 59: 177 – 191.

Taylor, C.R., and Rodriguez-Kábana, R. 1999 b. Optimal rotation of peanuts and cotton to manage soil-borne organisms. *Agricultural Systems* 61: 57 – 68.

Ter Braak, C.J.F. and Vrugt, J.A. 2008. Differential evolution Markov Chain with snooker update rand fewer chains. *Statistics and Computing* 18: 435 – 446.

Termorshuizen, A.J. and Rouse, D.I. 1993. Towards a mechanistic model for the *Verticillium* dahliae - potato system. *European Journal of Plant Pathology* 99 (Suppl. 3): 201-218.

Tixier, P., Risède, J.M., Dorel, M., Malézieux, E. 2006. Modelling population dynamics of banana plant-parasitic nematodes: a contribution to the design of sustainable cropping systems. *Ecological Modelling* 198: 321 – 331.

Thrall, P.H., Bever, J.D., Mihail, J.D., Alexander, H.M. 1997. The population dynamics of annual plants and soil-borne fungal pathogens. *Journal of Ecology* 85: 313 – 328.

United Nations. 2013. The future we want. Resolution adopted by the General Assembly, 123rd plenary meeting, 27 July 2012 (A/RES/66/288). Reissued for technical reasons on 17 January 2013. http://daccess-dds-ny.un.org/doc/UNDOC/GEN/N11/476/10/PDF/N1147610.pdf?OpenElement. Accessed on June12, 2013.

van den Berg, W. and Rossing W.A.H. 2005. Generalized linear dynamics of a plant-parasitic nematode population and the economic evaluation of crop rotations. *Journal of Nematology* 37: 55-65.

van den Berg, W., Rossing W.A.H., Grasman, J. 2006. Contest and scramble competition and the carry-over effect in *Globodera* spp. in potato-based crop rotation using an extended Ricker model. *Journal of Nematology* 38: 210 – 220.

Wakelin, S.A., Warren, R.A., Kong, L., Harvey, P.R. 2008. Management factors affecting size and structure of soil *Fusarium* communities under irrigated maize in Australia. *Applied Soil Ecology* 39: 201 – 209.

Summary

During the last decades, agricultural farming systems faced an intensification process, associated with an increased use of fossil fuel energy due to the intensive use of agrochemicals, mechanization and irrigation. This intensification resulted in an increase in soil erosion rates, a decrease in soil organic matter content and associated losses of soil structure and water holding capacity, an increase in pest and disease problems and biodiversity losses, and an increase in vulnerability to climate change and related extreme events. As a consequence, many of these farming systems are not sustainable. There is an urgent need for development of farming systems where farm productivity increases by drawing on internal ecological cycles, by reducing the dependency on non-renewable and external inputs and by optimizing the use of water and energy. Moreover, these strategies should also prevent further land and environment degradation and support farmers to achieve long-term goals of sustainability.

Sustainable farming systems have often been associated with relatively long crop rotations, spanning a period of five to eight years, and with low frequency of individual crop species and high species diversity over time. An adequate crop rotation provides essential ecosystem services, among them provisioning services such as production of food and fibre; regulating services such as disease, weed and pest control, supporting services such as nutrient cycling, soil formation and water retention. The design of crop rotation is a complex process, where farmers' long-term objectives and ambitions at the whole farm level, local resource availability, climate, and shorter-term socio-economic conditions need to be considered. Models can support the design of crop sequences and help to reveal synergisms and trade-offs among objectives.

Despite their importance, pathogen dynamics are rarely taken into account in cropping system models. Soilborne pathogen dynamics in a crop sequence can be described by interlinked quantitative population models where different equations describe the increase and decline of the pathogen population during and after a crop and intercrop activity, respectively. In order to calibrate and evaluate the models, quantitative information on soilborne pathogen dynamics in the presence of different crops and intercrop activities (e,g. green manures, fallow) is needed. However, crop rotation experiments are resource demanding in time, labour and space, and usually only involve few experimental factors. Thus new research strategies are required.

The aim of this thesis was to develop a research approach where experimental data and model simulations were combined to explore crop sequences that minimize soilborne pathogen inoculum build up, to subsequently include this information into models for designing sustainable crop rotations. To achieve this aim, experimental data from greenhouse pot experiments, microplot experiments and commercial farm fields was combined and used to study the dynamics of the pathogens and to calibrate simple pathogen population models for *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *cepae (Foc),* two ecologically distinct and relevant pathogens in vegetable production systems. Then, *S. rolfsii* and *Foc* population dynamics were simulated to explore crop sequences that minimize soilborne pathogen inoculum build up.

The dynamics of *S. rolfsii* sclerotia was studied in two different processes: survival in crop debris amended and un-amended soil (greenhouse pot experiment) and population changes under three cropping sequences: sweet pepper (*Capsicum annuum* L.) monocrop, sweet pepper – black oat (*Avena strigosa* Schreb.) and sweet pepper - onion (*Allium cepa* L.) (microplot experiment) (Chapter 3). Sclerotia survival in soil after winter green manure incorporation and decomposition during summer was generally lower than after summer green manure incorporation and decomposition during winter. But the most important result was that the incorporation of the residues of various legume crops (black beans (*Phaseolus vulgaris* L.), cowpea (*Vigna unguiculata* (L.) Walp.), hairy vetch (*Vicia villosa* Roth) and lupines (*Lupinus* spp. L.)) allowed multiplication of sclerotia while various grasses (sudangrass (*Sorghum* × *drummondi* (Steud.) Millsp. & Chase), foxtail millet (*Setaria italica* (L.) P. Beauvois), oats (*Avena* spp. L.) and wheat (*Triticum aestivum* L.)) as well as sunhemp (*Crotalaria juncea* L.) resulted in a reduction of sclerotia in the soil. The build-up of sclerotia populations in the microplots was dependent on the crop sequence, resulting in greater multiplication by sweet pepper after black oat than after onion or fallow.

