# Soil-borne Pathogens in Intensive Legume Cropping - *Aphanomyces* spp. and root rots

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# Abstract

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Root diseases caused by soil-borne pathogens are often main constraints in legume crop production. Changes towards organic farming practices have recently contributed to an increase in legume cropping, mainly for nitrogen supply purposes, and these have raised concerns about unacceptable build-up of soil-borne pathogen inocula. This study aimed to evaluate the impact of frequent legume cropping on such inocula build-up, and had emphasis on *Aphanomyces euteiches*, an important pathogen causing pea root rot in Sweden. Field experiments with legume monocultures were established, and the effect of these monocultures on disease development and yields in subsequent pea, broad bean and snap bean was measured. Isolates of *Aphanomyces* spp., from several legumes, were tested for host ranges and characterised by means of morphological, biochemical and molecular methods. A survey of legume-specific soil-borne pathogens in fields under frequent legume cropping in northern Spain was also undertaken.

Several legumes were found to be hosts for *A. euteiches*, and this pathogen was isolated from field-grown alfalfa, snap bean, pea, sweet clover and vetch. The Swedish isolates of *A. euteiches* were assigned two putative pathotypes, pea- and vetch-specific. Other species of *Aphanomyces* had a wide host range among legumes, but these did not induce disease symptoms. Sequencing of ITS1 - 5.8S - ITS2 rDNA region and RFLP of AT-rich DNA allowed appropriate delineation of these *Aphanomyces* spp. Monocultures of the tested legume crops affected the inoculum potential of *A. euteiches* differently. Pea, broad bean, snap bean, vetch and sweet clover were almost equally efficient in inoculum build-up and markedly affected subsequent pea and broad bean yields. Monocultures of alfalfa, birdsfoot trefoil, red, white, and Persian clover had lower impact on disease development and yields in subsequent crops. *Thielaviopsis basicola* and *Rhizoctonia solani* were the most prevalent pathogens in pea and snap bean fields in Spain and significantly affected yield. Climatic factors and soil properties favoured prevalence of these pathogens in Spain, whereas *A. euteiches* was most prevalent under Swedish conditions. It is concluded that intensive legume cropping will, on many soil types not be sustainable in the long-term due to the build-up of soil-borne pathogen inoculum.

*Keywords*: Broad bean, *Fabaceae*, *Fusarium* spp., Fungi, Host range, *Oomycetes*, Organic farming, Pea, *Rhizoctonia solani*, *Thielaviopsis basicola*, Vetch.

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*To my loved wife Jolanta.*

# Contents

# Appendix

## Papers I-IV

The present thesis is based on the following papers, which will be referred to by their Roman numerals.

I. Levenfors, J.P., Wikström, M., Persson, L. and B. Gerhardson, 2003. Pathogenicity of *Aphanomyces* spp. from different leguminous crops in Sweden. *European Journal of Plant Pathology*, In press.

II. Levenfors, J.P., Wikström M. and B. Gerhardson. Soil-borne root pathogens, disease development and yield effects in legume crops grown in short crop rotations. (Manuscript).

III. Levenfors, J.P. and J. Fatehi. Molecular characterization of *Aphanomyces* spp. associated with legumes. (Submitted for publication).

IV. Levenfors, J., Wikström, M. and I. Castresana. Pea and bean root rot pathogens in fields under intensive pea/bean cropping in northern Spain. (Manuscript).

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# 1. Background

Leguminous crops have been cultivated since ancient time. They are a source of food for humans and feed for domestic animals as well as provide nitrogen for subsequent crops in the crop rotations. Nutritionally the legumes are complementary to cereals as a source of the amino acid lysine, which is limited in cereals, whilst cereals supply the amino acids methionine and cysteine, which are limited in legumes (Salunkhe & Deshpande, 1991). This has little importance in modern agriculture, but can be of significance in developing countries where animal proteins are often expensive, or not readily accepted (Deshpande & Damodaran, 1990). In animal husbandry, legumes are widely used as protein sources, especially those with extensive foliage such as clovers and alfalfa (*Medicago sativa* L.), and Sinha (1977) states that the dairy industry in many countries is directly related to the atmospheric nitrogen fixation through legumes. Processed soybean is another important source of fodder protein, particularly in western countries.

In Sweden, the acreage that is sown with leguminous crops raised substantially in the mid 1980s. This was partly due to subsidiaries (Anonymous, 2001) but also because of an increased practice of organic farming where legume-rich crop rotations are an important way for substituting commercial, easy-soluble N-fertilisers. Therefore, in numerous organic farming crop rotation systems, legume crops in some form might occur as often as four out of six years. Since many of these crops are recognised to have soil-borne diseases in common (Salt & Delaney, 1984), such high frequencies of their occurrence in crop rotation systems increases concerns about a fast build up of inocula of soil-borne pathogens affecting these crops.

Following the above, three main questions are raised; i) To what extent do legume-rich crop rotations contribute to the build up of inoculum concentration and inoculum potential of specific soil-borne pathogens, and ii) in what way can an alternation between different legume crops in the rotation affect such a build up? Since suitable crop rotations are widely accepted as one of the fundamental tools for managing soil-borne plant pathogens, this kind of inoculum build up might endanger the long term sustainability of such cropping systems, in addition to contributing to significant yield losses. Flint & Roberts (1988) recognise the following three characteristics of a soil-borne pathogen, which allow its control by means of crop rotation practises: i) the inoculum of the pathogen must originate from the field itself, ii) the host range of the pathogen must be quite narrow or at least not include plant crops, which are commonly cultivated in the field, iii) the pathogen must be incapable of survival for longer periods without the presence of host plants.

An important question initiating these thesis studies was to what extent the legume pathogens in the areas investigated meet the above mentioned criteria? The study was limited to soil-borne pathogens, and was focused on the oomycete *Aphanomyces euteiches* Drechsl., a causal agent of pea root rot, which is recognised as causing severe losses in pea (*Pisum sativum* L.) plantations all over

Sweden (Olofsson, 1967). Since *A. euteiches* is a strictly soil-borne pathogen and survives in soil in absence of a host plant for long periods (Papavizas & Ayers, 1974), the pathogen host range among leguminous crops grown in the area, and its effect on these crops were of special interest as was the effect of these leguminous crops on *A. euteiches* propagation.

# 2. Aims and outline of the study

The aforementioned increased production of leguminous crops in Sweden, and the concerns about maintaining long term sustainability in organically farmed fields, contributed significantly to the start of this project. As pea is one of the most important legume crops, the main aim set was to determine the impact of crop rotations with high frequencies of leguminous crops on yields of pea. Furthermore, since *Aphanomyces euteiches* is recognised as the most damaging pea pathogen in the areas of interest, the study focused on this pathogen.

The following hypotheses were set up at the start of the project (1997):

- Various leguminous crops grown as monocultures affect the inoculum potential of *A. euteiches*, which in turn affects yields of subsequent pea, snap bean (*Phaseolus vulgaris* L.) and broad bean (*Vicia faba* L.) crops.
- *A. euteiches* colonizes and proliferates in leguminous crops other than pea (*Pisum sativum* L.) and might also be parasitic to these crops.
- The Swedish population of *A. euteiches* consists of subpopulations or pathotypes, which are defined by host preferences for different leguminous crops.

Laboratory and field experiments were the main means for testing these hypotheses. Four field experiments were established at different locations in Sweden. In these experiments a number of annual, biannual and perennial leguminous crops were monocultured for 3 to 4 years. After this period of monoculture, subsequent pea, bean and broad bean crops were established and the influence of the previous monocultures on disease development and yields were measured. Attempts to isolate *Aphanomyces* spp. from all the legumes sown were made during the first season of monoculture, and a number of isolates that originated from a range of leguminous crops, were obtained and then tested for pathogenicity towards the main host pea and towards several other legumes.

Efforts to conclusively identify these *Aphanomyces* isolates on the basis of pathogenicity tests, observed morphological characters and isozyme analysis were not successful and as a result, a fourth hypothesis was presented set up:

- Characterisation of intra-specific variation observed among Swedish *Aphanomyces* isolates can be improved/achieved with the help of molecular methods.

In order to test this hypothesis and to better characterise the Swedish population of *Aphanomyces* spp. infecting legumes two independent methods were used: i) sequencing of Internal Transcribed Spacers (ITS1 and ITS2) with 5.8 r-DNA, and

ii) Restriction Fragment Length Polymorphism (RFLP) of AT-rich (assumingly mitochondrial) genomic DNA.

In addition to this and as a result of reports about observed yield losses in fields under intensive pea and snap bean cropping in northern Spain, a survey aiming to identify the pathogenic fungi involved in root rot of these crops was performed. The following contradictory assumptions were then tested:

- *A. euteiches* is as prevalent in northern Spain as it is in Sweden and yield losses are due to infections of this pathogen.
- Other pathogens of root rot complex in pea and snap bean occur in northern Spain and these are responsible for observed yield losses.

# 3. Legume cropping, cropping systems and legume soil-borne pathogens – a short overview

## 3.1. The effect of legume cropping on soil properties and nitrogen balance.

The use of legume cropping for improving the properties of cultivated soils is a highly recognised strategy/method in agriculture. Already Theophrastus (372-287 BC) described the significance of utilising legumes as mulches (Caamal-Maldonado *et al.*, 2001). Besides providing protein rich grain yields, the forage legumes are also known to improve agricultural sustainability (Thiessen Martens *et al.*, 2001). Their ability to decrease soil erosion (Hargrove *et al.*, 1984), to maintain soil organic matter, and to improve the soil structure (Frye *et al.*, 1988; Sainju *et al.*, 2001; Sainju *et al.*, 2002) is much appreciated. Maintenance of soil organic matter by use of legume cropping might also help to reduce $CO_2$ and $N_2O$ in the atmosphere through, first fixing these *in planta* and then sequestering them in soils (Lal & Kimble, 1997; Sainju *et al.*, 2002). Furthermore, the interest of using both annual and perennial legumes to reduce weed density and biomass has recently increased in organic farming and in areas with poor soils (Caamal-Maldonado *et al.*, 2001; Fisk *et al.*, 2001; Teasdale *et al.*, 1991; Teasdale 1996). However, in modern organic farming, the main reason for cropping legumes is for their ability to fix atmospheric nitrogen (Giller & Wilson, 1991; Wortmann *et al.*, 2000).

The atmospheric nitrogen in the form of $N_2$ is virtually an inexhaustible source of nitrogen, but it is at the same time not generally accessible to plants. The formation of plant usable nitrogen through symbiotic nitrogen fixation is energy consuming and takes place mainly when the specific symbiotic associations with nitrogen fixing soil bacteria are formed (Lafay & Burdon, 1998; Patriarca *et al.*, 2002). The specialised $N_2$-fixing nodules are typical for the *Fabaceae-Rhizobium* union, and make the plants autotrophic for external nitrogen sources (Patriarca *et al.*, 2002). This, in turn, makes many members of the plant family *Fabaceae* of

considerable agricultural and ecological importance (Allen & Allen, 1981). In the nodules, *Rhizobium* bacteria are able to convert gaseous $N_2$ into plant available $NH_4^+$ by means of the nitrogenase enzyme complex (Subba Rao, 2001) and produce $NH_4^+$ from nitrate and amino acids (Patriarca *et al.*, 2002). The plants in turn deliver photosynthetic carbon compounds to the *Rhizobium* bacteria (Mithöfer, 2002). This loss of fixed carbon is considered as an important reason why legume yields are relative low, when compared to e.g. cereals (Roughley *et al.*, 1983), even though enzymatic N-fixation is less energy consuming than present industrial N-fixation from $N_2$, which requires a substantial amount of energy to form ammonia from hydrogen and nitrogen. In agriculture world-wide, the annual $N_2$-fixation is estimated to 90 Mt, of which 50 Mt is assimilated by forage legumes through their associations with $N_2$-fixing soil bacteria, whereas the estimate of fertiliser N applied is 60 Mt (Frame *et al.*, 1998).

There are many reports estimating the amount of nitrogen, which can be fixed by leguminous crops. For example, Giller and Wilson (1991) and Wortmann *et al.* (2000) estimate $N_2$ fixation in a range of 20 to 250 kg N $ha^{-1}$ for grain legumes while Peoples *et al.* (1995) reports that symbiotic N-fixation effects in input of nitrogen to cropping systems in a range between 50 and 350 kg N $ha^{-1}$. Roughley *et al.* (1983) conclude that differences, which are often reported in the rate of N fixation (e.g. 45-552 kg for broad bean) are due to other factors such as a use of various analyse methods and the influence of environmental stress. The leguminous crops, including grain legumes, are not only essentially self-supporting with respect to nitrogen (Bohlool & Ladha, 1992), but they can also reduce the amount of N-fertilisation needed for subsequent crops (Hargrove, 1986; Kuo *et al.*, 1996; Sainju *et al.*, 2001). However, this strongly depends on the type of leguminous crop and grain legumes, e.g. peas may negatively affect N-balance in the soil due to significant nitrogen removal by a rich grain harvest (Karlen *et al.*, 1994).

## 3.2. Legume crops in Swedish cropping systems

In Sweden, the production of grain legumes substantially increased in the mid 1980´s, partly due to subsidisation from the government but also due to an increasing interest in organic farming. Broad bean was for example a very marginal crop in Sweden between the 1960s and 1980s, but has then steadily increased and is presently cropped on an acreage of approximately five thousands hectares. The acreage of pea has increased similarly (Table 1; Anonymous, 1965-2002) and the same tendency has been observed worldwide. Figure 1 presents data on grain legume production over a period of 40 years, also confirming the rising importance of these crops worldwide.

Table 1. Acreage (thousands of hectares) of leguminous crops and leys in Sweden from 1964 to 2001.

| Year | Grainlegumes | Leys | Total acreage under agriculture |
|---|---|---|---|
| 1964 | 12(<1)[1] | 1355(41) [1] | 3304 |
| 1970 | 6(<1) | 1031(34) | 3032 |
| 1980 | 16(<1) | 914(31) | 2951 |
| 1990 | 33(1) | 929(33) | 2845 |
| 2001 | 36(1) | 900(28) | 3153 |

Source: Anonymous. 1965-2002. Yearbook of Agricultural statistics. Statistics Sweden.
[1]Values in brackets are percentages of total acreage under agriculture.

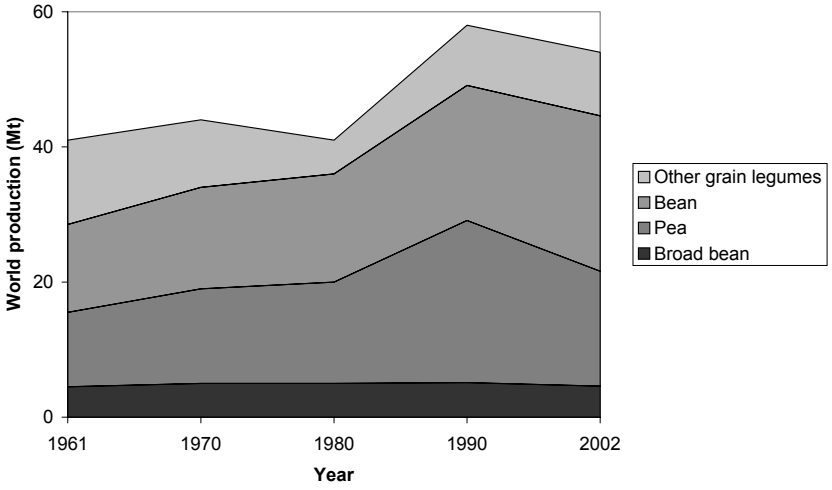


Figure 1. Worldwide production of grain legumes during the period 1961 to 2002. Grain legumes comprise species of *Phaseolus* spp., *Pisum* spp., *Vicia* spp. and *Lupinus* spp.

The goal for the impending organic farming in Sweden is to produce higher quality food products using crops that are locally available and produced in an environmentally friendly manner as described in the program of The Swedish Ecological Farmers Association (Det nödvändiga systemskiftet. Ekologiska Lantbrukarnas jordbrukspolitiska program, mars 1996). In order to rely on local and renewable resources, in mixtures of animal fodder, protein from imported soybean is supposed to be exchanged to protein mainly from locally produced pea or broad bean. Both these crops might be of the same value as soybean when used as a protein source, but the cost of cultivation may be lower, since they are grown locally. Furthermore, according to this program ecological agriculture should also aim in maintaining the fertility of the soil and maximise re-circulation of plant nutrients and organic matter. Therefore easy-soluble nitrogen fertilisers are banned in organic plant production and should be replaced by a crop rotation system,

which allow nitrogen levels to be maintained using ecological sustainable methods. These circumstances have, especially, raised the demands of frequent occurrence of leguminous crops in organic crop rotations, both in order to keep the nitrogen balance in the cropping system and to maintain the protein balance in animal food. The use of grass/legume mixtures, and particularly grass/clover mixtures, ensures proper plant nutrients are supplied to other crops following them in the crop rotation cycle. In addition to providing fodder, legumes also help control pests and weeds, as well as improve soil structure. However, the main reason for frequent culturing of legumes in organic farming is for their nitrogen fixing ability.

Although in Sweden the acreage of leys has decreased, ley production has become an important feature of organic farming where the acreage of ley legumes has increased. Table 2 presents four crop rotations, commonly practiced in Swedish organic farming. The frequency of legume crops in these rotations differs depending on the type of farm production. When animal production is not present, grain legumes may appear as often as every fourth year. In longer rotation systems, the leys (clover/grass mix) are used to provide extra nitrogen (Table 2) and in dairy farms, leys may comprise 40 to 67 % of the crops in the rotations (Table 2).

Table 2. Examples of crop rotations practised by organic farmers in Sweden.

| Farm without animal production | | Dairy farm | |
|---|---|---|---|
| I | II | I | II |
| Barley or oats | Barley or oats | Barley or oats | Barley or oats |
| Green manure | Green manure | Ley I | Ley I |
| Winter wheat | Winter wheat | Ley II | Ley II |
| Pea or broad bean | Pea | Winter wheat | |
| | Barley or oats | Pea | |
| | Clover | | |

Source: Jan Hill, Länsstyrelsen i Västra Götaland and Ann-Charlotte Wallenhammar, Hushållningssällskapet i Örebro.

The market for organically produced products is also steadily increasing. The overall world market for ecological products has increased by approximately 20 % and in Sweden by 20-30 % each year and there is a strong possibility that such demand will continue to increase (http://www.ekolantbruk.se; read 14th of February 2003). Because of market demand, the interest in growing leguminous crops has gradually increased amongst the Swedish farmers. The acreage of leguminous grain crops, in terms of the pea, broad bean and snap bean (*Phaseolus vulgaris* L.), have increased from 21 000 hectares in 1995 to 37 000 in 2000. The acreage of leys, including ley-legumes, was approximately 900 000 hectares in 2000, which represented around one-third of the total arable land used in Sweden (Anonymous, 2001). The increased acreage of leguminous crops does appear to mirror the increased interest in organic farming amongst Swedish farmers. According to the data illustrated by the Swedish Farmers Association, around 15

% of Swedish farmers have already converted at least part of their farms into organic production.

It is mainly this change towards legume rich crop rotations that has raised concerns about soil-borne and other diseases associated with these crops, as they might constitute one of the major threats in keeping the legume-intensive growing system sustainable. The word sustainability in this thesis is defined as "the successful management of resources to satisfy changing human needs while maintaining or enhancing the quality of the environment and conserving resources" (TAC, CGIAR, 1978). Referring to soil-borne diseases, sustainability of the growing system would thus mean that the inoculum density of certain soil-borne pathogens is kept at such a low, or acceptable level over an extended time period that, as a result, provides good conditions for cultivating various crops and obtaining satisfactory yields. Any long-term unbalance in this system may cause the loss of sustainability.

