Development of methods improving precision of risk assessment of *Sclerotinia* stem rot in oilseed rape

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Abstract

The disease forcasting of *Sclerotinia* Stem Rot in oilseed rape available for Swedish farmers today is a regional risk assessment, based on local climate and field data. Precision in risk assessment can be improved by determining whether the pathogen is present in the field. In a study carried out in 1998 and 1999 three different methods were evaluated. Identification of inoculum on petals was performed by two serological kits available on the market, Alert®-On-Site detection kit (Neogen corporation, Lansing MI 48 912, USA) and IDENTIKIT™ (Adgen LTD. Auchincruve, AYR, KA6 5 HW, UK) and was compared with the petal test on agar (Turkington *et. al*., 1988). Altogehter 119 fields of spring oilseed rape, spring oilseed turnip rape and winter oilseed rape were tested. The results of the Alert- test and the Identikit did not correspond reliably with the results of the agar petal test. With the Agar test a quantitative measure was achieved indicating the future disease risk. In areas where humidity was high during flowering a relationship between percentage of infected petals and disease incidence in the field was found. At the level of 20 % infected petals disease incidence exceeded 20 %, the damage threshold, in several fields. In areas where dry weather prevailed during flowering low disease incidence was found also at high levels of infected petals.

Key words: Oilseed rape, *Sclerotinia* stem rot, Agar test, Alert-On-Site, Identikit.

Introduction

Sclerotinia stem rot, caused by the phytopathogenic fungus *Sclerotinia sclerotiorum* (Lib.) de Bary, is a major disease of spring oilseed rape in Sweden. The pathogen survives in the soil for long periods as sclerotia. Ascospores are produced during moist conditions by carpogenic germination and constitute the major source of inoculum for infection by *S. sclerotiorum* (Willets and Wong, 1980). An exogenous source of nutrients as senescent plant parts (petals) or pollen is required for ascospore germination. The infection occurs when the ascospore- infected petals, during flowering, are falling and thus sticking to the leaves, allowing the pathogen to penetrate the petiole, leading to infection of the stem. The impact of *Sclerotinia* stem rot is dependent on weather conditions and the timing of ascospore release and causes severe damage from year to year. Fungicides applied to oilseed rape for the control of *Sclerotinia* stem rot have to be timed accurately to prevent infection, since once the disease is established within the plant it cannot be effectively cured. Timing of spray application is generally attained at mid- flowering to ensure an efficient fungicide cover of the petals (Hardwick et al 1991). The disease forecasting available for Swedish farmers today is a regional risk assessment, based on local climate and field data. However, precision in risk assessment can be improved by determining whether the pathogen is present in the field. The aim of the research presented here was to evaluate serological based detection methods on the market of *S. Sclerotiorum* on petals of spring oil seed brassicas and winter oilseed rape and to assess the corresponding disease incidence at each site. These investigations were conducted in 1998 and 1999.

Material and methods

Sampling

Petals were collected from 119 oilseed fields in south and central Sweden (table 1). Petals were collected about 50 meters from the edge of the field, prior to full bloom (stage 65) as 10 inflorescences were cut with a knife, placed in individual plastic bags and kept in a coolbox and stored cool overnight. In 1999 the samples were stored in a freezer prior to analysing.

Serological tests

Identification of inoculum on petals was performed by two serological kits available on the market, based on the use of a monoclonal antibody in a plate-trapped antigen ELISA. From each inflorescence two petals were removed with a pair of tweezers from the lower part of the inflorescence. Alert®-On-Site Disease Detection Kit (Neogen Corporation, Lansing MI 48 912, USA) was conducted in 1998 in all of the samples according to the instructions of the manufacturer. Alert®-On-Site cross reacts with *Botrytis cinerea*. The readings were visually classified into three levels; positive, possibly positive ? and negative. In 1999 another kit recently introduced on the market, IDENTIKIT™ (Adgen LTD. Auchincruive, AYR, KA6 5HW, UK) was used on samples from 20 randomly selected fields. This test has according to the manufacturer a high specificity for *S. Sclerotiorum* with little or no cross reactivity with *B. Cinerea*. IDENTIKIT™ demands according to our experiences assess to a well equipped laboratory and requires by a series of incubations a day of work whereas Alert-on-Site was conducted within an hour. To interpret the results of Identikit a plate reader /spectrophotometer at 621 nm is required, and a sample is

determines as positive or negative according to the absorbance of the positive and negative control.

Table 1. Distribution of fields sampled in 1998 and 1999.