The dynamics of *Foc* was studied at two different levels: multiplication in single plants (greenhouse pot experiment, Chapter 4) and survival or growth in different crop sequences (microplot experiment and farm fields, Chapter 5). *Foc* colonized and multiplied in the root systems of the 13 non *Allium* plant species tested without inducing disease symptoms or growth retardation, concluding that all the plant species tested are “reservoir-hosts” for *Foc*. The lowest *Foc* levels per g of dry weight of root were found in wheat, sunflower (*Helianthus annus* L.) , cowpea and foxtail millet; intermediate levels in onion, oat, black oat, white lupine, blue lupine, tomato (*Solanum lycopersicum* L.), sudangrass, corn and sweet corn (*Zea mays* L.); whereas the highest *Foc* level was found in black bean (Chapter 4). *Fusarium* pathogen

dynamics was strongly affected by the cropping history in a particular field. *Fusarium* populations increased from transplant to harvest when another onion crop was planted in the same field the previous winter whereas *Fusarium* populations decreased when a winter green manure was planted (Chapter 5).

Pathogen dynamics in crop sequences was simulated for the two soilborne pathogens *S. rolfsii* and *Foc*. The model for *S. rolfsii* described the build-up of the pathogen within a crop n ($Pf_{(n)} = Pi_{(n)} / (\alpha + \beta\ Pi_{(n)})$), followed by the decline of inoculum after incorporation of crop debris into soil ($Sf = Si \exp(-bd)$, where $Si = Pf$), and the model was calibrated using quantitative data generated in pot and microplot experiments (Chapter 3). The model for *Fusarium* described the build-up of the pathogen within a crop n ($Pf_{(n)} = Pi_{(n)} / (\alpha + \beta\ Pi_{(n)})$) followed by gradual pathogen release from plant roots after harvest ($Pf_{(n,t)} = Pf_{(n,0)} (1-\exp(-bt))$). The model was calibrated with data from greenhouse pot experiments (Chapter 4) and validated with data collected in microplots and on farm fields (Chapter 5). Regardless of the simplicity and limitations of the population models described (Chapter 3, Chapter 5) differences among crop sequences and alternating cycles of increasing and decreasing soil pathogen populations were detected. Also differences at equilibrium populations were related to host frequency and to cropping history, particularly important for *Foc* dynamics. The developed methodology facilitates the exploration of crop sequences that limit pathogen build-up and supports the selection of a limited number of rotation options to be tested in farmers' fields.

This thesis delivers a methodological approach to the design of crop rotations and their effects on soil borne pathogen dynamics. The combination of data from controlled experiments, novel analytical tools (Bayesian analysis, modelling and simulation) and on-farm observations can lead to the identification of optimal crop rotations without extensive field experiments that require a lot of time, space and economic resources.

Samenvatting

Gedurende de laatste decennia heeft de landbouw te maken gehad met een proces van intensivering, die was geassocieerd met een toename in het gebruik van fossiele energie als gevolg van het intensief gebruik van bestrijdingsmiddelen, kunstmest, mechanisatie en irrigatie. Deze intensivering heeft geresulteerd in bodemerosie, een afname van bodemorganische stof en daarmee samenhangend verlies aan bodemstructuur en watervasthoudend vermogen van bodems, een toename van problemen met ziekten en plagen en verlies aan biodiversiteit, en een toename in de gevoeligheid voor klimaatverandering en daaraan gerelateerde extreme weersgebeurtenissen. Als gevolg hiervan zijn veel landbouwsystemen in bedrijfsverband (verder te noemen bedrijfssystemen) niet duurzaam. Er bestaat een acute noodzaak om bedrijfssystemen te ontwikkelen waarin productiviteitstoename is gebaseerd op gebruik van bedrijfsinterne ecologische cycli, door vermindering van de afhankelijkheid van niet-hernieuwbare en externe inputs, en door het optimaliseren van de inzet van water en energie. Bovendien dienen deze strategieën ook verdere degradatie van land en milieu te voorkomen en boeren te ondersteunen bij het bereiken van lange-termijn doelen van duurzaamheid.

Duurzame bedrijfssystemen worden vaak geassocieerd met relatief lange gewasrotaties van vijf tot acht jaar, waarin de frequentie van individuele soorten laag is bij een hoge soortendiversiteit in tijd en ruimte. Een adequate gewasrotatie levert essentiële ecosysteemdiensten, waaronder productverstrekking zoals de productie van voedsel en vezels, regulerende diensten zoals ziekte-, onkruid- en plaagbeheersing, en ondersteunende diensten zoals hergebruik van plantenvoedingsstoffen, bodemvorming en retentie van water. Ontwerpen van gewasrotaties is een complex proces waarbij lange-termijn doelen van boeren, doelen op bedrijfsniveau, lokale beschikbaarheid van hulpmiddelen, klimaat, en korte termijn sociaaleconomische omstandigheden in ogenschouw moeten worden genomen. Modellen kunnen het ontwerpen van gewasopvolgingen ondersteunen, en helpen inzicht te krijgen in synergie en uitruil tussen doelstellingen.

Ondanks hun belang wordt de dynamica van ziekteverwekkers zelden in ogenschouw genomen in modellen van gewasopvolging. Het verloop in de tijd van de omvang van populaties bodemgebonden ziekteverwekkers kan worden beschreven met gekoppelde kwantitatieve populatiemodellen waarin verschillende vergelijkingen de toe- en afname van de populatie van een ziekteverwekker beschrijven tijdens, respectievelijk, de gewasactiviteit en de activiteit tussen opeenvolgende gewassen. Om de modellen te kalibreren en te

evalueren is kwantitatieve informatie nodig over de veranderingen in de populatie van een ziekteverwekker in aanwezigheid van verschillende gewassen en tussen-gewas activiteiten (bv. groenbemesters, braak). Experimenten met gewasrotaties vragen echter een grote inzet van tijd, arbeid en ruimte, en gewoonlijk kunnen slechts enkele proeffactoren worden toegepast. Dit maakt nieuwe onderzoeksstrategieën noodzakelijk.