## 3.3. Important fungal and *Oomycete* pathogens associated with leguminous crops

In the initial phase of this work, isolations of pathogenic fungi and *Oomycetes* from grain and ley legumes grown in Swedish fields, and also from pea and snap bean fields in Spain were carried out (**I; IV**). *Fusarium* species were often isolated from Swedish ley legumes (**II**; Lager, 2002), pea and broad bean, although no severe symptoms caused by these fungi were observed, except for red clover (*Trifolium pratense* L.). Most of the pea plants showing distinct, aboveground symptoms were infected with *A. euteiches*. Conversely, in Spain *A. euteiches* was rarely detected, while *Thielaviopsis basicola* (Berk. & Broome) Ferraris and *Rhizoctonia solani* Kühn were found to be the most important and yield reducing pathogens of pea and snap bean (**IV**). Based on these results and on other studies showing that *A. euteiches* is the most important pathogen on peas in Scandinavia (Persson *et al.*, 1997), *A. euteiches* was emphasised in further experimental studies. However, since other pathogens present in Swedish soils also contribute to pea and snap bean yield losses all over the world, these have also been taken into consideration.

### *3.3.1* Fusarium *spp.*

The economically important legume pathogens commonly occurring in Sweden are either seed- or soil-borne but some of the seed-borne ones, and among them several *Fusarium* spp., have part of their life cycle in the soil. As such, they also often infect several susceptible crops (Lager 2002). The *Fusarium* resting structures are chlamydospores, altered mycelial fragments or microsclerotia (Beckman & Roberts, 1995; Price, 1984). The plant pathogenic *Fusarium* spp. are often divided into three groups; i) commonly attacking cereals, e. g. *F. avenaceum* (Fr.) Sacc., *F. graminearum* Schwabe and *F. culmorum* (Smith) Sacc., ii) causing root rots, mainly *F. solani* (Mart.) Sacc. and iii) the wilt causing *F. oxysporum* Schl. group (Price, 1984). Many *Fusarium* spp. attack also a wide range of legume crops such as: clovers (Lager & Gerhardson, 2002; Lim & Cole, 1984; Skipp *et*

*al.*, 1986) (**II**), green bean (Hall, 1994) and broad bean (Majchrzak *et al.*, 1996). Legume root rot is often associated with infections caused by *F. oxysporum F. solani* and *F. avenaceum* (Salt, 1983). In field surveys of red clover fields in Sweden (Lager, 2002; Rufelt, 1986) the most frequently isolated *Fusarium* species was *F. avenaceum*. (Persson *et al.*, 1997) regularly isolated *F. avenaceum*, *F. culmorum*, *F. oxysporum* and *F. solani* from root tissue of peas. They report that, among these, *F. avenaceum* and *F. solani* induce the highest disease severity rating when challenging peas in pathogenicity testing. *Fusarium* pea wilt was first described in 1925 by Jones & Linford (1925), and associated with *F. oxysporum* f. sp. *pisi* (e.g. Wade, 1929). This pathogen penetrates the host plant into the vascular tissue and spreads throughout the plant by mycelial growth or microconidia, produced in the xyleme (Kraft, 1994). Recently even *F. graminearum*, another typical cereal pathogen, is proposed to be associated with infections of peas and beans (Chongo *et al.*, 2001). Beuselinck *et al.* (1984) and Chao *et al.* (1995) report root rot in Birdsfoot trefoil (*Lotus corniculatus* L.), caused by *Fusarium*, *Sclerotinia* and *Rhizoctonia* spp. in areas with warm and humid weather conditions. In a field survey of Spanish fields sown with snap bean or pea, *F. oxysporum* and *F. solani* were frequently isolated from both pea and snap bean. However these pathogens were not of the highest importance when considering their impact on yield losses (**IV**).

### *3.3.2* Thielaviopsis basicola *(synanamorph* Chalara elegans *Nag Raj & Kendrick, 1975).*

This pathogen has a wide host range amongst economically important crops (Lucas, 1975; Otani, 1962). Similarly to *A. euteiches*, it seems to lack saprophytic ability and should therefore be considered as an obligate parasite (Hood & Shew, 1997). Harman (2001) concludes that, generally, the pathogen is favoured by arid soils with high temperatures. However, Bødker *et al.* (1993b) surveyed pea fields in Denmark and reported 19 % lower yields of pea in fields where *T. basicola* was found in pea plants when compared to fields where *T. basicola* was not present. A 20 % yield loss of pea was recorded also in a similar study conducted in pea fields in Spain (**IV**). In this study, also a slightly lower yield of snap bean was also observed in fields infected with *T. basicola*. This pathogen causes black root in various legumes such as alfalfa, pea and snap bean (Bødker *et al.*, 1993b; Hall, 1994; Oyarzun *et al.*, 1993; Reddy & Patrick, 1989). Broad bean is also reported to be its host (Moore, 1959). In an English survey, Salts (1983) classify black root as an uncommon root rot. In this study, chlamydospores of *T. basicola* were observed in root tissue of white clover (*T. repens* L.) collected in only one field experimental site, but the roots displayed only slight discoloration (**II**).

### *3.3.3* Rhizoctonia solani*, with the teleomorph phase* Thanatephorus cucumeris *(A. B. Frank) Donk.*

The pathogen is distributed worldwide and has a very wide host range (Salt, 1983). Generally, disease symptoms are most severe when the temperature is high (>20° C) and under conditions of high moisture and in light or sandy soils (Harman, 2001; Kraft *et al.*, 1997; Sinclair, 1997). *R. solani* can survive in the

absence of a living host for a long period time in the soil as hyphae, sclerotia and through saphrophytic growth on organic matter. The pathogen causes seed and seedling rot of peas (Harman, 2001) and might contribute to significant yield reductions in snap bean (Harman, 2001; Mathew & Gupta, 1996). In a field survey of Spanish fields sown with pea and snap bean, *R. solani* was found in 47 % of the snap bean fields surveyed and in 14 %of the pea fields surveyed. Furthermore, snap bean fields, which showed the presence of *R. solani*, averaged 17 % less yields when compared to snap bean fields which, did not show the presence of *R. solani* (**IV**). Additionally, isolates of *R. solani* recovered from sugar beet and various legumes can be pathogenic to broad bean (Engelkes & Windels, 1996) but infections in fields is often accompanied by other species such as *Pythium* spp., *Fusarium* spp., *Cylindrocarpon destructans* and other fungi (Salt, 1983). Although several anastomosis groups (AG) were reported in association with peas, the AG 4 seems to be the most important in peas (Harman, 2001).

### *3.3.4* Pythium *spp.*

These pathogens cause root rot or seedling rot in a range of legumes (e. g. Allen *et al.*, 1998; Harman, 2001). Seed infection and pre-emergence damping-off, which can severely reduce seedling stands of pea and other legumes is often connected to the presence of one out of its two causal agents *P. ultimum* and/or *R. solani* (Bødker *et al.*, 1993a; Tu, 1987; Xi *et al.*, 1995). However, the incidence of damping-off caused by *P. ultimum* and *R. solani* alone, might be significantly lower when the substantial amounts of *P. ultimum* and *R. solani* inocula are present at the same infection site (Pieczarka & Abawi, 1977; Xi *et al.*, 1995). Pieczarka and Abawi (1977) proposed that lower disease incidence is an effect of competition for the infection site between these two pathogens. In Sweden *Pythium* spp. were isolated from 33 to 47 % of fields surveyed by (Persson *et al.*, 1997). Moreover, in that study *P. irregulare* was found highly pathogenic to peas, when assayed in greenhouse pathogenicity tests. The severity of infections caused by this *Oomycete* in field conditions is, however, clearly less significant and/or often masked by significant infections caused by its relative, *A. euteiches*.

### *3.3.5 Some other legume pathogens*

Clover rot, *Sclerotinia trifoliorum* Erikss., is a disease which often limits the persistence of red clover (Williams, 1984). The symptoms are expressed as brown spots on the leaves in autumn and as black patches on rotting plant during. The common legume pathogen *C. destructans* is reported to be associated with broad bean (Salt, 1983), but in this study was isolated from red- and white clover roots (**II**). Other fungal pathogens occurring in Sweden are: i) *Botrytis cinerea* and *B. fabae* causing lesions on broad bean (Sundheim, 1973), and ii) *Phoma medicaginis* var. *pinodella* (L. K. Jones) Boerema, and *Mycosphaerella pinodes* (Berk. & Blox) Vestergr., which have peas as their host (Nasir & Hoppe, 1991). None of these pathogens is recognised as important in Sweden. In certain areas, the *Ascochyta* blight complex may cause serious yield losses, up to 20 %, especially when in combination with *M. pinodes* (Moussard *et al.*, 1998; Tivoli *et al.*, 1996; Wroth, 1998). The downy mildew of pea, caused by *Peronospora viciae* (Berk.) de Bary also regularly occur in Sweden (Stegmark, 1990), and in 1999

downy mildew was also observed in commercial grown broad bean in Västra Götalands län, mid Sweden.

# 4. *Aphanomyces* spp. as legume pathogens

## 4.1. *A. euteiches* - the causal agent of pea-root-rot

At present, the Eukaryote *Aphanomyces* spp. is classified within the kingdom *Chromista*, the class *Oomycetes*, order *Saprolegniales* and the genus *Saprolegniaceae*. *Oomycetes* resemble fungi, both morphologically and physiologically, but they are phylogenetic relatives to diatoms, chromophyte algae and other heterokont protists (Petersen & Rosendahl, 2000; Tyler, 2001). This algal affinity was already postulated by Bessey (1971) and Dick (1969) and is nowadays supported by analysis of r-RNA tandem (Petersen and Rosendahl, 2000; Wainright *et al.*, 1993). *Oomycetes* are known to be saphrophytes, commensals or parasites on many types of hosts including nematodes, algae, fish, insects, other fungi and plants (Deacon & Donaldson, 1993; Schneider & Robertson, 1975; Sherwood & Hagedorn, 1962). Some of them cause major epidemics, e.g. *Aphanomyces astaci* (Unestam, 1981) and *A. invadans* (Vogelbein *et al.*, 2001) in crayfish and fish, respectively. Several species of the related genera *Phytophthora*, *Pythium*, *Plasmodiophora*, *Bremia*, and *Peronospora* are economically important pathogens of crop plants (Deacon and Donaldson, 1993; Jones & Drechsler, 1925; Tyler, 2001). *A. euteiches*, a causal agent of pea root rot belongs to the latter group and is recognised as one of the most damaging soil-borne pathogens of this important leguminous crop (Jones and Drechsler, 1925). In addition to peas, it infects several other legumes (Grau *et al.*, 1991).

*A. euteiches* is known to be a true soil-borne pathogen. Wallace (1978) defines a pathogen to be soil-borne when a part of its life cycle takes place in the soil. The plant associated *Aphanomyces* species have their entire life cycle in the soil. As a strict soil-borne pathogen, the only way it can be dispersed is through transportation of soil; soil associated material and infected plant tissues that are moved from one place to another. Scott (1961) reports that *A. euteiches* is ubiquitous in the soil. Therefore, in areas where the environment is suitable for this pathogen, its migration as a possible way of its spread is of less significance. Winner (1966) reports that *A. euteiches* has no saprophytic ability of importance whereas other legume pathogens, such as e.g. *Fusarium* spp. often survive as saprophytes (Beckman and Roberts, 1995) on plant debris. It also has a narrower host range when compared to e.g. many *Fusarium* pathogens. Additionally, its oospores might survive for many years in a non-host environment without being damaged.

The entire life cycle of this pathogen takes place in the plant rhizosphere with exception that mycelium colonizes the hypocotyl or epicotyl of the pea host plant. The oospores are the source of primary inoculum and the survival structures in a non-host environment. When a host plant is present the oospores have the opportunity to germinate and a life cycle of the pathogen may be completed. The

asexual stage of the life cycle starts with oospore germination, the oospore forms a germ tube and a long, terminal zoosporangium. Shang *et al.* (2000) show that oospore germination is stimulated by placing them on the surface of several plant roots, including non-hosts when compared to exposing them to only root exudates. That study shows moreover, that oospores germinated better when placed on lateral roots of pea and bean as opposed to taproots. The zoosporangium can release more than 300 primary zoospores (Scott, 1961). The primary zoospores are pyriform and have two whiplash flagella. The primary zoospores may form round cysts (approximately 10 µm in diameter). Secondary zoospores are reniform, have one long tinsel and one short whiplash flagella laterally attached. They can alternate between primary phase and secondary phase in cycles. Zoospores are the infecting agent, which attach to the epidermal cells of host, encyst, germinate and form coenocytic hyphae in the root tissue. The zoospores are dependent on free liquid for motility and infection. Zoospores gather and encyst in response to attractants and recognition of a host surface. When adhered to the host surface, the cyst germinates by cyclic release and absorption of calcium ions (Deacon & Saxena, 1998; Donaldson & Deacon, 1992). Haploid antheridia and oogonia are formed and, if they are compatible, an antheridium penetrates an oogonium with fertilization tubes, which deliver male nuclei to the oogonium for formation of a diploid oospore. Oogonia are terminal, approximately spherical in shape and have a diameter ranging between 25 to 35 µm (Scott, 1961).

## 4.2 Disease symptoms and pathogenicity tests

Symptoms of infected plants with disease commence with the yellowing of the root tissue. At a later stage, the root tissue becomes brown and watery and in severe cases the hypocotyl or epicotyl becomes watery, darkened and constricted at the soil line. Vetch roots infected with *A. euteiches* demonstrated similar symptoms as infected pea roots. Pea plants usually start yellowing from the lower nodes and in severe cases wilt entirely. When uprooting diseased plants from field soil, the cortical cells are often left behind, indicating that the cells are infected and rotten. In this study, the symptoms of pea root rot in plants dug up from naturally infested fields correlated strongly with the symptoms induced on peas sown in manually infested soil, in pathogenicity greenhouse tests. However, the root systems were generally more intact in pea plants used in pathogenicity tests than those in the field grown pea plants.

Besides the use of naturally infested soil, two types of inoculum for pathogenicity tests are commonly utilised in host range studies: 1) zoospores suspended in water (Mitchell & Yang, 1966; Papavizas and Ayers, 1974) and 2) inoculum consisting of oospores mixed in a dry medium (Schneider, 1978). The zoospore inoculum is often preferred because it is more expedient to produce and zoospores are the primary source of infection in natural systems. Several factors influence the zoospore production. These are age of culture type, quantity and temperature of water used for washing cultures as well as time of washing and aeration during the washing (Llanos & Lockwood, 1960). Optimisation of all these parameters might significantly increase zoospore yield. Llanos and Lockwood

(1960) also indicated that more zoospores are produced when a combination of tap and distilled water was used as a medium replacement. In this study, both types of inocula were used; zoospores were produced as described by Papavizas and Ayers (1974) and oospore inoculum was made according to Persson *et al.* (1999). However, in some cases zoospore suspensions were produced by the modified method of Mitchell and Yang (1966) in attempts to obtain higher yield of zoospores. Furthermore, zoospore production by isolates, which yielded too low concentrations, was usually enhanced by the use of filtered river water and distilled water mix (50:50) (data not shown). Whereas zoospore inoculum needs to be produced just in time for use in pathogenicity tests, one advantage of the oospore inoculum is that it can be stored for a certain period before its use in these tests.

When vermiculite was used as a plant growing substrate in pathogenicity tests, problems to estimate the symptom development on broad bean roots, caused by *A. euteiches* pathogenic to pea, appeared. The common natural blackening of root tissue in these plants might conceal root rot symptoms caused by *A. euteiches*. Therefore, an alternative test method was designed for broad bean plants. A sandy loam soil was steam sterilised at 110º C for 12 h and ventilated in open boxes for at least two days. The soil was then mixed with talcum powder amended with oospores of *A. euteiches* (isolate "R"; (**I**)) until a concentration of about 800 oospores per gram soil was obtained, as described by Persson *et al.* (1999). Seeds of broad bean (cv. 'Aurora') were surfaced sterilised in 1.5 % NaOCl for 4 min, and 10 seeds were then sown per plastic pot (500 ml each) filled with this mix to a depth of 3 cm. The pots were watered to the same capacity they would receive when growing in fields. After plant emergence, the number of plants per pot was adjusted to five. The pots were kept in the greenhouse, maintained at a temperature of 25° C during the day and 19°C during the night. Symptoms were assessed four weeks after sowing as described in (**I**). Treatments comprised of six pots containing talcum/oospore mix and six pots containing an equal amount of talcum without oospores. Symptoms on broad bean roots appeared different to those on peas. Small and well-separated areas of the root tissue were black in contrast to healthy areas, which appeared as white tissue. No disease symptoms on hypocotyl were observed. Root mass of infected plants was obviously lower than for untreated controls, but no differences in green mass weight between infected plants and the control were determined (data not shown). The presence of *A. euteiches* in the root tissue showing disease symptoms was confirmed by microscopic observation of oospores and by re-isolation of the fungus on semi-selective medium.

## 4.3. Isolation and primary identification of *Aphanomyces* spp.

One of the characteristics for some isolates of the *A. euteiches* species is its pathogenicity to peas (Jones and Drechsler, 1925). Therefore, baiting of soil samples with presumable infestation of *A. euteiches* by sowing peas is used in many studies to obtain isolates for further tests (e.g. Beute & Lockwood, 1967; Wicker *et al.*, 2001). In addition, other leguminous crops such as alfalfa, snap bean or broad bean were used as bait plants to recover isolates of *A. euteiches* (e.

g. Grau *et al.*, 1991). The choice of bait host will result in different conclusions about the presence or absence of *A. euteiches*. Pea will bait out bean specific forms, but also pea will be inefficient in baiting alfalfa types and bean is the universal bait host. The seeds of susceptible leguminous crops are, in most of the bating studies, grown for approximately 10-14 days in soil samples collected from infested fields and *A. euteiches* is then isolated from roots displaying typical root rot symptoms (Beute & Lockwood, 1967; Wicker *et al.*, 2001). In order to avoid bacterial or fungal contamination, different approaches have been used to obtain pure isolates of *A. euteiches* from host tissue. Drechsler (1929) placed root pieces in Petri dishes with sterile water and the water was then exchanged several times until no visible contamination was present. After 12 to 24 hours, outgrown mycelia were harvested and dried between paper towels before being cultured on a suitable nutrient medium. Beute & Lockwood (1967) surface sterilised washed root pieces with 0.5 % sodium hypochloride and small root pieces were placed on 2 % water agar. Surface sterilisation with sodium hypochloride followed by rinsing with sterile water (e. g. Wicker *et al.*, 2001), or washing under running tap water (**I** and **II**) are still important methods to diminish contamination, even though isolations are made on semi-selective media.

Various semi-elective media for isolation of plant pathogenic *Aphanomyces* spp. were developed in the 1980´s and 1990´s (Larsson & Olofsson, 1994; Malvick *et al.*, 1994; Pfender *et al.*, 1982; Pfender *et al.*, 1984), and in this study the semi-selective medium described by (Larsson & Olofsson, 1994) was used. However, direct isolations from roots of field-grown leguminous plants showing symptoms of root rot were selected to obtain *Aphanomyces* isolates in this work, instead of the baiting method. Lamari & Bernier (1985) also used field grown broad bean to isolate *A. euteiches* for further pathogenicity tests. Since no direct comparisons between isolates recovered by baiting and isolated from field grown plant roots can be made, conclusive evidence of how the isolation method influences the outcome of pathogenicity tests is currently not available. The use of only peas as baiting plant (e.g. Wicker *et al.* 2001) might bias the recovery of isolates, which are specific towards only peas, or to peas and to certain other crops. Isolates with vetch specificity, as were demonstrated in **I**, might not be found when using peas as baiting plants.

