Relation between Spatial Variability of Plant Physiological Parameters in Oilseed Rape and Infection with *Leptosphaeria maculans*

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With 2 Figures

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Summary


Since the 1980s severe sulphur deficiency has been recorded in agricultural crops; this is meanwhile the most widespread nutrient disorder in northwestern Europe. An insufficient sulphur supply reduces not only crop yield and diminishes crop quality, but it also results in a higher susceptibility to certain diseases. In sulphur response trials with oilseed rape, potatoes and grapes, resistance to fungal pathogens could be induced by soil-applied sulphur. The identification of metabolites and pathways involved in sulphur-induced resistance (SIR) is crucial in order to prompt resistance mechanisms under field conditions.

Geostatistical analysis was applied to acquire information about the small-scale spatial variability of plant characteristics and the probability of severe infections of oilseed rape with *Leptosphaeria maculans* in a field experiment as a means to outline

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the impact and inter-relationship of unknown and given factors that influenced the resistance of the plants and that remain hidden when using traditional statistical procedures.

The fungal infection severity of oilseed rape with *Leptosphaeria maculans* varied spatially and the probability of a strong infection was higher for the cultivar *Lipton* than for the cultivar *Bristol*. Spatial patterns for the probability of severe fungal infections matched that of the sulphur status of the crop in such way that a higher risk was related to a lower sulphur status and lower glucosinolate content. An inverse pattern was found for the glutathione concentration.

**Introduction**

In 2001, a total 33.663 t of pesticides were consumed by German agriculture with 8.246 t being fungicides (Anonymous 2002). Today's con-
sumers are becoming more and more concerned about pesticide residues in food. Research on nutrient induced resistance is a great challenge to reduce or forgo pesticide applications. Interactions between mineral elements and plant diseases are well known for essential macro and micro plant nutrients, and aluminium and silicone (Datnoff & al. 2004). Though the significance of individual nutrients for maintaining or promoting plant health saw some interest in the 1960s and 1970s (Bergmann 1983), research in the field of nutrient induced resistance mechanisms has been scarce because of its complexity and limited practical significance due to the availability of effective pesticides.

The problem of increased infections of Brassica napus with Pyrenopeziza brassicae started when atmospheric sulphur (S) depositions were drastically reduced after clean air acts came into force and the cultivation of double low oilseed rape cultivars. Double low cultivars differ from single low cultivars in their glucosinolate content (Schnug 1988), the leaf myrosinase activity (Porter & al. 1991), the ability to accumulate glucosinolates in response to damage by fungal pathogens (Doughty & al. 1991) and the restricted potential to recycle S bonded in glucosinolates (Schnug & Haneklaus 1994). Thus, double low cultivars are particularly sensitive to insufficient S supply (Schnug & Ceynowa 1990, Paul 1992, Schnug & Haneklaus 1994). Under the conditions of S deficiency in field experiments soil-applied S fertilisation significantly reduced the infection of oilseed rape with light leaf spot (Pyrenopeziza brassicae), of grapes with powdery mildew (Uncinula necator) and of potato with stem cancer (Rhizoctonia solani) (Schnug & al. 1995a, Bourbos & al. 2000, Klikocka & al. 2004). Soil-applied S reduced infection rate and infection severity with Rhizoctonia solani on potato tubers by 41% and 29%, respectively (Klikocka & al. 2004). Sulphur fertilisation reduced the number of spots on leaves of grapes and the rate of infected berries with Uncinula necator by more than 80% (Bourbos & al. 2000). These results clearly indicated that S metabolism of plants is closely linked with their natural defence against diseases. However, the mechanisms of sulphur-induced resistance (SIR) are not yet fully understood. Metabolites and processes, which are putatively involved in SIR, are glutathione, phytoalexins and glucosinolates, and the release of reduced gaseous S compounds (Haneklaus & al. 2004).

