Plant Pathology and Biocontrol Unit, Swedish University of Agricultural Sciences

Effects of Lime and Organic Amendments on Soilborne Pathogens, especially *Aphanomyces* spp. of Sugarbeet and Spinach

by

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Supervisor:

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Master Thesis 2004

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The master thesis study is an obligatory part in the education of Masters in science of agriculture. It aims at under supervision give the students training to solve a task in an independent and scientific way. The

present case is consequently a student work and the content, results and conclusions should be judged against this background.

Abstract

This study examined the effect of factory and slaked lime, cruciferous plants, poultry, liquid swine and liquid cattle manure on infection of soilborne pathogens, especially *Aphanomyces* spp., on sugarbeet and spinach in greenhouse and field experiments. Soil samples collected from infected fields were brought to the laboratory and used for current greenhouse experiments. Lime or manure were mixed with soil, filled into plastic pots and sown with sugarbeet or spinach seeds. After four weeks the infections of the plants were assessed by using a disease severity index (DSI). The study did also investigate how different placements of lime in soil effected soilborne pathogens, and how soil pH was affected by different lime applications.

The field experiments were carried out in 2003 on spinach fields at two commercial farms in southern Sweden. In late July, two weeks before seeding, liquid manure (swine, 20 ton/ha; cattle, 40 ton/ha) were applied and cultivated with a harrow or a plough. On one experimental site were cruciferous plants grown as precrop. Moving of the cruciferous plants was done about a week prior to tillage. The effect of the treatments on the infection of the soilborne pathogens was determined by counting the plant appearance in two occasions and by assessment of a disease severity index of the roots. Cattle manure reduced root rot the most and lowered the DSI from 40 to 30, but swine manure and cruciferous plants had also good effect on soilborne pathogens in the field experiment. Both plant appearance and spinach yield were benefited by cattle and swine manure.

All organic amendments and lime treatments suppressed the severity of root rot in the experiments. Liquid swine manure lowered the DSI in sugarbeet between 12% to 34% in the greenhouse experiments and the plants fresh weight increased with up to 239%. Six ton slaked lime per hectare had best effect on soilborne pathogens among the lime treatments and reduced the DSI up to 21% compared to the control. The limes placement in soil turned out to be of great importance on the effect of soilborne pathogens. According to this study should lime be applicated below seed depth for best effect against the soilborne fungi. The effect on soil pH by different lime applications was measured during 72 days. Slaked lime and factory lime increased the soil pH several pH units during the first days, which creates an unfavourable environment for many pathogens.

The results from this study suggest that applications of organic amendment or lime in certain rates and conditions to infested soils have the potential to control and decrease the infection of several soilborne pathogens.

Content

Introduction

Damping off and root rot of spinach (*Spinacia oleracea* L.) and sugarbeet (*Beta vulgaris* L.) are causing problems in commercial production and are seriously limiting the yield in several countries, among others Sweden (Larsson and Gerhardson, 1992; Papavizas and Ayers, 1974). Several different fungi are involved in the damping off and root rot complex; species in *Aphanomyces, Pythium and Phytophthora* are pathogens that often occur, and are found in soils in southern Sweden (Gerhardson, 2003). Some of the other pathogenic fungi that are found in the complex are species in the genera*, Fusarium, Cylindrocarpon and Rhizoctonia* (Larsson and Olofsson, 1994).

Many of the important soilborne pathogens like *Aphanomyces, Pythium* and *Phytophthora* belong to the group *Oomycetes*. They are fungal like, often-called pseudofungi, and belong to the kingdom *Chromista*. *Chromistas* elongated mycelium has no cross walls and contains cellulose and glucans. They produce oospores as resting spores and zoospores or zoosporangia as asexual spores (Scott, 1961). *Oomycetes* belong to two orders, *Saprolegniales* or *Peronosporales*. *Aphanomyces* is the only genus of *Saprolegniales*, with important plant pathogens. include several of the most important plant pathogens*, Pythium, Phytophthora* and pathogens causing downy mildews; *Plasmopara, Bremia, Peronospora, Pseudoperonospora and Sclerospora* (Agrios, 1997).

Blackroot of sugarbeets and root rot of spinach are both complex diseases caused by *Aphanomyces* spp. and by various other pathogens. It is established that more than one pathogen usually is involved in the root rot complexes, but it is very difficult to ascertain what role is taken by each pathogen and what is the nature of the interrelationships of the pathogens (Papavizas and Ayers, 1974). Even though there are many pathogens involved in root rot diseases there is a considerable agreement among plant pathologists that the chief agent concerned is A*phanomyces cochlioides* Drechs. in sugarbeet (Scott, 1961) and A*phanomyces cladogamus* Drechs. in spinach (Larsson and Olofsson, 1994).

In resent investigations about pathogens both *Aphanomyces* spp. and *Pythium* spp. have been found to dominate and to occur in surprisingly high numbers in soils in southern Sweden (Gerhardson, 2003; Larsson and Gerhardson, 1992). These fungi are soilborne and benefits from intensive production with short crop rotations, high soil water content and low pH (Larsson and Gerhardson, 1992) and are believed to cause even more damage in the future.

Soilborne fungi are difficult to control chemically and there are at present no resistant varieties. Many researchers have therefore tried to find alternative ways to suppress the fungi. Many experiments have indicated that organic amendments suppress the infection of soilborne fungi (Conn and Lazarovits, 1999; Conn and Lazarovits, 2000; Tsao and Oster, 1981; Lazarovits, 2001; Nilsson, 2002; Widmer *et al.*, 1998). Another way to control the infection of pathogens might be with the use of lime. An application of lime changes the chemistry and physiology of the soil, and is also affecting the soil biological flora. When lime is added to soil is many positive effects achieved. The lime increases the soil pH and several nutrients, mostly macronutrients, will get more available to the crop (Barrows *et al*., 1968; Tisdale *et al*., 1999). The high soil pH is also creating an

unfavorable environment and many pathogens can not propagate and exist in soil solution with pH above 8 (Papavizas and Ayers, 1974). When lime with a high CaO content is used the ion cover on the soil particles will be exchanged from being mostly negative, which binds a thick layer of water around the particles, to consist of Ca^{2+} ions which give a mostly positive and much thinner water layer around the particle. The soil gets a better structure that drain the water (Berglund and Blomquist, 2003). Since several of the soilborne fungi requires a high soil moister content or a film of water to be able to move towards their hosts, these effects will reduce the severity (Papavizas and Ayers, 1974).

Goals and hypothesis
The main reasons for the initiation of these investigations were to evaluate if manure or lime had a suppressing effect on infection of soilborne pathogens in sugarbeet and spinach in greenhouse experiments and in experiments in commercial fields in southern Sweden, and also if the placement of the lime in the soil gave different effect on fungi. The study was also measuring pH changes in different soil types after applications of different amounts and kinds of lime. It was also of interest to see whether there were any differences in effect on infection of soilborne pathogens by manure or lime in different types of soils. Since sugarbeet and spinach are closely related, both belonging to the *Chenopodiaceae* family, the experiments were designed similarly in regards to nutrients, lime and soilborne pathogens.

The goal for this study was to find a way to reduce the severity of root rot in sugarbeet and spinach. The main hypotheses are:

- Manure application to soil can reduce the infection of soilborne pathogens in sugarbeet and spinach.
- Lime application to soil can reduce the infection of soilborne pathogens in sugarbeet and spinach.
- There is a difference in effect on root rot pathogens between different kinds of lime.
- A higher amount of lime will more effectively reduce the infection of a pathogen than a lower.

Pathogens in the root rot complex

***Aphanomyces* spp.**
In 1860 DeBary described the genus *Aphanomyces* for the first time. The generic epithet *Aphanomyces* was chosen from the Greek meaning “imperceptible-fungus”, because of the very delicate, almost imperceptible, appearance of the vegetative hyphae as it spreads radially from a submerged substratum in water. Drechsler, Jones and Kendrick had all made investigations about *Aphanomyces* in the 1920’s and could together describe a closely related group of *Aphanomyces* species, all pathogenic on the roots of phanerogam seedlings (Scott, 1961). The species *A. cochlioides* and *A. cladogamus* were established in 1929 by Drechsler (Scott, 1961). There are about thirty different species in the genus *Aphanomyces*. Sugarbeet is infected by *A. cochlioides* Drechs. and spinach by *A. cladogamus* Drechs, but their biology is very similar (Papavizas and Ayers, 1974).

Another well-known and economically important species is *Aphanomyces euteiches* Drechs., pathogenic to pea (Hall, 1989).

Aphanomyces is a water mold, presence of an abundant moister promotes the development of the fungi. *Aphanomyces* spp. occurs in nature as parasites of algae, aquatic animals and phanerogams, but they are to be considered only facultative parasites. *Aphanomyces* spp. has a delicate hyaline mycelium in both water and on solid substrate. On a substrate the hyphae ramify over and through the agar in a sparse way with little or no aerial development and after several weeks the surface might be covered with thick white mycelium. Hyphae are 3-9µ in diameter and sparingly or moderately branched in almost right angles (Papavizas and Ayers, 1974).

Two types of zoospores are produced in the asexual stage, and this is called diplanetism. This asexual stage occurs when young vigorous hyphae become zoosporangia. The Primary zoospores (6 to 15µ in diameter) produced by the sporangia may vary from very few to 300 per sporangium. The entcystment last for 1-3 hours and then secondary zoospores emerge from the primary zoospore cysts. The secondary zoospores are about 13µ long and 7 to 8µ in diameter and they possess two 24µ long flagella. Zoospores remain motile for 12 hours, and then they loose their flagella and round up (Papavizas and Ayers, 1974). Under favourable conditions they will germinate into one to three slender germ tubes which complete the cycle of asexual reproduction (Drechsler, 1929).

The sexual stage with oospore formation is generally considered to occur when the thallus of *Aphanomyces* is exposed to adverse conditions or environmental stress. The vegetative phase represent a rather short period in the life cycle of *Aphanomyces*. The female "oogonium" and the male "antheridium" are produced on the vegetative mycelium. The oogonium is sub spherical, 20-29µ (*A. cochlioides*) and 19-33µ (*A. cladogamus*) in diameter, the wall is irregularly thickened with a smooth outer surface and a sinuous inner contour. Oogonia are terminal on short lateral branches (Scott, 1961). The oogonia contain a single oosphere (unfertilized egg), which becomes an oospore upon fertilization, provided by one to five antheridia that wraps around the individual oogonia. *A. cochlioides* has a characteristic crowded condition of the antheridial apparatus, while *A. cladogamus* are less crowded with 2-3 antheridia wrapped around the oogonia (Papavizas and Ayers, 1974).