Doel van dit proefschrift was een onderzoeksstrategie te ontwikkelen waarbij gegevens van experimenten werden gecombineerd met modelsimulaties voor het verkennen van gewasopvolgingen die de opbouw van inoculum in de bodem minimaliseren, en het vervolgens gebruiken van deze informatie in modellen voor het ontwerp van duurzame gewasrotaties. Om dit doel te bereiken zijn gegevens van potexperimenten in kassen, van experimenten op micro-veldjes en van commerciële bedrijven gecombineerd en gebruikt om het tijdsverloop van de ziekteverwekkers te bestuderen en eenvoudige populatiemodellen te kalibreren voor *Sclerotium rolfsii* and *Fusarium oxysporum* f.sp. *cepae (Foc),* twee ecologisch verschillende ziekteverwekkers in productiesystemen van groentegewassen. Met deze modellen werd het tijdsverloop van *S. rolfsii* en *Foc* gesimuleerd om te verkennen welke gewasopvolgingen tot minimale opbouw zouden leiden van inoculum van bodemgebonden ziekteverwekkers.

Het tijdsverloop van *S. rolfsii* sclerotia is bestudeerd voor twee verschillende processen: de overleving in grond waaraan al dan niet was gewasresten waren toegevoegd (potexperiment in de kas) en de verandering van de populatiedichtheid bij drie gewasopvolgingen in micro-veldjes: paprika (*Capsicum annuum* L.) als monocultuur, paprika – Japanse haver (*Avena strigosa* Schreb.) en paprika – ui (*Allium cepa* L.) (Hoofdstuk 3). Overleving van sclerotia in de bodem na inwerken van een winter groenbemester en decompositie tijdens de zomer was in het algemeen lager dan na inwerken van een zomer groenbemester en decompositie tijdens de winter. Het belangrijkste resultaat was dat inwerken van gewasresten van verschillende leguminose gewassen (zwarte boon (*Phaseolus vulgaris* L.*)*, cowpea (*Vigna unguiculata* (L.) Walp.), bonte wikke (*Vicia villosa* Roth) en lupine (*Lupinus* spp. L.)) leidde tot toename van het aantal sclerotia, terwijl het inwerken van verschillende grasachtigen (soedangras (*Sorghum* × *drummondi* (Steud.) Millsp. & Chase), vogelgierst (*Setaria italica* (L.) P. Beauvois), haver (*Avena* spp*.* L.) en tarwe (*Triticum aestivum* L.)) en Bengaalse hennep (*Crotalaria juncea* L.) resulteerde in een afname van de dichtheid sclerotia in de bodem. De toename van de sclerotia in de microveldjes hing af van de gewasvolgorde, en leidde tot sterkere vermeerdering in paprika na Japanse haver dan na ui of na een braakperiode.

Het tijdsverloop van *Foc* is bestudeerd op twee niveaus: vermenigvuldiging in individuele planten (potexperiment in de kas; Hoofdstuk 4) en overleving of groei in verschillende gewasopvolgingen (experimenten in microveldjes en waarnemingen op bedrijfspercelen; Hoofdstuk 5). *Foc* koloniseerde de wortels van de 13 niet-Allium soorten en vermenigvuldigde zich daar, zonder dat de planten ziektesymptomen vertoonden of groeiachterstand opliepen. Hieruit werd geconcludeerd dat alle geteste plantensoorten "reservoir-waardplanten" zijn voor *Foc*. Het laagste gehalte aan *Foc* per g wortel drooggewicht werd gevonden in tarwe, zonnebloem (*Helianthus annus* L.), cowpea en vogelgierst. Tussenliggende gehalten werden gevonden voor ui, haver, Japanse haver, witte lupine, blauwe lupine, tomaat (*Solanum lycopersicum* L.), soedangras, mais (*Zea mays* L.) en suikermais. De hoogste gehaltes Foc werden aangetroffen in zwarte boon (Hoofdstuk 4). Het populatieverloop van *Fusarium* werd sterk beïnvloed door de gewasgeschiedenis van een perceel. Populaties *Fusarium* namen toe van planten tot oogst indien in de voorgaande winter een ui gewas was geteeld in hetzelfde veld, terwijl populaties afnamen na teelt van een winter groenbemester.

Het populatieverloop in gewasopvolgingen is gesimuleerd voor de twee bodemgebonden ziekteverwekkers *S. rolfsii* and *Foc*. Het model voor *S. rolfsii* beschreef de toename van de ziekteverwekker in een gewas *n* ($Pf(n) = Pi(n) / (\alpha + \beta\ Pi(n))$), gevolgd door een afname van het inoculum na inwerken van gewasresten in de bodem ($Sf = Si \exp(-bd)$, waar $Si = Pf$), en het model is gekalibreerd met kwantitatieve gegevens uit pot en microveld experimenten (Hoofdstuk 3). Het model voor *S. rolfsii* beschreef de toename van de ziekteverwekker in een gewas *n* ($Pf(n) = Pi(n) / (\alpha + \beta\ Pi(n))$), gevolgd door geleidelijk vrijkomen van van de ziekteverwekker uit de plantenwortels na oogst ($Pf(n,t) = Pf(n,0) (1-\exp(-bt))$). Het model was gekalibreerd met gegevens uit potexperimenten in de kas (Hoofdstuk 4) en gevalideerd met gegevens verzameld in microveldjes en op bedrijfspercelen (Hoofdstuk 5). Ondanks de eenvoud en de beperkingen van de gebruikte modellen (Hoofdstuk 3, Hoofdstuk 5) werden verschillen tussen gewasopvolgingen en cycli van afwisselend toenemende en afnemende bodempopulaties door de modellen gereproduceerd. Ook waren verschillen in evenwichtsdichtheden toe te schrijven aan frequentie van de waardplant, en aan gewashistorie. Deze waren vooral van belang voor *Foc*. De ontwikkelde methodologie maakt het mogelijk gewasopvolgingen te identificeren die de opbouw van ziekteverwekkers beperken en een beperkt aantal rotaties te selecteren voor nader uittesten in boeren percelen.

