#### Ecology and Epidemiology

# Disease Progression and Yield Losses from Root Diseases Caused by Soilborne Pathogens of Spinach

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Accepted for publication 4 November 1991 (submitted for electronic processing).

### **ABSTRACT**

Larsson, M., and Gerhardson, B. 1992. Disease progression and yield losses from root diseases caused by soilborne pathogens of spinach. Phytopathology 82:403-406.

Severity of spinach diseases caused by soilborne pathogens, mainly Aphanomyces cochlioides, but also Phytophthora cryptogea, Pythium spp., and Fusarium spp., was determined in a total of 23 commercial fields located in southern Sweden. Disease progress curves increased rapidly from emergence to when the cotyledons and the first pair of true leaves were fully developed. Thereafter, disease did not increase. A field disease severity index (ranging from 0 to 100), read after plant developmental stage 2 (one true leaf pair), was correlated with spinach

yield. An incremental increase in field disease severity index corresponded to a yield loss of approximately 1% or 210 kg/ha. Soil treatment with dazomet, which breaks down to release methyl isothiocyanate in soil, resulted in yield increases of 100-150% in fields with established pathogen populations. However, only small yield increases were obtained in fields in which spinach was grown for the first time. Average field disease severity indices for spinach grown in monoculture, in rotation with other crops, and for the first time in a field were 39, 31, and 19, respectively.

Additional keyword: Spinacia oleracea.

Soilborne diseases in spinach (Spinacia oleracea L.) are a serious constraint to spinach production in the area of intensive production in southern Sweden. Most often, these diseases cause symptoms of seedling damping-off, stunted growth, foliar chlorosis, and dark roots in older plants. Severe infestations result in decreased spinach yield and inferior crop quality. Observations in commercial growings give strong indications that weather conditions as well as compacted soil play a decisive role for disease outbreaks. Numerous pathogens have been implicated as causal agents with this soilborne disease and yield decline problem. Those reported are several Pythium spp. (2,5,11,13,20), which cause preand post-emergence damping-off; Rhizoctonia solani Kühn, which may cause both seedling damping-off and foot rot (2,14,20); Aphanomyces cochlioides Drechs. and Phytophthora cryptogea Pethybr. & Lafferty, which cause root rots of older plants (1,2,10). In addition, isolations of Fusarium oxysporum Schlechtend.:Fr. f. sp. spinaciae (Sherb.) W. C. Snyder & H. N. Hans. from wilting spinach plants have been reported from Japan (14), the United States (4,20), Canada (e.g., 17), and Australia (21).

Extensive surveys (to be published), in which root pieces from field plants were plated on selective agar media, have shown that A. cochlioides is the predominating spinach root rot pathogen in Sweden. It was isolated from about 90% of the fields and about 40% of the roots sampled. Further, in a typical field, P. cryptogea, pathogenic F. oxysporum, and Pythium spp. (mainly P. ultimum Trow and P. sylvaticum W. A. Campbell & J. W. Hendrix) each occurred on 5-10% of the roots. The pathogen frequencies were rather stable from year to year, and the distributions within fields showed small variations. Multiple infections with two or more of the pathogens mentioned are common, but the importance of interacting pathogen populations is unknown.

The purpose of this paper was to determine the disease progression of spinach root rot during the growing season, yield loss caused by the disease in commercial spinach production, and potential effects of soil fumigants and cropping history on disease severity.

## MATERIALS AND METHODS

Field surveys. Plant samples were taken during 1981–1982 and 1987–1991 from a total of 23 fields in which spinach was grown commercially. Normally, two crops per year are grown; the first crop is sown in April and the second in late July or early August. The soils in sampled fields were sandy loams or, in a few cases, sands. Soil pH values were between 6.5 and 7.0. All the fields were sown with the cultivars F1, F3, F4, and F9 (supplied by the spinach breeding program of Nordreco AB, Bjuv, Sweden) by using commercial seed drills with a row spacing of 12.5 cm, which gave a plant density of about 40 plants per meter of row. All harvesting in the commercial fields was done with a spinach reaping machine.