Blackroot disease starts in an early seedling stage and occurs in two phases; an early acute phase of short duration, pre- and post-emergence damping-off, and later a chronic phase (Olsson, 2001; Whitney and Duffus, 1995). Species of *Aphanomyces* are considered to cause no or little pre-emergence damping off, but cause extensive post-emergence damping off, where entire fields might be destroyed, especially in warm, wet soil (Whitney and Duffus, 1995). The fungus seldom infects seedlings in soil at temperatures below 13°C and the optimum temperature for infection is between 17-25°C. The post-emergence damping-off are evident from the time of emergence to the first true leaves have developed. The chronic phase appears in late June to August, when the lowest leaves turns yellow and the pathogen stunts the plant (Papavizas and Ayers, 1974).

The motile zoospores move towards the plant by chemotaxis. Sugarbeet root exudate is reported to contain 14 amino acids, three organic acids and nine sugars. This specific pattern of exudate allows the pathogen to locate the host plant (Rai and Strobel, 1966). The plant is often infected at ground level where the motile zoospores infect the hypocotyl through stomata. The most typical symptom induced by *Aphanomyces* is a brownish root discoloration that extends from the roots up to the hypocotyl and sometimes evens above the soil surface. The cortex of the hypocotyl dries and the stem and hypocotyl shrink to a dark, slender thread (Larsson and Olofsson, 1994; Papavizas and Ayers, 1974). The hypocotyl is weakened and the seedling falls over. Some plants may later recover from infection, but will be dwarfed. Others continue to show symptoms ranging from slight to complete necrosis (Larsson and Olofsson, 1994; Whitney and Duffus, 1995).

***Pythium* spp.**

Pythium species occur in waters and soils throughout the world. They live on dead plant and animal matters as saprophytes or as parasites of roots of plants (Agrios, 1997). *Pythium* spp. cause both pre- and post-emergence damping off and the losses vary due to the soil moisture content, soil temperature and other factors. The severity in field is probably often underestimated, because most losses occur pre-emergence and can not be distinguished from other causes like waterlogging, soil crapping and pest damage (Williams and Asher, 1996). The greatest damage is done to the seed and seedling roots during germination under conditions of high soil temperature and excessive soil moisture (Sumner *et.al,* 1976). Seeds in the seedbed are most often killed by *Pythium* spp., while infection of older plants often results in root and stemlensions, root rot and consequently a retarded growth (Whitney and Duffus, 1995). Infested seeds of sugar beet and spinach become brown, soft and mushy and fail to germinate. The fungi spreads quickly in young seedlings, the cells collapses and the seedling will be overrun by the fungus and die. Emerged plants are often attacked at the roots or at the stem in the vicinity of the soil line. The fungus will soften the basal part of the plant and even make it thinner than the uninvaded part above it, which results in a plant fall (Whitney and Duffus, 1995).

Pythium spp. have white mycelium, which grows very fast and gives rise to sporangia, which can develop directly into a germtube ready to infect, or into a secondary sporangium, a vesicle. The temperature decides what the sporangia will develop into. At temperatures above 18°C the sporangia germinates mostly with germtubes and at temperatures between 10-18°C with a vesicle containing zoospores. The vesicle produces about 100 zoospores, which are released and after a few minutes of swarming they will round up and create an encysted zoospore. The zoospores will germinate with a germtube and cause infection. The sporangia might also produce an oogonium and an antheridium that develops into a resting structure, an oospore. The germination of oospores is stimulated by seed or root exudates, chiefly sugars and amino acids (Whitney and Duffus, 1995).

The fungi reaches the most severe outcome in fields where the same crop is planted year after year and when the soil is kept wet for a prolonged time and when there is an excess

of nitrogen in the soil. No commercial varieties of sugarbeet or spinach with resistans to *Pythium* spp. exist yet, therefore is the use of cultural practices very important to reduce the infection. A well-planed crop rotation might reduce a great deal of the disease problems. Good soil drainage and air circulation among plants are important as are planting when temperatures are favourable for fast plant growth and the optimizing of nitrogen fertilizers (Whitney and Duffus, 1995). One way to control *Pythium* spp. in fields is to use chemicals. A systemic fungicide, with the composition Propamocarb or Oxadixyl, will have the best effect on damping off and can be applied as soil or seed treatment. The use of fungicides against *Pythium* and damping off is very limited in Sweden but occur more frequently abroad. Most common is a seed treatment followed by a spraying of the seedlings (Agrios, 1997).

Observations made by Larsson (1994) indicate that *P. sylvaticum* Campbell and Hendrix and *P. ultimum* var. *ultimum* are the most serious threat among the *Pythium* spp. in the pathogen complex causing spinach root rot. But the importance of *Pythium* spp. is probably low in comparison with the other major root pathogens such as *A. cladogamus* and *Phytophthora cryptogea* Pethybr. & Lafferty (Larsson and Olofsson, 1994). In sugarbeet production *P. sylvaticum* was found to be the most dominating *Pythium* spp. among the root rot disease fungi in southern Sweden (Gerhardson, 2003). Another fungi that occur in the disease complex is *P. ultimum* Trow, this fungi attacks seedlings at a temperature favourable for the germination of beet seed (Whitney and Duffus, 1995).

***Fusarium* spp.**

Fusarium genera is wide-spread and well-known for its large number of pathogenic species. Over twenty are known to be pathogenic agents to plants in different agroclimatic areas. (Nelson *et al*., 1983). *Fusarium* spp. cause vascular wilts, damping-off, rotting of roots, lower stems, and crowns, and rots of corms and tubers. The losses may be severe by reduced stands, growth and yield of infected plants even though the fungus often occur on the plant root surface without being pathogenic to the plant (Agrios, 1997).

The pathogen survives as a non-motile resting spore in the soil for several years and the spore germinates when it gets in contact with root exudate. A hypha will grow towards the root and the chief attack is below ground where it penetrate the root tissue, beginning with the finest feeding roots and continuing into the taproot (Chupp and Sherf, 1960). Symptoms of *Fusarium* spp. are root rots and vascular wilts, the foliage turns yellow, beginning with the older leaves and progressing toward the center, death of older leaves and curly heart leaves. Seed plants maybe killed by the disease, but even if mature plants seldom are killed, the yield loss can nevertheless be considerable (Chupp and Sherf, 1960).

Most characteristic for *Fusarium* are the colourless spores, which in several species are canoe-shaped in side view, have a distinct "foot cell" at the lower end, and are divided by several cross-walls. The conidiophores are often clustered to form sporodochia and produce large pasty masses of spores from tapered phialides. Two other spore forms may

occur, microconidia resembling spores and phialides and chlamydospores, thick-walled swellings along the filaments. (Nelson, *et al*., 1983).

Among the most common plant pathogenic species are *F. solani* and *F. oxysporum* Snyder and Hansen (Chupp and Sherf, 1960). Fusarium wilt caused by *F. oxysporum* occur on both sugarbeet and spinach, each of these diseases is caused by a different special form of the fungus (Nelson *et al*., 1983). As with many other soilborne fungi there are no adequate control methods in the field. An aspect of diseases caused by *Fusarium* that is different from other soilborne disease of sugarbeet and spinach, is the effect of soil moisture. The non-motile conidia do not require free water to infect the roots, as *Aphanomyces* or *Pythium* species do. A good crop rotation, early planting, use of resistant crops and disease free or fungicide treated seed, applications of organic material and use of fertilizers in nitrate form might reduce the severity of *Fusarium* (Agrios, 1997).

***Phytophthora* spp.**

Phytophthora exist in a variety of species. Most of them cause root and lower stem-rots but also damping off of seedlings on numerous species of plants, ranging from seedlings of annual vegetables to fully developed forest trees (Agrios, 1997). Typical symptoms observed in infected sugarbeet and spinach plants are rotted and dark root tips, wilting of cotyledon and lower leaf, which is spreading upward until plant death (Larsson and Gerhardson, 1990). In a disease survey, carried out in commercial spinach fields in southern Sweden by Larsson and Olofsson (1994), *P. cryptogea* was determined to be the root rot fungi that caused the most severe damage in spinach. *P. cryptogea* is very similar both morphological and serological to *P. drechsleri* Tucker, pathogenic in sugarbeet (Larsson and Gerhardson, 1990).

Sugarbeet (*Beta vulgaris* L.)

In Sweden has sugarbeet (*Beta vulgaris* L.) been cultivated since 1870 with the largest extent in 1920 (Fogelfors, 2001). Today is sugarbeet mostly grown in the southernmost part of Sweden where the conditions are favorable for sugarbeet production. It is an economic important crop in Sweden. About 4500 Swedish farmers are cultivating 55 000 hectares and produces 430 000-ton sugar each year which approximately correspond to the Swedish consumption (Jordbruksstatistisk årsbok, 2003). Sugarbeets are grown on contract with Danisco Sugar AB (Fogelfors, 2001).

Sugarbeet is attacked by several soilborne pathogens and most serious is *A. cochlioides*. This fungi is present more or less frequent in sugarbeet soils and is responsible for yield losses every year (Pers. com. Persson, L.) During the first weeks after seeding might early attacks by soilborne pathogens be prevented if the beet seeds are fungicide treated. The possibilities to control later root rot attacks with chemicals are very limited (Whitney and Duffus, 1995). Several sugarbeet varieties are offered by the market, the variety with best resistance against *A. cochlioides* at the present is Syngentas variety Sapporo (Pers. com. Nihlgård, M.). In order to avoid disease problems, sugarbeet is usually not grown in crop rotation more frequently than once in three years. In case *Brassicaceae* crops occur

in the crop rotation should the sugarbeet not be grown more frequently that once every fourth year to avoid yield losses by nematodes (Odlingsanvisningar, 2004).

Sugarbeet is planted as early as possible to give the plants a possibility to outrun weeds and fungi. The planting is often carried out in the beginning of April. The beet seed requires a germination temperature of three degrees but optimal growth temperature is about 25 degrees. Four seeds per meter, about 90 000 plants per hectare, are planted with a precision machine, which also often bands fertilizers at the same time. Sugarbeet has a long growth period, about eight months, which lasts until freezing. Danisco Sugar decides the day the sugarbeet crop should be delivered to the factory, which might be between September and January. Sugarbeet can be grown in all kinds of soils, the best soils are free from stones and have pH above 6.5 in sand soils and above 7.0 in clay soils (Odlingsanvisningar, 2004).

A sugarbeet crop on 45 ton/ha uses about 230 kg nitrogen, 30 kg phosphorus, 280 kg potassium, 145 kg sodium and 50 kg magnesium per hectare (Betboken, 1988). That is far more nitrogen and potassium than the crop usually is fertilized with. Normal fertilization is about 90-120 kg N, 30 kg P, 50 kg K, 70 kg Na, and 15 kg Mg per hectare (Odlingsanvisningar, 2004). The very deep root system of sugarbeet, that penetrates the whole soil profile, and the long growth period allow the great uptake of minerals (Betboken, 1988).

Spinach (*Spinacia oleracea* L.)

