

Risk-factors for the occurrence of *Verticillium longisporum* in winter oilseed rape in Germany

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Abstract

In a two-year monitoring the occurrence of *Verticillium*-wilt in oilseed rape could be confirmed for all surveyed regions in Germany. However, there are some regional differences in the infestation frequency. In North- and Northeast- Germany, where traditionally oilseed rape is cultivated intensively, the disease is more frequently diagnosed. The obtained data show a clear effect of the percentage of oilseed rape in the crop rotation on the occurrence of infections by the pathogen *V. longisporum*. By performing the oilseed rape crop rotation in longer intervals (more than three years) and an additional growing of non-host plants in close cereal/oilseed rape crop rotations, the risk of an infestation by this pathogen could be reduced. In addition, a longer cultivation interval of oilseed rape had a positive effect on its yield. The influence of the sowing time became clear, too. The earlier the sowing/the longer the growing-period in autumn, the higher the risk of an infection by *V. longisporum*. The influence of the tillage on the occurrence of the disease cannot be assessed from the monitoring data. For this statement, long-term studies of a soil-cultivation field trial were consulted. The results of the studies point out the importance of the cultivation method. The influence of the harvesting machines could also be determined as the most important source of spreading the pathogen. For the evaluation of the influence of the variety characteristics on the occurrence of the pathogen and on the resulting measure of harm, data were consulted from additional trials, too. During several years, tolerances of certain varieties could be determined. The effect of organic nitrogenous fertilizer could not be assessed finally. These investigations showed also no effect of the factors soil type, pH-value and the application of fungicides on the occurrence of *Verticillium*-wilt disease.

Key words: *Verticillium*-wilt disease, *Verticillium longisporum*, oilseed rape, crop rotation.

Introduction

There have been reports on the occurrence of the *Verticillium*-wilt in the traditional oilseed rape cultivation areas in the North and Northeast of Germany for some time. The number of reports from other parts of Germany increased during the end of the 90's. Therefore, the German Phytomedical Society (DPG) committee on oilseed rape carried out a monitoring for this disease, and subsequently an occurrence of the disease in all German regions could be proved. In addition, the incidence of the infestation in the years 2000 to 2003 was increasing. However, the surveyed locations were not selected randomly, but by being "suspicion locations" and areas with new variety tests. Higher incidences were determined in the North (Schleswig-Holstein) and Northeast (Mecklenburg-Vorpommern), compared to other regions of Germany (Steinbach et al. 2005). These results led to the examination of the reasons for the differences in incidences and to a subsequent research project where the monitoring was continued for additional 2 years (2002, 2003).

Further results for risk factors of the disease could be obtained by the analysis of a long-term field trial for tillage systems. The results pointed out the influence of the intensity of tillage on the occurrence of the pathogen and gave an indication to avoid the spreading by combine harvester.

Because the pathogen cannot be effectively controlled by plant protection products, the search for resistances and/or tolerance in the spectrum of varieties of winter oilseed rape is of special interest. On this problem an investigation started at 2005 at a field contaminated homogeneously with *V. longisporum*, to evaluate the resistance of winter oilseed rape varieties.

Material and methods

For monitoring, stubble samples of winter oilseed rape (GS 92-98) were collected during 2002 and 2003 after harvest from everywhere in Germany. The samples were examined for infestation with *Verticillium* sp. by visual estimation centrally at the Federal Biological Research Centre for Agriculture and Forestry. Afterwards they were tested by using an ELISA-test which was developed and carried out at the University of Göttingen (Cernusko 1995). Additionally, at each location a soil sample was taken which was examined for the occurrence of microsclerotia of *Verticillium* sp. by a Petri-dish-test containing a selective medium (Termorshuisen 1997). The Petri-dish-test was performed at the Center for Plant protection Service of Mecklenburg-Vorpommern. This test is able to detect the amount of microsclerotia in the soil but it can not differentiate between *Verticillium dahliae* and the rapeseed pathogen *Verticillium longisporum*. The possibility for estimating the risk of a *Verticillium*-infestation in a field was also evaluated. For the monitoring only locations were selected where a contamination

with *V. longisporum* was suspected. Therefore, the frequency of the infestation is not a measure for the absolute occurrence in Germany. Beside the potential occurrence of *V. Longisporum*, the locations should exhibit an as stable crop rotation as possible, and should also permit a comparison between conservation tillage and ploughing. At the surveyed locations, data about the crop rotation, cultivation, fertilization, sowing date, variety, soil type, pH value and the plant protection measures were raised by means of questionnaires. In cases where it was possible, a survey of the yield took place to estimate the relevance of the infestation towards the yield.