The high spatio-temporal variability of plant and environmental factors under field conditions hampers the identification of mechanisms involved in SIR, their interactions and possible triggers. Exploiting SIR in agriculture depends, however, on prompting SIR in the production field. In this study geostatistical analysis was performed to head straight to this target by assessing spatial patterns for the risk of severe
fungal infections and their link to the spatial variation of plant parameters.

Geostatistics have been successfully applied in mining for describing quantitatively the spatial distribution of ores, precious metals and diamonds (Krige 1966, Journel & Huijbregts 1997), in soil chemistry for assessing the spatial variability of soil characteristics (Webster & McBratney 1987), and in environmental pollution for estimating ozone exposure parameters (Lefohn & al. 1988). In plant pathology geostatistics were applied on plot and field scale for the analysis of the spatial variation of plant diseases (Chellemi & al. 1988, Larkin & al. 1995, Wu & al. 2001, Morgan & al. 2002) such as the evaluation of the spatial distribution and simulation of insect populations (Ribes-Dasi & al. 2001) and the prognosis of the spreading of plant virus diseases (Nelson & al. 1994).

It was the objective of this study to investigate coincidental spatial patterns of the risk of severe fungal infections of oilseed rape with Leptosphaeria maculans and the spatial variation of physiological plant characteristics for an advanced understanding of factors governing the pathogenesis in dependence on the S supply under field conditions.

Materials and Methods

A quadri-factorial field experiment was conducted on a Cambisol in 2001/2002 in Braunschweig, Germany (52°18' N, 10°27' E). The field trial was conducted in plots of 60 m² in seize with four replicates. Plots were arranged in a completely randomised block design.

Two oilseed rape (Brassica napus) cultivars were grown: Bristol, which was rated as susceptible to light leaf spot (Pyrenopeziza brassicae) and resistant to stem canker (Leptosphaeria maculans) (Gladders & al. 1998) and Lipton, which was rated as resistant to light leaf spot and susceptible to stem canker (HGCA 2003). For defining the growth stages (GS) of oilseed rape the BBCH scale was used (Strauss & al. 1994).

S was supplied as potassium sulphate (K₂SO₄) at rates of 0 and 150 kg S ha⁻¹. The S rates were split as follows: 40 kg S ha⁻¹ before sowing (GS 01), 40 kg S ha⁻¹ at growth stage 12 and 70 kg S ha⁻¹ in spring at the start of the vegetation period (GS 19). The K supply was balanced by adding adequate amounts of KCI. N was supplied as ammonium nitrate (NH₄NO₃) at rates of 100 and 200 kg N ha⁻¹. The N dose was split in two application rates, the first being applied at the start of vegetation period (GS 19) and the second at beginning of stem elongation (GS 50-53).

Fungicides with the active ingredients carbendazim plus flusilazole, vinclozolin and iprodion were used (Anonymous 2004). Flusilazole (250 g L⁻¹) plus carbendazim (125 g L⁻¹) was applied against infections with P. brassicae, vinclozolin against stem rot (Sclerotinia sclerotiorum) and iprodion against stem rot (Sclerotinia sclerotiorum), dark pod spot (Alternaria brassicae) and L. maculans. In the plus fungicide treatment of the experimentation the plots received all of these fungicides, while in the minus fungicide treatment no flusilazole nor carbendazim was applied. There-
fore, the plus fungicide plots received 1 L ha\(^{-1}\) flusilazole and carbendazim in autumn (GS 14–15), and at the beginning of flowering (GS 50–53) all plots were treated with 1.5 kg ha\(^{-1}\) vinclozolin and 3 L ha\(^{-1}\) iprodion.

In autumn 2001 infection of oilseed rape with \textit{L. maculans} was observed in all plots. The infection severity of oilseed rape with \textit{L. maculans} was visually rated in the field at growth stage GS 14–15 by counting the number of fungal lesions on leaves in an area of 1.0 x 0.5 m. For each plot 5 separate counts were made and the mean score value was determined. The infection severity was scaled as follows: 1–2 = low (1–2 spots per leaf); 3 – 5 = medium (3–5 spots per leaf); 6 = high (>6 spots per leaf).