Spinach *(Spinacia oleracea* L.*)* is grown in the southernmost part of Sweden. Findus Sverige AB contracts about 170 ha each year for commercial spinach production and the 18 growers produce about 2 350-ton spinach every year. Most of the spinach is sold as processed spinach and a small amount as leaf spinach. Spinach grows quickly and reaches edible maturity in 37-45 days. Normally, two crops per year are grown; the first crop is sown in April and the second in late July or early August. All fields are located close to the company to preserve the quality of the spinach. Growers will mostly use the same spinach fields for both spring and fall spinach, and in some cases the spinach will be grown on the very same field every year. The short crop rotation allows fungi to propagate, and the production is often limited by damping of and root rot, but the short rotations makes the weed control easier (Pers. com. Gunnarsson, B.).

Spinach requires a high level of fertility, especially of nitrogen. Spring spinach usually requires a larger quantity of fertilizers than fall crops because some nutrients remains in the fall since spring fertilization. The requirements for spring spinach are approximately 130 kg N, 35 kg P and 125-150 kg K per hectare and about 65 kg N, 0-15 kg P and 55-75 kg K in fall spinach. Due to the health requirements it is not allowed to use bio slurry or amendments with high cadmium content in the crop rotation (Pers. com. Gunnarsson, B.). The use of proper varieties is very important and they are chosen to have a vigorous growth but also to prevent bolting. Spinach quickly bolts and produces seed under conditions of long day and warm weather. Sowing dates are therefore important regarding the bolting behavior and normally spinach is not sown in July (Hägnefelt and

Olsson, 1999). Sowing dates are also important for distributing the harvest on several days which gives the processing and freezers possibility to take care of the yield (Pers. com. Wikström, M.). 40-50 kg spinach seeds are sown per hectare with a precision seeder and the aim is approximately 35 appeared plants per meter. Field viability is 70-95% and the yield is about 14 ton per hectare in spinach for processing and approximately 10 ton/ha for leaf spinach (Pers. com. Gunnarsson, B.).

Spinach can be grown successfully on a variety of soils, but a fertile sandy loam high in organic matter is preferred. To be able to develop high yields, spinach needs organic matter and high, uniform moisture content. Spinach requires abundant moisture because it is so shallow rooted and quick growing. It is especially important to keep the soil moist until seedlings have emerged. Irrigation will be necessary if precipitation is absent to insure a high plant appearance and quality product (Pers. com. Gunnarsson, B.). It is important to use an irrigation process that do not splash soil onto the spinach leaves or damage them (Ingvarsson, 1992). Spinach is also very sensitive to low pH and grow very poorly at pH below 6.0 and for optimum growth the soil pH should range from 6.2-7.0 (Tisdale *et al.*, 1999).

Material and Methods

Material

Soils used in the experiments were chosen with regard to different textures and clay content, to evaluate if there were any differences in the effect of organic amendments or lime on the severity of root rot in soils. SBU AB has done studies on soil texture and disease severity index in sugarbeet fields in Sweden and by using this data it was possible to choose fields with different inoculum content and soil texture to the experiments. The characteristics of the soils used in this study are excerpt from these studies and are detailed in Table 1.

Table 1. Soil characteristics

Sugarbeet soils	CEC meq/ 100 g dry soil[1]	Org.C[1] %	DSI[2] Green house	pH[3]	P-AL[3] mg/ 100g soil	K-AL[3] mg/ 100g soil	Mg-AL[3] mg/ 100g soil	Ca-AL[3] mg/ 100g soil	Clay[3] %	Silt[3] %	Sand[3] %	Gravel[3] %
Barkåkra	1.4	1.74	80	5.9	14	7.2	5.0	110	3	44	46	7
Boserup	4.6	1.99	52	7.7	31	11.0	6.2	470	11	30	49	10
Fjärestad	2.6	1.53	54	7.2	23	12.0	9.7	210	10	29	55	6
Kärrarp	-	4.60	-	6.0	9.1	30.0	21.0	320	41	54	3	2
Mjöhult	3.4	2.81	72	7.3	14	11.0	9.8	280	15	34	50	1

Selleberga	-	1.80	-	6.3	26	20.0	5.8	120	11	36	45	8

[1] Analysis made by Siv Olsson, Geochimica.
[2] Analysis made by Lars Persson, SBU AB. Disease severity index on sugar beet.
[3] Analysis made by AnalyCen Nordic AB, Kristianstad, ammonium lactate extractable.

Organic amendments

Sampling of the organic amendments, used in the experiments, was done at one occasion. The amendments were kept cool in a refrigerator until usage. Samples were sent for analysis of nutrients at AnalyCen Nordic AB, Lidköping. The results from the analysis are presented in Table 2.

Table 2. Chemical characteristics of amendments applied in field and greenhouse experiments, analysis values in kg/10 ton manure

Manure	Dry mass %	Total nitrogen (N)	Ammonium nitrogen (NH_4^+)	Potassium (K)	Magnesium (Mg)	Phosphorus (P)
Swine[a]	4.3%	-	27	23	9	20
Swine	4.5 %	60	25	44	35	88
Cattle	4.5 %	30	7	19	43	85
Poultry	21.5 %	140	137	64	-	30

[a]Liquid swine manure used only in field experiment 2. Chemical characteristics from 1999.

Fertilizer

Probeta NPKS (15-4-7-2), with the common name Probeta NPK, was used as fertilizer in the experiments. Probeta NPK contains important micro- and macronutrients for the sugarbeet crop. Probeta NPK is produced by Hydro Agri (Köping) and consist of; 15.0% nitrogen, 3.6% phosphorus, 7.8% potassium, 0.9% magnesium, 1.8% sulfur, 9.5% sodium, 0.12% boron and 0.55% manganese all provided in weight %. The fertilizer is vaguely acidic.

Lime

The raw material to all kinds of lime is limestone ($CaCO_3$). The efficiency of the lime products depends on the geological origin, which decides the solubility of the lime younger lime is more soluble than older, and by the grain size of the product. All kinds of lime increase the pH, but finely ground materials react more rapidly than coarse materials due to larger surface area. The ability to neutralise acid depends on the Calcium oxide (CaO) content in the lime product (Kihlstrand and Lundström, 1997). Lime with a high amount of pure calcium oxide (CaO) or calcium oxide bound to water (CaO,H_2O) also improves soil structure and tilth. The content depends on the origin of the limestone, but also the process for developing the product. Slaked lime ($Ca(OH_2$) is developed by heating $CaCO_3$ until it splits up into calcium oxide (CaO) and carbon dioxide (CO_2), and then add water. Factory lime is a widely used product by the farmers in southern Sweden. It is a left over product from the sugar production and consists mostly of $CaCO_3$ but also

of vegetabilian substances. The lime contains some extra minerals after the purification; nitrogen 0.6%, phosphorous 0.7%, potassium 0.05%, magnesium 1.1% (% of dry mass) they are extracted from the sugarbeet and are valuable as soil enhancers (Produktfaktablad, 2002). The market supplies several kinds of lime, with different reaction times and nutrient content. The lime that was used in this study is presented in Table 3.

Table 3. Lime

	Factory lime[2]	Slaked lime[1]	Limestone[1]
Common name	Factory lime	Nord Kalk Struktur	Nord K Bas
Chemical	$CaCO_3$	$Ca(OH_2)$	$CaCO_3$
Grain size, mm	0-0.06	0-0.06	0.032-150
CaO %	30	73	54
N kg/ton	4	-	-
P kg/ton	4.5	-	-
K kg/ton	<0.6	-	-
S kg/ton	2.5	-	-
Mg kg/ton	7	7	5
Cd g/ton	0.45	<0.1	0.1

[1] Analysis figures from Nordkalk (Liberg, 2003).
[2] Analysis figures from Produktfaktablad (2002).

Greenhouse experiments

The soil samples were taken with a spade to a depth of 20 cm during the time June to August and brought in plastic bags into the laboratory. Organic amendments and lime were mixed into the soil by shaking the treatment and soil in a plastic bag for one minute. The mixture was then filled into plastic pots (500 ml) and sown with 10 seeds of the spinach cultivar F9 or the sugarbeet cultivar Envol. Six replicates were made of each treatment. The pots were placed in the greenhouse in a randomised block design for four weeks. The greenhouse temperature was kept at 23°C during the day and 19°C at night. Extra light, by Osram HQI-T 400 W, gave approximately 8000 lux at plant height and was supplied for 16 hours per day. The plants were watered daily to keep the moisture content optimal for infection. After four weeks the roots were removed from the soil and gently washed in water. The severity of root rot was then visually examined and the plants were divided into six disease severity classes, ranging from 0 (healthy plants) to 100 (dead plants) according to Larsson and Gerhardson (1990) (Table 4). Every plant was given a disease severity index (DSI) and an average disease severity index was then calculated for each treatment. Finally the root system and the green mass were parted and the fresh plant weight was measured.

Table 4. Disease Severity Index (DSI) by Larsson and Gerhardson (1990)

Plant reaction observed	Disease Severity Index
No visible symptoms.	0
About 10 % of the root system were dark and affected.	10
About 50 % of the root system were dark and affected.	25

The whole root system was dark and affected but no symptoms on hypocotyl or leaves.	50
The whole root system, as well as the hypocotyl, was dark and affected. No clear wilting of the leaves.	75
Plants were dead, or the whole root system, as well as the hypocotyl, was dark and affected and the leaves were wilted.	100

The amount of organic amendments added in the greenhouse experiments was adjusted according to the total nitrogen content in the manure. Chemical characterization of amendments used in the experiments is provided in Table 2. The aim was to reach a level of 120-kg nitrogen/ha in each treatment. The amounts of manure applied in each treatment were calculated by using the pot area and corresponded to 20-ton liquid swine manure, 40-ton liquid cattle manure and 9-ton poultry manure per hectare. Three different kinds of lime were used in the experiments. Slaked lime, limestone and factory lime (Table 3.).

Experiments 1 and 2

Greenhouse experiments were initiated to test the effect of organic amendments on root rot of spinach and sugarbeet. Three different kinds of organic amendments were tested: liquid swine manure, liquid cattle manure and poultry manure. A treatment with a mineral fertilizer, Probeta NPK, and a control treatment were also included in the experiments (Table 5 and 6). The soil pH was measured with a MiniLab IQ125 Professional pH Meter (IQ Scientific Instruments, Inc, Canada) the day the amendments were mixed into the soil and the day when the pots were sown in an attempt to evaluate the influence of the manure on pH.

Table 5. Arrangement for greenhouse experiment 1, spinach in Strövelstorp soil and different kinds of manure

	Treatment	Amount/ha	grams/pot
A	Control	-	-
B	Swine manure	20 ton/ha	13.0
C	Cattle manure	40 ton/ha	26.3
D	Poultry manure	9 ton/ha	5.6
E	Probeta NPK	0.8 ton/ha	0.5

Table 6. Arrangement for greenhouse experiment 2, sugarbeet in Fjärestad soil and different kinds of manure

	Treatment	Amount/ha	grams/pot
A	Control	-	-
B	Swine manure	20 ton/ha	13.0
C	Cattle manure	40 ton/ha	26.3
D	Poultry manure	9 ton/ha	5.6
E	Probeta NPK	0.8 ton/ha	0.5

Experiment 3 and 4

In experiments three and four (Table 7 and 8), both lime and manure treatments were included. Two different kinds of lime were used, slaked lime in the amounts of 3-ton /ha and 6-ton /ha and factory lime in 8-ton /ha. The lime treatments did not achieve any fertilization.