The effects of different tillage systems on the occurrence of *V. longisporum* were investigated in a long-term field trial near Brunswick. The trial was established in 1995 with an oilseed rape-wheat-wheat crop rotation. Three types of tillage systems were tested: ploughing as the standard (25-30 cm deep), conservation tillage by grubbing (15-20 cm deep) and no-till/direct drilling. The lay-out of the field trial was a split-plot design with two repetitions. Every plot size is 2000m². The occurrence of *V. longisporum* was assessed as the degree of infection at 100 randomly chosen plants per plot at growth stage 92.

For the investigation of the susceptibility of winter oilseed rape varieties, a field trial was carried out in the years 2005 and 2006 at a location with homogeneous contamination by *V. longisporum*. The trial was accomplished in a randomized block layout with four repetitions and a single plot sizes of 50m². The varieties were provided by the companies DSV, KWS, NPZ and Syngenta Seeds. The plots were harvested in both years. The evaluation of the susceptibility of the varieties was done by visual estimation of the roots and stalks at growth stage 81 and of the stubble at growth stage 92-98. A scale from 1 (healthy plant) to 9 (plant with >75% totally discoloured - blue-grey or black - tissue of root and stalk, infiltrated with microsclerotia) was used (STEINBACH unpublished).

Results and discussion

The evaluation of the two-year investigations showed that the disease occurs in all important rapeseed cultivation regions in Germany. However, the regional spreading is very different and a safe diagnosis of the disease is still a problem for farmers. Although samples were to be sent in from suspicion locations, on average over the years only 58% of the samples exhibited an infestation with *Verticillium*. Apart from the locations with a minimum infestation, which was not covered by the sampling, a certain percentage of locations remained, where an assumed infection was not found. This confirms the statements of WOLF and WEINERT (2003) that the infection is often visually misjudged. The highest certainty for the proof is offered so far by the ELISA-test. Also, a small percentage of the samples in the project was classified visually as not being infected, but was recognized afterwards by means of the ELISA as weakly infected.

The evaluation of the questionnaires, the assessments and the tests confirm *Verticillium longisporum* as a pathogen of crop rotation disease. As table 1 shows, the occurrence of infestation increases with a higher frequency of rape cultivation in crop rotation. The decrease of the interval of rapeseed by one year did already cause an increase of the *Verticillium* infestation of around 45% and a duplication of soil inoculum. If "non-host plants" were added to the crop rotation, the infestation and the soil inoculum were reduced to a lower level.

Beside the effects of crop rotation, the growing period had influence on the occurrence of the disease. An extension of the vegetation period by earlier sowing already increased the soil inoculum. Therefore, the visible infestation was increased only after a two-week vegetation extension. However, an influence of the soil tillage on the occurrence of the disease could not be deduced from the monitoring data. In conservation tillage systems the incidence of infestation was slightly higher than in ploughing systems. The influence of the factor variety is present, but is also weak. The available monitoring data record is not sufficient here to provide solid statements.

Table 1. Effects of arable farming and crop growing factors on the infestation incidence of the *Verticillium*-wilt in oilseed rape

Factor	influence (%)	
	infestation	Soil inoculum
Oilseed rape in the crop rotation (cr)		
25 %	100	100
33 %	145	213
C r cereals-rape		
75%: 25%	100	100
66%: 25%	142	234
Cr cereals/potato/ leguminous crops	46	90
Growing period (45 weeks = 315 days)	100	100
+ 1 week	117	214
+ 2 weeks	184	210
+ 3 weeks	154	195
Tillage system		
ploughing	100	100
conservation tillage	114	129

For the factor nitrogen only small effects on the infestation were shown. Therefore, not the amount of the N-fertilization

seems to be crucial, but the kind of fertilizer: i. e. a portion of organic nitrogen fertilization in the entire N-quantity led to a smaller infestation but the soil inoculum was increased. Here, further investigations must take place, in order to be able to secure the few existing data. This includes also the influence on the structure of population of *Verticillium* sp. and on the decrease rate of the microsclerotia in the soil. The factors soil type, pH value and also fungicide application did not influence the occurrence of the disease during the monitoring.

The yield was negatively affected only as a consequence of a high frequency of rape cultivation within the crop rotation. Since this can be accompanied by the incidence of other rape diseases, the effect cannot be attributed exclusively to the *Verticillium* infestation. Therefore, negative yield effects were clearly shown only in those areas which had an infestation frequency of over 75%. Although the results of the tests are preliminary, there is the conclusion that the oilseed rape population must have a high balance ability in those cases where the infestation level is to be classified as small up to medium. Finally, there has to be examined to what extent the yield is reduced by the late development of the disease.