Morphological and physiological features are still commonly used as the main tool for identification of isolated pathogens. They provide primary identification of many destructive pathogens, and among them also many *Aphanomyces* spp. The problems inherent in applying predominantly morphologically characters for classification purposes are emphasised especially for the genus *Phytophthora* (Brasier, 1991), but they concern in my opinion also the genus *Aphanomyces*. For example, only a limited number of morphological characters useable for separation of taxa (e. g. variation in sporangial size, oospore dimensions) are available, and these are usually plastic and variable depending on environmental conditions (Kuan & Erwin, 1980). Overlapping of useful characters among isolates of different closely related species is, thus common (Brasier, 1991).

In the species of *Aphanomyces*, like in other *Oomycetes*, the primary identification of an isolate to species level is mainly based on a combination of

morphological features including colony morphology, as well as dimensions and structures of oogonia, oospores, and antheridia. The number of antheridia per oogonium and the dimension of hyphae are also essential characters (Dick, 1969; Scott, 1961). However, identification can be difficult due to variations in these characters within the same species. Many of the isolates obtained from different leguminous crops, in this study, followed the species description of *A. euteiches* (Scott, 1961). However, some of them did not fit morphologically to the accepted description of *A. euteiches*. One set of isolates had smaller dimension of oospores and oogonia than typical *A. euteiches*. Moreover, the colony morphology of these isolates appeared to be different when grown on corn meal agar. A combination of these two characters suggested that this group of isolates was distinct from *A. euteiches* (**I**). Additionally, a few isolates had larger dimensions of oospores and oogonia compared to *A. euteiches* species description (**I**). As a result of this they could not be readily classified as *A. euteiches*, and were subjected for more careful identification by using biochemical and molecular methods as described below.

## 4.4. Host ranges and pathogenicity of *Aphanomyces* taxa infecting legumes

The host range as constituted by the ability to infect and proliferate in multiple crops is of essential importance for evaluating the impact of a soil-borne pathogen on the sequence of crops in a crop rotation. Generally, there are two ways for a pathogen to interact with a host plant: the interaction may result in commensalism or in parasitism. Commensalism is defined as the association between two organisms of different species that live together and share food resources, one species benefiting from the association without harming the other (Lawrence, 2000). Plant parasitism defines an association of a parasite organism with a plant; during this association the parasite multiplies or grows at the expense of the host. The host is harmed by this interaction (Agrios, 1997). Obligate parasites require a living host to complete a life cycle whereas facultative parasites may find alternative paths (Beckman & Roberts, 1995) for survival or propagation. In contrast to e.g. several *Fusarium* spp., which can complete their life cycle even on dead plant debris, *A. euteiches* is dependent, as far as it is known, on a living host plant for both the oospore germination and/or for completion of its life cycle. This also means that crop rotation practices and frequency of the host plant may be of significant importance for this obligate parasite to propagate and survive in the soil.

Pathogenicity pattern to selected test plants and/or pathogenicity for specific plant varieties is considered as one of the crucial taxonomic characters, which are used to delineate pathotypes, *formae speciales* or races of isolates of a pathogen classified within the same taxa. A pathotype is defined as “an infrasubspecific subdivision characterised by a pathogenic reaction in one or more hosts" (Hawksworth *et al.*, 1995). A *forma specialis* is defined as a preliminary grouping of strains according to physiological characters such as host preferences and pathogenicity properties. A *forma specialis* may have preference for more than one host (Irwin & Dale, 1982). A race is defined by Agrios (1997) as “a

subspecies group of pathogens that infect a given set of plant varieties". Thus, a *forma specialis* is not an established taxonomic level and to recognize a race, the pathogen needs to be tested on different varieties of a certain crop species. Pfender & Hagedorn (1982) applied the concept of *formae speciales* to distinguish two types *A. euteiches* isolates; one type which was pathogenic to peas, were assigned *A. euteiches* f. sp. *pisi* whereas the other type, which induced pathogenicity to solely snap bean was assigned *A. euteiches* f. sp. *phaseoli*. The latter type was also reported to have larger oospores and oogonia than the former type. However, since the dimensions of oospores and oogonia may vary within a wide range and overlap between species (Scott, 1961), the use of these characters alone to classify certain isolates as *formae speciales* have often been questioned. For example, Kuan and Erwin (1980) questioned the use of oospore and oogonial size to characterize formae speciales or varieties of the *Oomycete*, *Phytophthora megasperma* Drechs. The concept of the race has been applied to characterize certain specialization of *A. euteiches* isolates from pea and alfalfa. Considering variation in virulence to pea, 2 to 4 races are distinguished among isolates from Michigan, New Zealand and Norway (Beute & Lockwood, 1967; Manning & Menzies, 1984; Sundheim, 1972). Moreover, different levels of resistance within pea germ plasms to multiple strains of *A. euteiches* could also be identified when using this concept (Malvick & Percich, 1999). Variation in virulence is also reported for *A. euteiches* isolates pathogenic to alfalfa, which are now designated to race 1 (R1) and race 2 (R2) (Malvick & Grau, 2001). Representative isolate of *A. euteiches* f. sp. *phaseoli* as well as alfalfa specific isolates race 1 and 2 were included, in this study, and confirmed to infect their own host of origin. However, some differences in disease severity and broader pathogenicity pattern then earlier described were detected for these isolates when compared to the original studies (**I**).

Despite the problems to characterise *Aphanomyces* isolates on a basis of host range studies, or pathogenicity profiles on leguminous crops, these features have often been used for classification of *A. euteiches* and other *Aphanomyces* spp. (Delwiche *et al.*, 1987; Jones and Drechsler, 1925; Larsson, 1994; Malvick *et al.*, 1998). Linford (1927) found that, besides peas, other leguminous crops such as alfalfa and sweet clover (*Melilotus officinalis* L.) are hosts for *A. euteiches*. *A. euteiches* was also isolated from symptom-less root tissue of alfalfa, white clover, and weeds belonging to the genera *Brassicaceae*, *Caryophyllaceae* and *Violaceae* (Chan & Close, 1987). Furthermore, it was obtained from roots of field grown broad bean and vetch plants displaying disease symptoms (Lamari & Bernier, 1985; Tsvetkova & Kotova, 1985; **I**). *A. euteiches* isolates recovered by using soil-baiting technique in greenhouse, (e. g. Beute & Lockwood, 1967; Holub *et al.*, 1991), are reported to be parasitic to a broad range of leguminous crops commonly used in agricultural production (Grau *et al.*, 1991; Malvick *et al.*, 1998; Papavizas & Ayers, 1974; Pfender & Hagedorn, 1982; Schmitthenner, 1964; Tofte *et al.*, 1992; Wicker *et al.*, 2001).

Several pathotypes are also distinguished based on pathogenicity data. Delineation of studied isolates to certain pathotype group differs, however, depending on threshold levels used to evaluate disease symptoms. Thus, seven pathotypes of *A. euteiches* that express different degrees of pathogenicity to selected legume crops are distinguished from studies by Grau *et al.* (1991) and

Holub *et al.* (1991). Six pathotypes are pathogenic to varying leguminous crops and one is non-pathogenic. Beside non-pathogenic group of isolates, the six pathogenic pathotypes is characterised by the host preference for alfalfa, pea, snap bean, vetch, bean/alfalfa, pea/alfalfa, red clover/alfalfa. Wicker *et al.* (2001) describes four pathotypes that are distinguishable among French *A. euteiches* isolates, all pathogenic to at least two leguminous crops. The four distinguished pathotype groups are: "broad host range", "pea/vetch/alfalfa/broad bean", "pea/vetch/alfalfa" and "pea/vetch". Non-pathogenic isolates were not found in that study. In this respect, the Swedish set of isolates studied clearly differed from the French and American sets of isolates because it comprised only two putative pathotypes, pea- and vetch specific (**I**). In concordance with the American study, a non-pathogenic group of *Aphanomyces* isolates was also discriminated among Swedish isolates. Moreover, the non-pathogenic isolates were those, which showed different morphological features than typical *A. euteiches* isolates. Therefore, these isolates were denoted as *Aphanomyces* sp1 and *Aphanomyces* sp2 (**I**).

In general, the isolates baited in North America seem to have a broader host spectrum than those obtained in Europe. Although slightly more isolates of pea origin were studied both in North America and in France, the Swedish population of pea *A. euteiches* isolates was confirmed to be different from the American and French populations (**I**). *A. euteiches* is considered as a variable organism and therefore conclusions based on the data from only 2-3 isolates per host of origin (**I**) should be verified by additional experiments. However, the presence of vetch-specific isolates among Swedish *A. euteiches* is supported by other circumstances: i) the disease symptoms on greenhouse vetch plants corresponded to symptoms on field grown vetch roots, which the isolates originated from (Koch's postulates), ii) the symptoms resembled those these appearing on pea plants infected with *A. euteiches*, iii) oospores were present in root tissue and iv) vetch-specific isolates separated from other isolates in principal component analysis of pathogenicity data (**I**).

Data of disease ratings from pathogenicity tests are often subjective since disease rating is based on visual judgements of root discoloration. In certain crops, especially broad bean, a natural darkening of root tissue may occur which may interfere with the disease rating. A change of temperature regime in a narrow range can also affect the pathogenicity of *A. euteiches* isolates (Holub *et al.*, 1991). Thus, the finding that alfalfa specific isolates, (MF-1 and MHA 41) obtained from the United States (Malvick & Grau, 2001), which induced lower disease rating in this study (**I**) than in the original study, although both studies considered the isolates as pathogenic to alfalfa, probably has an origin in testing differences. In this case, the difference in temperature regimes, and the alfalfa genotypes used in the pathogenicity tests are probably the main reasons for different disease ratings. In addition, the definition of pathogenicity might be questioned. Wicker *et al.* (2001) used a lower threshold level to consider an isolate as pathogenic than it was used in studies by e. g. Malvick *et al.* (1998) and by us (**I**). This resulted in that Wicker *et al.* (2001) assigned the isolate "Ae 5" within a pathotype with a broader host range than it appeared to be in our study (**I**). These differences make exact comparisons between various studies difficult.

Furthermore, *A. euteiches* is considered as a variable pathogen (Beute & Lockwood, 1967; Holub *et al.*, 1991), which shows a wide range of pathogenicity to several leguminous crops, indicating that many subpopulations might occur. Thus, studies on host range and pathogenicity profiles of plant pathogenic *Aphanomyces* show variable results, which leads to difficulties with proper analysis and interpretation of data. Pathogenicity tests and morphological studies of *Aphanomyces* isolates are therefore often combined with biochemical and molecular genetic tools.

## 4.5. Characterisation of *Aphanomyces* spp. by biochemical and molecular methods

Isozyme analysis is one of the additional techniques commonly used to estimate genetic diversity, among field-originating isolates of *Oomycetes* and other pathogens. An advantage of this technique over those using total protein visualisation is that the obtained banding patterns are not too complex and therefore easier to differentiate and interpret (Nygaard *et al.*, 1989). This technique is often used to clarify the taxonomic relationships between closely related microorganisms that are difficult to distinguish morphologically (Beakes & Ford, 1983; Mills *et al.*, 1991), and also to reveal phylogenetic relationships between organisms at different levels (Bonde *et al.*, 1984; Micales *et al.*, 1988). Nygaard *et al.* (1989) illustrate the value of isozyme analysis for differentiating and grouping isolates of the *Oomycete P. megasperma* at species level as well as the potential of this technique within the genus. Larsson (1994) describes the technique as useful to differentiate among isolates of *Aphanomyces* spp. at species level. In that study, the enzyme systems, glucose-6-phosphate dehydrogenase (G6PDH) and malate dehydrogenase (MDH) were found to be particularly useful for analysis of *Aphanomyces* spp. when samples were separated using polyacrylamid gel electrophoresis on Pharmacia's PhastGel Gradient 8-25 gels. The studied isolates were found to comprise four distinct groups and species of *A. euteiches*, *A. cochlioides* and *A. cladogamus* were clearly distinguished.

Isozyme analysis was employed in this study in order to verify differences in morphological characters and pathogenicity data for isolates obtained from leguminous crops and expected to belong to the species of *A. euteiches*. The analysis of MDH banding patterns revealed three distinct isozyme groups among tested isolates. Additionally, a reference isolate of *A. cochlioides* comprised a separate fourth isozyme group. All isolates pathogenic to pea or vetch displayed the same MDH-pattern and thus were considered to belong to species of *A. euteiches*. These isolates that were non-pathogenic to leguminous crops, formed two separate isozyme groups. The pattern of isolates denoted *Aphanomyces* sp1 resembled the pattern of the reference isolate of *A. cladogamus*, whereas the pattern of isolates denoted *Aphanomyces* sp2 was unique. However, the data accumulated from morphological studies, pathogenicity profiles and from isozyme patterns of Swedish *Aphanomyces* isolates originating from legumes did not allow their clear delineation. Therefore, molecular techniques were additionally applied in order to further characterise and classify these isolates.

Molecular approaches, especially methods employing PCR, provide the most sensitive means for characterising and classifying plant pathogenic *Oomycetes* and fungi as well as for their direct detection in environmental samples (Bailey *et al.*, 2002; Lee & Taylor, 1992; Vandemark *et al.*, 2000). The variation found in sequences of ribosomal RNA genes and spacers regions has become one of the most popular methods recently used in phylogenetic studies (Hillis & Dixon, 1991). Ribosomal DNA (rDNA) tandem coding for production of 16S-18S, 5.8S, 25S-28S and 5S rRNA genes in addition to ITS 1 and ITS 2 regions between these genes allow assessments and comparisons of phylogenetic relationships of all organisms (Bruns *et al.*, 1991; Olsen *et al.*, 1986). Moreover, this data lead to the proposal for the new division of living organisms into three major domains: *Archaea*, *Bacteria* and *Eucarya* (Woese *et al.*, 1990). The rDNA array is useful in analysis of both closely and distantly related organisms because different regions evolved at different rates and it comprises highly conserved as well as highly variable sequence regions, and according to the theory of "concerted evolution", all r-DNA arrays evolve with equal speed in most of the organisms (Hillis & Dixon, 1991; Olsen *et al.*, 1986). Thus, both sequencing and RFLP analysis of rDNA arrays are nowadays well established and provide taxonomical data on all sorts of organisms including *Oomycetes* (Lee & Taylor, 1992; Petersen & Rosendahl, 2000).

The RFLP-analysis of mitochondrial and genomic DNA in addition to analysis of rDNA have also been used to estimate intra- and inter-specific relatedness of different *Oomycetes*, especially *Phytophthora* spp. (Förster *et al.*, 1988; Förster *et al.*, 1990). In the case of *Oomycetes*, the RFLP of mitochondrial DNA provides a more reliable data set than the RFLP of genomic DNA because of predominantly sexual reproduction in *Oomycetes*. Mitochondrial DNA is inherited from only one mating partner, which makes it more conserved and secure to use in phylogenetic analysis. Mitochondrial DNA of *Oomycetes* is considered to have several AT rich regions, which enable the use of specific GC-cutting restriction enzymes to obtain reliable and reproducible banding patterns (Karlovsky & Fartmann, 1992).

Among the *Oomycetes*, molecular studies are again most advanced for the genus *Phytophthora*, especially *P. infestans* and *P. sojae*, for which detailed genetic maps have been constructed, primarily using molecular techniques such as Restriction Fragment Length Polymorphism (RFLP); Random Amplified Polymorphic DNA (RAPD) and Amplified Fragment Length Polymorphism (AFLP) (Lee *et al.*, 1997; Whisson *et al.*, 1995). Besides constructing genetic maps, several other molecular tools are commonly used to clearly classify *Oomycetes* including *Aphanomyces* species. These include mitochondrial DNA (mtDNA) RFLP analysis, RFLP analysis and sequencing of 5.8S rRNA gene with surrounding Internal Transcribed Spacer regions 1 and 2 (ITS 1 and ITS 2) in addition to 18S and 28S rRNA genes (Hudspeth *et al.*, 2000; Leclerc *et al.*, 2000; Lee & Taylor, 1992; Malvick *et al.*, 1998; Petersen & Rosendahl, 2000). These regions are also commonly used for resolving taxonomic relationships among other groups of Eukaryots (Hillis & Dixon, 1991).

Since little is known about inter-specific molecular diversity of members of the *Aphanomyces* genus, selected isolates of *Aphanomyces* spp., although not

representatives of all species, were used to determine the taxonomical position of this genus in comparison to other genera of *Oomycetes* (Hudspeth *et al.*, 2000; Leclerc *et al.*, 2000; Petersen & Rosendahl, 2000; Riethmueller *et al.*, 1999). Taxonomic and phylogenetic analyses in these studies were mainly inferred from sequence comparisons of 28S rDNA (Leclerc *et al.*, 2000; Petersen & Rosendahl, 2000; Riethmueller *et al.*, 1999). Additionally, Leclerc *et al.* (2000) used the ITS sequence data and Hudspeth *et al.* (2000) inferred phylogeny of *Peronosporomycetes* from sequence data of mitochondrial locus (COX2) encoding subunit (COII) of the oxidase of cytochrome C. Based on this data, different *Aphanomyces* isolates clustered together and were well separated from other *Peronosporomycetes* (Hudspeth *et al.*, 2000; Leclerc *et al.*, 2000; Petersen & Rosendahl, 2000; Riethmueller *et al.*, 1999). The exception was an isolate of *Aphanomyces laevis*, which was separated from isolates of *Aphanomyces stellatus* and *Aphanomyces* sp. in studies by Riethmueller *et al.* (1999). Additionally, about 40 nucleotide sequences can be resolved when searching National Center for Biotechnology Information (NCBI) taxonomy database for *Aphanomyces* (January 2003) and twelve of these are sequences of *Aphanomyces invadans* genes. These include sequences of ITS 1 and ITS 2 as well as sequences of all rRNA genes. Only four nucleotide sequences are found for *A. euteiches* including two taxonomically informative sequences of the 28S rRNA gene. Morover, species-specific PCR primers were constructed to allow detection of *A. euteiches* and *A. cochlioides* from environmental samples. One of the primer sets is based on phylogenetically informative portion of the actin gene (Weiland & Sundsbak, 2000) and another set was created on the basis the ITS region of rDNA (Vandemark *et al.*, 2000).

Malvick *et al.* (1998) used RAPD analysis to study the intra specific genetic variation within the group of 68 *A. euteiches* and four *A. cochlioides* isolates. Three genotypically different groups of *A. euteiches*, which corresponded to their host of origin and host preferences, were distinguished. Furthermore, non-pathogenic isolates were found to form a separate genotypic group. However, this method was not sensitive enough to distinguish between non-pathogenic isolates of *A. euteiches* and the sugar beet pathogen *A. cochlioides*.

The lack of reliable molecular method to analyse variation of *A. euteiches* isolates at species level contributed to the choice of additional methods used in this study, and which have not earlier been described. These methods were RFLP of AT-rich, presumably mitochondrial, DNA and sequencing of the ITS 1 - 5.8S - ITS 2 rDNA region. About 30 isolates comprising the species of *A. euteiches*, *A. cochlioides*, *A. cladogamus* as well as unidentified *Aphanomyces* sp1 and sp2 were selected for analysis. Specific isolates such as *A. euteiches* f sp. *phaseoli* (ATCC46688), two *A. euteiches* alfalfa specific isolates ("MF1", "MHA41") (Malvick and Grau, 2001) and a type isolate of *A. cladogamus* (CBS108.29) were also included to test the sensitivity of these new methods (**III**).