Younger, fully differentiated leaves of oilseed rape were taken randomly from each plot at the beginning of stem elongation (GS 50–53). Rating of the fungal infection severity and plant sampling were carried out at different dates for the following reason: S induced resistance is supposedly highest at a stage of high metabolic activity and should be distinctly expressed in young, fully expanded leaves in spring (SCHNUG & HANEKLAUS 1998). Metabolic modifications produced by infections with \textit{L. maculans} in the early growth phase, and varying in dependence on the S supply should therefore be pronounced in the sampled plant tissue.

Leaf samples were either shock-frozen in liquid nitrogen and freeze-dried, or dried in a ventilated oven at 60° C until constancy of weight. The glutathione and glucosinolate content were determined by HPLC analysis according to HELL & BERGMANN 1990 and ROSA 1992, respectively.

The oven-dried samples were ground to a particle size < 0.12 mm using a Retsch ultra-centrifugal mill for further analysis. The total S content was determined by X-ray fluorescence spectroscopy (X-RF) according to SCHNUG & HANEKLAUS 1999.

For statistical analysis the SPSS software package version 10 was used (BÜHL & ZÖFEL 1994). Statistically significant differences between means were determined by the t-test.

Geostatistics (variography) describe the spatial relationship between the measurements of variables at different sampling locations (MATHERON 1971, JOURNEL & HUIJBREGTS 1997). Basic information about geostatistics were provided by ISAAKS & SRIVASTAVA 1989, JOURNEL 1989 and GASSNER & SCHNUG 2003.

The semi-variogram provides a measure of spatial auto-correlation by describing how sample data are related depending on distance and direction (ISAAKS & SRIVASTAVA 1989). The basic theory behind geostatistical analysis is that with increasing distance between two sampling points the variance increases (Fig. 1). The average squared differences between paired data values (y(h)) is plotted against h, the distance or lag separating the pairs (Fig. 1). Crucial criteria of a variogram are the nugget effect (C\(_0\)), the sill (C\(_0\) + C\(_j\)) and the range (a) (Fig. 1). The variance at the distance zero reveals the unexplained variance and is called nugget effect (Fig. 1; KRIE 1966). The minimum distance at which the maximum variance in the entire sample population can be found is called range (Fig. 1).

For the semi-variogram analysis the Variowin software package version 2.2 was employed (PANNATIER 1996). For each plot (3 x 20 m) coordinates of the centre point were taken for geocoding and the determined value for individual parameters assigned.
A quantification of the contribution of random variation to the semi-variance observed in a data set can be estimated from the ratio of nugget semi-variance to sill semi-variance (NR) (TRANGMAR & al. 1985):

\[ \text{NR} = \frac{C_0}{C_0 + C_1} \times 100 \]

This ratio was used to describe the strength of the spatial dependency within the field (CAMBARDELLA & al. 1994), and NR values of ≤25% indicate a strong, values of 25% < NR < 75% a moderate and values of NR ≥75% a weak spatial dependency.

Based on the results from (semi)variogram analysis, data points between sampling locations can be interpolated (ISAAKS & SRIVASTAVA 1989). The semi-variogram model information was used to create maps showing the spatial variability of plant parameters by employing the kriging algorithm in the Surfer software package (KECKLER 1995). For geo-referenced data that showed no autocorrelation, such as glucosinolate and total S content, maps were created by using the inverse distance algorithm. For the infection severity with L. maculans, which is a descriptive, categorical variable, the indicator kriging procedure was applied and a so-called probability map was produced revealing the probability of severe infections with L. maculans (JOURNEL 1983). For the indicator kriging procedure each observed score value for fungal infections z(x) was transformed to an indicator value I(x, score) according to MARINONI 2003:

\[ I(x, \text{score}) = \begin{cases} 1, & \text{if } z(x) = \text{score} \\ 0, & \text{otherwise} \end{cases} \]