Yield and crop rotation data from sampled fields were obtained from field managers for Swedish Nestlé AB. For analysis of correlation between cropping frequency and disease severity indices, the fields were divided into three groups: monoculture (spinach had been grown continuously twice a year for at least 5 yr; n = 20); crop rotation (spinach had been grown at least once previously, but at least one other crop had preceded the spinach crop that was sampled; n = 21); and virgin (spinach had never been grown before; n = 4).

Assessments of root damage on field plants. The plants assessed were sampled during 1987-1990 by carefully digging up all plants along a 0.5 m stretch of row (about 20 plants) at 10 randomly chosen locations in each field sampled. The uprooted plants were placed in plastic bags and taken to the laboratory, where the roots were washed thoroughly by hand. After this, severity of root rot was assessed by giving each spinach plant a field disease severity index between 0 (= healthy plants) and 100 (= dead plants) according to the procedure described by Larsson and Gerhardson (12). The same person made all disease ratings, and a mean value of the severity index was calculated for each field. In the 1987-1989 seasons, plant samples were taken two to four times in each growing season at various plant developmental stages. In spring 1990, four fields with pathogens occurring in frequencies typical for the area were chosen for sampling in shorter intervals. These fields were sampled every second or third day during the early plant developmental stages and, later on, twice a week (a total of 9-11 samples per field). The plant developmental stages were designated as number of leaf pairs fully developed; the fully developed cotyledons were designated as plant developmental stage 1. Harvest normally occurred when seven to eight leaf pairs were developed.

Relationship between disease severity and yield. In determining the relationship between disease severity and yield data from commercial fields, only disease ratings obtained from plants sampled after developmental stage 2 were used, because after this developmental stage, values of severity indices remained relatively stable. Also, the relationship between disease severity and fresh weights of individual plants was determined. Samples of 150 plants per field were taken in two different fields in spring 1990 and in autumn 1991 at normal harvest time. Disease severity index of each plant was read, and the whole above-ground portion of the plants was weighed. From one of these fields, there were plants from the rating classes with indices 25, 50, and 75, and from the other field all rating classes with indices 0–100 were represented.

Field experiments with soil treatment. To assess yield losses caused by soilborne pathogens, the chemical compound dazomet (3,5-dimethyltetra-hydro-1,3,5(2H)-thiadiazine-2-thione), Basamid formulation, was used for disinfestation of experimental plots. The compound hydrolyzes to methyl isothiocyanate (MIT), formic aldehyde, and hydrogen sulfide in contact with moist soil (22). The dazomet was incorporated into soil to a depth of 20-25 cm with a rotovator, and no soil covering or packing was used after treatment. Similarly, control plots were cultivated with a rotovator. Dazomet-treated soil was considered to be free of the compound and its decomposition products, when seeds of cress (*Lepidium sativum* L.) germinated normally in soil sampled periodically after treatment. Most fields were considered to be safe for sowing spinach about 4 wk after treatment.

The experiments had a randomized block design with a plot size of 500 m² (1981), 200 m² (1982), or 25 m² (1988 and 1989). Three experiments were in fields in which spinach was grown for the first time (virgin), and six were in fields with documented root rot in previous spinach crops (monoculture). In 1981 and 1982, spinach yields were obtained by weighing the fresh spinach from two subplots, 20 m² each, randomly marked out in each plot and harvested with a mowing machine. The yields were obtained in the same way in 1989, but then only one subplot per plot was used. In 1988, all plants along 0.5 m of a row at

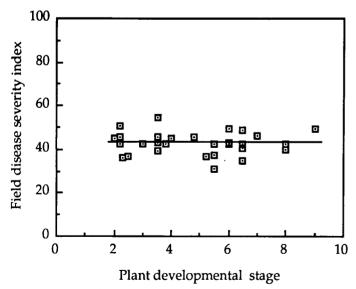


Fig. 1. Relationship between field disease severity index after growth stage 2 and plant developmental stage. The curve shown is a regression line calculated from disease progress curves obtained from 16 fields read in 1987-1989 with two to four readings per field. Before the regression line was drawn, all readings done before plant developmental stage 2 were omitted, and the disease progress curves were transformed to the average disease level of all these fields to eliminate differences in disease levels between fields. The coefficient of determination is 0.0003.