Dit proefschrift levert een methodische benadering voor het ontwerpen van gewasrotaties en

hun effecten op bodemgebonden ziekteverwekkers. De combinatie van gegevens uit gecontroleerde experimenten, nieuwe analytische instrumenten (Bayesiaanse analyse, modelbouw en simulatie) en waarnemingen op bedrijven kan leiden tot de identificatie van optimale gewasrotaties zonder kostbare veldexperimenten die een groot beslag leggen op tijd, ruimte en geld.

Resumen

En las últimas décadas, los sistemas de producción agrícola han sufrido procesos de intensificación asociados a un aumento del uso de energía fósil por el incremento en el uso de agroquímicos, mecanización y riego. Como consecuencia de la intensificación, se constatan importantes pérdidas de suelo por erosión, disminución de los niveles de materia orgánica asociado a pérdidas de estructura y capacidad de almacenamiento de agua en el suelo, incrementos en los problemas de plagas y enfermedades y biodiversidad, y un aumento de la vulnerabilidad al cambio climático y los eventos extremos asociados. Todos estos cambios resultan en sistemas de producción no sustentables, lo que hace necesario desarrollar sistemas de producción alternativos. Los nuevos sistemas de producción deberán incrementar la productividad del sistema mediante el fortalecimiento de los ciclos ecológicos, la reducción de la dependencia de insumos externos no renovables y la optimización del uso de agua y energía. Estas estrategias de producción además deberán prevenir una mayor degradación del ambiente y ayudar a los productores a alcanzar los objetivos de sustentabilidad de largo plazo.

La sustentabilidad de los sistemas de producción agrícolas se asocia a rotaciones de cultivos que duran 5 a 8 años, con una baja frecuencia de una especie particular y una alta diversidad de especies en el tiempo. Una adecuada rotación de cultivos presta servicios ecosistémicos esenciales, entre ellos la provisión de alimentos y fibras, la regulación de enfermedades, plagas y malezas, y el soporte del ciclado de nutrientes, la formación de suelo y la retención de agua. El diseño de las rotaciones de cultivos es un proceso complejo, en donde los objetivos a largo plazo y las aspiraciones de los productores a nivel predial, se deben combinar con la disponibilidad de recursos locales, el clima y las condiciones socio-económicas de corto plazo. El uso de modelos contribuye al diseño de secuencias de cultivos y ayuda a identificar las sinergias y competencias entre objetivos.

A pesar de su importancia, la dinámica de los patógenos de suelo rara vez es considerada en los modelos de simulación de rotaciones. En una secuencia de cultivos la dinámica de los patógenos de suelo se puede describir mediante la combinación de modelos poblacionales cuantitativos, donde diferentes ecuaciones describen el aumento y la reducción de la población del patógeno en el suelo durante un cultivo y en el período entre cultivos. Para calibrar y evaluar dichos modelos, es necesario disponer de información cuantitativa sobre la dinámica de los patógenos durante la fase de cultivo o inter-cultivo (ej. abonos verdes, barbecho). Dicha información podría ser obtenida en experimentos de rotaciones de cultivos,

pero debido a la cantidad de tiempo, trabajo y espacio demandada por dichos experimentos la dinámica de los patógenos rara vez es priorizada. Por tanto, es necesario explorar nuevas estrategias de investigación que posibiliten la combinación de varios factores experimentales.

El objetivo de esta tesis fue desarrollar una metodología de investigación para explorar secuencias de cultivos que minimicen el incremento del inóculo de los patógenos de suelo. Esta metodología combina datos experimentales y modelos de simulación, para luego incluir dicha información en modelos que contribuyen al diseño de rotaciones de cultivos sustentables. Para ello, información generada en experimentos en macetas conducidos en invernáculos, experimentos de microparcelas y mediante el monitoreo de cultivos comerciales, se combinaron y emplearon para estudiar la dinámica de *Sclerotium rolfsii* y *Fusarium oxysporum* f.sp. *cepae* (*Foc*) y calibrar modelos poblacionales sencillos para los dos patógenos, ecológicamente diferentes y relevantes en los sistemas de producción hortícolas. Luego, las dinámicas poblacionales de *S. rolfsii* y *Foc* fueron simuladas para explorar secuencias de cultivos que minimicen el incremento del inoculo de estos patógenos en el suelo.

La dinámica de los esclerotos de *S. rolfsii* se estudió en dos procesos: sobrevivencia de los ecleorotos en el suelo con o sin incorporación de abonos verdes (experimento en macetas en invernáculo), y cambios en la población de esclerotos en el suelo en tres secuencias de cultivos: monocultivo de morrón (*Capsicum annuum* L.), morrón – avena negra (*Avena strigosa* Schreb.) y morrón – cebolla (*Allium cepa* L.) (experimento de microparcelas) (Capítulo 3). La sobrevivencia de los esclerotos en el suelo luego de la incorporación de un abono verde de invierno y su descomposición durante el verano fue generalmente menor que luego de la incorporación de un abono verde de verano y su descomposición durante el invierno. Pero el resultado más importante fue que la incorporación de leguminosas (poroto negro (*Phaseolus vulgaris* L.), caupí (*Vigna unguiculata* (L.) Walp.), vicia peluda (*Vicia villosa* Roth) y lupinos (*Lupinus* spp. L.)) permitió la multiplicación de los esclerotos, mientras que la incorporación de gramíneas (sudangras (*Sorghum* × *drummondi* (Steud.) Millsp. & Chase), moha (*Setaria italica* (L.) P. Beauvois), avenas (*Avena* spp. L.) y trigo (*Triticum aestivum* L.)) y crotolaria (*Crotalaria juncea* L.) resulto en una reducción del número de esclerotos en el suelo. El incremento de las poblaciones de esclerotos en las microparcelas fue dependiente de la secuencia de cultivos, donde la mayor multiplicación se dio durante el cultivo de morrón luego de la avena negra, comparado con la multiplicación de los esclerotos en el cultivo de morrón luego de un cultivo de cebolla o luego de un barbecho.