I(x, score) is the transformed indicator at location x for a specified score value. Following the above transformation, measured values for the infection severity were transformed to indicator variables by scoring them either as 1 or 0. At every location
where the observed fungal infection severity $z(x)$ had a specified score value of 6, an indicator value of 1 was assigned, which is equivalent to a 100% risk of a severe infection. All other score values were assigned the indicator value of 0 (0% probability of a severe infection). The spatial continuity of the transformed indicator values was modelled by variography, and then used to create a kriged interpolation surface.

**Results**

Putative metabolites involved in SIR include among others glutathione and glucosinolates. Glutathione is one regulating chemical in defence mechanism and a relationship between glutathione content and the extent of protection against fungal diseases was found (GULLNER & KÖMIVES 2001). Glucosinolates are low-molecular-mass N and S-containing secondary compounds, which are synthesized by some plant species for increasing their resistance against unfavourable effects of predators, competitors and parasites because they exhibit toxic or repellent effects (MITHEN 1992).

The influence of S and N fertilisation, cultivar and fungicide treatment on fungal infection rates, total S, GSH and GSL content was determined and results are presented in Table 1.

**Table 1**

<table>
<thead>
<tr>
<th>Treatment/Variable</th>
<th>Total S (mg g$^{-1}$)</th>
<th>GSH (μmol g$^{-1}$)</th>
<th>GSL (μmol g$^{-1}$)</th>
<th>Infection severity$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td>S (kg ha$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>4.78</td>
<td>23.4</td>
<td>5.45</td>
<td>4.18</td>
</tr>
<tr>
<td>150</td>
<td>9.29</td>
<td>20.4</td>
<td>5.92</td>
<td>4.21</td>
</tr>
<tr>
<td>N (kg ha$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>100</td>
<td>7.00</td>
<td>23.3</td>
<td>6.06</td>
<td>4.17</td>
</tr>
<tr>
<td>200</td>
<td>7.07</td>
<td>20.4</td>
<td>5.31</td>
<td>4.22</td>
</tr>
<tr>
<td>Fungicide</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>-</td>
<td>7.04</td>
<td>22.9</td>
<td>5.70</td>
<td>4.19</td>
</tr>
<tr>
<td>+</td>
<td>7.03</td>
<td>20.8</td>
<td>5.67</td>
<td>4.19</td>
</tr>
<tr>
<td>Cultivar</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bristol</td>
<td>6.93</td>
<td>22.2</td>
<td>5.46</td>
<td>3.73</td>
</tr>
<tr>
<td>Lipton</td>
<td>7.14</td>
<td>20.5</td>
<td>3.91</td>
<td>4.66</td>
</tr>
<tr>
<td>LSD$_5$ %</td>
<td>0.35</td>
<td>5.5</td>
<td>1.31</td>
<td>0.36</td>
</tr>
</tbody>
</table>

*Note: *scores for infection severity (6 = maximum; 1 = minor)

Sulphur fertilisation increased significantly the total S content in the vegetative tissue of oilseed rape. The total S content increased from 4.8 mg g$^{-1}$ in the control plots to 9.3 mg g$^{-1}$ in S fertilised plots. On the other hand, the glutathione and glucosinolate content, and the infection severity were not significantly influenced by S fertilisation. Nitrogen fertilisation and fungicide treatment had no significant influence on the investigated para-
meters. No significant differences were found between the susceptible cultivar *Lipton* and the resistant cultivar *Bristol* with respect to the total S content, GSH and GSL concentration. But, the infection severity of the cultivar *Lipton* was significantly stronger than that of *Bristol*. The correlation analysis revealed no significant interactions between factors. The results from traditional statistical analysis reveal no involvement of glutathione and glucosinolates in pathogenesis.

Geostatistical analysis was carried out in order to determine spatial patterns for the risk of severe infections with *L. maculans* and plant parameters. The results from variography are summarised in Table 2.