Province	Winter oilseed rape	Spring oilseed rape	Spring oilseed turnip rape
1998			
Skåne	12		
Östergötland	17	12	3
Örebro county	3	9	4
1999			
Skåne	8		
Östergötland	15	15	0
Örebro county	1	12	8
Total	56	48	15

Agar test

The petal test was conducted according to Turkington et al. (1988, 1991). In total 80 petals were examined for each field, as four petals were placed on each agar plate. The agar plates were incubated and placed in room temperature, and infected petals were assessed after 5-6 days for colonies of *S. sclerotiorum* and *B cinerea*. A second reading was undertaken a week later. In 1998 all of the spring oilseed rape and spring oilseed turnip rape fields and 8 fields of winter oilseed rape were examined, and in 1999 all of the 59 fields were investigated with the agar method.

Field assessment

Assessment of plants in the sampled fields was conducted prior to harvest in winter oilseed rape in the middle of July and in spring oilseed rape and in spring oilseed turnip rape in the middle of August. 100 plants in each field were investigated situated 20, 40, 60, 80 and 100 m from the edge of the field. At every station 20 plants were pulled out in the row and disease incidence was determined as the proportion of plants with infested main stalks.

Results

The Alert-on-Site test showed a positive or possibly positive reaction in 65% of the winter oilseed rape fields sampled in the south of Sweden 1998. However, the agar test was not performed in all of these fields. Disease incidence was low as 2 % of the plants were infested. The relationship between the Alert test and the Agar test is poor (figure 1) since 51 % of the Agar tests show a negative reaction.

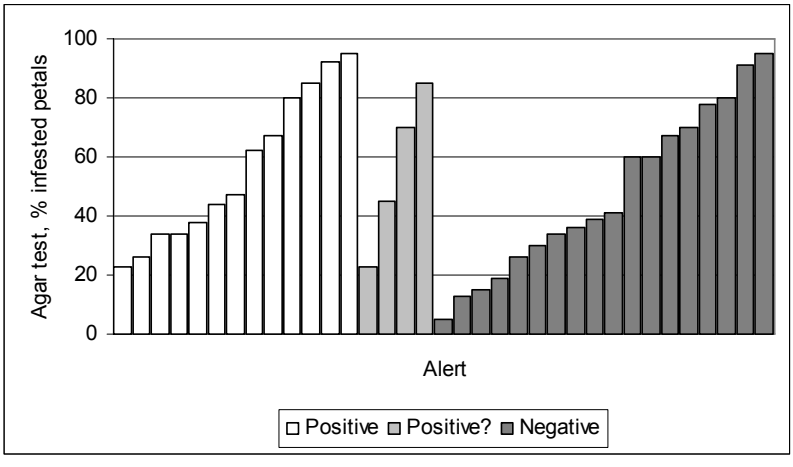

Figure 1. Comparison of the results of Alert test and Agar test. The Alert tests were recorded as positive, probably positive or negative. The Agar tests were determined as the percentage of petals with colonies of *S.sclerotiorum*.

Nor has the Identikit tests shown reactions that corresponds with the petal test (figure 2). 41 % of the Identikit tests were determined as negative while the Agar test in some of the samples showed high levels of infestation.

The Agar test showed a great occurrence of petals infested with *S. Sclerotiorum*, ranging from 4 to 95 % and *B. cinerea* ranging from 1 to 90 %. The level of infestation was on average higher in the county of Östergötland compared to Örebro county (table 2). Relationships between infested petals and disease incidence are presented in figure 3 and figure 4.

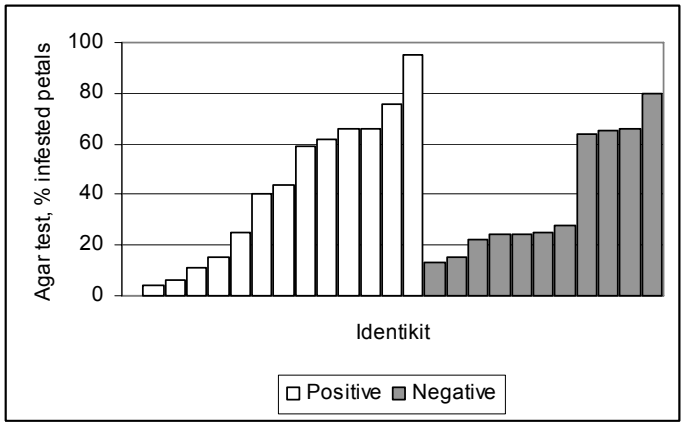

Figure 2. Comparison of the results of Identikit test and Agar test.