La dinámica de *Foc* se estudió a dos niveles: multiplicación en la planta (experimento en macetas en invernáculo, Capítulo 4) y sobrevivencia o multiplicación en diferentes secuencias de cultivos (experimento de microparcelas y seguimiento de productores, Capítulo 5). *Foc* colonizó y se multiplicó en el sistema radicular de 13 especies no pertenecientes al género *Allium*, sin causar síntomas de enfermedad ni retardar el crecimiento, concluyendo que todas las especies evaluadas constituyen "hospederos de reserva" para *Foc*. Los niveles más bajos de *Foc* por gramo de raíz seca se encontraron en trigo, girasol (*Helianthus annus* L.), caupí y moha; valores intermedios en cebolla, avena, avena negra, lupino blanco, lupino azul, tomate (*Solanum lycopersicum* L.), sudangrass, maíz (*Zea mays* L.)y maíz dulce; mientras que el nivel más alto se encontró en poroto negro (Capitulo 4). La dinámica de *Fusarim* en el suelo fue fuertemente afectada por la historia de cultivos del sitio. Las poblaciones de *Fusarium* aumentaron entre transplante y cosecha cuando en el mismo sitio el invierno anterior se plantó cebolla, mientras que las poblaciones de *Fusarium* decrecieron cuando en el mismo sito se plantó un abono verde de invierno (Capitulo 5).

Mediante modelos, se simuló la dinámica poblacional de los patógenos de suelos *S. rolfsii* y *Foc* bajo diferentes secuencias de cultivos. El modelo para *S. rolfsii* describe el incremento de la población del patógeno durante el cultivo n ($Pf_{(n)}= Pi_{(n)} / (\alpha + \beta\ Pi_{(n)})$), seguido de la disminución de la población luego de la incorporación del abono verde en el suelo ($Sf = Si \exp(-bd)$, donde $Si = Pf$), y el modelo fue calibrado empleando los datos cuantitativos obtenidos en los ensayos en macetas y en microparcelas (Capitulo 3). EL modelo para Fusarium describe el incremento de la población del patógeno a lo largo de un cultivo n ($Pf_{(n)}= Pi_{(n)} / (\alpha + \beta\ Pi_{(n)})$), seguido de la liberación gradual del patógeno de las raíces del cultivo luego de la cosecha ($Pf_{(n,t)} = Pf_{(n,0)} (1-\exp(-bt))$). El modelo se calibró con información cuantitativa generada en experimentos en macetas en invernáculo (Capitulo 4) y luego se validó con información generada en microparcelas y chacras de productores (Capitulo 5). A pesar de la simplicidad y limitaciones de los modelos poblacionales descriptos (Capitulo 3, Capitulo 5), los mismos detectaron diferencias entre las secuencias de cultivos y ciclos de crecimiento y reducción de la población de patógenos en el suelo. También se identificaron diferencias en los niveles de equilibrio alcanzados asociados a la frecuencia del hospedero y la historia de los cultivos, de particular importancia en la dinámica poblacional de *Foc* en el suelo. La metodología propuesta facilita la exploración de secuencias de cultivos que limiten el incremento del inóculo en el suelo y apoya a la selección de un limitado número de rotaciones que deberán ser evaluadas posteriormente en cultivos comerciales.

Esta tesis propone un abordaje metodológico para el diseño de rotaciones de cultivos y sus efectos sobre los patógenos de suelo. La combinación de información generada en experimentos controlados, el uso de nuevas herramientas de análisis (análisis Bayesiano, uso de modelos y simulación) y la obtención de información generada en cultivos comerciales, contribuirá con la identificación de rotaciones de cultivos apropiadas, sin la alta demanda de tiempo, espacio y recursos económicos requerida por los experimentos de campo de largo plazo.

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While following a sandwich PhD programme, I had to combine the thesis work with INIA's responsibilities as a researcher. This was only possible thanks to the collaboration of many research assistants, colleagues and authorities at INIA. First I'd like to acknowledge Alfredo, Beatriz, Diego, Lucía, María José, Mariana, Nora and Wilma for their collaboration during the experimental activities and for taking care of the experiments while I was in Wageningen. Second, I acknowledge the authorities of INIA for the financial support and for the freedom given to organize the multiple activities for the thesis and INIA's work, in particular to Alfredo Albín, Jorge Sawchik, José Terra and Roberto Zoppolo. Finally my special thanks to Stella Zerbino, not only for her valuable collaboration and fruitful technical discussions, but mainly for her friendship.

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Curriculum Vitae

Carolina Leoni was born on 29 December 1966 in Montevideo, Uruguay. She studied at the Faculty of Agronomy (Facultad de Agronomía – Universidad de la República (UdelaR) – Uruguay), where she graduated as agronomy engineer in 1996. From 1996 to 1999 she joined the National Institute of Agricultural Research (Instituto Nacional de Investigación Agropecuaria - INIA) as a Research Assistant at Tacuarembó Research Station to work on the development of vegetable and fruit tree production in North-eastern Uruguay, a region traditionally devoted to cattle production and more recently to forestry. In February 2000 she started the M.Sc. programme on Plant Pathology at the University of Sao Paulo (Brazil), from which she graduated in March 2002. During her Master programme she studied the effect of sewage sludge on *Phytophthora nicotianae* management in citrus orchards, under the supervision of Dr. Raquel Ghini (EMBRAPA Environment– Brazil). In March 2001, she became a full time researcher in Plant Pathology at the INIA Las Brujas Research Station in Southern Uruguay, the main region for fruit and vegetable production. As INIA researcher, she participated actively in the development of the National Programmes on Integrated Production of Vegetables and Fruits, and Organic Production, jointly with researchers from UdelaR and farmers, and coordinated by the Ministry of Agriculture. In December 2007 she started a 'sandwich' Ph.D. programme within the former Biological Farming Systems group, currently Farming Systems Ecology group of Wageningen University. At present she is working at INIA for the National Production and Environmental Sustainability Research Program, the National Family Farm Production Research Program and the National Fruit Research Programme as an Assistant Researcher.