The sequencing of the ITS 1 - 5.8S - ITS 2 rDNA region and RFLP of AT-rich DNA revealed intra- and inter-specific variation among plant root associated *Aphanomyces* species and were useful to delineate phylogenetic relationships between tested isolates (**III**). Sequencing of other rDNA units such as 28S rRNA

gene were previously reported to allow differentiation of certain *Aphanomyces* at the species level (Leclerc *et al.*, 2000; Petersen and Rosendahl, 2000). However, only certain plant parasitic *Aphanomyces* spp. were included in each of those studies and usually formed separate clade when compared to fish-parasitic and plant saprophytic *Aphanomyces* spp. This study (**III**) showed that methods applied also allowed delineation of relationships between *Aphanomyces* isolates originating from different plant hosts. Parsimony analysis of the ITS region sequence data allowed their separation into two main clades. The first main clade included all plant pathogenic *A. euteiches* regardless of their geographical- and host of origin what suggests that the population of pathogenic *A. euteiches* is rather homogenous. The second main clade comprised isolates of *A. cladogamus* and non-pathogenic *Aphanomyces* isolated from legumes. Furthermore, *A. cochlioides* and one non-pathogenic isolate, '65', did not show similarity with any of other clades. In addition, the fish pathogen *A. invadans* was clearly different from the plant associated *Aphanomyces* (**III**).

Likewise the ITS sequence data, RFLP of AT-rich DNA showed that pea- and vetch-pathogenic isolates of *A. euteiches* had a unique restriction pattern, regardless of their geographical origin and host of origin. However, using this method they were differentiated from other *A. euteiches*, including alfalfa and snap bean specific isolates (**III**). In this respect, RFLP of AT-rich DNA was more sensitive than sequencing of ITS region (**III**) and supported assignment of alfalfa- and snap bean-specific isolates into races or *forma specialis*, respectively (Malvick & Grau, 2001; Pfender & Hagedorn, 1982). This method was also more informative in delineating isolates within ITS clade of *A. cladogamus* and allowed differentiation of non-pathogenic Swedish *Aphanomyces* isolates from legumes from *A. cladogamus*. Furthermore, *A. cochlioides* and the *Aphanomyces* isolate recovered from barley formed unique patterns.

Interestingly, the non-pathogenic *Aphanomyces* isolate '65' formed an own unique clade. RFLP of AT-rich DNA also confirmed its specificity by an exclusive restriction pattern (**III**). Furthermore, morphological characters and isozyme analysis (**I**), supported its exclusiveness among studied isolates. Possibly, it can be proposed as a new species of *Aphanomyces*. However it needs to be compared to other known *Aphanomyces* species, e.g. to the soil saprophyte *A. laevis* and to radish associated *A. raphani*. Besides this, the attempt to obtain more isolates with similar ITS sequence and restriction pattern of AT-rich DNA have to be undertaken.

# 5. Control regimes for pea soil-borne diseases

## 5.1. Soil management and plant health

Soil quality in relation to plant health has long been an issue of economic importance, and has lately also been emphasised because of politically driven interest towards decreased use of chemicals, and the maintaining of biological diversity. These processes resulted in accepting the international agreement of banning chemically based methods for soil disinfestations by e.g. ethylene

dibromide, and 1,2 dibromochloropropane (United Nations Environment Program, 1992). Moreover, increasing occurrence of pest resistance to various popular pesticides, for example the resistance in potato late blight fungus to commonly used fungicides (Quintanilla, 2002), has raised concern for maintaining crop production levels by application of pesticides. Development and improvement of methods alternative to the use of pesticides are, thereby, increasingly emphasised topics in agriculture (Chellemi & Porter, 2001), and better accessing of soil quality in relation to plant health is here one important possibilities.

In this context, soil quality is commonly defined by a number of physical, chemical and biological properties that determine the potential to maintain plant health, and the soil biological productivity (Council, 1993; Doran & Parkin, 1994; Larsson & Pierce, 1994). Various biological, chemical and physical parameters are used to indicate soil quality (Chellemi & Porter, 2001). Among those, the following are often reported to be of importance: i) biological - microbial biomass, community structure, plant health; ii) chemical - soil pH, C/N ratio, cation exchange capacity, extractable minerals and iii) physical - bulk density, water infiltration and rooting depth, texture (Chellemi & Porter, 2001). All these factors influence also the plant health as an important factor that limits optimisation of yield and its quality (Cook, 2000). Improvement of soil quality and plant health depends, however, of the cropping system, and overall agricultural practices e.g. use of pesticides, organic amendments or tilling (Chellemi & Porter, 2001; Cook, 2000). In contrast to utilisation of organic amendment, chemical treatments have destabilising effects on the soil properties and often on crop quality (Sturz *et al.*, 1997). The implementation of chemicals e. g. herbicides such as dinoseb, trifluralin or dinitramine to control pea root rot was surveyed in field experiments with positive results (Grau & Reiling, 1977; Jacobsen & Hopen, 1981). However, such treatments are not economically and environmentally feasible. Therefore other methods including selecting pea lines with resistance to *A. euteiches*, identifying and assessing soil suppressiveness, as well as using organic amendments and biocontrol organisms and estimating the potential of root rot in field soils are more realistic practical control measures.

## 5.2. Host resistance in pea

Commercial pea cultivars are considered to be derived from a restricted gene pool and usually do not show any resistance to root rot pathogens, including *A. euteiches* (Kraft *et al.*, 1981), whereas some of non-commercial pea genotypes might show resistance reactions to one or more root pathogens (Kraft, 1986). There are several reports about assaying pea genotypes for resistance, specifically against pea root rot. Already, Lockwood (1960) challenged pea lines to *A. euteiches* in pathogenicity tests and found differences in resistance to pea root rot among the tested pea lines. Differential response of pea lines to a challenge with a range of *A. euteiches* isolates demonstrated in several studies the existence of some resistance to this pathogen (Beute & Lockwood, 1967; Malvick & Percich, 1998; Manning & Menzies, 1984; Sundheim, 1972; Wicker & Rouxel, 2001). Wicker & Rouxel (2001), working with similar tests concluded that virulence properties of *A. euteiches* isolates might strongly influence screening results and

should be considered in breeding programs. In that study two pea lines, PI180693 and 552, with partial resistance were also suggested as an interesting source of resistance to *A. euteiches*. Indeed, five root rot resistant germplasm pea genotypes are reported to be registered: Gritton (1990), Malvick and Percich (1999) describe 20 pea accessions from the *P. sativum* Plant Introduction Collection, which show resistance to multiple strains of *A. euteiches*.

Kraft & Boge (1996) describe some characters associated with resistance in pea to *A. euteiches* root rot. These are reduced production of *A. euteiches* oospores and germination of zoospores, hampered pathogen proliferation and slower development of lesions. Additionally, attempts to introduce an *in vitro* plantlet test for differentiation of partially resistant and susceptible to *A. euteiches* genotypes of pea were made, and this test was used for defining resistance to other root rot pathogens such as *F. solani* f.sp. *pisi* (Gretenkort & Helsper, 1993). However, no commercial cultivars with an adequately high resistance to *A. euteiches* are so far available. Nevertheless, sucessful breeding for resistance to other soil-borne pathogens such as *Fusarium oxysporum* f.sp. *pisi* (Kraft, 1994) and downy mildew (*Peronospora viciae* f.sp. *pisi*) (Stegmark, 1988) has been made.

Similarly as for pea, attempts to find alfalfa germ plasm resistant to *A. euteiches* were undertaken. Variation in virulence among alfalfa originating isolates is reported by Grau *et al.* (1991). That study differentiates a group of isolates, which are highly virulent on the susceptible cultivar 'Saranac' and show low virulence to the resistant line 'WAPH-1' from another group of isolates virulent on both 'Saranac' and 'WAPH-1'. These two groups of isolates were finally separated as distinct races 1 and 2, on a basis of these results (Grau *et al.*, 1991). The possible benefits of this observed resistance of alfalfa cultivars to *A. euteiches* were tested in field studies (Munkvold *et al.*, 2001). However, since only the currently available race 1 resistant alfalfa cultivars were used, that limited the yield benefits in fields where predominantly race 2 isolates were detected. Therefore, authors conclude: "Incorporation of race 2 resistance is likely to improve the performance of alfalfa cultivars in *A. euteiches* infested soils" (Munkvold *et al.*, 2001).

## 5.3. Microbial antagonism as a pea root rot-controlling factor

Microbial antagonism is commonly connected with the activity of soil populations of bacteria, actinomycetes, fungi, protozoa and algae, and has been intensively studied in relation to soil-borne pathogens, especially in the plant rhizosphere (Lynch, 1990). Numerous studies demonstrated ability of microbial antagonists to suppress diseases caused by several plant pathogens (Burr & Caesar, 1984; Weller, 1988). Other types of described plant beneficial microorganisms include plant growth promoting rhizobacteria (PGPR), and symbionts such as nitrogen-fixing *Rhizobium* spp. and vesicular arbuscular mycorrhiza (VAM) (Sturz *et al.*, 1997). Dehne (1982) and Hooker *et al.* (1994) report that plants colonized by mycorrhizal fungi are, additionally, less susceptible for infections by soil-borne pathogens. The possibility of applying integrated control of *F. solani* f. sp. *phaseoli*, *R. solani* and *F. oxysporum* fungi causing root rot of beans, with a

fungal-antagonistic *Bacillus subtilis* strain and *Rhizobium* bacteria was also recently proposed by Estevez de Jensen *et al.* (2002). In that study, the control effect of *B. subtilis* was evident in greenhouse and field experiments and inoculation with *Rhizobium tropici* had a positive effect on bean yield.

Attempts to apply antagonistic microorganisms in order to obtain biological control in pea crops against root rot caused by *A. euteiches* have also been undertaken. Microorganisms such as *Pseudomonas cepacia* (now *Burkholderia cepacia*), *Pseudomonas flourescens*, *Streptomyces lydicus*, *Glomus intraradices* and *G. mosseae* are reported to have certain ability to control infections caused by *A. euteiches* (Kjøller & Rosendahl, 1996; Parke *et al.*, 1991; Slezack *et al.*, 1999; Yuan & Crawford, 1995). The most studied isolate of *B. cepacia*, applied as a seed treatment, was shown to significantly increase emergence and yield of several pea cultivars when *Aphanomyces* root rot was present in the soil (King & Parke, 1993; Parke *et al.*, 1991), and the isolate also had an effect against *Pythium* damping off in pea. In field experiments it influenced the disease incidence at harvest by 11-19 % (Bowers & Parke, 1993). The introduction of bacterial cells to seeds of various pea cultivars was found not to change the indigenous population of *B. cepacia* (King & Parke, 1996). When tested for zoospore homing and infection events, the isolate caused zoospore lysis, prevented cyst germination and inhibited germ tube growth of *A. euteiches* as well as of *Pythium aphanidermatum in vitro*. The isolate is postulated to control *Pythium* infections through antibiosis and competition for plant exudates attracting zoospores (Heungens & Parke, 2000) and it is at high densities able to reduce the colonization of taproots by the mycelium as well as formation of oogonia by *A. euteiches* (Heungens & Parke, 2001).

Antagonistic effects against a range of root rot fungal pathogens, including *A. euteiches* are also demonstrated for the actinomycete *Streptomyces lydicus* when tested *in vitro* (Yuan & Crawford, 1995). Studies conducted with *P. ultimum* indicate that the isolate is capable of destroying oospores and damaging the cell walls of fungal hyphae which makes it a potentially useful biocontrol agent. Additionally, Kjøller and Rosendahl (1996) show that pea plants treated with the mycorrhizal fungus *G. intraradices* were more tolerant to *A. euteiches* infections. Furthermore, Slezack *et al.* (1999) reported that pea pre-inoculated with *G. mosseae* was protected against this pathogen. Recently, Bødker *et al.* (2002) used pea to study the interactions between *A. euteiches* and indigenous arbuscular mycorrhizal fungi in field. Their results suggest that mycorrhizal fungi influence the production of oospores rather than the vegetative stage of pathogen growth. However, neither of the organisms here mentioned has, so far, been tested on a larger scale or been further developed into commercial biocontrol product against *A. euteiches*.

## 5.4. Pea root rot control by soil organic amendments

Organic amendments in the form of composts, green manures or sewage sludges are known to suppress several soil-borne pathogens such as: *R. solani*, *F. oxysporum*, *Sclerotinia sclerotiorum* (Lib.) de Bary, *Verticillium dahliae* and *T. basicola* (Blok *et al.*, 2000; Candole & Rothrock, 1997; Gorodecki & Hadar, 1990; Hoitink & Fahy, 1986; Thaning, 2000). Also several *Oomycetes*, and among them *Phytophthora* spp (Vaughn *et al.*, 1954), *Pytium* spp. (Lewis *et al.*, 1992) and *A. euteiches* (Fritz *et al.*, 1995; Papavizas, 1966) are reported to be suppressed by various soil organic amendments.

Several reports suggest that compounds released from decomposition of plant residue in soil can affect the pathogen survival and thus disease expression in a subsequent host crop (Schippers *et al.*, 1990; Sturz *et al.*, 1997). One example is the saponins produced by roots of oats (*Avena sativa* L.) and among these especially the avenacin, which is known to cause lysis of zoospores of *Aphanomyces* and other oomycetous genera. This type of compounds prevent formation of cyst walls of zoospores (Deacon & Mitchell, 1985) and might affect the growth of hyphae, zoospores and oogonia (Engelkes & Windels, 1994). Similarly, several sulphur-containing volatiles such as mercaptans, sulfides and isothiocyanates are released from decomposing plant tissue of species within the genera *Brassicaceae* and *Sinapis* (Bailey *et al.*, 1961; Kjaer, 1960; MacLeod & MacLeod, 1968), and these substances also have demonstrated toxicity to a wide range of fungal species e.g. (Lewis & Papavizas, 1971; Walker *et al.*, 1937). Mulch or sewage sludge applied to the soil may stimulate microorganisms, which is often regarded as beneficial for plant development and suppressive for many plant pathogens. Furthermore, several amendments benefit soil quality by improving its physical properties and nutrient levels (Benedict *et al.*, 1988; Chang *et al.*, 1983).

Fritz *et al.* (1995) and Williams Woodward *et al.* (1997) demonstrated in greenhouse and field experiments that oat sown prior to pea reduces *Aphanomyces* root rot in pea. In this study (**II**), oat was not used as a typical amendment but was grown in a mixture with pea, and even though oat constituted only 20 % of the mixture, planting pea together with oat resulted in a retarded development of pea root rot in pea when compared to pea grown in pure stand. Papavizas (1966) reports that plant species of the genus *Brassicaceae* incorporated in soil can reduce expression of pea root rot under greenhouse conditions. In that study, soil amended with 0.6 % of cabbage leafs showed a clear suppressive effect to pea root rot up to at least 15 weeks after incorporation of the cabbage leafs. White mustard (*Sinapis alba* L.) as green manure is also reported to have suppressive effect on *Aphanomyces* infections in the following pea crop (Muehlchen *et al.*, 1990). In a study of Lumsden *et al.* (1983) it was likewise found that soil amendment with sewage sludge suppressed *Aphanomyces* pea root rot in pea. However, *Fusarium* root rot of pea and *Thielaviopsis* black root rot of bean increased in the sewage sludge amended soil when compared to non-amended soil, showing that soil amendments might be either disease suppressing or disease promoting depending

on conditions. Soil amendments have, as far as I am aware off, not been extensively used for practical pea root rot control.

## 5.5. Soil disease suppressiveness

The expression of a soil inoculum potential of a certain pathogen is, to great extend, dependant on the prevailing weather, cropping and several other conditions. Certain fields, or soils, have also been found to tolerate frequent cropping of a susceptible crop and still show a low risk of disease outbreaks, whereas other fields require long time span between susceptible crops in order to avoid build-up of high inoculum potential (Persson *et al.*, 1999). Factors inherent in the soil here seem to affect the inoculum potential of soil-borne pathogen. This is often denoted as receptivity of a soil; low receptivity gives low disease risk and *vice versa*. Alabouvette *et al.* (1982) defines soil receptivity as the impact of field soils on the pathogen ability to cause disease in a susceptible crop. Physical, chemical, or biological characteristics of the soil may contribute to the soil receptivity. Soil receptivity can be quantified in a scale ranging from disease conducive to disease suppressive (Oyarzun *et al.*, 1997). These definitions imply that the concept "soil receptivity" is a magnitude quantified to a level of "soil conducive" or "soil suppressive". Consequently, every soil can be assigned a potential of soil receptivity for a certain pathogen.

This phenomenon may be a useful tool for controlling various soil-borne pathogens (Alabouvette *et al.*, 1979), which is also a reason why it is commonly referred to as soil disease suppressiveness rather than soil receptivity. Soil disease suppressiveness is usually classified in two groups, constitutive and acquired, depending on its background. Constitutive suppressiveness is stable over time and not obviously affected by the cropping system, whereas acquired suppressiveness is a result of the cropping system, e.g. monoculture in the take-all decline in wheat (Alabouvette, 1990; Hornby, 1979). Additionally, Dobbs & Hinson (1953) introduced the term soil fungistasis in connection to soil receptivity. This term describes a process when fungal spores enter a resting period in contact with soil particles. Generally, heavier soils enforce fungistasis better than coarse-textured soils (Filonow & Lockwoood, 1983), although other factors such as microbial activity and content of organic matter can have a strong impact on soil fungistasis (Chinn, 1967). Common means for inducing disease suppressiveness in soils are organic amendments or other cultural practices such as monoculture, mulching, etc. (Baker & Chet, 1982; Sturz *et al.*, 1997).

Many reports relate soil disease suppressiveness to specific microbiological activities in soil, and an important role of microorganisms for soil suppressiveness to *Fusarium* wilts was demonstrated by eliminating microorganisms with methyl bromide or electromagnetic irradiation (Alabouvette, 1990). The soil disease suppressiveness could then be restored after adding non-treated soil to the treated soil (Sher & Baker, 1980). The active microorganisms may compete with the soil-borne plant pathogens for nutrients, or essential elements, or they may produce antifungal compounds. For example, rhizobacteria have been shown to compete with fungal pathogens by reducing the amount of nitrogen and carbon available

for fungal spores and pre-infection growth (Elad & Baker, 1985), and bacteria producing siderophores, metabolites with high affinity to sequester iron, were found to reduce growth of soil-borne plant pathogens and promote plant growth in iron-limited soils (Kloepper *et al.*, 1980). Antagonism or competition between avirulent and virulent *Fusarium* spp. was also proposed to contribute to soil suppressiveness (Schneider, 1984). The production of antifungal compounds, e. g. 2,4-diacetylphloroglucinol by certain fluorescent *Pseudomonas* strains is another possible mechanism of antagonism, especially in soils suppressive to take-all decline (Leyns *et al.*, 1990; Raaijmakers & Weller, 1998).