**Table 2**

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model</th>
<th>Nugget ((C_0))</th>
<th>Sill ((C_0 + C_1))</th>
<th>Range ((a))</th>
<th>Nugget/Sill ((C_0/C_0 + C_1))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glutathione</td>
<td>Spherical</td>
<td>52.2</td>
<td>102.2</td>
<td>8.26</td>
<td>0.510</td>
</tr>
<tr>
<td>Probability of severe infections</td>
<td>Spherical</td>
<td>0.060</td>
<td>0.130</td>
<td>7.60</td>
<td>0.460</td>
</tr>
</tbody>
</table>

For the parameters total S and glucosinolate content, no spatial correlation was found, which reveals that these parameters vary over shorter distances than the given lag distance. For the variables glutathione and infection severity a range of about 8 meters was determined (Table 2). The nugget/sill ratio provides an estimation of the random variation in the data and with NR values of 51% and 46% for glutathione and the risk of severe fungal infections, respectively a moderate spatial dependency is revealed.

Figure 2 reflects the small-scale spatial variability of the total S, glutathione and glucosinolate content and the probability of severe fungal infections in oilseed rape with *L. maculans* on an experimental field. The digital maps were created by interpolating data for glutathione by kriging, for total S and glucosinolates by employing the inverse distance procedure, and for the risk of high infection severity by indicator kriging (Fig. 2).

The probability of a severe infection differed widely on the experimental site with values from 20 to 60%. In the southeastern parts of the field the risk was distinctly higher than in the northern and western area. In general, the risk of severe infections was higher for the cultivar *Lipton*. On plots where *Lipton* was grown, a high risk of severe infections coincided with increased glutathione levels in the vegetative plant tissue. Such relationships were less pronounced for the cultivar *Bristol*. By comparison, spatial patterns for total S and glucosinolates revealed no significant differences between cultivars.
Overlaying the maps, areas with high glutathione content matched those with a high probability for severe fungal infections, while inverse relationships were found for the total S and glucosinolate content. Apparently plants responded to fungal infections with an increased glutathione synthesis, whereby the onset seemed to be dependent on some threshold infection (Fig. 2). There was no consistent influence of S fertilisation on the glutathione content (Table 1, Fig. 2), so that it can be assumed that the fungal infection caused an increased glutathione synthesis in plants (Fig. 2).

The fact that a low S nutritional status occurred at the same locations where an enhanced risk of severe infections with *L. maculans* was found indicates that an insufficient S supply promotes the sensitivity of plants to fungal infections. The significance of the concurrence of low glucosinolate contents and a high risk for fungal infections remains obscure, but suggests that higher glucosinolate content had some protective function.

**Discussion**

Interactions between mineral elements and plant diseases are well known for essential macro and micro plant nutrients, and aluminium and silicone (DATNOFF & al. 2004). In the case of S, the fungicidal effect of foliar-applied S has been exploited since the end of the nineteenth century (HOY 1987). In comparison, the significance of soil-applied S, independent of the S form, for disease resistance only became evident a century later. The S metabolism of plants offers several options to combat fungal attacks (HANEKLAUS & al. 2004). Glutathione and glucosinolates are S-containing compounds, which are putatively involved in SIR. Though triggers and effectiveness of specific S metabolites that are involved in SIR are not known yet, these could be released in a chain reaction that is initiated by the pathogen and controlled by the S supply of the plant (HANEKLAUS & al. 2004).

The total glucosinolate concentration in seeds as well as in vegetative parts of oilseed rape is strongly related to the S nutritional status of the plant (SCHNUG 1988, SCHNUG & al. 1995b). The S supply of oilseed rape is a major factor for maintaining the glutathione content (SCHNUG & al. 1995b). Sulphur deficient plants have very low glutathione concentrations and S fertilisation strongly increases the free thiol content (DE KOK & al. 1981, SCHNUG & al. 1995b).