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List of publications

Scientific papers

Alaniz, S., **Leoni, C.**, Bentancur, O., Mondino, P. Elimination of summer fungicide sprays for apple scab (*Venturia inaequalis*) management in Uruguay. *Scientia Horticulturae* (accepted).

Arboleya, J., Gilsanz, J.C., Alliaume, F., **Leoni, C.**, Fallero, M., Guerra, S. 2012. Minimum tillage and vegetable crop production. *Agrociencia* 16: 62-70 (special issue).

Cabrera, D., Soria, J., Nuñez, S., **Leoni, C.**, Feippe, A., Maeso, D., Zoppolo, R. 2007. El cultivo del peral en Uruguay. Pautas tecnológicas. *Fruticultura Profesional* 168:33-40.

Gilsanz, J. C., **Leoni, C.**, Schelotto, F., Acuña, A. Uso potencial de los lodos urbanos en la producción agrícola. *Agrociencia* (accepted).

Larrañaga, P., Díaz-Dellavalle, P., Cabrera, A., Alem, D., **Leoni, C.**, Almeida Souza, A.L., Giovanni-De-Simone, S., Dalla-Rizza, M. 2012. Activity of naturally derived antimicrobial peptides against filamentous fungi relevant for agriculture. *Sustainable Agriculture Research* 1: 211-221.

Leoni, C. and Ghini, R. 2003. Efeito do lodo de esgoto na indução de supressividade in vitro a *Phytophthora nicotianae*. *Fitopatologia Brasileira* 28: 67-75.

Leoni, C. and Ghini, R. 2006. Sewage sludge effect on management of *Phytophthora nicotianae* in citrus. *Crop Protection* 25: 10-22.

Leoni, C., de Vries, M., ter Braak, C.J.F., van Bruggen, A.H.C., Rossing, W.A.H. 2013. *Fusarium oxysporum* f.sp. *cepae* dynamics: in-plant multiplication and crop sequence simulations. *European Journal of Plant Pathology* 137: 545-561. doi: 10.1007/s10658-013-0268-6.

Leoni, C., Rossing, W.A.H., van Bruggen, A.H.C. Crop Rotation. In: Finckh, M., Tamm, L., van Bruggen, A.H.C. (Eds) *Plant Disease Management in Organic Agriculture*. St. Paul, MN., American Phytopathological Society, (*in press*).

Leoni, C., ter Braak, C.J.F., Gilsanz, J.C., Dogliotti, S., Rossing, W.A.H., van Bruggen, A.H.C. *Sclerotium rolfsii* dynamics in soil as affected by crop sequences. *Applied soil Ecology* (accepted).

Mondino, P., Casanova, L., Celio, A., Bentancur, O., **Leoni, C.**, Alaniz, S. Sensitivity of *Venturia inaequalis* to trifloxystrobin and difenoconazole in Uruguay. *Crop Protection* (submitted).

Scholberg, J.M.S., Dogliotti, S., **Leoni, C.**, Cherr, C.M., Zotarelli, L., Rossing, W.A.H. 2010. Cover crops for sustainable agrosystems in the Americas. Pages 23-58. In: Genetic Engeneering, Biofertilization, Soil Quality and Organic Farming. Lichtfouse, E., (Ed).

Sustainable Agriculture Reviews, Vol. 4. Springer.

Scholberg, J.M.S., Dogliotti, S., Zotarelli, L., Cherr, C.M., **Leoni, C.**, Rossing, W.A.H. 2010. Cover crops in agrosystems: innovations and applications. Pages 59-97. In: Genetic Engeneering, Biofertilization, Soil Quality and Organic Farming. Lichtfouse, E., (Ed). *Sustainable Agriculture Reviews*, Vol. 4. Springer.

Conference Papers and abstracts

Alaniz, S., Mondino, P., **Leoni, C.** 2003. La eliminación de las aplicaciones de fungicidas en el verano: su incidencia sobre el control de la sarna del manzano (*Venturia inaequalis*). IX Congreso Nacional de Horticultura y Simposio de Producción y Comercialización de Manzana – Resúmenes. Montevideo, Uruguay. p.87.

Arboleya, J., Gilsanz, J.C., Alliaume, F., **Leoni, C.**, Falero, M., Guerra, S. 2010. Manejo sustentable en la producción hortícola intensiva. Evolución del contenido de agua, de propiedades físicas, químicas, biológicas de suelo y del rendimiento en el cultivo de zapallo. 1° Congreso de Co-innovación de Sistemas Sostenibles de Sustento Rural, April 2010, Minas, Uruguay. p. 307-310.

Arboleya, J., Gilsanz, J.C., Alliaume, F., **Leoni, C.**, Fallero, M., Guerra, S. 2012. Minimum tillage and vegetable crop rotations. Proceedings of the 19th ISTRO Conference, September 2012, Montevideo, Uruguay.

Campelo, E., **Leoni, C.**, Arboleya, J., Paullier, J., Martínez, N., Carrega, E., Mondino, P., Núñez, S., Buschiazzo, M., de Lucca, R., Merino, N., Carbone, F., Scattoni, I., González, P., Ferrando, P., Rabellino, F., Moizo, A., Tellis, V. 2005. Producción Integrada en Uruguay. X Congreso Nacional de la Sociedad Uruguaya de Hortifruticultura – Resúmenes. Montevideo, Uruguay.