Table 7. Arrangement for greenhouse experiment 3, sugarbeet in Barkåkra soil and different kinds of manure and lime

	Treatment	Amount/ha	grams/pot
A	Control	-	-
B	Swine manure	20 ton/ha	13.0
C	Cattle manure	40 ton/ha	26.3
D	Poultry manure	9 ton/ha	5.6
E	Probeta NPK	0.8 ton/ha	0.5
F	Slaked lime	6 ton/ha	4.0
G	Slaked lime	3 ton/ha	2.0
H	Factory lime	8 ton/ha	5.3

Table 8. Arrangement for greenhouse experiment 4, sugarbeet in Mjöhult soil and different kinds of manure and lime

	Treatment	Amount/ha	grams/pot
A	Control	-	-
B	Swine manure	20 ton/ha	13.0
C	Cattle manure	40 ton/ha	26.3
D	Poultry manure	9 ton/ha	5.6
E	Probeta NPK	0.8 ton/ha	0.5
F	Slaked lime	6 ton/ha	4.0
G	Factory lime	8 ton/ha	5.3

Experiment 5

In experiment 5, lime was mixed into the soil at different days before sowing to evaluate if the time between liming and sowing effected the infection of soilborne pathogens (Table 9). Lime was mixed into the soil at the chosen period before sowing by similar procedure as in previous experiments and put in a 500-ml pot in the greenhouse. Four replicates were made of each treatment. On the same day all pots were each sown with ten seeds of the sugarbeet cultivar Envol. The pots were then placed in a randomised block design in the greenhouse for four weeks.

Table 9. Arrangement for greenhouse experiment 5, sugarbeet in Fjärestad soil and different kinds of lime, mixed into the soil different days before sowing

	Treatment	Amount/ha	Number of days before sowing	Sowing at day
A	Control	-	23	0
B	Slaked lime	6 t/ha	23, 17, 9, 6, 4, 2, 0	0

C	Factory lime	8 t/ha	23, 17, 9, 6, 4, 2, 0	0

Experiment 6

Experiment 6 (Table 10) was an attempt to evaluate if the placement of lime had any effect on the severity of root rot. The lime was either mixed into the whole volume of soil or mixed into the topsoil layer (5-cm). This experiment was first done with 9-ton slaked lime per hectare but the plants grew very poorly and became stunted. Plants turned dark green and the roots were dark, but without the typical Aphanomyces symptoms. The plant reactions were probably due to the rather high amount of slaked lime. The experiment was repeated, but with 6-ton slaked lime per hectare.

Table 10. Arrangement for greenhouse experiment 6, sugarbeet in Fjärestad soil, 6-ton slaked lime per hectare mixed into the topsoil, or into all soil in a one-litre pot

	Treatment	Amount/ha	grams/pot	Mixed in
A	Control	-	-	-
B	Slaked lime	6 t/ha	4.0	soil
C	Slaked lime	6 t/ha	4.0	topsoil

Experiment 7

A pilot study was done on the effects of the lime placement in the soil. Soil from Selleberga was used in experiment 7, where corresponding 6-ton slaked lime per hectare was placed in a layer in 1000-ml pots. The different trials were done with lime one centimetre above the seed, two centimetres below the seed and in the last trial the seed was placed directly into a layer of slaked lime. There were no replicates in the treatments. The pots were placed in the greenhouse for four weeks before visual examination.

Experiment 8

A pilot study on effects of banded slaked lime on root rot of sugarbeet in greenhouse was done on two soils. The goal was to find a way to reduce the cost for lime application but to retain the effect on the soilborne fungi. The amount of lime applied was calculated to correspond to 6-ton slaked lime per hectare on the banded area, measuring five cm, which would decrease amounts of lime needed to about one-tenth. Soils from Fjärestad and Boserup were used in the experiment. 6-ton slaked lime per hectare was banded two cm below the seeds in 30 cm long rows. Four replicates were used and placed in the greenhouse and treated the same as the previously described experiments for four weeks.

Experiment 9

The lime-soil experiment was done to evaluate how lime reacted in soil over time. Three different kinds of lime; slaked lime (3- and 6-ton/ha), factory lime (8-ton/ha) and limestone (4-ton/ha) were added to three different kinds of soils. Soil characteristics for the used soils, Kärrarp (clay soil), Barkåkra (sandy loam) and Boserup (loam), are provided in Table 1. The lime treatments were mixed into the soil in plastic bags and

poured into plastic pots. The pots were put in the greenhouse for constant temperature and daily watering. Measurements were done with the MiniLab IQ125 Professional pH Meter. From mixing date, pH was measured everyday for a week and then every second day in ten days. Some further measuring was done to evaluate if the pH stabilised over time. Last measuring was done two and a half-month after mixing date.

Field experiments

The experiments were initiated to evaluate the effects of organic amendments on root rot in spinach fields in southern Sweden. Two field experiments were conducted on commercial fields owned by farmers at the locations Strövelstorp (Field exp. 1) and Fjärestad (Field exp. 2). The fields had known occurrence of soilborne pathogens. Liquid swine and cattle manure, cruciferous plant and two kinds of tillage to work the amendment into the soil were tested. Both experiments were constructed using a block design, with amendment type as the main treatment effect in experiment one and tillage type as the main treatment effect in experiment two. The soil texture and DSI on the locations are characterized in Table 1.

Field experiment 1 - Strövelstorp

This experiment consisted of one application of each treatment, 100m * 12m. Within each treatment, four plots were put out and used as replicates for observation on infection and yield and these plots measured 1.5m * 10m. The treatments consisted of liquid swine manure, liquid cattle manure and cruciferous plants (*Sinapis alba*). The manure were obtained from farmers in the neighbourhood. The chemical characterisations of amendments used in field experiments are provided in Table 2. Amendments were applied at 20-ton liquid swine manure per hectare and 40-ton liquid cattle manure per hectare to generate approximately 100 % of the total spinach nitrogen requirement on 120 kg/ha. The amendments were applied with a manure spreader and cultivated with a plough approximately two weeks prior to planting. The cruciferous plants were grown on the experimental site for about six weeks before the green part were moved and ploughed into the soil, which was done about tree weeks prior to planting. A fertilized, non-amended treatment also measuring 100m*12m, was used as a control. This treatment was ploughed in the same manner as the other treatments to avoid the confounding effects of tillage. Cruciferous plants and control plots received on the 6th of August, 350 kg NPK (11-5-18) per hectare as a starter fertilizer. Spinach was seeded on the 8th of August 2003, using 55-kg seed per hectare.

Prior to the second nitrogen fertilization, composite soil samples were taken from each manure treatment (0-25 cm) to determine soil fertility status. The soil samples were analysed by Lennart Månsson International (LMI), Helsingborg, for soil N; ammonium and nitrate using the Spurway method. Both manure treatments and the control were fertilized with kalksalpeter (ksp), containing 15.5% nitrate nitrogen, based on soil test recommendations for spinach. 100-kg ksp per hectare was added to the treatment swine manure, 450-kg ksp per hectare to the cattle manure treatment and 320-kg ksp per hectare

to the control plot. The farmer managed the spinach for weed and insect pests according to recommendations.

Plant appearance was investigated when the spinach plants had reached the maturity stage with two pair of true leaves. Half a meter of plants just outside each plot corner were counted and marked. Two weeks later when the spinach had approximately four pair of true leaves the marked plants were taken into the laboratory. The roots were gently washed and the severity of root rot was examined. Each plant in the experiments was given a disease severity index (DSI) ranging from 0 (healthy plants) to 100 (dead plants) (Table 4).

The spinach was harvested on the third of October. Yield measurements were made using a special spinach harvester where the spinach yield from each plot (1.5 by 10 m) was harvested directly into a large bag, and the weight was measured by hanging the bag in scales on a tractors front lift.

The microbiological flora on the spinach leaves was analysed in Findus Sverige AB's microbiological laboratory to evaluate if the applied amendments would affect the quality of the spinach. The use of animal manure is always accompanied by risks of contamination by human or animal pathogens. A composite spinach sample from each treatment was delivered to laboratory for analysis.

Field experiment 2 - Fjärestad

A second field experiment was established in Fjärestad. The experiment consisted of four replications per treatment with similar design as in field experiment one. The objective of this experiment was to investigate if there were any differences in root rot severity after swine manure, and if the liquid swine manure was ploughed or harrowed into the soil.

Twenty-ton liquid swine manure per hectare was applied the 11th of July with a manure spreader and cultivated with a plough. The harrow treatment area was ploughed right before the manure was applied, the manure was then cultivated into the soil with a harrow. Also in this experiment a ploughed, fertilized, non-amended treatment was used as a control. The spinach hybrid, Falcon, was sown on the 9th of August 2003 in the amount of 55 kg per hectare with a precision seeder that also banded the 300 kg NPK (15.5% N, 2.4% P, 11.3% K) start fertilizer per hectare. Spinach was managed for weed and insect pests as recommended.

As in field experiment one, composite soil samples were taken from each treatment and sent to LMI for analysis. There was no need for special fertilization in any of the treatments and all treatments were refertilized on the 6th of September with 250-kg kalksalpeter per hectare. Plant appearance, disease severity index and the microbiological flora were investigated as in field experiment one. The harvest was carried out the 14th of October as described in field experiment one.

Isolation of fungi

The DSI index is based on visual symptoms and it is hard to evaluate which fungi that caused the symptoms on the root. It is important to keep in mind, that even symptom that very much look like *Aphanomyces* spp. could arise from other fungi species. Larsson (1994) describes *P. sylvaticum* and *P. heterothallicum* to cause similar symptoms as those induced by *A. cladogamus*. Isolations of fungi from infected plants were therefore made to determine present pathogenic fungi in the experiments. To get rid of dirt and bacteria the roots were placed under running tap water for two hours. Segments (5 mm long) were taken from parts of the roots showing symptoms and plated directly on different agar media. The media were SMA, a selective medium for *Aphanomyces* spp. (Larsson and Olofsson, 1994), PDS selective for *Fusarium* spp. and other fungi (Persson *et al*., 1997), SMP a semi selective medium for *Pythium* spp. (Larsson, 1994) and SMPH, selective for *Phytophthora* spp. (Larsson and Gerhardson, 1992). Plates were left in the laboratory with a temperature about 22°C for 3-5 days. Mycelia tips from the developed fungi were moved to new nonselective agar plates, CMA (corn-meal agar) and PDA (potato-dextrose agar). These plates were left for about two weeks in the laboratory before the fungal identification was made with microscope. Lars Persson did the identification of fungi. Present *Aphanomyces* spp. were determined to *A. cochlioides* or *A. cladogamus* depending on their morphological characteristics.