The results of the field experiment revealed that soil-applied S significantly increased the total S content. The cultivar *Lipton* proved to be more susceptible towards infections with *L. maculans* than *Bristol*. This finding agrees with those of GLADDERS & al. 1998 and HCGA 2003. Under field conditions no significant influence of S fertilisation and fungicide treatment on the glutathione and glucosinolate content was found. This is
most likely related to the impact of other abiotic and biotic factors influencing their concentration under field conditions. One factor is for instance the small-scale, spatio-temporal variability of plant available sulphate contents in soils (Bloem & al. 2001).

SIR runs parallel to other defence mechanisms, which are activated after fungal attack so that a clear differentiation is difficult, particularly under field conditions. Geostatistics were applied in order to capture the influence of the S nutritional status on S metabolites against the background of other factors that had an impact on them and to set these maps in relation to infections with *L. maculans*. Geostatistics have been applied before in epidemiological studies (Ribes-Dasi & al. 2001, Nelson & al. 1994) for the analysis of the spatial variation of plant diseases on plot and field scale (Chellemi & al. 1988, Larkin & al. 1995, Wu & al. 2001, Morgan & al. 2002). Using this approach Morgan & al. 2002, Barclay & al. 1973 and Mackenzie 1981 found that the controlled N fertiliser management could reduce infection rate and severity of early blight (*Alternaria solani*) in potatoes in small plot experiments.

The results from the (semi)variogram analysis revealed that no spatial auto-correlation existed for the parameters total S and glucosinolate content and that only a moderate spatial dependency was found for glutathione and the probability of severe infections with *L. maculans* (Table 2). For this reason variability was uncovered within single plots, which indicates that micro-environmental conditions influenced the host-pathogen relationship.

The ranges for spatial correlations are related to the sampling interval and pathogen involved. In the presented study the range was about 8 meter for the parameters probability of severe infections with *L. maculans* and glutathione content (Table 2). In field experiments Farias & al. 2002 found a range of 8.5 m for Rotylenchulus reniformis populations in a cotton field and Chellemi & al. 1988 determined ranges of about 2 meter for infections with Phytophthora nicotianae in pineapple. On field scale with larger sampling distances Munkvold & al. 1993 reported ranges of 15–25 m for Eutypa dieback disease of grapes, Lecoultre & al. 1989 found ranges of > 120 m for the African cassava mosaic virus and Webster & Boag 1992 determined a range of 60 m for soil contaminations with the nematodes Globodera rostochiensis and Heterodera avenae on potato fields.

The susceptibility of both cultivars against infections with *L. maculans* was obviously related to the S status (Fig. 2). In areas where the risk of severe infections was higher, a lower total S and glucosinolate content was found. Both cultivars responded to infections with *L. maculans* by an increased glutathione synthesis, whereby the onset of the synthesis seemed to be dependent on some threshold infection (Fig. 2). These findings provide further evidence that a rapid accumulation of glutathione in response
Fig. 2. Small-scale spatial variability of the probability of severe infections of oilseed rape with *L. maculans*, glutathione, glucosinolate, and total S content.
to pathogen attack is one response mechanism to fungal infections (VANACKER & al. 2000), and that elevated levels of glucosinolates contribute to the resistance against the fungus (Fig. 2). For this a sufficient S supply during plant growth is required (Fig. 2). For glutathione, it might be speculated that the rapid and strong increase in glutathione synthesis due to fungal infections (VANACKER & al. 2000) is significantly higher than that caused by S fertilisation. For the involved metabolic processes an adequate availability of plant available sulphate is as well as a sufficient S supply presumably conditional so that the required S rates may be higher than the physiological demand (HANEKLAUS & al. 2004).

The results of this work show that geostatistical analysis can be an effective tool to reveal relations between plant physiological parameters and fungal infections in field experiments.

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References


HCGA 2003. HCGA Recommended list winter oilseed rape. – UK.