Leoni, C. and Ghini, R. 2001. Efeito dos biossólidos na sobrevivência in vitro de *Phytophthora nicotianae* (sin: *P. parasitica*). *Summa Phytopathologica* 27: 127.

Leoni, C. and Ghini, R. 2001. Potencialidade dos biossólidos na indução de supressividade a *Phytophthora nicotianae* em plántulas de citros em casa de vegetção. *Fitopatologia Brasileira* 26 (Suppl.)

Leoni, C. and Ghini, R. 2003. Indução de supressividade de solo a *Phytophthora nicotianae* (sin = *P. parasitica*) com lodo de esgoto. *Summa Phytopathologica* 29: 127.

Leoni, C., de Vries, M., Gilsanz, J.C.., van Bruggen, A.H.C., Rossing, W.A.H. 2012. Screening crops for their ability to host *Fusarium oxysporum* f.sp*. cepae.* Proceedings of the 19th ISTRO Conference, September 2012, Montevideo, Uruguay.

Leoni, C., ter Braak, C.J.F., Gilsanz, J.C., van Bruggen, A.H.C., Rossing, W.A.H. 2010. Dynamics of *Sclerotium rolfsii* survival in green manure amended soil. 1° Congreso de

Co-innovación de Sistemas Sostenibles de Sustento Rural, April 2010, Minas, Uruguay. p.177-180.

Mondino, P. and **Leoni, C.** 2005. El manejo de las enfermedades del manzano y duraznero en Uruguay. X Congreso Nacional de la Sociedad Uruguaya de Hortifruticultura – Resúmenes. Montevideo, Uruguay.

Montelongo, M.J., **Leoni, C.**, Mondino, P. 2009. Primer reporte de *Pseudomonas syringae* pv. *papulans* causando "Blister spot" en manzanas Fuji en Uruguay. XV Congreso Latinoamericano de Fitopatlogía y XVIII Congreso Chileno de Fitopatlogía. Fitopatología - Libro de resúmenes. p. 253.

Pagani, M.C., **Leoni, C.**, Silvera, E., Ritchie, D.F. 2001. Characterization of fruit infection periods for bacterial spot on peach. *Phytopathology* 91 (Suppl.): 68.

Pombo, C., Scarlatto, M., Bacigalupe, G.F., Dogliotti, S., Rossing, W.A.H., Abedala, C., Aguerre, V., Albín, A., Alliaume, F., Alvarez, J., Barreto, M., Chiappe, M, Dieste, J.P., García, M., Guerra, S., **Leoni, C.**, Malan, I., Mancasola, V. Pedemonte, A., Peluffo, S. 2010. Co-innovando para una agricultura más sostenible. 1° Congreso de Co-innovación de Sistemas Sostenibles de Sustento Rural, April 2010, Minas, Uruguay. p. 7-10.

Scarlatto, M., Peluffo, S., Dogliotti, S., Dieste, J.P., Abedala, C., Aguerre, V., Albín, A., Alliaume, F., Alvarez, J., Bacigalupe, G.F., Barreto, M., Chiappe, M., Garcia, M., **Leoni, C.**, Malán, I., Mancasola, V., Pedmonte, A., Salvo, G. 2010. Un enfoque sistémico y participativo para el desarrollo de sistemas de producción familiares más sostenibles: la experiencia realizada en dos predios de Canelones en el marco de un proyecto de investigación. 12 Congreso Nacional de la Sociedad Uruguaya de Horti-Fruticultura, Montevideo, Uruguay. Resúmenes. p. 32.

Other publications

Alaniz, S., Delgado, D., **Leoni, C.**, Mondino, P. 2012. Situación actual de los cancros del manzano: distribución, descripción de síntomas, agentes causales, pautas de manejo. Seminario de Actualización Técnica Frutales de Pepita, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 687, p 29-34.

Arboleya, J., Gilsanz, J.C., **Leoni, C.** 2008. Manejo sustentable en la producción hortícola intensiva. Revista INIA 24: 21-24.

Arboleya, J., Gilsanz, J.C., Alliaume, F., **Leoni, C.**, Fallero, M., Guerra, S. 2010. Manejo sustentable en la producción hortícola intensiva. Seminario de Actualización Técnica. Manejo de suelos para Producción Hortícola Sustentable, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 624, p. 9-19.

Conde, P., Montelongo, M.J., **Leoni, C.** Enfermedades del olivo. 2013. Pages: 183 – 213. In: Grompone, M.A., Villamil, J. (Eds.). *Aceite de oliva: de la planta al consumidor*. Montevideo, Editorial Agropecuaria Hemisferio Sur and INIA.

Ghini, R. and **Leoni, C.** 2005. Uso de lodo de esgoto para indução de supressividade de solos a *Phytophthora nicotianae* em citros. Circular Técnica EMBRAPA Meio Ambiente N° 10. 5 pp.

Leoni, C. 2012. Indicadores biológicos para evaluar los agroecosistemas. Actividad microbiana del suelo – respiración. Seminario de Cierre del Proyecto INIA SA04, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 674, p. 61-65.

Leoni, C. and de Vries, M. 2012. Indicadores biológicos para evaluar la sustentabilidad de los agroecosistemas. Comunidades microbianas - Índice de patogenicidad del suelo - Ejemplo para secuencias hortícolas con aliáceas. Seminario de Cierre del Proyecto INIA SA04, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 674, p. 66-72.

Leoni, C. and Ghini, R. 2006. Efeito do lodo de esgoto na indução de supressividade de solos a *Phytophthora nicoantiae* em citros. In: Bettiol, W., Camargo, O.A. (Eds). Lodo de Esgoto: Impactos ambientais na agricultura. Jaguariúna, SP, EMBRAPA Meio Ambiente. p. 255-291.