two randomly selected places in each plot were dug up, placed in plastic bags, and taken to the laboratory in which the harvestable portion of the plants was cut off and fresh and dry weights (after drying at 105 C for 24 h) were recorded. Disease ratings were not done in 1981-1982, but in 1988 and 1989 the severity of root rot was examined at normal harvest time. In one of the experiments in 1988 and in two in 1989, the yields and the severity indices were also determined twice before normal harvest time, at plant developmental stages 2-3, and at stage 4-4.5. The procedure for determining these early yields was the same as for the final yields in 1988.

### RESULTS

Field surveys. Disease severity increased rapidly up to about plant developmental stage 2 and then leveled off in all the fields tested (Figs. 1,2). The average disease severity indices for all fields

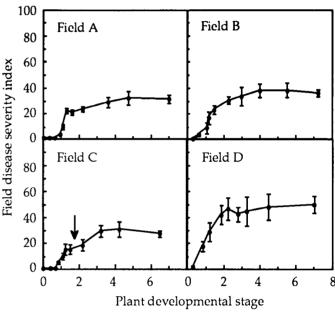


Fig. 2. Disease progress curves for four spinach fields sampled in spring 1990. Each value is an average of 10 means, each based on about 20 plants. The arrow in field C indicates the time of an irrigation followed by a heavy rain. Bars show standard deviation.

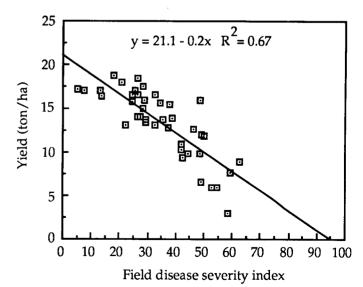


Fig. 3. Relationship between field disease severity index and spinach yield. Severity index read after plant developmental stage 2 compared with yields obtained from commercial spinach production in 20 fields in spring and autumn crops in 1987–1990.

TABLE 1. Effect of soil treatment with dazomet on spinach yield and disease severity index in treated and control plots in nine field experiments

Field type	Year	Season	Number of replicates	Rate of dazomet (kg/ha)	Yield increase from dazomet treatment (%)	Disease severity index	
						Treated plots	Control plots
Virgin	1982	Autumn	2	300	$8.9 \pm 7.4^{a}$	NR <sup>b</sup>	NR
	1988	Spring	8	350	$29.8 \pm 6.2$	$23.6 \pm 1.9^{a}$	$27.2 \pm 1.7^{a}$
	1988	Spring	8	350	$20.0 \pm 7.3$	$12.4 \pm 1.5$	$13.6 \pm 2.2$
Monoculture	1981	Autumn	2	300	$455.0 \pm 9.0$	NR	NR
	1982	Autumn	2	300	$81.8 \pm 2.4$	NR	NR
	1988	Spring	8	350	$53.2 \pm 5.8^{\circ}$	$29.5 \pm 3.0$	$79.7 \pm 0.7^{\circ}$
	1988	Spring	8	350	$74.8 \pm 12.5$	$36.4 \pm 1.9$	$62.8 \pm 0.9$
	1989	Autumn	4	350	$87.5 \pm 6.7^{\circ}$	$39.4 \pm 3.5$	$72.3 \pm 1.4^{\circ}$
	1989	Autumn	4	350	$167.0 \pm 24.7^{\circ}$	$37.0 \pm 2.8$	$75.9 \pm 1.6^{\circ}$

<sup>&</sup>lt;sup>a</sup>Standard error of the mean.

surveyed during 1987-1989 were 30.8 for spring crops and 36.1 for autumn crops. The slightly higher average for autumn crops compared to spring crops occurred every year but was not statistically significant (P = 0.349). Average yields for the same fields were 14,300 and 11,900 kg/ha for spring and autumn crops, respectively; this difference in yields was not significant (P = 0.053).