Plates with *Pythium* spp. were further analysed to identify the species. Plates with oospores were identified by shapes and sizes of antheridia, oogonia and oospores in microscope. Mycelia tips from plates without oospores were moved to PCA (potato-carrot agar) (Van der Plaats-Niterink, 1981) and as soon as the fungi stated to grow, the growth during 24 h in 25°C was measured. Plates with a fungal growth of 25 mm or more during 24 h were crossed on CMA with compatible male and female strains of *P. sylvaticum* obtained from Findus R&D.

Unidentified plates with oospores were tested in greenhouse with a pathogenicity test, two replicates per isolate were used. The plate content (CMA and fungi) was divided in two parts and placed one centimetre below the seed in a soil mixture consisting of 50% sand and 50% "greenhouse" soil. For curiosity also a strain of *P. sylvaticum* (No. 02800, male) pathogenic on dill was included. The control was prepared with the same soil mixture and a fresh CMA plate. The pots were sown with ten seeds of the beet variety Envol each and put in the greenhouse with light, temperature and watering as described in previous greenhouse experiment.

Statistical analysis

Treatment effects on DSI, yield, plant weight and plant appearance were analysed using analysis of variance the PROC GLM (General Linear Models) procedure of SAS (SAS Institute, Cary, NC). Duncan's multiple range test and Fisher's least significant difference (LSD) test at $P \leq 0.05$ were used to separate treatments.

Results

Greenhouse experiments

Greenhouse studies were carried out to evaluate if manure or lime had a suppressing effect on root rot and if there were any differences in suppression of soilborne pathogens in different kinds of soils by applied manure or lime. Soils from four sites were selected to determine the effect of manure application on soilborne pathogens in spinach and sugarbeet. Three of the soils came from sugarbeet fields; Fjärestad, Barkåkra and Mjöhult, all sandy loams with a range of pH from 5.9-7.3 (Table 1) and the fourth from a spinach field, Strövelstorp, a sandy loam with a soil pH of 6.0.

Experiment 1 and 2

There were significant differences in DSI between treatments in both experiment 1 with P=0.0229* (Table 11) and in experiment 2, P=0.0002*** (Table 12) Poultry manure reduced the root rot the most and gained the lowest DSI in both experiments, 25% respective 32% lower compare to the control. Swine and cattle manure decreased the DSI approximately to the same disease level, which in both experiments was lower than the DSI in the Probeta NPK treatment.

A significant difference in yield was also found in the experiments P=0.0436* (Table 11) respective P=0.0001*** (Table 12). Liquid swine manure increased the plant weight the most in spinach closely followed by liquid cattle manure. In experiment 2 Probeta NPK gave a significant increase in plant weight but did not eliminate the root symptoms to the extension as the manure treatments did.

Soil pH was measured at seeding time, the results showed varying pH changes from the treatments applications. In the spinach soil from experiment 1, organic amendments increased the soil pH while the pH was decreased in the sugarbeet soil from Fjärestad. In both soils did Probeta NPK decrease the soil pH.

Table 11. Disease severity index (DSI), soil pH at sowing date and plant fresh weight in spinach, Strövelstorp soil, greenhouse experiment 1

	Treatment	DSI		pH	Fresh weight (g/plant)	
A	Control	80	A[a]	6.0	0.26	C
B	Swine manure 20t/ha	66	B	6.4	0.45	A
C	Cattle manure 40t/ha	65	B	6.2	0.41	AB
D	Poultry manure 9t/ha	60	B	6.4	0.34	ABC
E	Probeta 700 kg/ha	70	B	5.9	0.29	BC

[a] Means in each column followed by the same letter are not significantly different (P≤0.05) according to Duncan's multiple range test.

Table 12. Disease severity index (DSI), soil pH at sowing date and plant fresh weight in sugarbeet, Fjärestad soil, greenhouse experiment 2

	Treatment	DSI	pH	Fresh weight (g/plant)
A	Control	53 A[a]	6.9	0.28 C
B	Swine manure 20t/ha	37 B	6.6	0.95 B
C	Cattle manure 40t/ha	38 B	6.8	1.07 B
D	Poultry manure 9t/ha	36 B	6.8	0.90 B
E	Probeta 700 kg/ha	41 B	6.2	1.29 A

[a] Means in each column followed by the same letter are not significantly different (P≤0.05) according to Duncan's multiple range test.

Experiment 3

Greenhouse experiment 3 was done with soil from Barkåkra. This soil had a high content of inoculum and induced a very high disease severity index, which is shown in Table 13. The different treatments gave significant difference P=0.0003*** in DSI. Liquid swine manure had a good effect on DSI and lowered it 34% compared to the control. The treatment 6-ton slaked lime per hectare had the lowest DSI among lime treatments and was 21% lower than the control.

Table 13. Disease severity index (DSI) in sugarbeet, Barkåkra soil, greenhouse experiment 3

	Treatment	DSI
A	Control	76 A[a]
B	Swine manure 20 ton/ha	50 C
C	Cattle manure 40 ton/ha	69 AB
D	Poultry manure 9 ton/ha	61 B
E	Probeta NPK 800 kg/ha	63 B
F	Slaked lime 6 ton/ha	60 BC
G	Slaked lime 3 ton/ha	70 AB
H	Factory lime 8 ton/ha	70 AB

[a] Means in each column followed by the same letter are not significantly different (P≤0.05) according to Duncan's multiple range test.

Experiment 4

There were significant differences between treatments in both DSI, P=0.0012** and yield, P=0.0001*** in experiment 4 (Table 14). All treatments had significant lower DSI compared to control. Probeta NPK and liquid swine manure had good effect in this soil and decreased DSI most in this experiment. Probeta did also significantly increase the plant weight. As in experiment 3, slaked lime, 6-ton/ha, had the lowest DSI among lime treatments.

Table 14. Disease severity index and plant fresh weight in sugarbeet, Mjöhult soil, greenhouse experiment 4

	Treatment	DSI	Fresh weight (g/plant)
A	Control	56 A	0.19 D
B	Swine manure 20 ton/ha	49 B	0.39 B
C	Cattle manure 40 ton/ha	52 B	0.44 AB

D	Poultry manure 9 ton/ha	52	B	0.36	BC
E	Probeta NPK 800 kg/ha	49	B	0.52	A
F	Slaked lime 6 ton/ha	50	B	0.36	BC
G	Factory lime 8 ton/ha	52	B	0.28	CD

[a] Means in each column followed by the same letter are not significantly different (P≤0.05) according to Duncan's multiple range test.

Experiment 5

This experiment was carried out to evaluate if the time passed between liming and sowing had any effect on the severity of root rot. However, very little infection was achieved in the experiment, and the DSI was low in all treatments (Table 15). The control had a lower DSI compared to the treatments with slaked lime and factory lime, and there was a significant difference P=0.0001*** between treatments in DSI. There was also a significant difference in yield between the treatments P=0.0001***. Slaked lime 6 ton/ha increased the plant weight with 92% compared to control and with 42% compare to factory lime.

Table 15. Experiment 5. Mean DSI and fresh weight in sugarbeet with different lime treatments

Treatments	Amount/ha	DSI	Fresh weight (g/plant)
Control	-	20 B[a]	0.51 C
Slaked lime	6 ton/ha	29 A	0.98 A
Factory lime	8 ton/ha	28 A	0.69 B

[a] Means in each column followed by the same letter are not significantly different (P≤0.05) according to Duncan's multiple range test.

There was a significant difference in mean DSI after mixing slaked lime and factory lime into soil at different time before sowing. Lowest infection was achieved when the lime had been applied 23 and 2 days before the sowing. Most infection was noticed at day 0, when the lime was mixed into the soil and then immediately sown with seeds. The time that passed between mixing day and sowing day did not seem to have much effect on the plant weight, but a tendency towards a lower plant weight at day 0 can be seen in Table 16.

Table 16. Mean DSI and fresh weight depending on the different days slaked and factory lime were mixed into the soil before sowing

Numbers of days mixed before sowing	Mean DSI (Slaked and Factory lime)	Fresh weight (g/plant)
23	23 B[a]	0.73 A
17	25 B	0.76 A
9	26 AB	0.74 A
6	27 AB	0.72 A
4	25 AB	0.72 A
2	23 B	0.72 A

0	31 A	0.68 A

[a] Means in each column followed by the same letter are not significantly different (P≤0.05) according to Duncan's multiple range test.

The treatments 6-ton slaked lime versus 8-ton factory lime per hectare were statistically tested within mixing days. Significant differences were found in plant weight (Fig 1) but not in DSI.

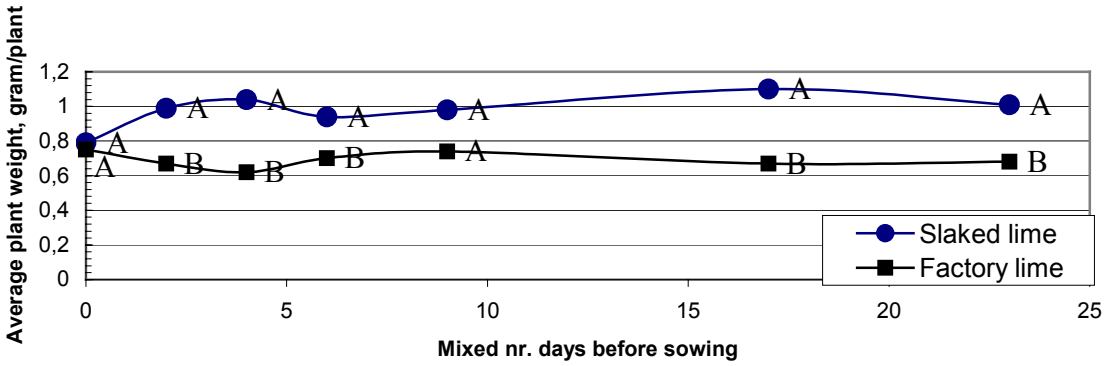

Figure 1. Plant fresh weights (g/plant) of two different lime treatments mixed into the soil at increasing numbers of days before sowing. Means within fresh weight with the same letters are not significant significantly different (P≤0.05) according to Duncan's multiple range test.

Experiment 6

The highest plant weight and the lowest DSI, 31% lower compared to control pot, was found in the treatment where the lime was mixed into the whole soil amount. The pots with lime in the topsoil had almost the same DSI as the control and also had the lowest number of appeared plants, 16 compared to the control, which had 20 plants. Both lime treatments increased the plant weight significantly (Fig. 2).