The data of our monitoring could not answer the question which effect on the occurrence is due to the tillage system, because a lot of fields were not consequently cultivated by conservation tillage. The data could only demonstrate a tendency for a higher risk by the practise of conservation tillage. Clear effects were achieved in a long-term field trial comparing the three soil cultivation systems: ploughing, conservation tillage and no-till.

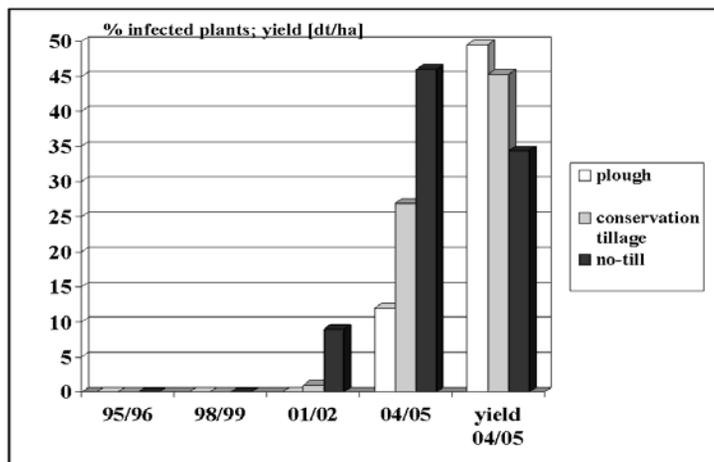


Figure 1. Effect of the tillage-system on the incidence of infected plants caused by *V. longisporum*

Table 2. Susceptibility of different varieties for *Verticillium*-wilt, GS 92

variety	2005		2006		2005		2006	
	average score stalk	average score root	average score stalk	average score root	% infected plants stalk	% infected plants root	% infected plants stalk	% infected plants root
Express	—	—	1,4	1,7	—	—	11	27
Smart	1,68	2,04	—	—	22	48	—	—
Roxet	4,92	4,04	—	—	76	78	—	—
NK Fair	1,52	1,71	1,84	1,84	21	36	28	28
NK Bravour	3,52	3,76	—	—	68	92	—	—
NK Nemax	—	—	1,08	1,28	—	—	4	14
NK Beamer	—	—	1,80	1,84	—	—	20	32
Remy	—	—	1,80	1,72	—	—	20	28
Courage	—	—	1,08	1,20	—	—	2	8
Allure	1,32	1,52	—	—	16	52	—	—
Alkido	2,96	2,36	1,68	1,56	50	54	16	20
Mika	2,36	2,52	2,08	2,24	58	64	26	44
Caracas	3,40	3,48	—	—	72	82	—	—
Litonic	2,40	2,40	—	—	42	52	—	—
Oase	1,36	1,36	1,24	1,24	12	14	8	10
Licorne	4,36	3,54	—	—	76	80	—	—
Lilian	—	—	1,16	1,72	—	—	6	30
Lioness	—	—	1,12	1,72	—	—	4	30
Talent	2,84	2,71	—	—	51	58	—	—
Trabant	2,84	2,00	1,84	2,08	54	34	24	44

The trial was established at a field where no oilseed rape had grown before. When oilseed rape was cultivated for the third time, the first plants infected by *V. longisporum* were detected. The degree of infection was very low. The highest amount was found in the no-till-system with 9% frequency (s. figure 1). Here, a clear tendency was noticed due to the intensity of tillage. The maximum of infected plants three years ago in the fourth rotation was 46% in the no-till-system again. The plough-system showed the lowest degree of infected plants. The data demonstrate also that there was no big effect on the spreading of the pathogen by the soil preparation equipment. This can be accepted, because the highest infection frequency was detected in the less cultivated area. As the main reason for the spreading of the disease, the combine harvester can be assumed. It was the only machine which was able to spread the microsclerotia homogeneously.

Because there is no possibility to control the Verticillium-wilt by plant protection products, the meaning of less susceptible varieties could be very important. For this reason a field trial was carried out in 2005 and 2006. The investigations of the varieties produced partly considerable differences of susceptibility for the disease (s. table 2). In the year 2005, the degrees of infection were highest with a score of 3.5 (variation between 1.32 to 2.44 average score). The differences in the second year were less by a score of about 1.3 (variation between 1.0 to 2.44). Varieties with less susceptibility in 2005 were Allure, Oase, NK Fair and in 2006 Courage, NK Nemax, Oase and Express. In 2006, the variety NK Fair was more susceptible than the standard Express. The infestation incidences reached 92% in maximum in 2005 and 48% in 2006. In the second year 2006, the roots were more infected than the stalks. Yield-losses were only found in the year 2005 if there were more than 70% infected plants or a score higher than 3.5. In the year 2006 no yield-differences were found.

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