Little is known to what extent the specific microbial antagonists are, themselves, involved in inducing soil disease suppressiveness, to what extend they depend on soil abiotic factors, and to what extend abiotic factors might be important, alone. Sher & Baker (1980) found that lowering of soil-pH from 8.0 to 6.0 diminished the suppressive effect of a sandy loam soil known to be suppressive to *Fusarium* spp. Peng *et al.* (1999) suggest that physical and chemical factors such as soil temperature, water content, pH, as well as amounts of calcium and iron in the soil influence chlamydospore germination and *Fusarium* wilt when suppressive soils are compared to conducive soils. Moreover, the data of (Domínguez *et al.*, 2001) shows that the structural stability of soil aggregates, clay fraction, concentration of soluble Na and electric conductivity are of importance for conduciveness or suppressiveness to banana wilt caused by *F. oxysporum* f.sp. *cubense* in Canary Island, Spain. Based on greenhouse bioassays, Persson and Olsson (2000) reported that higher Ca content and soil pH correlate with higher suppression of *Aphanomyces* root rot in pea and indicated that content and ratio of soil minerals may affect soil suppressiveness to this pathogen.

Studies of soil suppressiveness to *Aphanomyces* root rot in pea have been conducted in The Netherlands and in Sweden. By assaying soil receptivity in the Netherlands, Oyarzun *et al.* (1997) found that the soils tested were, in general, suppressive to diseases caused by *T. basicola*, but conducive to diseases caused by *A. euteiches*. The same soils showed reactions from being conducive to being suppressive in their response to diseases caused by *F. solani* f.sp. *pisi*. Persson *et al.* (1999), demonstrated a great variation in disease suppression to pea root rot in soils collected in southern Sweden. Some of the soils were strongly suppressive to pea root rot, and development of root rot was clearly different between experimental fields. Both biotic and abiotic factors were suggested to affect soil disease suppressiveness to pea root rot (Persson, 1998; Persson *et al.*, 1999).

## 5.6. Disease risk forecasting by estimation of inoculum potential

Since no efficient and commercially feasible chemical or biological control agents are available for protecting pea crops against *A. euteiches*, methods based on prediction of the inoculum potential in soil samples have been widely adopted. The virulence of the inoculum, its density and various environmental factors, such as soil disease suppressiveness, which affect the inoculum, determine the potential of the inoculum to infect host plant and to cause disease. However, the inoculum potential as such is not easy to determine, and (Bouhot, 1979) concludes that

prediction of the inoculum concentration in a soil sample provides a static observation of the inoculum potential in the root zone, which makes prediction of disease risk difficult. The pathogen population is, furthermore, dynamic and cycling through several stages: dormant, inactive phase; pre-colonisation phase; host colonisation phase; and reproduction phase (Mitchell, 1979). This implies that the method and period of soil sampling are of importance for the outcome of any field soil assay. Sherwood & Hagedorn (1958) propose that a representative sample should be taken by collecting soil from many parts of the field, as field parts often have different soil types and hydrologic properties. For collecting a representative sample large areas should be divided in smaller areas. Furthermore, areas, which have recently been grown with peas, should be sampled separately.

A number of different approaches have been addopted for estimating the inoculum potential of *A. euteiches* in field soil samples. Sherwood and Hagedorn (1958) developed a method based on a greenhouse bioassay. Soil samples were collected from fields, mixed, and then sown with pea in the greenhouse. Disease incidence was scored after a period of about four weeks and compared to a threshold level according to which a field should, or should not, be accepted for pea production. Positive correlation coefficients between greenhouse indices and field indices ranging between 0.56 and 0.74 were found in that study. Moreover, Biddle (1984) reports negative correlation coefficients between similar disease rating in greenhouse and yield of field-grown pea in the range of -0.47 to -0.89 from experiments in United Kingdom. He thereby concludes that the greenhouse test used was a good method for predicting the potential of pea root rot in the tested fields.

In addition to the greenhouse bioassay, two alternative methods for estimation of *A. euteiches* inoculum density in field soils have also been developed; a rolled towel (RT) bioassay and a most probable number (MPN) bioassay. For the RT bioassay, field soil samples are placed on roots of pea seedlings and then rolled into wet paper towels. The percentage of plants showing symptoms of pea root rot is used to measure the inoculum potential (Kraft *et al.*, 1990; Williams Woodward *et al.*, 1998). For the MPN method, pea seeds are planted in a series of dilutions comprising infested and steamed soil. After incubation for 16 days, the proportion of plants infected at the different dilutions is calculated (Pfender *et al.*, 1981). In another type of direct counting test Boosalis and Scharen (1959) were able to quantify the presence of oospores in plant debris obtained from field soil by repeatedly washing and sieving the soil samples. The plant debris was macerated to fragments of maximum size of (700 x 100) µm, mixed with 2 % water agar and then examined microscopically. The number of infested particles per 100 gram of soil was calculated. A positive correlation between root rot index of pea in greenhouse bioassay, and number of infested particles per 100 gram of soil was found.

The greenhouse bioassay is technically easier to apply, and its variability is lower compared to the RT and MPN methods. The MPN bioassay requires less soil and laboratory space than the greenhouse bioassay, but it is more complicated and labour consuming than the greenhouse bioassay. The RT method is technically simpler than the MPN method and requires less soil and laboratory space than the

other two methods (Malvick *et al.*, 1994). In this study (**II**), the greenhouse bioassay developed by Sherwood and Hagedorn (1958) was used to test inoculum potential of soil from field experimental sites where pea root rot was prevalent. In general, greenhouse bioassay showed higher disease rating than direct disease rating of the field-grown pea at flowering stage (data not shown).

# 6. Soil-borne pathogens in legume-rich crop rotations

## 6.1. Long history of practising crop rotations

A cropping system can be defined as the sequence of crops grown in a single field including either monoculture or alternating crops in the same field (Cook & Baker, 1983). The value of planned crop rotation as a method for disease control has been known since ancient time, and is also the issue of several recent studies (Bruehl, 1987). Well-balanced crop rotations were proposed to enhance agricultural production in ancient Rome. For rich soils a sequence of wheat, millet (*Panicum miliaceum* L.), emmer wheat (*Triticum dicoccon* Schrank), and fallow followed by spring beans was recommended (White, 1970). For poorer soils, the recommended rotation was either wheat followed by beans or another legume or emmer wheat and beans or other legume followed by a fallow period. Options during the fallow period were also other legumes such as lupins (*Lupinus albus* L.), vetch or beans used as a green manure. Moreover, according to Brehaut (1933) translation of Cato´s De Agricultura, the use of rotations was already prevalent and lupins, beans and vetch were recognised as being beneficial.

Little is known about crop rotation throughout the middle ages, however Franklin (1953) suggests, that the prevalent practice was most likely a crop fallow system with exception for alternating two years of wheat with five years of grass. The Norfolk rotation, or similar rotations, were widely practiced in England in the 18th century. This comprised of turnips (*Brassica* sp.), barley (*Hordeum vulgare* L.), clover and wheat (*Triticum aestivum* L.) grown in a four-year sequence (Martin *et al.*, 1976). During this time the use of "artificial manure" such as lime and soil minerals to supplement organic manures had also become a common practice (Parker, 1915). Practicing crop rotation also appeared to be prevalent in the United States. Karlen *et al.* (1994) cites that the US president T. Jefferson in the letter to G. Washington mentions the following rotation; 1 wheat, 2 corn (*Zea mays* L.), potato (*Solanum tuberosum* L.) or pea, 3 rye (*Secale cereale* L.) or wheat (*Triticum aestivum* L.) and 4, 5 and 6 clover or buckwheat (*Fagopyrum esculentum* Moench). Finally, during the 19th century researchers discovered the leguminous plants ability to utilise nitrogen from the atmosphere (Hall, 1905). This disoveryled to a better understanding of benefits brought about by crop rotation and furtherresearch was made into this area. In Sweden, legume rich crop rotations as shown in Table 2, became popular mainly due to an increased acreage

of organic farming. Short crop rotations with legumes are also practiced in the area surveyed in Spain with rotations comprising pea, snap bean and corn (**IV**).

## 6.2. Crop rotation and soil-borne pathogen management

As mentioned above, the beneficial effects of rotating crops in front of crop continuity (monoculture) have been well known since ancient time, although the reasons for these have not beenunderstood until recently (Karlen *et al.*, 1994). In modern farming and especially in organic farming practices, planned crop rotations are widely used and appreciated as measures to reduce the inoculum potential of soil-borne plant pathogens, as well as to maintain the quality of the soil quality (see also 5.1). The interaction between populations of a soil-borne pathogen and its plants can be described by a model where the inoculum density decreases in the soil in the absence of suitable host plants, and increases in the presence of a host or hosts. The disease potential of a host is defined by its susceptibility to a pathogen in combination with environmental factors (Baker *et al.*, 1967). For certain hosts, the disease potential is, to a great extent, determined by the plant genotype. Furthermore, Baker *et al.* (1967) define the disease severity as the product of the inoculum potential and the host disease potential. In this context, the host range of a soil-borne pathogen is of great importance; a broad host range signifies greater opportunities for the pathogen to propagate in different crops in the rotation. However, this model is not valid where decline-effects in prolonged monocultures as described by Hornby (1979) are at hand. Such decline-effect seems to apply to certain pathogens, and especially for the take-all fungus (*Gaeumannomyces graminis* var. tritici) attacking cereals (Hoestra, 1975), whereas for *Aphanomyces* root rot of pea there has been no reported case (Bødker, 1995).

From the perspective of plant pathology, crop rotation can be used as an effective method to control various plant diseases. It can also be considered as a biological method of disease management and therefore can be relevant to organic farming practices. As stated by Cook and Veseth (1991): “rotation allows time for natural enemies to destroy the pathogens of one crop while one or preferably two unrelated crops are grown”. However, there are certain requirements or conditions that must be fulfilled in order fordisease control methods to be effective throughout crop rotation: i) the source of the pathogen must be from the field itself, ii) the host range of the pathogen needs to be narrow and iii) the pathogen survival in absence of suitable host(s) should be limited over time. These requirements imply that suitable crop rotations are effective mainly to control soil-/residue-borne pathogens with a narrow host range. It may not be, for example, an effective strategy for controlling diseases such as *Sclerotinia* stem rot, which has an extensive host range, that include common weeds and many crops including bean (Steadman, 1983) and pea (unpublished data). In addition, it may not affect those pathogens that produce long-lived resting structures or that survives in infested plant tissue, which is resistant to decay. Some examples of such pathogens are *Sclerotinia sclerotiorum* that produce long-lived sclerotia (Adams & Ayers, 1979; Thaning, 2000) and *Leptosphaeria maculans*, which causes black leg

disease and that can survive for extended periods in tissue of its host, canola e.g. (Petri, 1986).

Considering these three requirements, neither of the soil-borne root rot pathogens, studied in this thesis work can easily be controlled by crop rotation. Both *T. basicola* and *R. solani* identified as important pathogens on pea and snap bean in Spain (**IV**) have wide host ranges (Engelkes and Windels, 1996; Lucas, 1975; Rothrock, 1992; Xi *et al.*, 1995). *A. euteiches*, the most important pea pathogen in Sweden, is recognised as long-lived (Papavizas and Ayers, 1974), and has multiple hosts among legumes (I; II) (Delwiche *et al.*, 1987; Linford, 1927; Tsvetkova and Kotova, 1985). On the other hand, the benefits of including several leguminous crops in crop rotations as a means of disease control in other crops are widely recognised (Karlen *et al.*, 1994; Sturz *et al.*, 1997).

## 6.3. Legume-rich crop rotations – benefits and problems

### *6.3.1. Beneficial effects of legume crops*

Many reports have emphasised the beneficial effects of legumes in the crop rotations on soil properties (Frye *et al.*, 1988; Sainju *et al.*, 2001; Thiessen Martens *et al.*, 2001), and for lowering soil-borne pathogen attacks (e.g. Bruehl, 1987; Karlen *et al.*, 1994). These effects may have a physical, chemical or biological background. Perennial legumes such as alfalfa, red clover, sweet clover and birdsfoot trefoil form taproots, which allow them to transport and recycle plant nutrients from deeper soil levels, which are normally not available to crops with shallow root systems. A good example is the root system of alfalfa that has been shown to utilise nitrogen from a depth greater than 5.5 meters in some soils (Asseng *et al.*, 1998; Karlen *et al.*, 1994), however the penetration depth may vary considerably due to differences in soil type and degree of soil compaction. The penetration depth of alfalfa taproots in one of the field experiments undertaken in this study was only one third of the penetration depth of alfalfa grown in another field nearby although the soil type was classified as clay in both fields (unpublished data). Together these studies show that the soil nitrogen balance can be affected by factors other than the nitrogen fixation by legume crops. Furthermore, the formation of a substantial root system has often been regarded as a highly beneficial effect on the soil porosity (Karlen *et al.*, 1994).

The positive effects of legumes in suppressing soil-borne diseases of other crops have been demonstrated in several studies. Reeves *et al.* (1984) report that the inclusion of lupin in the crop rotation one or two years before wheat reduced the disease rate of take-all in the subsequent wheat crop when compared to a continuous wheat rotation. Reduction of take-all incidence is also reported after precrops such as pea and oat (Cotteril & Sivasithamparam, 1988). Felton *et al.* (1998) shows that crown root of wheat, caused by (*F. graminearum*), can be reduced and wheat yields increased in a chickpea-wheat versus a wheat-wheat crop rotation. In addition, disease management in barley crop is reported to benefit from precrop legumes. Dyke & Slope (1978) concludes that take-all was a minor

problem in barley after bean or red clover when compared to barley after barley. Another specific legume, hairy vetch (*Vicia villosa* Roth) is often used as a cover crop and has suppressive effect on *T. basicola* infections in subsequent cotton crops (Rothrock *et al.*, 1995). In addition, it reduces soil erosion and improves soil properties (Candole & Rothrock, 1997). The disease suppressive effect of hairy vetch is associated with the presence of ammonia in hairy vetch amended soils at concentrations sufficient to influence viability of the chlamydospores of *T. basicola* (Candole & Rothrock, 1997). Populations of *T. basicola* are also reduced in soils amended with alfalfa as reported by Baard & Laubscher (1983). Addditionally, Sturz *et al.* (1998) show that clover can benefit a subsequent potato crop by sharing specific associations of bacterial endophytes, in addition to its beneficial effects of residual nitrogen and organic matter added.

### *6.3.2. Negative effects of legume crops in relation to soil-borne pathogens other than* Aphanomyces *spp.*

Conversely to the decrease of *T. basicola* soil inoculum as an effect of cropping hairy vetch, this legume is known to increase soil population of *Rhizoctonia* spp. and *Pythium* spp. (Rothrock *et al.*, 1995). Also other legumes such as vetch, clovers and lupine are reported to increase densities of these pathogens when compared to grasses or fallow (Sumner *et al.*, 1995). Moreover, populations of *T. basicola* are reported to increase in soils planted with snap bean, one of its common hosts (Reddy & Patrick, 1989). The results of our field survey in pea and snap bean fields in Northern Spain indicate that *T. basicola* and *R. solani* are predominant root rot pathogens in this area (**IV**). High *T. basicola* infestation of pea plants in fields surveyed might be connected with a high frequency of pea and snap bean in the crop rotations with pea appearing more often than every sixth year. As both crops are hosts for this pathogen, a propagation of soil inoculum might occur. Also abiotic soil properties such as pH (Rothrock, 1992), moisture (Bhatti & Kraft, 1992b) and compaction (Bhatti & Kraft, 1992a; Burke & Holmes, 1972) were in this case favourable for *T. basicola* propagation. Out of these factors, soil compaction might have been the most important since a very low field disease severity index (FDSI) was detected for plants sown in two sandy soils (**IV**). *R. solani* and *Pythium* spp. were frequently isolated from especially snap bean suggesting that *R. solani*/*Pythium* damping off complex occurs in surveyed fields. The predominance of *R. solani* over *Pythium* spp. might be explained as a result of competition between these pathogens for the infection site as was suggested by Pieczarka and Abawi (1977). Pea and snap bean are hosts for *R. solani* and its additional plant hosts such as e.g. sugar beet (Engelkes & Windels, 1996) are included in analysed rotation system, which might contribute to inoculum build-up. The crop rotations allowing a build-up of these inocula potential resulted in high FDSI on pea and snap bean and in significant yield losses (**IV**). Similarly, yield losses in pea due to *T. basicola* infection were detected in Denmark (Bødker *et al.*, 1993b).

### *6.3.3. Variable effects of legume crops in relation to* Aphanomyces *infections*

The practice of frequent legume cropping as one of the means for managing soil nitrogen supply has been widely adopted in intensive crop production systems of organic farming only recently. Proportions of legumes in crop rotations have increased significantly as an effect of this development to especially provide sufficient N-fertilization for the following crops. In this context frequent growing of legumes should, as input, provide enough nitrogen to get an output of satisfactory yields. However, other factors than plant nutrient balance might also strongly influence the expected output. Among these other factors, the effect of inoculum build-up of certain devastating soil-borne pathogens raises concerns about the feasibility of intensive legume cropping in a longer perspective. As shown in many reports (e.g. Kraft *et al.*, 1997; Lager, 2002), and this doctoral work, several soil-borne pathogens infect a number of legume crops. In Scandinavia, *A. euteiches* is regarded as the most serious pea pathogen (Olofsson, 1967; Persson *et al.*, 1997; Sundheim & Wiggen, 1972) and has thus been emphasized here. In this connection, the recent report of synergistic effects between seemingly non-pathogenic *Fusarium solani* and *A. euteiches* seems to be interesting because such synergism can contribute to differences of pea root rot severity between fields (Peters & Grau, 2002).

Concerning *A. euteiches*, the results from field experiments carried out in this study imply that, under Swedish conditions, broad bean, pea, snap bean, sweet clover and vetch should be regarded as equally disease proliferating crops when planning crop rotations. In general, FDSI in these crops steadily increased during their monoculture, and they also negatively affected the yields of subsequent pea or broad bean (**II**). The pathogen *A. euteiches* was isolated from pea, snap bean and sweet clover grown in the field (**I**), whereas broad bean was confirmed as an *A. euteiches* host in greenhouse experiments. Symptoms in broad bean were only detected on the roots, and neither green mass weight loss, nor visual symptoms on above ground plant parts were found. These results suggest that broad bean is most probably a host for *A. euteiches* in field, but is not or much less affected than pea. The same might be true for alfalfa: *A. euteiches* was isolated from tissue displaying only slight discolorations, whereas these isolates induced typical disease symptoms on pea (**I**). Alfalfa as a monocultured precrop did not have clearly negative effects, on yields of subsequent pea, broad bean and snap bean, when compared to other monocultures (**II**). Conversely, studies from the United States report a parasitic interaction between alfalfa and isolates of *A. euteiches* (Delwiche *et al.*, 1987; Malvick & Grau, 2001).

The isolation of vetch-specific *A. euteiches* in this study (**I**), and the significant development of FDSI in vetch monoculture (**II**) implies that vetch is a main host of *A. euteiches* in Sweden and not an alternative host, beside pea, as suggested by Wicker *et al.* (2001). Swedish vetch specific isolates could not be separated from pea specific isolates with molecular methods applied, whereas alfalfa specific isolates obtained from the United States were clearly distinguished by both sequencing of ITS 1 - 5.8S - ITS 2 rDNA array and by RFLP of AT rich DNA (**III**). Cropping history might have contributed to the evolution of these

pathotypes. Alfalfa is not commonly grown in the studied areas of Sweden, whereas alfalfa is an important crop in the United States. Vetch was on the other hand a common crop in surveyed areas in southern Sweden during, at least, the last century and was reported to be grown in Sweden already in the 16th century (Linnaeus, 1749). The influence of the cropping history and practices on *Aphanomyces* root rot potential were reported in several studies (Bødker *et al.*, 1993a; Oyarzun *et al.*, 1993; Pfender & Hagedorn, 1983; Temp & Hagedorn, 1967), but there is no clear report of such influences on pathotype development.