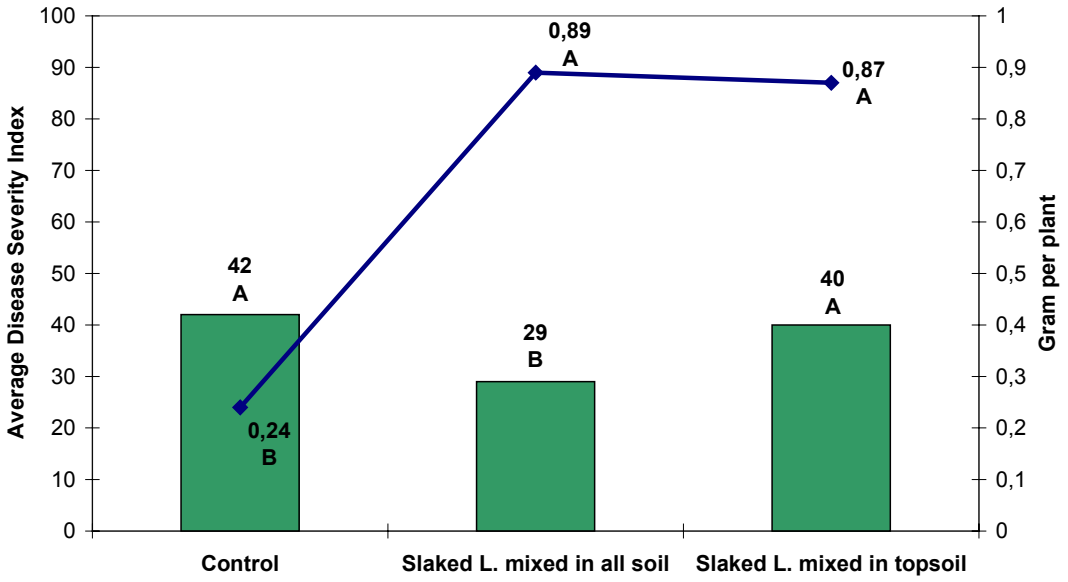

Figure 2. Disease severity index (DSI) and plant weight with significance levels for sugarbeet in experiment 6 with 6-ton slaked lime per ha mixed into the soil either in all pot soil or in topsoil layer. Means within DSI or fresh weight with the same letters are not significant significantly different ($P \leq 0.05$) according to Duncan's multiple range test.

Experiment 7

This pilot study indicated that the placement of slaked lime had impact on soilborne pathogens but also on the germination of sugarbeet seeds. Seeds placed directly into the lime layer got an extremely low plant appearance, five appeared plants, compared to lime above the seed, 10 plants, and lime below the seed, 11 plants. Pots with lime placed above the seed had a DSI on 54. Slaked lime placed below the seed had the lowest DSI, index 17, and the treatment with seeds placed in a lime layer gave a DSI of 24.

Experiment 8

The lime applied in band decreased the infection in the soil from Fjärestad compared to the control (Table 17). Unfortunately, there was very little infection in the Boserup soil, resulting in a low DSI both in the control and in the treatment with banded lime. However, in both experiments differences in plant growth were observed. Plants grown in banded lime appeared later than the control plants, they also got a blue-green colour and became stunted in growth.

Table 17. Disease severity index and plant appearance in sugarbeet grown in banded lime, corresponding to 6-ton slaked lime per hectare

Treatment	Soil	DSI	Plant appearance
Control	Fjärestad	36	19

Banded lime	Fjärestad	30	19
Control	Boserup	20	19
Banded lime	Boserup	22	19

Experiment 9

The pH curves of the three types of lime in the different soils seem to develop in the same direction, with a high pH top that last for one to two days and then stabilises some units over start pH (Fig. 3, 4 and 5). Slaked lime 6 ton/ha increased the soil pH the most and remained at a higher pH value than the other lime treatments in all soils. Slaked lime 3 ton/ha and factory lime 8 ton/ha raised soil pH some units. Limestone gave the lowest pH raise and the control increased slightly. The same tendency was found in all soils.

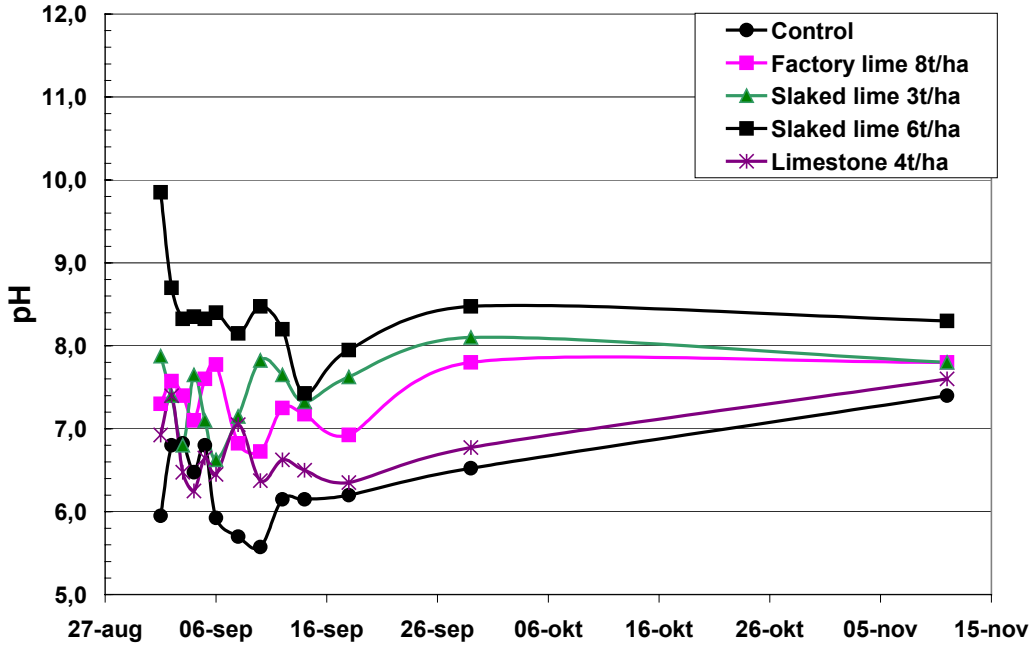

Figure 3. Effects on pH by application of different limes to a sandy loam (Barkåkra; Table 1). The measuring started at the day lime was applied and went on with a decreasing intensity for 72 days.

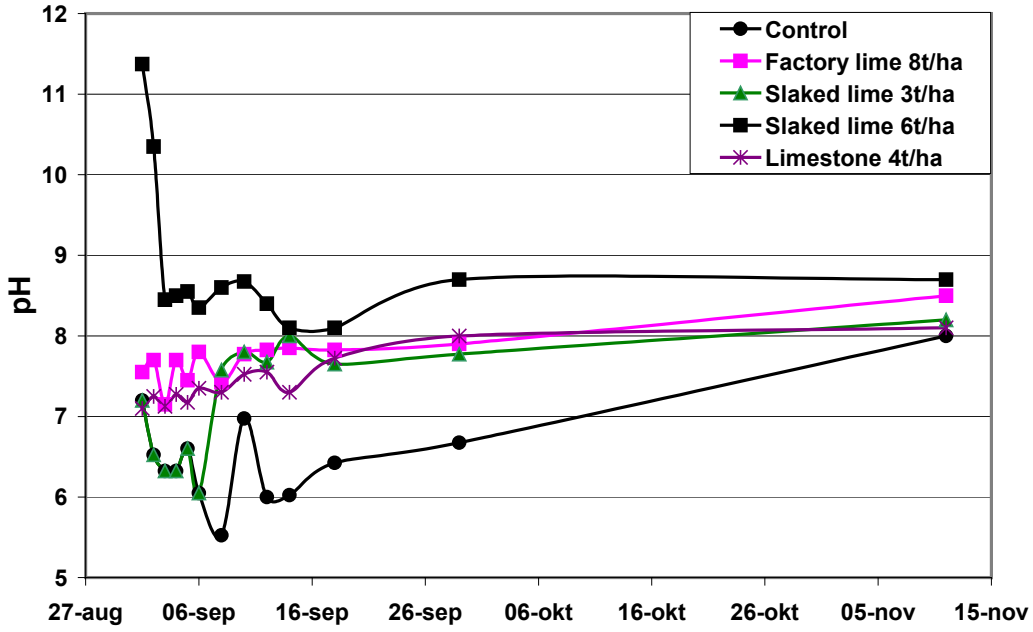

Figure 4. Effects on pH by application of different limes to a loam (Boserup; Table 1). The measuring started at the day lime was applied and went on with a decreasing intensity for 72 days.

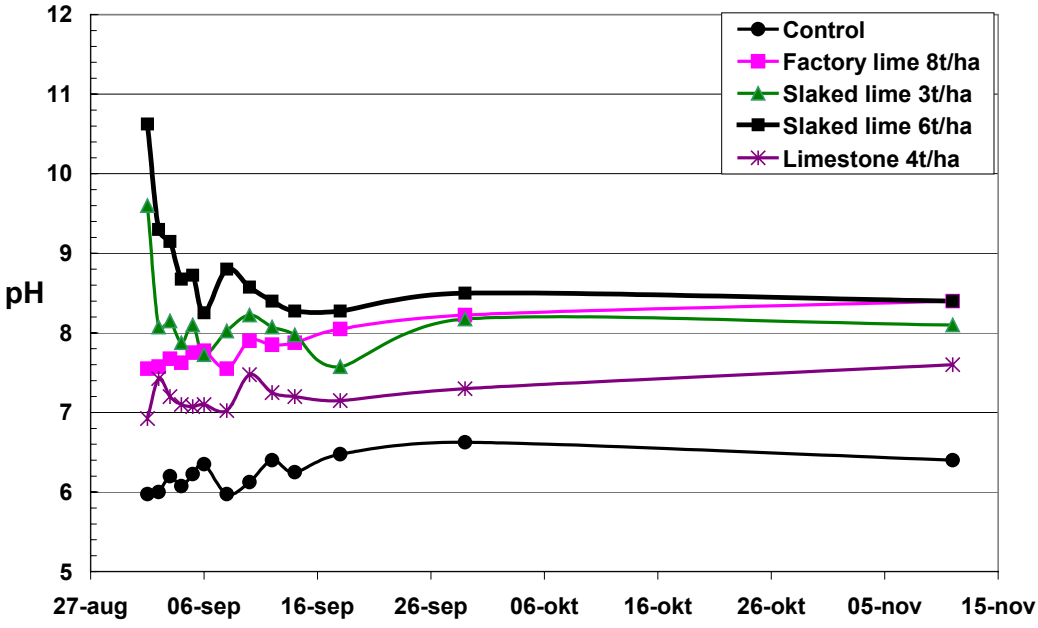

Figure 5. Effects on pH by application of different limes to a clay (Kärrarp; Table 1). The measuring started at the day lime was applied and went on with a decreasing intensity for 72 days.

Field experiment

Field experiment 1 - Strövelstorp

The field experiment in Strövelstorp gave a significant difference in DSI between treatments, P=0.0068** (Table 18). Liquid cattle manure lowered DSI 25% and liquid swine manure with 23% compared to the control. There was also a significant difference in yield between treatments, P=0.0009***. The liquid cattle manure increased the yield with 18% compared to the control. A relation between DSI, yield and plant appearance was found in the liquid cattle manure treatment, with the lowest DSI, high number of plants and the highest yield in the field experiment. Cruciferous plants had the lowest number of appeared plants in the experiment and gave also the lowest yield.