Leoni, C., Conde, P., Paullier, J., Montelongo, M.J., Mondino, P. 2013. *Manual para la identificación de las principales enfermedades y plagas del olivo en Uruguay*. Boletín de Divulgación N° 102. Montevideo, INIA. 49 p.

Leoni, C., de Vries, M., Gilsanz, J.C., van Bruggen, A.H.C., Rossing, W.A.H. 2012. Efecto de la biodiversidad vegetal sobre la dinámica poblacional de patógenos de suelo en sistemas hortícolas. Seminario de Cierre del Proyecto INIA SA04, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 674, p. 94-100.

Leoni, C., Gilsanz, J.C., de Vries, M. 2011. Abonos verdes: una herramienta para el manejo de patógenos de suelo. Jornada de Divulgación en Producción Orgánica, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 662, p. 7-12.

Leoni, C., Maeso, D., Paullier, J., Banchero, L., Cabrera, S., Campelo, E., Martínez, D., González, P. 2003. Producción Integrada. Guía de monitoreo de plagas y enfermedades para cultivos hortícolas: tomate, cebolla, ajo, lechuga, frutilla. Montevideo, Uruguay. Artes Gráficas S.A. 125 p.

Mondino, P., Alaniz, S., **Leoni, C.** 2010. Manejo integrado de las enfermedades del duraznero en Uruguay. In: Soria, J (Ed.). Manual del Duraznero - Manejo integrado de plagas y enfermedades. Boletín de Divulgación N° 99. Montevideo, Hemisferio Sur, p. 45-76.

Mondino, P., Casanova, L., Celio, A., **Leoni, C.**, Alaniz, S. 2012. Situación de la resistencia a

trifloxystrobin y difenoconazole de poblaciones de *Venturia inaequalis* en Uruguay, estrategias de manejo antirresistencia. Seminario de Actualización Técnica Frutales de Pepita, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 687, p. 35-44.

Paullier, J., **Leoni, C.**, Baraibar, A., Folch, C., Núñez, P. 2007. Desarrollo de bioinsecticidas para el control de plagas agrícolas. Revista INIA 11: 45-47.

Scattoni, I., Mondino, P., Gepp, V., Bentancourt, C., Alaniz, S., Mujica, V., Núñez, S., **Leoni, C.**, Carbone, F., Merino, N., Buschiazzo, M. 2003. Producción Integrada. Guía de monitoreo de plagas y enfermedades para cultivos frutícolas: frutales de carozo, frutales de pepita, uva de mesa. Montevideo, Uruguay. Artes Gráficas S.A. 92 p.

Zerbino S. and **Leoni, C.** 2012. Importancia de la biodiversidad para el funcionamiento de los agroecosistemas. Seminario de Cierre del Proyecto INIA SA04, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 674, p. 6-19.

Zerbino, S., **Leoni, C.**, Altier, N., Zarza, R. 2012. Indicadores biológicos para evaluar los agroecosistemas. Integración de indicadores para la evaluación de la sustentabilidad de los agroecosistemas. Un ejemplo en sistemas lecheros del litoral oeste. Seminario de Cierre del Proyecto INIA SA04, INIA Las Brujas, Uruguay. Serie Actividades de Difusión N° 674, p. 80-86.

PE&RC PhD Training Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (6 ECTS)
- Management of soil-borne pathogens by crop rotation: a review (2010)

Writing of project proposal (4.5 ECTS)
- Modelling soil-borne pathogen dynamics in organic and conventional farming systems in Southern Uruguay from a whole-farm perspective (2008)

Post-graduate courses (4.5 ECTS)
- Advanced statistics course; PE&RC (2008)
- Multivariate analysis course; PE&RC (2008)
- Soil ecology course; PE&RC, E&E, SENSE (2010)

Laboratory training and working visits (3 ECTS)
- Molecular techniques to assess and quantify soil microbial communities; Julius Khün Institute, Federal Research Centre for Cultivated Plants, Braunschweig, Germany (2010)

Deficiency, refresh, brush-up courses (3 ECTS)
- Nematology (2008)
- Basic taxonomy of plant parasitic nematodes (2008)
- System analysis, simulation and system management (2008)

Competence strengthening / skills courses (1.8 ECTS)
- Competence assessment; PE&RC (2007)
- Scientific writing; Language Services, WUR (2012)

PE&RC Annual meetings, seminars and the PE&RC weekend (1.2 ECTS)
- PE&RC Day (2007)
- PE&RC Weekend (2008)

Discussion groups / local seminars / other scientific meetings (7.5 ECTS)
- 78th Meeting of the KNPV working group Soil-borne Pathogens and Soil Microbiology (2008)
- EULACIAS Project; monthly meetings and 2 international workshops (2008-2010)
- Seminars at INIA; Uruguay; 4 each year (2009-2013)
- Annual meetings of the Uruguayan Phytopathological Society, Sociedad Uruguaya de Fitopathología – SUFIT (2010-2012)

International symposia, workshops and conferences (7.8 ECTS)
- 9th International Congress of Plant Pathology; Torino, Italy (2008)
- 1st Latin American and European Congress on Co-innovation of Sustainable Rural Livelihood Systems; oral presentation and partly extended summary for proceedings; Minas, Uruguay (2010)
- Latin-American Regional Workshop ISTRO; poster presentation; Colonia del Sacramento, Uruguay (2010)
- 19th ISTRO Conference; poster presentation and complete paper for proceedings; Montevideo, Uruguay (2012)

Supervision of MSc students (9 ECTS)
- The effect of onions and oats on *Fusarium oxysporum* f. sp. *ceapae* populations in organic and conventional soils
- Can soil management reduce FBR in onion bulbs caused by *Fusarium oxysporum* f. sp. *cepae*?; a study carried out in Uruguay
- Population analysis of *Fusarium oxysporum* f. sp. *cepae*

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