With exception for birdsfoot trefoil and pea, *Aphanomyces* spp. other than *A. euteiches* were also isolated from a wide range of the legumes. The molecular characterisation showed that these isolates clearly differ from the *A. euteiches* isolates obtained from Sweden, France, Spain and the United States but some of them resembled *A. cladogamus* (**III**). In general, these isolates were non-pathogenic to any of tested legumes in greenhouse pathogenicity tests (**I**). Similarly, Malvick *et al.* (1998) found a group of non-pathogenic *Aphanomyces* isolates in the United States, which by RAPD analysis were shown to be genetically distant from both *A. euteiches* and *A. cochlioides*. Since *A. cladogamus* was not included in that study, it is difficult to relate these non-pathogenic isolates with Swedish non-pathogenic isolates. The possible host range, effects in crop rotations and the agronomic importance of these *Aphanomyces* spp. are still unclear. However, *A. cladogamus* is reported as a prevalent spinach pathogen in southern Sweden (Larsson & Olofsson, 1994) and associated with tomato (Drechsler, 1929). Therefore, this spinach pathogen is certainly present in surveyed fields in southern Sweden and legumes may possibly act as alternative host for this organism.

The monocultures of birdsfoot trefoil, red, white and Persian clover seemingly did not proliferate *A. euteiches* under the conditions of the field experiments carried out. The fact that FDSI development over time in monocultures of these crops was low and that the yields of subsequent pea, broad bean and snap bean were not negatively affected compared to other tested crops (**II**), strongly suggest that these crops belong to low risk group considering build-up of *A. euteiches* soil inocula. The increase of FDSI in red clover was clearly connected to *Fusarium* infections, which might have been a part of the root rot complex also in other crops and possibly contributed to the FDSI development and yield losses. Interesting in this connection is also that certain legume-infecting *Fusarium* pathogens might infect both legumes and cereals. Elmholt (1996) and Knudsen *et al.* (1999) suggest that the build-up of *Fusarium* inoculum potential in organic leys might contribute to lower disease suppression against foot rot of wheat in these soils when compared to soils cultivated with conventional methods. Also Lager (2002) concludes that several clover soil-borne pathogens cause diseases in various legumes and that in legume intensive crop rotations including pea, red clover and wheat *Fusarium* spp. are potentially severe pathogens.

### *6.3.4. Legume soil-borne diseases and long term cropping system sustainability*

The presented findings of significant yield reductions in cropping systems with high frequencies of legumes in the rotations, which were in addition exceptionally high (II), raise questions about on the long-term sustainability of these systems. Characteristic features of the most of high-yielding, rational cropping systems are the use of uniform plant genotypes, adequate or high nutrient supply, and usually alternation between a few economically valuable crops in the rotations. All this constitutes good prerequisites for soil-borne pathogens to proliferate where other conditions, such as climate and presence of hosts are also fulfilled. These characteristics are in contrast to the more extensive cropping systems that were prevalent in most of western countries before Word War II, and nowadays are still used in many developing countries. Therefore, extensive cropping systems with low nitrogen supply through using e.g. 25 % or less of legume crops in rotations might well be long term sustainable. In modern, more rational cropping systems, for example in certain Swedish organic farming systems without animal integration into the production, the need for intensive legume cropping might lead to an unacceptable and accelerating build-up of inoculum potential of several soil-borne pathogens, and among these especially *A. euteiches*. Therefore, the conclusion of Salt and Delaney (1985) that all legume crops should be regarded as one crop when considering soil-borne diseases and crop rotation is clearly supported also by this study.

Several measures and cultural practices, such as use of tillage, various composts and mulches, cover crops, green manures in combination with the careful analysis of soil inocula potential and cropping history of the field, as well as careful planning of the crop rotations should help to design soil and crop management systems that are sufficiently well suppressive to soil-borne pathogens. The results and reasoning presented in this thesis are thought to contribute to the knowledge needed for doing this.

# 7. Conclusions

The following conclusions are drawn from the results obtained during the course of this thesis work:

• In Sweden, several leguminous crops are hosts of the pea root rot pathogen *A. euteiches*, but some of its legume hosts do not develop aboveground disease symptoms.

• Broad bean develops root rot symptoms due to *A. euteiches* infections after manual inoculation with oospores in greenhouse tests, and oospores are formed in its root tissue.

• Several leguminous crops are hosts for species of *Aphanomyces*, other than *A. euteiches*, without showing disease symptoms.

• Swedish isolates of *A. euteiches* can be assigned two putative pathotypes, with host preferences for pea and vetch, respectively.

• Monocultures of various leguminous crops affect the inoculum potential of *A. euteiches* pathotypes pathogenic to pea in Sweden.

• In Swedish crop rotations pea, broad bean, snap bean, vetch and sweet clover crops should be considered equally efficient in building up of soil inoculum inducing pea root rot.

• Monocultures of alfalfa, birdsfoot trefoil, Persian clover, red and white clover have lower impact on subsequent yields of pea and broad than have pea, broad bean, snap bean, vetch and sweet clover. For pea grown in fields infested with pea root rot, this effect can be explained by the differences in the development of *Aphanomyces* root rot.

• *T. basicola* and *R. solani* are prevalent pathogens of pea and snap bean, respectively, in the areas of the Castilla y Leon, Rioja and Navarra regions in northern Spain. These two pathogens also are also the cause for significant yield losses in these crops.

• Sequencing of ITS1 - 5.8 - ITS2 rDNA region and RFLP of AT-rich DNA are useful methods for delineating relationships between various plant pathogenic and plant associated *Aphanomyces* spp. RFLP of AT-rich DNA is however a more sensitive method in detecting within-species variation.

• Cropping systems with intensive legume cropping will, on many soil types, not be sustainable in the long-term due to the build up of soil-borne pathogen inoculum.

# 8. References


Adams P.B. & Ayers W.A. (1979) Ecology of *Sclerotinia* species. *Phytopathology* 69: 896-899.

Agrios G.N. (1997) Plant Pathology. London: Academic Press.

Alabouvette C., Rouxel F. & Louvet J. (1979) Characteristics of *Fusarium* wilt-suppressive soils and prospects for their utilization in biological control. In *Soil-Borne Plant Pathogens*, edited by Schippers B. & Gams W. London.

Alabouvette C., Couteaudier Y. & Louvet J. (1982) Comparaison de la réceptivité de différents sols et substrats de culture aux fusarioses vasculaires. *Agronomie* 2: 1-6.

Alabouvette C. (1990) Biological control of *Fusarium* wilt pathogens in suppressive soils. In *Biological Control of Soil-borne Plant Pathogens*, edited by Hornby D. Wallingford: CAB International.

Allen O.N. & Allen E.K. (1981*) The leguminosae. A Source Book of Characteristics. Uses and Nodulation*. London: Macmillan.

Allen D.J., Buruchara R.A. & Smithson J.B. (1998) Diseases of Common Bean. *In The Pathology of food and Pasture Legumes*, edited by Allen D.J. & Lenné J.M. Cambridge, UK: CAB International.

Anonymous (1965-2002) The Yearbook of Agricultural Statistics Sweden. Halmstad.
Asseng S., Fillery I.R. & Gregory P.J. (1998) Wheat response to alternative crops on duplex soil. *Australian Journal of Experimental Agriculture* 38: 481-488.
Baard S.W. & Laubscher C. (1983) Effect of crop residues and alternative crops on the survival of *Thielaviopsis basicola* in blackhull-including soil. *Phytophylactica* 15: 145-148.
Bailey S.D., Bazinet M.L., Driscoll J.L. & McCarthy A.I. (1961) The volatile sulphur components of cabbage. *Journal of Food Science* 26: 163-170.
Bailey A.M., Mitchell D.J., Manjunath K.L., Nolasco G. & Niblett C.L. (2002) Identification to the species level of the plant pathogens *Phytophthora* and *Pythium* by using unique sequences of the ITS1 region of ribosomal DNA as capture probes for PCR ELISA. *FEMS Microbiology Letters* 207 (2): 153-158.
Baker R., Maurer C.L. & Maurer R.A. (1967) Ecology of plant pathogens in soil. VII. Mathematical models and inoculum density. *Phytopathology* 57: 662-666.
Baker R.F. & Chet I. (1982) Induction of suppressiveness. In *Suppressive Soils and Plant Disease*, edited by Schneider R.W. St. Paul, MN, USA: American Phytopathological Society.
Beakes G. & Ford H. (1983) Esterase isozyme variation in the genus *Saprolegnia* with particular reference to the fish-pathogenic *S. diclina-parasitica* complex. *Journal of General Microbiology* 129: 2605-2619.
Beckman C.H. & Roberts E.M. (1995) On the Nature and Genetic Basis for Resistance and Tolerance to Fungal Wilt Diseases of Plants. In *Advances in Botanical Research*, edited by Andrews J.H. & Tommerup I.C. London: Academic Press.
Benedict A.H. Epstein E., & Alpert J. (1988) *Composting Municipal Sludge: A Technological Evaluation*. Park Ridge, NJ: Noyes Data Corporation.
Bessey E.A. (1971) *Morphology and taxonomy of fungi*. Edited by Blakiston. New York: Hafner.
Beuselinck P.R., Peters E.J. & McGraw R.L. (1984) Cultivar and management effects on stand persistence of birdsfoot trefoil. *Agronomy Journal* 76: 490-492.
Beute M.K. & Lockwood J.L. (1967) Pathogenic variability in *Aphanomyces euteiches*. *Phytopathology* 57: 57-60.
Bhatti M.A. & Kraft J.M. (1992) Influence of soil bulk density on root rot and wilt of chickpea. *Plant Disease* 76: 960-963.
Bhatti M.A. & Kraft J.M. (1992) Influence of soil moisture on root rot and wilt of chickpea. *Plant Disease* 76: 1259-1262.
Biddle A.J. (1984) A prediction test for pea footrot and the effects of previous legumes. Paper read at Proc. Br. Crop. Prot. Conf. Pest Dis.
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. *Phytopathology* 90: 253-259.
Bohlool B.B. & Ladha J.K. (1992) Biological nitrogen fixation for sustainable agrigulture: A perspective. *Plant and Soil* 194: 11-14.
Bonde M.R., Peterson G.L., Dowler W.M. & May B. (1984) Isozyme analysis to differentiate species of *Peronosclerospora* causing downy mildews of maize. *Phytopathology* 74: 1278-1283.
Boosalis M.G. & Scharen A.L. (1959) Methods for microscopic detection of *Aphanomyces euteiches* and *Rhizoctonia solani* and for isolation of *Rhizoctonia solani* associated with plant debris. *Phytopathology* 49: 192-198.
Bouhot D. (1979) Estimation of inoculum density and inoculum potential: techniques and their value for disease prediction. In *Soil Borne Plant Pathogens*, edited by Schippers B. & Gams W. London, UK: Academic Press.
Bowers J.H. & Parke J.L. (1993) Epidemiology of *Pythium* damping-off and *Aphanomyces* root rot of peas after seed treatment with bacterial agents for biological control. *Phytopathology* 83: 1466-1473.
Brasier C.M. (1991) Current Questions in *Phytophthora* Systematics the Role of the Population Approach. Paper read at British Mycological Society Symposium, 17. *Phytophthora*; Symposium of The British Mycological Society, The British Society for

Plant Pathology and The Society of Irish Plant Pathologists, Dublin, Ireland September 1989.
Brehaut E. (1933) "*Cato the Censor on Farming*" (translation). New York: Columbia University Press.
Bruehl G.W. (1987) *Soilborne plant pathogens*. New York.
Bruns T.D., White T.J. & Taylor J.W. (1991) Fungal molecular systematics. *Annual Review of Ecology and Systematics* 22: 525-564.
Burke D.W., & Holmes L.D. (1972) Counteracting bean root rot by loosening the soil. *Phytopathology* 62: 306-309.
Burr T.J. & Caesar A. (1984) Beneficial plant bacteria CRC. *Critical Reviews in Plant Sciences* 2: 1-20.
Bødker L., Leroul N. & Smedegaard Petersen V. (1993a) Influence of pea cropping history on disease severity and yield depression. *Plant Disease* 77: 896-900.
Bødker L., Leroul N. & Smedegaard-Petersen V. (1993b) The occurrence in Denmark of black root rot of pea caused by *Thielaviopsis basicola*. *Plant Pathology* 42: 820-823.
Bødker L. (1995) Prevention of soil-borne diseases. Paper read at 12th Danish Plant Protection Conference.
Bødker L., Kjøller R., Kristensen K. & Rosendahl S. (2002) Interactions between indigenous arbuscular mycorrhizal fungi and *Aphanomyces euteiches* in field-grown pea. *Mycorrhiza* 12: 7-12.
Caamal-Maldonado J.A., Jiménez-Osornio J.J., Torres-Baragán A. & Anaya A.L. (2001) The use of allelopathic legume cover and mulch species for weed control in cropping systems. *Agronomy Journal* 93: 27-36.
Candole B.L. & Rothrock C.S. (1997) Charaterzation of the suppressiveness of hairy vetch-amended soils to *Thielaviopsis basicola*. *Phytopathology* 87: 197-202.
Chan M.K.Y. & Close R.C. (1987) *Aphanomyces* root rot of peas 2. Some pasture legumes and weeds as alternative hosts for *Aphanomyces euteiches*. *New Zealand Journal of Agricultural Research* 30: 219-223.
Chang A.C., Page A.L. & Warneke J.E. (1983) Soil conditioning effects of municipal sludge compost. *Journal of Environmental Engineering* 109: 574-583.
Chao L., De Battista J. & Santinaque F. (1995) Incidence of birdsfoot trefoil crown and root rot in west Uruguay and Entre Rios (Argentina). *In Proceedings of the First International Lotus Symposium*, edited by Beuselinck P.R. & Roberts C.A. St Louis, USA: University of Missouri-Columbia Press.
Chellemi D.O. & Porter I.J. (2001) The role of plant pathology in understanding soil health and its application to production agriculture. *Australasian Plant Pathology* 30: 103-109.
Chinn S.H.F. (1967) Differences in fungistasis in some Saskatchewan soils with special reference to Cochliobolus sativus. *Phytopathology* 57:2 24-226.
Chongo G., Gossen B.D., Kutcher H.R., Gilbert J., Turkington T., Fernandez M.R. & McLaren D. (2001) Reaction of seedling roots of 14 crop species to *Fusarium graminearum* from wheat heads. *Canadian Journal of Plant Pathology* 23: 132-137.
Cook R.J., & Baker K.F. (1983) *The Nature and Practice of Biological Control of Plant Pathogens*. St Paul, MN, U.S.A: The Amercian Phytopathological Society.
Cook R.J. & Veseth R.J. (1991) *Wheat health management, Plant Health Management Series*. APS Press.
Cook R.J. (2000) Advance in plant health management in the Twentieth Century. *Annual Review of Phytopathology* 38: 95-116.
Cotteril P.J. & Sivasithamparam K. (1988) Reduction of take-all inoculum by rotation with lupins oats or field peas. *Journal of Phytopathology* 121: 125-134.
Council N.R. (1993) "*Soil and water quality: An agenda for agriculture*". Washington DC: National Academy Press.
Deacon J.W. & Mitchell R.T. (1985) Toxicity of oat roots, oat root extracts, and saponins to zoospores of *Pythium* spp. and other fungi. *Transactions of the British Mycological Society 84*: 479-487.
Deacon J.W. & Donaldson S.P. (1993) Molecular recognition in the homing responses of zoosporic fungi, with special reference to *Pythium* and *Phytophthora*. *Mycological Research* 97: 1153-1171.

Deacon J.W. & Saxena G. (1998) Germination triggers of zoospore cysts of *Aphanomyces euteiches* and *Phytophthora* parasitica. *Mycological Research* 102: 33-41.
Dehne H.W. (1982) Interaction between vesicular-arbuscular mycorrhizal fungi and plant pathogens. *Phytopathology* 72: 1115-1132.
Delwiche P.A., Grau C.R., Holub E.B., & Perry J.B. (1987) Characterization of *Aphanomyces euteiches* isolates recovered from alfalfa in Wisconsin. *Plant Disease* 71: 155-161.
Deshpande S.S. & Damodaran S. (1990) Food legumes: Chemistry and technology. *Advanced cereal Science technology* 10: 147-241.
Dick M.W. (1969) Morphology and taxonomy of the *Oomycetes* with special reference to *Saprolegniaceae*, *Leptomitaceae* and *Pythiaceae*. *New Phytologist* 68: 751-755.
Dobbs C.G. & Hinson W.A. (1953) A widespread fungistasis in soils. *Nature* 172: 197-199.
Domínguez J., Negrín M.A. & Rodríguez C.M. (2001) Aggregate water-stability, particle-size and soil solution properties in conducive and suppressive soils to *Fusarium* wilt of banana from Canary Islands (Spain). *Soil Biology & Biochemistry* 33: 449-455.
Donaldson S.P. & Deacon J.W. (1992) Role of calcium in adhesion and germination of zoospore cysts of *Pythium*: a model to explain infection of host plants. *Journal of General Microbiology* 138: 2051-2059.
Doran J.W., & Parkin T.B. (1994) Defining and assessing soil quality. *In "Defining soil quality for a sustainable environment*" Soil Science Society Special Publication no. 35, edited by Doran J.W., Coleman D.C., Bezdicek D.F. & Steward B.A. Madison, Wisconsin.
Drechsler C. (1929) The beet water mold and several related root parasites. Journal of Agricultural Research 38: 309-361.
Dyke G.V. & Slope D.B. (1978) Effects of previous legume and oat crops on grain yield and take-all in spring barley. *Journal of Agricultural Science in Cambridge* 91: 443-451.
Elad Y. & Baker R. (1985) The role of competition for iron and carbon in suppression of chlamydospore germination of *Fusarium* spp. *Phytopathology* 75: 1053-1059.
Elmholt S. (1996) Microbial activity, fungal abundance and distribution of *Penicillium* and *Fusarium* as bioindicators of a temporal development of organically cultivated soils. *Biological Agriculture and Horticulture* 13: 123-140.
Engelkes C.A. & Windels C.E. (1994) ß-escin (saponin), oat seedlings and oat residue in soil affects growth of *Aphanomyces cochlioides* hyphae, zoospores and oogonia. *Phytopathology* 84: 1158.
Engelkes C.A. & Windels C.E. (1996) Susceptibility of sugar beet and beans to *Rhizoctonia solani* AG-2-2 IIIB and AG-2-2 IV. *Plant Disease* 80: 1413-1417.
Estevez de Jensen C., Percich J.A. & Graham P.H. (2002) Integrated management strategies of bean root rot with *Bacillus subtilis* and *Rhizobium* in Minnesota. *Field Crops Research* 74: 107-115.
Felton W.L., Marcellos H., Alston C., Martin R.J., Backhouse D., Burgess L.W. & Herridge D.F. (1998) Chickpea in wheat-based cropping systems of northern New South Wales II. Influence on biomass grain yield, and crown rot in the following wheat crop. Australian *Journal of Agricultural Research* 49: 401-407.
Filonow A.B. & Lockwoood J.L. (1983) Mycostasis in relation to the microbial nutrient sinks of five soils. *Soil Biology and Biochemistry* 15: 557-565.
Fisk J.W., Hesterman O.B., Shrestha A., Kells J.J., Harwood R.R., Squire J.M. & Sheaffer C.C. (2001) Weed suppression by annual legume cover crops in no tillage corn. *Agronomy Journal* 93: 319-325.
Flint M.L. & Roberts P.A. (1988) Using crop diversity to manage pest problems: Some Californa examples. *American Journal of Alternative Agriculture* 3: 163-167.
Förster H., Kinscherf T.G., Leong S.A. & Maxwell D.P. (1988) Estimation of relatedness between *Phytophthora* species by analysis of mitochondrial DNA. *Mycologia* 80: 466-478.
Förster H., Oudemans P. & Coffey M.D. (1990) Mitochondrial and nuclear DNA diversity within six species of *Phytophthora*. *Experimental Mycology* 14 : 18-31.
Frame J., Charlton J.F.L. & Laidlaw A.S. (1998) *Temperate forage legumes*. New York: CAB International.