Table 18. Disease severity index (DSI), yield and plant appearance in spinach, Strövelstorp in 2003, field experiment 1

Treatment	DSI	Yield (kg/ha)	Plant appearance/m
Control (fertilized)	40 A[a]	27 580 B	31 AB
Liquid swine manure 20 ton/ha	31 B	29 500 AB	36 A
Liquid cattle manure 40 ton/ha	30 B	32 580 A	33 AB
Cruciferous plants	33 B	22 580 C	28 B

[a] Means in each column followed by the same letter are not significantly different (P≤0.05) according to Duncan's multiple range test.

Field experiment 2 - Fjärestad

The field experiment in Fjärestad had unfortunately very little infection and no significant differences were found. There is a tendency towards a higher plant appearance and a higher yield, 2 000 kg per hectare compared to the control, where the swine manure was harrowed into the soil (Table 19).

Table 19. Disease severity index (DSI) and yield in spinach. Field experiment 2, Fjärestad in 2003

Treatment	DSI	Yield (kg/ha)	Plant appearance/meter
Control (fertilized)	34 A[a]	29 920 A	33 A
Ploughed in liquid swine manure (20 ton/ha)	35 A	29 330 A	30 A
Harrowed in liquid swine manure (20 ton/ha)	36 A	32 080 A	38 A

[a] Means in each column followed by the same letter are not significantly different (P≤0.05) according to Duncan's multiple range test.

Microbial analysis
The microbiological laboratory of Findus Sverige AB, examined the microbiological flora of *Escherichia coli*, *Staphylococcus aureus*, *Bacillus cereus*, *Clostridium perfringens*, *Salmonella*, and *Listeria monocytogenes* on the spinach leaves. The levels of these microorganisms were all under threshold values, and the treatments did not affect the quality of the spinach in a negative way or in a way that could be a threat to human health.

Isolation of fungi
Pythium spp. were found in all soils and *Aphanomyces* spp. in nearly all soils used in the investigations. The species of A*phanomyces* were A. *cladogamus* or A. *cochlioides* depending on morphology and their host, spinach respectively sugarbeet. Fungi isolated from infected spinach and sugarbeet plants are presented in Table 20 respective Table 21.

Table 20. Fungi isolated from spinach plants in greenhouse- and field experiments with root rot symptoms

Fungal spp. in spinach soil	Strövelstorp	Fjärestad
Aphanomyces cladogamus	*	n.f.
Fusarium oxysporum	*	n.f.
F. redolens	*	n.f.
Pythium spp.	*	*
P. sylvaticum	n.f.	n.f.
P. ultimum	*	n.f.

* = found
n.f. = not found

Table 21. Fungi isolated from sugarbeet plants in greenhouse- and field experiments with root rot symptoms

Fungal spp. in sugarbeet soil	Fjärestad	Barkåkra	Boserup	Mjöhult
Aphanomyces cochlioides	*	*	*	*
Fusarium avenaceum	*	*	n.f.	n.f.
F. equseti	*	n.f.	n.f.	n.f.
F. oxysporum	n.f.	*	*	*
F. sambucinum	*	*	n.f.	*
F. redolens	*	n.f.	*	*
Pythium spp.	*	*	*	*
P. sylvaticum	n.f.	n.f.	*	n.f.
P. ultimum	*	n.f.	n.f.	n.f.

* = found
n.f. = not found

Results from the pathogenicity test, indicated that some of the tested isolates of *Pythium* gave a higher DSI in sugarbeet compared to the control (Table 22). The test was,

however, done with only two replications and therefore the results have to be interpreted with care.

Table 22. Isolates from sugarbeet and spinach plants in greenhouse and field experiments tested on sugarbeet in greenhouse

Host plant	Isolates	DSI
Control	-	25
Dill	*Pythium sylvaticum* male 02800	18
Spinach	*P. ultimum*?	25
Sugarbeet	*P. ultimum*?	40

Discussion

Manure

Suppression of soilborne pathogens, increased plant weight, yield and plant appearance was achieved with application of manure in this investigation. In all greenhouse experiments, did swine, cattle and poultry manure decrease the DSI. Treatments with liquid swine manure reduced soilborne pathogens the most. Swine manure lowered the DSI from 76 to 50 in greenhouse experiment 3 (Table 13). In another experiment with swine manure, the DSI was decreased from 53 to 37 and the fresh weight increased with 239% (Table 12). All manure treatments significantly increased the fresh weight of the plants compared to the control. Comparing the manure treatments with the mineral fertilizer Probeta NPK, indicates a higher reduction in DSI despite similar nitrogen levels. In greenhouse experiment 1 and 2, had all manure treatments lower DSI than the Probeta NPK treatment, and in experiment 3 were swine manure significant lower than Probeta NPK. However, in experiment 4, had Probeta NPK and swine manure the same DSI but the lowest among treatments.

The results from the field experiment in Strövelstorp indicated that the manure treatments had the same reducing ability in the field as seen in the greenhouse experiments. Both liquid swine and cattle manure reduced root rot in the field, liquid cattle manure had best effect and lowered DSI from 40 to 30. This DSI reduction on 25% was also visible when the yield was measured, which was increased with 18% compare to the fertilized control. Both manure treatments did also increase the plant appearance compared to the control. In the other field experiment at Fjärestad, little infection was observed, and the liquid swine manure did not reduce DSI. The grown field had no history of spinach production. A combination of the history and the favourable weather conditions for spinach production may explain the very low DSI and that no *A. cladogamus* infection was observed. There were also other factors that might have influenced the results in Fjärestad. The field was dry at germination stage, which gave an uneven plant appearance and the experiment was probably influenced by an edge effect when the experiment was located in the outline of the field.

There are also other explanations for varying results on disease suppression. Former experiments have shown that manures worked well in some soils, but had little effect impact in others (Lazarovits, 2001; Conn and Lazarovits, 2000; Conn and Lazarovits, 1999). The manure is a complex substrate that changes dramatically in chemistry and physical properties during storage (Conn and Lazarovits, 2000). In this study is the effect of manure on soilborne pathogens present in different kinds of soils, ranging from sandy loam to loam. But the fact that the effect on soilborne pathogens is manure specific has also been noticed in Swedish field experiments (Pers. com. Olsson, Å.). The results achieved in this study correlates well with the results Nilsson got in 2002, in the study about the effects of organic amendments on *Aphanomyces* root rot of peas. It was then established that a single application of organic amendments to soil could reduce *Aphanomyces* root rot in field experiments and that liquid swine manure had the best ability to reduce the Aphanomyces root rot. In this study on sugarbeet and spinach had also liquid cattle manure a good reducing effect on the soilborne pathogens. Experiment with liquid swine manure in *Aphanomyces* infested sugarbeet fields have been carried out in southern Sweden in 2003 by SBU AB. The results have been varying. In three of four experiments the application of liquid swine manure and Probeta N gave a lower DSI and a higher amount of plant appearance than the treatment with Probeta NPK. In one experiment out of four a higher extraction of sugar was achieved with liquid swine manure and Probeta N (Pers. com. Olsson, Å.). A problem with liquid swine manure application in spring is the narrow drilling window, which often is missed when the manure application is delaying the drilling date. The rain period will then come to close after drilling (Pers. com. Persson, P-O.). The advantages from manure applications are easily lost if the seeding is delayed.

Pythium spp. were found in the fungi isolations from Fjärestad (Table 21). *Pythium* are known to have a pre damping-off effect, which tend to occur before or soon after emergence. Even though no significant difference between the treatments was measured there seems to be a tendency towards a higher plant appearance and yield in the harrowed swine manure treatment. It is possible that the occurrence of *Pythium* was underestimated in the DSI because most losses are pre-emergence and not included. The higher manure content in the surface soil in the harrowed plots is also combined with a higher amount of the common root-inhibiting bacteria *Pseudomonas fluorescens*, which is antagonistic against *Pythium* (Williams and Asher, 1996). In German field experiments manure have proved to increase plant appearance in spinach with 20% and the yield with 77% compared to NPK fertilized control in *Phytophthora cryptogea* infected soil (Beckmann and Kröber, 1974). This might be one reason to the lesser extent of *Pythium* severity and the higher number of appeared plants in plots with harrowed swine manure.

The cruciferous plant treatment lowered DSI but did not increase the yield compare to the control in the field experiment, which can be due to the fact that the plant appearance was reduced compared to the control. It is established that cruciferous plants not just reduce soilborne pathogens but also are phytotoxic to weeds and crop plants planted soon after they have been cultivated into the soil. Plants in the family cruciferous contains glucosinolates, a compound that degrades enzymatically into a variety of different compounds as isothiocyanates, nitriles, thiocyanates, oxazolidinethiones and

epithionitriles, which all may be toxic to microorganisms and plants (Papavizas and Lewis, 1971). The use of cruciferous plants for elimination of soilborne pathogens is an effective way to control fungi, but it is important to use proper tillage and to allow the plants to degrade and become less toxic before seeding the crop plant.

The suppression of soilborne pathogens by manure is probably depending upon several mechanisms. One factor that is believed to reduce the survival of plant pathogens is the production of volatile fatty acids, consisting of acetic, propionic and isobutyric acids, at the degradation of the manure by microorganisms. The kill of microsklerotia and other plant pathogens, was shown by Tenauta and Lazarovits (2002), to be due to the biological production of ammonia (NH_3) and nitrous acid (HNO_2). When soil microorganisms degrade high-nitrogen manure, any nitrogen present in excess of the microorganisms needs is released into soil solution as ammonia. The ammonia converts to ammonium (NH_4^+) which rises the pH. At pH 8.5 or above is the ammonium converted back into the very toxic state, ammonia (Lazarovits, 2001; Tenauta and Lazarovits, 2002; Tenauta *et al*., 2002; Tsao and Oster, 1981). Ammonium is nontoxic to plants or pathogens even in high concentrations (Lazarovits, 2001; Tisdale *et al.,* 1999). The production of nitrous acids is also important for reduction of plant pathogenic organisms in soil. Through bacterial nitrification ammonium is converted into nitrite (NO_2^-) and then to nitrate (NO_3^-). The pH drops during this conversion, and if pH goes below pH 5.5 nitrite will take the chemical form HNO_2, which is extremely toxic to many plant pathogens but also to crop and weed seeds (Lazarovits, 2001). It is the acidity that promotes the protonation and generation of nonionized forms of short chain volatile fatty acids. In an investigation by Conn and Lazarovits (2000) was the effect of liquid swine manure eliminated when the pH of the soil was raised from 5 to 6.5 (Tenauta *et al*., 2002). The soil pH is consequently the crucial driving factor in the production of ammonia and nitrous acids, and the soils buffering capacity plays an important role in regulating whether the active compounds can form or not (Lazarovits, 2001). The active compounds is mostly present in films of moisture around soil or toxicant emanating amendment particles which results in a local soil pH difference and a variation in toxicant presence. An uneven manure distribution in the soil may also lower the toxicant effectiveness (Tsao and Oster, 1981). Even though the soil pH was measured in greenhouse experiment 1 and 2 (Table 11 and 12) and found to be between pH 5.5 and 8 is it not impossible that ammonia or nitrous acids have been produced.