A good linear relationship ( $R^2 = 0.67$ ) was found between the severity indices read after plant developmental stage 2 and the yields obtained in commercial spinach fields for the years 1987-1990 (Fig. 3). In all the fields investigated, the average severity index was 34, which corresponded to an average yield loss of about 35% (i.e., about 1% yield loss per incremental increase in severity index). A similar relationship was obtained between severity indices of individual plants sampled from the same field and plant yield. The resulting regression equations based on samples from two fields with 150 observations in each showed that an incremental increase in severity index approximately corresponded to a plant yield loss of 1% by weight ( $R^2 = 0.82$  and 0.68, respectively).

Field experiments with soil treatment. Soil treatment with dazomet resulted in an average yield increase of 100-150% within monoculture fields, whereas virgin fields averaged only a 20% increase with dazomet treatment (Table 1). Dazomet treatment similarly resulted in markedly decreased disease severity indices in the field group monoculture but not in the field group virgin. Dry matter was on average 8.2% in plants grown in untreated plots and 6.8% in the plants grown in dazomet-treated plots, giving a difference in dry matter of 1.4%, which was highly significant (P=0.0001). The difference in weight of the harvestable plant portion between dazomet-treated and control plots decreased with time (Fig. 4), indicating a compensating effect.

Influence of cropping history on disease severity. There was no statistically significant difference in severity indices between the monoculture (average severity index 39) and crop rotation (average severity index 31) field groups (P = 0.074), and neither between the crop rotation and virgin (average severity index 19) field groups (P = 0.138). However, a significant difference in severity indices was obtained between the monoculture and virgin fields (P = 0.007).

### DISCUSSION

The assessment of disease severity (the amount of tissue damaged), as used in this study for following disease progression, is a rather more subjective approach than readings of disease incidence (the number of plant units infected). However, a reading of disease incidence, which has been extensively used in studies of soilborne diseases (e.g., 6,7), would have been impracticable in this case, because a spinach root system without some root rot was very rare and in many fields the disease incidence would have become 100% before the cotyledon stage of the plants.

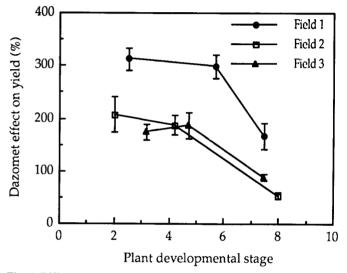


Fig. 4. Differences (%) in yield (fresh weight of the harvestable portion) between spinach plants from dazomet-treated plots and from control plots at various plant developmental stages. The three curves show the effect of dazomet treatment in three different field experiments, all placed in monoculture fields. Bars show standard error of the mean.

Further, the relationship between our severity indices and the fresh weights of the whole above-ground portion of individual plants was good ( $R^2 = 0.82$  and 0.68 in replicated experiments), which gives good reason to regard the method used as satisfactory.

The disease progress curves obtained from the four fields sampled at short intervals in 1990 show a sigmoid shape with a rapid increase at early developmental stages (Fig. 2). This curve form agrees with the curves found for other root rots (e.g., Aphanomyces root rot of peas) (16). The reduced rate of the disease progress after developmental stage 2 (Figs. 1,2) probably is the result of a balance between continual root production by the plant and new infection by the pathogen(s). Such a balance would be pathogen-dependent, and A. cochlioides, the main pathogen found in the fields sampled, may have a mode of infection fitting this theory (15). The high incidence of infection found gives some reason to assume that under environmental conditions favorable to disease (e.g., heavy rain or irrigation) disease progression might continue to proceed at a positive rate after plant developmental stage 2. An indication of this is seen at the arrow in the curve of field C (Fig. 2), which progresses more rapidly after an irrigation followed by a heavy rain. However, the disease progress curve for field C was an exception, and other dramatic rises in severity indices after developmental stage 2 were not observed during the 4 yr of investigation. Given this result, we conclude that it should be possible to use a severity index taken any time after developmental stage 2 for estimating yield.