Franklin T.B. (1953) *British Grasslands*. London: Faber & Faber.
Fritz V.A., Allmaras R.R., Pfleger F.L. & Davis D.W. (1995) Oat residue and soil compaction influences on common root rot (*Aphanomyces euteiches*) of peas in a fine-textured soil. *Plant and Soil* 171: 235-244.
Frye W.W., Blevins R.L., Smith M.S., Corak S.J. & Varco J.J.I.H. (1988) Role of annual legume cover crops in efficient use of water and nitrogen. In *Cropping strategies for efficient use of water and nitrogen*, edited by Hargrove W.L. Madison, WI: ASA Specific Publications 51 pp. 129-154.
Giller K.E. & Wilson K.J. (1991) *Nitrogen fixation in tropical cropping systems*. Oxon, UK: CAB International.
Gorodecki B. & Hadar Y. (1990) Suppression of *Rhizoctonia solani* and *Sclerotium rolfsi*i diseases in container media containing composted separated cattle manure and composted grape marc. *Crop Protection* 9: 271-274.
Grau C.R. & Reiling T.P. (1977) Effect of trifluralin and dinitramine on *Aphanomyces* root rot of pea. *Phytopathology* 67: 273-276.
Grau C.R., Muehlchen A.M. & Tofte J.E. (1991) Variability in virulence of *Aphanomyces euteiches*. *Plant Disease* 75: 1153-1156.
Grau C.R., Muehlchen A.M., Tofte J.E. & Smith R.R. (1991) Variability in virulence of *Aphanomyces euteiches*. *Plant Disease* 75: 1153-1156.
Gretenkort M.A. & Helsper J.P.F.G. (1993) Disease assessment of pea lines with resistance to foot rot pathogens: Protocols for *in vitro* selection. *Plant Pathology* 42: 676-685.
Gritton E.T. (1990) Registration of five root rot resistant germ plasm lines of processing pea. *Crop Science* 30: 1166-1167.
Hall A.D. (1905) *The Book of the Rothamsted experiments*. New York: Dutton.
Hall R. (1994) Compendium of bean diseases. Edited by Hall R., *The disease compendium series of the American Phytopathological Society*. St. Paul, Mn: The American Phytopathological Society.
Hargrove W.L., Langdale G.W., & Thomas Q.W. (1984) Role of legume cover crops in conservation tillage production systems. *ASAE Paper* ASAE, St. Joseph MI, 84: 2038.
Hargrove W.L. (1986) Winter legumes as a nitrogen source for no-till grain sorghum. Agronomy Journal 78:70-74.
Harman G.E. (2001) *Pythium* spp. In *Compendium of pea diseases*, edited by Kraft J.M. & Pfleger F.L. St Paul, Mn, USA: American Phytopathological Society.
Harman G.E. (2001) *Rhizoctonia* seed and seedling rot. *In Compendium of pea diseases*, edited by Kraft J.M. & Pfleger F.L. St Paul, Mn, USA: American Phytopathological Society.
Harman G.E. (2001) *Thielaviopsis* root rot. In *Compendium of pea diseases*, edited by Kraft J.M. & Pfleger F.L. St Paul, Mn, USA: American Phytopathological Society.
Hawksworth D.L., Kirk P.M., Sutton B.C. & Pegler D.N. (1995) *Dictionary of the fungi*. Cambridge: CAB International.
Heungens K. & Parke J.L. (2000) Zoospore homing and infection events: effects of the biocontrol bacterium *Burkholderia cepacia* AMMDR1 on two Oomycete pathogens of pea (*Pisum sativum* L.). *Applied and Environmental Microbiology* 66: 5192-5200.
Heungens K. & Parke J.L. (2001) Postinfection biological control of oomycete pathogens of pea by *Burkholderia cepacia* AMMMDR1. *Phytopathology* 91: 383-391.
Hillis D.M. & Dixon M.T. (1991) Ribosomal DNA: Molecular evolution and phylogenetic inference. *The Quarterly Review of Biology* 66: 411-452.
Hoestra H. (1975) Crop rotation, monoculture and soil ecology. *Bulletin OEPP* 5: 173-180.
Hoitink H.A.J. & Fahy P.C. (1986) Basis for control of soil-borne plant pathogens with composts. *Annual Review of Phytopathology* 24: 93-114.
Holub E.B., Grau C.R. & Parke J.L. (1991) Evaluation of the *forma specialis* concept in *Aphanomyces euteiches*. *Mycological Research* 95: 147-157.
Hood M.E. & Shew H.D. (1997) Reassessment of the role of saprophytic activity in the ecology of *Thielaviopsis basicola*. *Phytopathology* 87: 1214-1219.
Hooker J.E., Jaizme-Vega M. & Atkinson D. (1994) Biocontrol of plant pathogens using arbuscular mycorrhizal fungi. In *Impact of arbuscular mycorrhizas on sustainable*

*agriculture and natural ecosystems*, edited by Gianinazzi S. & Schuepp S. Bäsel, Switzerland: Birkhäuser-Verlagh.
Hornby D. (1979) Take-all decline: a theorists paradise. In *Soil-Borne Plant Pathogens*, edited by Schippers B. & Gams W. London.
Hudspeth D.S.S., Nadler S.A. & Hudspeth M.E.S. (2000) A COX2 molecular phylogeny of the *Peronosporomycetes*. *Mycologia* 92: 674-684.
Irwin J.A. & Dale J.L. (1982) Relationships between *Phytophthora megasperma* isolates from chickpea, lucerne, and soybean. *Australian Journal of Botany* 30: 199-210.
Jacobsen B.J. & Hopen H.J. (1981) Influence of herbicides on *Aphanomyces* root rot of peas. *Plant Disease* 65:11-16.
Jones F.R. & Linford M.B. (1925) Pea disease survey in Wisconsin. Wisconsin Agricultural Experiment Station Results Bulletin 64: 31pp.
Jones F.R. & Drechsler C. (1925) Root rot of peas in the United States caused by *Aphanomyces euteiches*. *Journal of Agricultural Research* 30: 93-325.
Karlen D.L., Varvel G.E., Bullock D.G., & Cruse R.M. (1994) Crop rotations for the 21st century. *Advances in Agronomy* 53: 1-45.
Karlovsky P. & Fartmann B. (1992) Genetic code and phylogenetic origin of oomycetous mitochondria. *Journal of Molecular Evolution* 34: 254-258.
King E.B. & Parke J.L. (1993) Biocontrol of *Aphanomyces* root rot and *Pythium* damping-off by *Pseudomonas cepacia* AMMD on four pea cultivars. *Plant Disease* 77: 1185-1188.
King E.B. & Parke J.L. (1996) Population density of the biocontrol agent *Burkholderia cepacia* AMMDR1 on four pea cultivars. *Soil Biology & Biochemistry* 28: 307-312.
Kjaer A. (1960) Natually derived isothiocyanates (mustard oils) and their parent glucosides. *Fortschritte der Chemie Organischer Naturstoffe* 18:122-176.
Kjøller R. & Rosendahl S. (1996) The presence of the arbuscular mycorrhizal fungus *Glomus intraradices* influences enzymatic activities of the root pathogen *Aphanomyces euteiches* in pea roots. *Mycorrhiza* 6 (6):487-491.
Kloepper J.W., Leong J., Tientze M. & Schroth M.N. (1980) Enhanced plant-growth by siderophores produced by plant growth promoting rhizobacteria. *Nature* 286: 885-886.
Knudsen I.M.B., Debosz K., Hockenhull J., Jensen D.F. & Elmholt S. (1999) Suppressiveness of organically and conventionally managed soils towards brown foot rot of barley. *Applied Soil Ecology* 12: 61-72.
Kraft J.M., Burke D.W. & Haglund W.A. (1981) *Fusarium* diseases of beans peas and lentils. In Fusarium *diseases biology and taxonomy*, edited by Nelson P.E., Tousson T.A. & R.J. C. London, UK: University Press.
Kraft J.M. (1986) Seed electrolyte loss and resistance to *Fusarium* root rot of peas. *Plant Disease* 70: 743-745.
Kraft J.M., Marcinkowska J. & Muehlbauer F.J. (1990) Detection of *Aphanomyces euteiches* in field soil from northern Idaho by a wet-sieving/baiting technique. *Plant Disease* 74: 716-718.
Kraft J.M. (1994) *Fusarium* wilt of peas (A review). *Agronomie* 14: 561-567.
Kraft J.M., & Boge W.L. (1996) Identification of characteristics associated with resistance to root rot caused by *Aphanomyces euteiches* in pea. *Plant Disease* 80: 1383-1386.
Kraft J.M., Larsen R.C. & Inglis D.A. (1997) Diseases of pea. In *The Pathology of Food and Pasture Legumes*, edited by Allen D.J. & Lenné J.M. Cambridge, CAB International, UK.
Kuan T.L. & Erwin D.C. (1980) *Formae speciales* differentiation of *Phytophthora megasperma* isolates from soybean and alfalfa. *Phytopathology* 70: 333-338.
Kuo S., Sainju U.M. & Jellum E.J. (1996) Winter cover cropping influence on nitrogen mineralization presidedress soil nitrate test and corn yields. Biology and Fertility of Soils 22: 310-317.
Lafay B., & Burdon J.J. (1998) Molecular diversity of of rhizobia occurring on native shrubby legumes in southeastern Australia. *Applied and Environmental Microbiology* 64: 3989-3997.
Lager J. & Gerhardson B. (2002) Pathogenicity of clover root pathogens to pea, bean and lucerne. *Journal of Plant Diseases and Protection* 2: 142-151.

Lager J. (2002) Soil-borne clover diseases in intensive legume cropping. *Acta Universitatis Agriculturae Sueciae*, Agraria 362 SLU, Uppsala, doctoral thesis.
Lal R. & Kimble J. (1997) Conservation tillage for carbon sequestration. *Nutrient Cycling in Agroecosystems* 49: 243-253.
Lamari L. & Bernier C.C. (1985) Etiology of seedling blight and root rot of faba bean (*Vicia faba*) in Manitoba. *Canadian Journal of Plant Pathology* 7: 139-145.
Larsson W.E. & Pierce F.J. (1994) The dynamics of soil quality as a measure of sustainable environment. In *Defining soil quality for a sustainable environment*. Soil Science Society Special Publication no. 35., edited by Doran J.W., Coleman D.C., Bezdicek D.F. & Steward B.A. Madison, Wisconsin.
Larsson M. (1994) Pathogenicity, morphology and isozyme variability among isolates of *Aphanomyces* spp. from weeds and various crop plants. Mycological Research 98: 231-240.
Larsson M. & Olofsson J. (1994) Prevalence and pathogenicity of spinach root pathogens of the genera *Aphanomyces*, *Phytophthora*, *Fusarium*, *Cylindrocarpon* and *Rhizoctonia* in Sweden. *Plant Pathology* 43: 251-260.
Lawrence E. (2000) *Henderson's Dictionary of Biological Terms*. Edited by Lawrence E. 12 ed. London: Prentice Hall.
Leclerc M.C., Guillot J., & Deville M. (2000) Taxonomic and phylogenetic analysis of *Saprolegniaceae* (*Oomycetes*) inferred from LSU rDNA and ITS sequence comparisons. *Antonie van Leeuwenhoek* 77: 369-377.
Lee S.B., & Taylor J.W. (1992) Phylogeny of five fungus-like protoctistan *Phytophthora* species, inferred from the internal transcribed spacers of ribosomal DNA. *Molecular Biology and Evolution* 9: 636-653.
Lee T.V.D., Witte I.D., Drenth A., Alfonso C., Govers F., Van der Lee T. & De Witte I. (1997) AFLP linkage map of the oomycete *Phytophthora* infestans. *Fungal Genetics and Biology* 21 (3): 278-291.
Lewis J.A. & Papavizas G.C. (1971) Effect of sulfur-containing volatile compounds and vapors from cabbage decomposition on *Aphanomyces euteiches*. *Phytopathology* 61: 208-214.
Lewis J.A., Lumsden R.D., Millner P.D. & Keinath A.P. (1992) Suppression of damping of peas and cotton in the field with composted sewage slugde. *Crop Protection* 11: 260-266.
Leyns F., Lambert B., Joos H., & Swing J. (1990) Antifungal bacteria from different crops. In *Biological Control of Soil-Borne Plant Pathogens*, edited by Hornby D. Wallingford, UK: CAB International.
Lim L.L. & Cole A.L.J. (1984) Infection and colonization of white clover seedling roots by fungi. *New Zealand Journal of Agricultural Researc*h 27: 459-465.
Linford M.B. (1927) Additional hosts of *Aphanomyces euteiches*. *Phytopathology* 17:133-134.
Linnaeus C. (1749) *Linnaeus skånska resa*. Stockholm: Wahlström & Widstrand.
Llanos C.M. & Lockwood J.L. (1960) Factors affecting zoospore production by *Aphanomyces euteiches*. *Phytopathology* 50: 826-830.
Lockwood J.L. (1960) Progress and problems in breeding peas resistant to root rot. *Michigan Agronomy Experimental Station Quarterly Bulletin* 43: 358-366.
Lockwood J.L. (1960) Pea introductions with partial resistance to *Aphanomyces* root rot. *Phytopathology* 50: 621-624.
Lucas G.B. (1975) *Diseases of Tobacco*. Edited by Raleigh N.C. Biological Consulting Associates, USA.
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.
Lynch J.M. (1990) *The Rhizosphere*. Chichester, UK: Wiley Inter-Science.
MacLeod A.J. & MacLeod G. (1968) Volatiles of cooked cabbage. *Journal of Science of Food and Agriculture* 19: 273-277.
Majchrzak B., Kurowski T.P. & Pszczolkowski P. (1996) Reaction of faba bean and pea cultivars to pathogenic fungi under different growing conditions. *Plant Breeding and Seed Science* 40: 65-78.

Malvick D.K., Percich J.A., Pflegger F.L., Givens J. & Williams J.L. (1994) Evaluation of methods for estimating inoculum potential of *Aphanomyces euteiches* in soil. *Plant Disease* 78: 361-365.
Malvick D.K., Grau C.R. & Percich J.A. (1998) Characterization of *Aphanomyces euteiches* strains based on pathogenicity tests and Random Amplified Polymorphic DNA analyses. Mycological Research 102: 465-475.
Malvick D.K. & Percich J.A. (1998) Genotypic and pathogenic diversity among pea-infecting strains of *Aphanomyces euteiches* from the Central and Western United States. *Phytopathology* 88: 915-921.
Malvick D.K. & Percich J.A. (1999) Identification of *Pisum sativum* germ plasm with resistance to root rot caused by multiple strains of *Aphanomyces euteiches*. *Plant Disease* 83: 51-54.
Malvick D.K. & Grau C.R. (2001) Characteristics and frequency of *Aphanomyces euteiches* races 1 and 2 associated with alfalfa in the midwestern United States. *Plant Disease* 85: 740-744.
Manning M.A. & Menzies S.A. (1984) Pathogenic variability in isolates of *Aphanomyces euteiches* from New Zealand soils. *New Zealand Journal of Agricultural Research* 27: 569-574.
Martin J.H., Leonard W.H. & Stamp D.L. (1976) *Principles of Field Crop Production*, 3rd Ed. New York: Macmillan.
Mathew K.A. & Gupta S.K. (1996) Studies on web blight of French bean caused by *Rhizoctonia solani* and its management. *Indian Journal of Mycology and Plant Pathology* 26: 171-177.
Micales J.A., Bonde M.R. & Peterson G.L. (1988) Isozyme analysis and aminopeptidase activities within the genus Peronosclerospora. *Phytopathology* 78: 1396-1402.
Mills S.D., Förster H. & Coffey M.D. (1991) Taxonomic structure of *Phytophthora cryptogea* and *P. drechsleri* based on isozyme and mitochondrial DNA analyses. *Mycological Research* 95 31-48.
Mitchell J.E. & Yang C.Y. (1966) Factors affecting growth and development of *Aphanomyces euteiches*. *Phytopathology* 56: 917-922.
Mitchell J.E. (1979) The dynamics of the inoculum potential of populations of soil-borne plant pathogens in the soil ecosystem. In *Soil Borne Plant Pathogens*, edited by Schippers B. & Gams W. London, UK: Academic Press.
Mithöfer A. (2002) Suppression of plant defence in rhizobia-legume symbiosis. *Trends in Plant Science* 7: 440-444.
Moore W.C. (1959) *British Parasitic Fungi*. Cambridge.
Moussard A., Tivoli B., Lemarchand E., Deneufbourg F., Roi S. & Sicard G. (1998) Role of seed infection by the Ascochyta blight pathogen of dried pea (*Mycosphaerella pinodes*) in seedling emergence, eaely disease development and transmission of disease to aerial plant parts. *European Journal of Plant Pathology* 104: 93-102.
Muehlchen A.M., Rand R.E. & Parke J.L. (1990) Evaluation of crucifer green manures for controlling *Aphanomyces* root rot of peas. Plant Disease 74: 651-654.
Munkvold G.P., Carlton W.M., Brummer E.C., Meyer J.R., Undersander D.J. & Grau C.R. (2001) Virulence of *Aphanomyces euteiches* isolates from Iowa and Wisconsin and benefits of resistance to *A. euteiches* in alfalfa cultivars. Plant-Disease. 85: 328-333.
Nasir M. & Hoppe H.H. (1991) Studies on pathotype differentiation within *Mycosphaerella pinodes* (Berk. & Bloxam) Vestergren, a component of the Ascochyta-disease-complex of peas (*Pisum sativum* L.). *Journal of Plant Disease Protection* 98: 619-626.
Nygaard S.L., Elliott C.K., Cannon S.J. & Maxwell D.P. (1989) Isozyme variability among isolates of *Phytophthora megasperma*. *Phytopathology* 79: 773-780.
Olofsson J. (1967) Root rot of canning and freezing peas in Sweden. *Acta Agriculturae. Scandinavica* 17: 101-107.
Olsen G.J., Lane D.J., Giovannoni J. & Pace M.R. (1986) Microbial ecology and evolution: ribosomal RNA approach. *Annual Review of Microbiology* 40: 337-365.
Otani Y. (1962) Studies on the black root rot disease caused by *Thielaviopsis basicola* (Berk & Br.) Ferraris. *Bulletin Okayama Tob. Experimental Station* 23: 1-118.

Oyarzun P., Gerlagh M. & Hoogland A.E. (1993) Pathogenic fungi involved in root rot of peas in the Netherlands and their physiological specialization. *The Netherlands Journal of Plant Pathology* 99: 23-33.

Oyarzun P., Gerlagh M. & Hoogland A.E. (1993) Relation between cropping frequency of peas and other legumes and foot and root rot in peas. *The Netherlands Journal of Plant Pathology* 99: 35-44.