Although manure reduce populations of plant pathogens, overall they lead to an increase in soil microorganisms populations by up to 1000-fold following application, indicating that the manure not is toxic to all microorganisms (Conn and Lazarovits, 1999; Lazarovits, 2001; Lazarovits *et al*., 1999). This population of microorganisms will benefit plant production by acting as reserves of nutrients to be released to the crop as the populations decline (Lazarovits, 2001). The manure is also improving the plant resistance against soilborne pathogens when it contains fertilizers and micronutrients which enhance the plant development and lead to a higher tolerance against fungi (Lazarovits, 2001; Nilsson, 2002).

Another possible reason to the decrease in root rot severity when manure is added to the soil is the microbial population. The general as well as the antagonistic microbial population is significantly increased with a manure application. This will lead to a higher competition for existing nutrients in the soil and increase the effect of antagonistic microorganisms (Campbell, 1989; Conn and Lazarovitz, 1999; Tsao and Oster, 1981). Many researchers have postulated that the mechanism for disease or pathogen control in amended soil is the result of microbial antagonism and biocontrol. In an investigation by Williams and Asher on biocontrol of *Pythium ultimum* and *Aphanomyces cochlioides* were seedling emergence and the proportion of healthy seedlings significantly improved with effective biocontrol isolates (Williams and Asher, 1996). Organisms found to increase, following application of amendments, are among others species of *Pseudomonas, Penicillium, Trichoderma, Bacillus* and *Streptomyces*, all implicated as potential biocontrol agents according to Lazarovits (2001). Conn and Lazarovits found in their investigation about the impact of animal manure's on verticillium, that in all manure treatments that caused a reduction in microsklerotia germination, the microsklerotia were colonized by other fungi, particulary *Trichoderma* spp. (Conn and Lazarovits, 1999).

Tenauta *et al*. (2002) established that volatile fatty acids, found in liquid swine manure, were highly toxic to microslerotia of the soilborne fungus *Verticillium dahliae*. But the effect of volatile fatty acids on survival of soilborne plant pathogens is still unknown, although it might be detrimental to them, acetic acid suppressed infection of citrus seedlings by *Phytopththora nicotianae* (Widmer *et al*., 1998).

The efficacy of manures for reducing soilborne diseases may also depend on many other factors such as soil pH, soil moisture level, buffering capacity and temperature (Conn and Lazarovits, 1999; Conn and Lazarovits, 2000). In literature from 2000 by Conn and Lazarovits it is established that the toxicity of liquid swine manure was highest in dry soil with low pH and reduced with increasing soil moisture and pH. Year 2003 was dry and no dilution effects were present in the field experiments. This theory will bring about less or no reduction of pathogens by the manure in wet years.

The results of this study, although based on just a few infected soils and manure kinds from one specific source each, indicate that manure application can have dramatic impact on the severity of soilborne pathogens. However the impact of manures on soilborne pathogens cannot easily be predicted and factors as manure composition, soil characteristics and numerous others influence the effect on the pathogen populations and their activities. So as long as we do not have effective tools that can rapidly and accurately predict the impact on the pathogens, I think that I agree with Hoitink *et al.* (1993) when they say, "Management of diseases with soil amendments remains an art rather than a science".

Lime

The lime experiments were carried out in greenhouse on sugarbeet. Every treatment with lime did suppress the infection of soilborne pathogens. The highest application of slaked lime six-ton per hectare gave best effect against the pathogens and resulted in the lowest

DSI among lime treatments (Table 13 and 14). DSI was decreased 21% by six-ton slaked lime per hectare compared to the control in experiment 3 (Table 13).

The lime had a large impact on the plant weight. All treatments with lime were unfertilized but the plant weight was increased compared to control in all lime experiments. This increase must be due to the lime application. In experiment three the plant weight increased with 189%. There was unfortunately very low infection in experiment five (Table 15), and the effect of lime on DSI could not be clarified here. Another purpose of experiment 3 was to determine if the time between liming and sowing effected the DSI, but when there was so little infection was it impossible to determine that in this experiment. However there was a massive increase in plant weight by both slaked lime and factory lime compare to the control. The amounts of roots did also increase with an application of lime. This increase was never measured by weight but was great enough to be distinguished by the eye. The increase in plant weight and root mass is probably due to a number of reactions caused by the lime.

Results from the lime experiment in the three years long 4T project, showed that all lime treatments increased the sugar yield even if the mean soil pH (12 experiment sites) was 7.7 and without chemical liming needs. Nine-ton slaked lime gave a stable increase in sugar yield every year. The lime applications did also enhance the soil structure. A positive remaining structure effect was seen in the crop following sugarbeet (Berglund and Blomquist, 2003). A three year long investigation about effects of lime on soilborne pathogens in sugarbeet is run by SBU AB in southern Sweden. Results from the first year, 2003, show that slaked lime is delaying the plant appearance compare to factory lime and control (Pers. com. Olsson, Å. and Persson, L.). Lime has positive effects on soilborne pathogens and the severity is reduced, but it is also affecting the crop plant, in both positive and negative ways. The plant weight is increased by lime but the plant appearance might be later and the number of appeared plants fewer with large lime applications.

A direct benefit of lime, which is very obvious in acid soils, is the reduced activity of Al^{3+} by precipitation as $Al(OH)_3$. Al^{3+} is toxic to plants and restricts the plant uptake of calcium and magnesium (Tisdale *et al*., 1999). The soils used in the experiment were neither acid nor in need for chemical liming. The increased plant weight might instead have depended on other mechanisms like the positive effect on macro nutrient availability, faster decomposition of organic material or the enhanced nitrification, which all occur at higher soil pH (Forbes and Watson, 1996; Tisdale *et al*., 1999). The increase in root masses is explained by the great application of calcium (Ca^{2+}). Calcium is necessary in calcium binding proteins and regulates the activity of a number of enzymes and structural proteins that play a key role in cellular regulation (Kafkafi, 2002).

Lime causes a temporary initial increase in soil pH as seen in experiment seven, which lasts for about 2 to 3 days (Figures 3, 4 and 5) and the same pattern was seen in different types of soils. The increase in soil pH depends on the soils chemical characterization and soil origin. Clay has large surface area and is able to bind many more positive ions than sand or gravel, which have smaller surface area. Organic matter is increasing the ability

to bind positive ions in the soil. That means that higher amounts of lime must be applicated to increase soil pH in soils with high organic matter content (Tisdale *et al*., 1999). The lime application six-ton slaked lime per hectare gave the highest rise in pH as expected because it has a high content of CaO and is a powder with very small grain size, which allows it to react fast and effectively with clay particles in soil. Four-ton limestone resulted in the lowest rise, which also was expected when limestone reacts slowly and consists of large particles (Barrows *et al*., 1968; Kihlstrand and Lundström, 1997).

This temporary initial increase in soil pH to 8 or higher, is also the upper limit for *Aphanomyces* spp. and many other soilborne pathogens to cause disease (Papavizas and Ayers, 1974). The reduction of disease severity in all lime treatments might be a result from previous described mechanisms but may also depend on the unfavorable and sometimes even deathly environment to many fungi, created by the lime. The increase in pH was maybe also accompanied with an increase in ammonia levels. The release of this volatile toxic gas can be involved in reducing population levels of soilborne pathogens in the experiments. It is hard to distinguish which mechanism that is of most importance and under which environmental conditions it works best, as well as it is hard to predict the outcome in reduction of the lime application. Independent of if the effects arose from better soil structure, higher nutrient availability, pathogen lysis or volatile gas production did lime reduce the severity of root rot and increase the plant weight.

There were clear results that the placement of lime in the soil had large impact on the severity of soilborne pathogens. Concentrations of lime should not be to high when it chemically damage the plant by inhibit the plant appearance and plant growth. This was proved in experiment six when 9-ton slaked lime per hectare gave an extremely low plant appearance. But also when 6-ton slaked lime was spread in the topsoil layer of the pot was the same symptom achieved, and the DSI was almost as high as for the control. Six-ton slaked lime evenly spread in the pot profile gave the lowest DSI and the highest plant weight. The results from these experiments show that it is important to use right amount of lime and to place it on at least seed depth where it will give effect against soilborne pathogens.

Applications of manure or lime and growth of cruciferous plants as pre-crop on soils infected with soilborne pathogens might be a way to control fungal diseases if the standards for efficacy can be as those used for chemical pesticides. The goal is to rise or secure the crop yield but can also be a way to reduce the inoculum in the soil for further crop production. It is important that the treatments give good results in quantity or quality or in both in the production, the effect on the yield should be great enough to cover the costs for the treatments. A way of predicting the outcome of the treatments is to make soil tests to establish the nutrient content in the soil and later be able to optimize the input of fertilizers or lime. Test growing in greenhouse, fungi isolations and manure characterisations are also tools that can be used to predict disease severity and the effect of manure in the field. The use of treatments that lowers DSI but not improve yield are not economically justifiably.

It is obvious that many more investigations about using manure or lime as a way to control soilborne pathogens need to be done before anything can be said for sure. But maybe will we use manure or lime in the future to reduce fungal infections by soilborne plant pathogens of our crops.

Conclusion

My conclusions, drawn from literatures and experiments in this study, are following:

- Manure applications have beneficial effects on plant diseases and can reduce soilborne pathogens, among others *Aphanomyces* spp.

- Liquid swine manure has best reducing ability among the tested manure types.

- Manure increase the number of appeared plants and the plant weight.

- Cruciferous plants have potential to reduce soilborne pathogens in field, but have also a phytotoxic effect on both crop plants and weeds.

- Lime applied to soil can reduce the severity of soilborne pathogens.

- Slaked lime, six-ton per hectare, has the best ability to reduce pathogens. But has also a phytotoxic effect on the crop plant that may delay plant appearance.

- The placement of lime in soil has a great impact on the effect on soilborne pathogens. To achieve best effect should lime be placed on at least seed depth.

- Lime applicated to soil increases soil-pH several units for some days, which creates an unfavourable environment for the soilborne fungi that even might be deathly to them.

Acknowledgements

In the spring of 2001 I took my first course in plant pathology and my interest in pathology was awakened. Since then questions about this subject has interested me the most and it was naturally to accomplish my master thesis in this subject. I also had the privilege to work with questions inquired by the commerce, which made it even more interesting.

I would like to thank my supervisor Doctor Lars Persson for his help in the practical area and for his help with the scientific writing. I also would like to thank Doctor Åsa Olsson, SBU AB, and Doctor Mariann Wikström, Findus R&D AB, for valuable guiding and advises during my experiments. And for the help with the statistical analyses I would like to thank my examinator Lennart Johnsson.

This study would not have been possible to do without access to greenhouse, laboratory, proper equipment and field machines. That is why I would like to thank Findus R&D AB. And I would also like to thank all employees at Findus R&D for important technical assistance.

Many people helped me to get materials to the experiments and with assistance in them and I would like to thank all of you very much.

Findus R&D AB and Sockernäringens BetodlingsUtveckling AB financed the experiments.

Anna Ingemarsson

Uppsala, May 2004

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