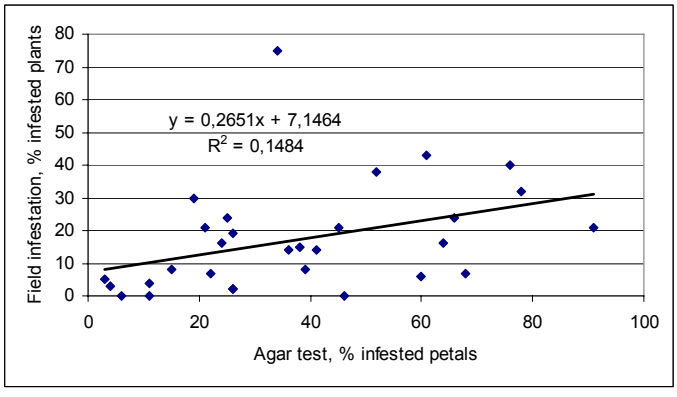

Figure 3. Relationship between Agar test and field infestation in spring oilseed brassicas 1998-1999, Örebro county.

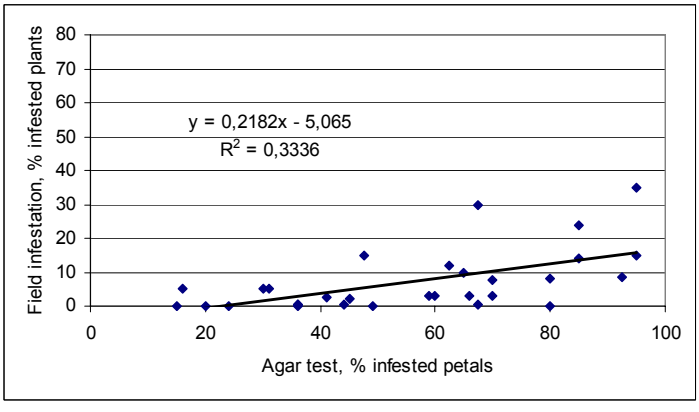

Figure 4. Relationship between agar test and field infestation in spring oilseed brassicas 1998-1999, Östergötland.

Discussion

The agar test is a simple method to quantify the prevalence of inoculum in a field, and to identify fields where a fungicide application is needed to control the disease. However, at least 5-6 days are needed to distinguish between colonies of *S. Sclerotiorum* and *B. cinera* (Turkington et al, 1988), and by the time the results are presented the optimal time of application is crossed.

Our results clearly show that the weather conditions during flowering has a determining influence on disease incidence. In several fields, particularly winter oilseed rape 1999 and spring oilseed rape in the province of Östergötland 1999 (table 2) a high level of infestation was showed by the Agar tests, whereas field infestation was low due to dry and hot weather during the flowering period (figure 3). In the county of Örebro field infestation was recorded at a considerably higher level (figure 4, table 2), due high humidity in the crop stand during the flowering period. At the level of 20 % infected petals, disease incidence exceeded 20 %, the damage threshold, in several fields. The prediction method used in Sweden is based on several parameters where knowledge of the previous oilseed crop in the field is the most important (Twengström et al, 1998). Survival of sclerotia in the soil has been observed for nine years in central Sweden (Twengström, 1999). The farm enterprises today are increasing in size rapidly, hence, the knowledge of the crop sequence of the fields is low (Redner, pers comm.), thus a

quantitative, fast and reliable method is required to identify the fields where a fungicide application is appropriate.

Table 2. *Sclerotinia* stem rot. average of agar test and field disease incidence.

	Agar test % infested petals	Field infestation % infested plants
1998		
Summer oilseed rape, Östergötland	69	13
Summer oilseed brassicas, Örebro	43	25
1999		
Winter oilseed rape, Skåne	45	6
Winteroilseed rape, Östergötland	38	5,1
Summer oilseed rape, Östergötland	42	3,2
Summer oilseed brassicas, Örebro	36	12,7

The results of our studies show that the Alert-test and the Identikit test did not correspond reliably with the Agar test, however, IDENTIKIT™ is further developed in a quantitative version. A PCR- assay for detection of airborne inoculum of *S. Sclerotiorum* is developed in UK (Freeman et al, 2002). In a project started in Sweden in 2006 a real time PCR- assay is developed from naturally infested petals and validated with the Agar test.

Acknowledgement

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