Oyarzun P.J., Dijst G., Zoon F.C. & Maas P.W.T. (1997) Comparison of soil receptivity to *Thielaviopsis basicola*, *Aphanomyces euteiches*, and *Fusarium solani* f. sp. *pisi* causing root rot in pea. *Phytopathology* 87: 534-541.

Papavizas G. (1966) Suppression of *Aphanomyces* root rot of peas by cruciferous soil amendments. *Phytopathology* 56: 1071-1075.

Papavizas G.C. & Ayers W.A. (1974) *Aphanomyces* species and their root diseases in pea and sugarbeet. Technical *Bulletin, United States Department of Agriculture* No.1485: 158pp.

Parke J.L., Rand R.E., Joy A.E. & King E.B. (1991) Biological control of *Pythium* damping-off and *Aphanomyces* root rot of peas by application of *Pseudomonas cepacia* or Pseudomonas fluorescens to seed. *Plant Disease* 75: 987-992.

Parker C.P. (1915) *Field Management and Crop Rotation*. St. Paul, Minnesota: Webb.

Patriarca E.J., Tatè R.. & Iaccarino M. (2002) Key role of bacterial $NH_4$+ metabolism in *Rhizobium*-plant symbiosis. *Microbiology and Molecular Biology Reviews* 66: 203-222.

Peng H.X., Sivasithamparam K., & Turner D.W. (1999) Chlamydospore germination and *Fusarium* wilt of banana plantlets in suppressive and conducive soils are affected by physical and chemical factors. *Soil Biology & Biochemistry* 31: 1363-1374.

Peoples M.B., Herridge D.F. & Ladha J.K. (1995) Biological nitrogen fixation: An efficient source of nitrogen for sustainable agricultural production. *Plant and Soil* 174: 3-28.

Persson L., Bødker L., & Larsson Wikstrom M. (1997) Prevalence and pathogenicity of foot and root rot pathogens of pea in southern Scandinavia. *Plant Disease* 81: 171-174.

Persson L. (1998) Soil suppressiveness to *Aphanomyces* root rot of pea. *Acta Universitatis Agriculturae Sueciae*, Agraria 131, SLU, Uppsala, doctoral thesis.

Persson L., Larsson-Wikström M. & Gerhardson B. (1999) Assessment of soil suppressiveness to *Aphanomyces* root rot of pea. *Plant Disease* 83: 1108-1112.

Persson L. & Olsson S. (2000) Abiotic characteristics of soils suppressive to *Aphanomyces* root rot. *Soil-Biology & Biochemistry*. 32: 1141-1150.

Peters R.D. & Grau C.R. (2002) Inoculation with nonpathogenic *Fusarium solani* increases severity of pea root rot caused by *Aphanomyces euteiches*. *Plant Disease* 86: 411-414.

Petersen Ann B., & Rosendahl S. (2000) Phylogeny of the *Peronosporomycetes* (*Oomycota*) based on partial sequences of the large ribosomal subunit (LSU rDNA). Mycological-Research. [print] November, 2000; 104: 295-1303.

Petri G.A. (1986) Consequences of survival of *Leptosphaeria maculans* (blackleg) in canola stubble residue through an entire crop rotation sequence. *Canadian Journal* of *Plant Pathology* 8: 356.

Pfender W.F., Rouse D.I. & Hagedorn D.J. (1981) A "most probable number" method for estimating inoculum density of *Aphanomyces euteiches* in naturally infested soil. *Phytopathology* 711169-1172.

Pfender W.F. & Hagedorn D.J. (1982) *Aphanomyces euteiches* f.sp. *phaseoli*, a causal agent of bean root and hypocotyl rot. *Phytopathology* 72: 306-310.

Pfender W., Delwiche P., Grau C. & Hagedorn D. (1982) A semiselective medium for isolating *Aphanomyces* from plant tissue. *Phytopathology* 72: 993.

Pfender W.F. & Hagedorn D.J. (1983) Disease progress and yield loss in *Aphanomyces* root rot of peas. *Phytopathology* 73: 1109-1113.

Pfender W.F., Delwiche P.A., Grau C.R. & Hagedorn D.J. (1984) A medium to enhance recovery of *Aphanomyces* from infected plant tissue. *Plant Disease* 68: 845-847.

Pieczarka D.J. & Abawi G.S. (1977) Effects of interaction between *Fusarium*, *Pythium* and *Rhizoctonia* on severity of bean root rot. *Phytopathology* 68: 403-408.

Price D. (1984) *Fusarium* and plant pathology: the reservoir of infection. In The applied mycology of *Fusarium*, edited by Moos M.O. & Smith J.E. Cambridge: Cambridge University Press.

Quintanilla P. (2002) Biological control in potato and tomato to enhance resistance to plant pathogens-especially against *Phytophthora* infestans in potato. *Acta Universitatis Agriculturae Sueciae*, Agraria 315, SLU, Uppsala, doctoral thesis.

Raaijmakers J.M. & Weller D.M. (1998) Natural plant protection by 2,4-diacetylphloroglucinol-producing Pseudomonas spp. in take-all decline soils. *Molecular Plant Microbe Interactions* 11: 144-152.

Reddy M.S. & Patrick Z.A. (1989) Effect of host, nonhost and fallow soil on populations of *Thielaviopsis basicola* and severity of black root rot. *Canadian Journal of Plant Pathology* 11: 68-74.

Reeves T.G., Ellington A. & Brooke H.D. (1984) Effects of lupin-wheat rotations on soil fertility, crop disease and crop yields. *Australian Journal of Experimental Agricultural and Animal Husbandry* 24: 595-600.

Riethmueller A., Weiss M. & Oberwinkler F. (1999) Phylogenetic studies of Saprolegniomycetidae and related groups based on nuclear large subunit ribosomal DNA sequences. *Canadian-Journal of Botany*. 77: 1790-1800.

Rothrock C.S. (1992) Influence of soil temperature, water, and texture on *Thielaviopsis basicola* and black root rot of cotton. *Phytopathology* 82: 1202-1206.

Rothrock C.S., Kirkpatrick T.L., Frans R.E. & Scott H.D. (1995) The influence of winter legume cover crops on soilborne plant pathogens and cotton seedling diseases. *Plant Disease* 79: 167-171.

Roughley R.J., Sprent J.I., & Day J.M. (1983) Nitrogen fixation. In *The Faba Bean*, edited by Hebblethwaite P.D. London: Butterworth.

Rufelt S. (1986) Studies on *Fusarium* root rot of red clover (*Trifolium pratense* L.) and the potential for its control. Department of Plant and Forest Protection, Swedish University of Agricultural Sciences, Uppsala, doctoral thesis.

Sainju U.M., Singh B.P. & Whitehead W.F. (2001) Comparison of the effects of cover crops and nitrogen fertilization on tomato yield, root growth and soil properties. *Scientia Horticulturae* 91:2 01-214.

Sainju U.M., Singh B.P. & Whitehead W.F. (2002) Long-term effects of tillage, cover crops, and nitrogen fertilization on organic carbon and nitrogen concentrations in sandy loam soils in Georgia. *Soil & Tillage Research* 63: 167-179.

Salt G.A. (1983) Root Diseases of *Vicia faba* L. In *The Faba Bean* (*Vicia faba* L.), edited by Hebblethwaite P.D. London: Butterworths.

Salt G.A. & Delaney K.D. (1984) Influence of previous legume crops on root diseases in peas and beans. Proc Easter Sch Agric Sci Univ Nottingham 40: 247-256.

Salt G.A. & Delaney K.D. (1985) Influence of previous legume crops on root diseases in peas and beans. In *The Pea Crop*, edited by Hebblethwaite P.P., Heath M.C. & Dawkins T.C.K. London: Butterworths.

Salunkhe D.K., & Deshpande S.S. (1991) *Foods of plant origin: production, technology, and human nutrition*. New York: Van Nostrand Reinhold.

Schippers B., Bakker A.W., Bakker P.A.H.M. & van Peer R. (1990) Beneficial and deleterious effects of HCN-producing pseudomonads on rhizospere interactions. *Plant and Soil* 129: 75-83.

Schmitthenner A.F. (1964) Prevalence and virulence of *Phytophthora*, *Aphanomyces*, *Pythium*, *Rhizoctonia* and *Fusarium* isolated from diseased alfalfa seedlings. *Phytopathology* 54: 1012-1018.

Schneider C.L. & Robertson L.S. (1975) Occurrence of diseases on sugarbeet in a crop rotation experiment in Saginow County, Michigan in 1969-1970-1971. *Plant Disease* 59: 194-197.

Schneider C.L. (1978) Use of oospore inoculum of *Aphanomyces cochlioides* to initiate blackroot disease in sugar beet seedlings. Journal of American Society of Sugar Beet Technology, 20: 55-62.

Schneider R.W. (1984) Effects of non pathogenic strains of *Fusarium oxysporum* on celery root infection by *F. oxysporum* f.sp. apii and a novel use of the Lineweaver-Burk double reciprocal plot technique. *Phytopathology* 74: 646-653.

Scott W.W. (1961) A monograph of the genus *Aphanomyces*, *Technical Bulletin (Virginia Agricultural Experiment Station)* 151. Blacksburg, Va.: Virginia Agricultural Experiment Station, 1961. 95 pp.Il.

Shang H., Grau C.R. & Peters R.D. (2000) Oospore germination of *Aphanomyces euteiches* in root exudates and on the rhizoplanes of crop plants. *Plant Disease* 84: 994-998.

Sher M. & Baker R. (1980) Mechanism of biological control in a *Fusarium* suppressive soil. *Phytopathology* 70: 412-417.

Sherwood R.T. & Hagedorn D.J. (1958) Determining common root rot potential of pea fields. *Wisconsin Experimental. Station. Bulletin* 531.

Sherwood R.T. & Hagedorn D.J. (1962) Studies on the biology of *Aphanomyces euteiches*. *Phytopathology* 52: 150-154.

Sinclair J.B. (1997) Diseases of Soyabean. In *The Pathology of Food and Pasture Legumes*, edited by Allen D.J. & Lenné J.M. Cambridge, UK: CAB International.

Sinha S.K. (1977) Food legumes: distribution, adaptability and biology of yield. In *FAO plant production and protection paper*. Rome: Food and Agriculture Organization of the United Nations.

Skipp R.A., Christensen M.J. & Nan Z.B. (1986) Invasion of red clover (*Trifolium pratense*) roots by soilborne fungi. *New Zealand Journal of Agricultural Research* 29: 305-313.

Slezack S., Dumas Gaudot E., Rosendahl S., Kjøller R., Paynot M., Negrel J. & Gianinazzi S. (1999) Endoproteolytic activities in pea roots inoculated with the arbuscular mycorrhizal fungus *Glomus mosseae* and/or *Aphanomyces euteiches* in relation to bioprotection. *New Phytologist*. 142: 517-529.

Steadman J.R. (1983) White-mold a serious yield-limiting disease of bean. *Plant Disease* 67: 346-350.

Stegmark R. (1988) Downy mildew resistance of various pea genotypes. *Acta Agriculturae Scandinavica* 38: 373-379.

Stegmark R. (1990) Variation for virulence among Scandinavian isolates of *Peronospora viciae* f. sp. *pisi* (pea downy mildew) and responses of pea genotypes. *Plant Pathology* 39: 118-124.

Sturz A.V., Carter M.R. & Johnston H.W. (1997) Review of plant disease, pathogen interactions and microbial antagonism under conservation tillage in temperate humid agriculture. *Soil & Tillage Research* 41: 169-189.

Sturz A.V., Christie B.R. & Matheson B.G. (1998) Associations of bacterial endophyte populations from red clover and potato crops with potential for beneficial allelopathy. *Canadian Journal of Microbiology* 44: 162-167.

Subba Rao N.S. (2001) Soil Microbiology Fourth Edition *of Soil Microorganisms and Plant Growth*. Plymouth: Science Publishers, Inc.

Sumner D.R., Phatak S.E., Gai J.D., Chalfant R.B., Brunson K.E. & Bugg R.L.M. (1995) Soilborne pathogens in a vegetable double-crop with conservation tillage following winter cover crops. *Crop Protection* 14: 495-500.

Sundheim L. & Wiggen K. (1972) *Aphanomyces euteiches* on peas in Norway. Isolation technique, physiologic races, and soil indexing. Norges Landbrukshogsk Meld, 51: 17 pp.

Sundheim L. (1972) Physiologic specialization in *Aphanomyces euteiches*. Physiological *Plant Pathology* 2 (3):301-306.

Sundheim L. (1973) *Botrytis fabae*, *Botrytis cinerea*, and *Ascochyta fabae* on broad bean (*Vicia faba*) in Norway. *Acta AgriculturaeScandinavica*, 23: 43-51

TAC CGIAR (1988) *Sustainable agricultural production: Implications for internatioal agricultural research*. CGIAR (Consultative Group on International Agricultural Research) meeting, Berlin, Germany

Teasdale J.R., Beste C.E. & Potts W.E. (1991) Response of wheats to tillage cover crop residue. *Weed Science* 39: 195-199.

Teasdale J.R. (1996) Contribution of cover crops to wheat management in sustainable agricultural systems. *Journal of Production Agriculture* 9: 475-479.

Temp M.V., & Hagedorn D.J. (1967) Influence of cropping practises on *Aphanomyces* root rot potential of Wisconsin pea fields. *Phytopathology* 57: 667-670.

Thaning C. (2000) Ways of managing *Sclerotinia sclerotiorum* inoculum. *Acta Universitatis Agriculturae Sueciae*, Agraria 240, SLU, Uppsala, doctoral thesis.
Thiessen Martens J.R., Hoeppner J.W. & M.H. E. (2001) Legume cover crops with winter cereals in Southern Manitoba: establishment, productivity and microclimate effects. *Agronomy Journal* 93: 1086-1096.
Tivoli B., Beasse C., Lemarchand E. & Masson E. (1996) Effect of ascochyta blight (*Mycosphaerella pinodes*) on yield components of single pea (*Pisum sativum*) plants under field conditions. *Annals of Applied Biology* 129: 207-216.
Tofte J.E., Smith R.R. & Grau C.R. (1992) Reaction of red clover to *Aphanomyces euteiches*. *Plant Disease*: 39-42.
Tsvetkova N.A. & Kotova V.V. (1985) Root rots of peas and vetch. Zashchita Rastenij 8: 50-51.
Tu J.C. (1987) Integrated control of the pea root rot disease complex in Ontario. *Plant Disease* 71: 9-13.
Tyler B.M. (2001) Genetics and genomics of the oomycete-host interface. *Trends in Genetics* 17: 611-614.
Unestam T. (1981) Fungal diseases of freshwater terrestrial crustacea. *In Pathogenesis of Invertebrate Microbial Diseases*, edited by Davidson E.W. Towota, NJ, USA: Allanheld, Osmun.
United Nations Environment Program (1992) *Montreal Protocol Assessment Supplement, Methyl Bromide: Its Science, Technology and Economics. Synthesis Report of the Methyl Bromide Interim Scientific Assessment and Methyl Bromide Interim Technology and Economic Assessment.*
Wade B.L. (1929) The inheritance of *Fusarium* wilt resistance in canning peas. *Wisconsin Agricultural Experimantal Station Bulletin* 97: 32pp.
Wainright P.O., Hinkle G., Sogin M.L. & Stickel S.K. (1993) Monophyletic origins of the metazoa: an evolutionary link with fungi. *Science* 260: 340-342.
Walker J.C., Morell S. & Foster H.H. (1937) Toxicity of mustard oils and related sulfur compounds to certain fungi. *American Journal of Botany*: 536-541.
Wallace H.R. (1978) Dispersal in time and space. In *Plant Disease*, *an Advanced Treatise*, edited by Horsfall J.G. & Cowlings E.B. New York: Academic Press.
Vandemark G.J., Kraft J.M., Larsen R.C., Gritsenko M.A. & Boge W.L. (2000) A PCR-based assay by sequence-characterized DNA markers for the identification and detection of *Aphanomyces euteiches*. *Phytopathology* 90: 1137-1144.
Vaughn E.K., Roberts A.N., & Mellenthin W.M. (1954) The influence of Douglas fir sawdust and certain fertilizer elements of the incidence of red stale disease in strawberry. *Phytopathology* 44: 601-603.
Weiland J.J. & Sundsbak J.L. (2000) Differentiation and detection of sugar beet fungal pathogens using PCR amplification of actin coding sequences and the ITS region of the rRNA gene. *Plant Disease* 84: 475-482.
Weller D.M. (1988) Biological control of soil borne plant pathogens in the rhizosphere with bacteria. *Annual Review of Phytopathology* 26: 379-407.
Whisson S.C., Drenth A., MacLean D.J. & Irwin J.A.G. (1995) *Phytophthora sojae* avirulence genes, RAPD, and RFLP markers used to construct a detailed genetic linkage map. Molecular Plant Microbe Interactions 8: 988-995.
White K.D. (1970) Fallowing, crop rotation, and crop yieds in Roman Times. *Agricultural History* 44: 281-290.
Wicker E., Hullé M. & Rouxel F. (2001) Pathogenic characteristics of isolates of *Aphanomyces euteiches* from pea in France. *Plant Pathology* 50: 433-442.
Wicker E. & Rouxel F. (2001) Specific behaviour of French *Aphanomyces euteiches* Drechs. populations for virulence and aggressiveness on pea, related to isolates from Europe, America and New Zealand. *European Journal of Plant Pathology* 107: 919-929.
Williams Woodward J.L., Pfleger F.L., Fritz V.A. & Allmaras R.R. (1997) Green manures of oat, rape and sweet corn for reducing common root rot in pea (*Pisum sativum*) caused by *Aphanomyces euteiches*. Plant and Soil 188: 43-48.

Williams Woodward J.L., Pfleger F.L., Allmaras R.R. & Fritz V.A. (1998) *Aphanomyces euteiches* inoculum potential: a rolled-towel bioassay suitable for fine-textured soils. *Plant Disease* 82:3 86-390.
Winner C. (1966) Untersuchungen über parasitogene Schäden an Wurzeln der Zuckerrübe insbesondere durch *Aphanomyces*, und über Möglichkeiten ihrer Verhütung. *Phytopathologische Zeitschrift* 57: 105-120.
Woese C.R., Kandler O. & Wheelis M.L. (1990) Towards a Natural System of Organisms Proposal for the Domains Archaea Bacteria and Eucarya. *Proceedings of the National-Academy of Sciences of the United States of America* 87: 4576-4579.
Vogelbein W.K., Shields J.D., Haas L.W., Reece K.S. & Zwerner D.E. (2001) Skin ulcers in estuarine fishes: A comparative pathological evaluation of wild and laboratory-exposed fish. *Environmental-Health-Perspectives* 109: 687-693.
Wortmann C.S., McIntyre B.D. & Kaizzi C.K. (2000) Annual soil improving legumes: agronomic effectiveness, nutrient uptake, nitrogen fixation and water use. *Field Crop Research* 68: 75-83.
Wroth J.M. (1998) Variation in pathogenicity among and within *Mycosphaerella pinodes* populations collected fro field pea in Australia. *Canadian Journal of Botany* 76: 1955-1966.
Xi K., Stephens J.H.G. & Hwang S.F. (1995) Dynamics of pea seed infection by *Pythium ultimum* and *Rhizoctonia solani*: Effects of inoculum density and temperature on seed rot and pre-emergence damping-off. *Canadian Journal of Plant Pathology* 17: 19-24.
Yuan W.M., & Crawford D.L. (1995) Characterization of *Streptomyces lydicus* WYEC108 as a potential biocontrol agent against fungal root and seed rots. *Applied and Environmental Microbiology* 61: 3119-3128.

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