<sup>&</sup>lt;sup>b</sup>Disease severity index not read.

<sup>&</sup>lt;sup>c</sup>Control plots fertilized with an extra 40 kg of nitrogen per hectare to compensate for the nitrogen supplied by killed organisms in the dazomettreated plots.

The good relationships found between severity indices and fresh weights of individual spinach plants and between severity index and yield obtained in the commercial growing of spinach (Fig. 3) offer a possibility to extrapolate and in this way estimate a "disease-free" yield (i.e., yield of visibly healthy plants with severity index = 0) and also to calculate a theoretical yield loss. Assuming a straight line, such an extrapolation of the curve in Figure 3 gives an estimated disease-free yield in commercial spinach fields of about 21 tons/ha. The data from the control plots in the dazomet field experiments indicate that the disease-free yield could be up to 3 tons more per hectare. This difference probably depends on a more efficient cutting technique with lower stump height in the experiments. An estimated disease-free yield of healthy plants in the dazomet-treated plots gives a value of about 30 tons/ha (i.e., about 6 tons higher than in the control plots a difference that has no obvious explanation). It may depend on increased nutrient levels in the dazomet-treated plots, due to higher mineralization in these plots. It may also depend on the occurrence of pathogens causing minor or invisible disease symptoms (i.e., minor pathogens sensu Salt (19), which are affected by dazomet but do not affect the severity index used). Experimental evidence favouring any of these theories is, however. lacking.

The average yield increase of 100-150% after dazomet treatment in the monoculture fields (Table 1) corresponded to yield losses of 50-60%. However, this figure is probably an underestimation, because the severity indices in the dazomet-treated plots were never zero, but regularly about 35 (Table 1), which points to survival of some pathogens. In estimating the same yield losses from the severity index levels in untreated plots (approximately 70 from Table 1), a somewhat higher loss is also found. Assuming one severity index unit approximately corresponds to a yield loss of 1% by weight (Fig. 3), the estimate gives a yield loss of around 70%. These estimated losses correspond well to the yield losses of about 50% or more obtained in other crops in which comycetous pathogens within the genera Aphanomyces, Phytophthora, and Pythium have caused root rots (e.g., 3,9,16,18,23).

The yield of plants grown in dazomet-treated plots expressed as a percentage of control plot yield markedly decreased with time (Fig. 4). This decrease may be caused by a reinfestation of the treated plots. However, as the severity indices did not show a corresponding increase, we regard this explanation as unlikely. Another more probable explanation is that the competition for space between plants became stronger in the dazomet-treated plots because these plants grew better, and more plants survived. If true, this explanation also means that our measures of using the same amount of seeds in all plots and harvesting the experiments when the untreated plots are ready for harvest may both have resulted in an underestimation of the effect of the dazomet treatment on yield. The significant difference in percentage of dry weight (1.4%) found between the plants grown in dazomet-treated plots and those grown in control plots probably is a result of an impaired water uptake in the more diseased plants in control plots, as was earlier indicated for P. cryptogea

The disease severity is partly dependent on cropping history, because there was a significant difference between the severity indices in monoculture fields and in virgin fields (average severity indices 39 and 19, respectively). However, the lack of significant differences between the field groups monoculture and crop rotation supplies some evidence that a crop rotation has a limited influence on the inoculum of the soil pathogens. This in turn implies that the pathogens involved are able to survive for a long time in soil and/or have a wide host range. Several pathogens within the genera concerned, Aphanomyces, Phytophthora, Pythium, and Fusarium, have one or both of these characters (8). Recent research has further shown that P. cryptogea, in addition to spinach, also infects crops like wheat, oats, oil-seed rape, and peas (12), and that the spinach pathogen, A. cochlioides, has some common weeds as hosts (unpublished data). From this, we conclude that the pathogens concerned are only partly dependent on cropping frequency and accordingly, unless very long intervals are used, crop rotation is not an effective means of controlling spinach root rot.

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