



Slutrapport

Epidemiology and development of a forecasting method for *Acrothecium* rot in stored carrots

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Del 1: Utförlig sammanfattning

Den allvarligaste lagringssjukdomen i svensk morotsodling är *Acrothecium*-röta, som orsakas av svampen *Acrothecium carotae* (syn. *Rhexocercosporidium carotae*). Syftet med detta projekt var att klarlägga biologi och spridningsvägar för svampen samt att utveckla en prognosmetod. Ett flertal DNA-analyser mha TaqMan-PCR har utförts på kommersiella fröpartier, på egenproducerat frö, och på rötter och blad av morotsplantor; allt från småplantor till skördefärdiga plantor. Resultaten visar att det kan finnas spår av *Acrothecium*-DNA på kommersiella morotsfrön, men det var inget samband mellan detta och det slutliga angreppet. *Acrothecium*-DNA har påträffats i höga mängder på egenproducerade frön som kommer från kraftigt infekterade morötter. DNA fanns också både i rötter och blad från mycket tidigt i plantans utveckling fram till skörd, vilket innebär att det är mycket svårt att bekämpa svampen. Det har visat sig att den viktigaste faktorn som påverkar angreppets storlek är förekomst av mekaniska skador på rötterna. Utöver de mekaniska skador som uppstår vid skörd har vi nu också indikationer på att skador orsakade av insekter, till exempel morotsbladloppa, eller av nematoder, kan utgöra en inkörsport till svampen och medföra ökade angrepp. Man ska därför försöka undvika alla möjliga skador på morötterna för att minska angreppen. Även skördetidpunkten har stor betydelse för angreppet – ju längre morötterna står i marken, desto större blir angreppet. Därför bör man inte vänta för länge att skörda morötterna på hösten. Svampen finns överallt i alla morotsodlingsområden i Sverige. De viktigaste infektkällorna är smittad jord och andra flockblomstriga värdväxter såsom vildmorot, vildpersilja och hundkåx. Dessa växter bör bekämpas i närheten av morotsfälten. Ett flertal fältförsök har lagts ut där fröna har behandlats med kemiska eller biologiska produkter, eller med värmebehandling, ThermoSeed. Det bästa sättet att bekämpa svampen hittills har varit genom att behandla utsädet med den biologiska produkten Cedress. En prognosmetod har utvecklats för att kunna förutsäga angreppet på hösten. Även en mer användarvänlig DNA-baserad metod, LAMP, har utvecklats för detektion av svampen. Resultaten av undersökningarna har presenterats på ett flertal möten med morotsodlare och rådgivare.

Projekt har fått finansiering genom:

Del 2: Rapporten

BACKGROUND

Black spots on carrots have become an important threat to production of cold stored carrots. The fungus *Acrothecium carotae* (syn. *Rhexocercosporidium carotae*) is found to be the dominating pathogen causing this problem. Symptoms developing during storage are brown to black superficial spots, which later develop to larger black areas on the roots. The pathogen was first described in Norway (Årsvoll, 1965). Later it has been reported also from Denmark (Hobolth, 1983), Sweden (Ewaldz, 1992; Pettersson, 1992), Canada (Shoemaker *et al.*, 2002), The Netherlands (Kastelein *et al.*, 2003), Poland (Jeske *et al.*, 2014), France (Tailleur *et al.*, 2014) and the United States (Hay *et al.*, 2017). This disease is by far the most important disease of carrots in Sweden and the losses after storage have been substantial in carrots from many fields. Dutch investigations have shown that a higher occurrence of black spots may be linked with harvest conditions, such as temperature at harvest date and mechanical damage at harvest (Kastelein *et al.*, 2003). Also, the presence of umbelliferous plants in or near carrot fields may be linked with the occurrence of black spots.

Basic knowledge on *A. carotae* is very limited and important information needed for designing efficient control methods is lacking. In an earlier joint Swedish-Dutch project financed by SLF (Wikström *et al.*, 2009; Wikström *et al.*, 2007) a TaqMan-PCR method for detection and quantification of the pathogen was developed. In our previous project, also extensive field inventories were performed. Results from our earlier studies have shown that the disease occurred in all investigated fields during the latest 14 years. Carrot, wild carrot (*Daucus carota* ssp. *carota*), fool's parsley (*Aethusa cynapium*), and cow parsley (*Anthriscus sylvestris*) have been shown to be hosts for this pathogen. Multiple regression analysis of data showed that the two most important factors for damage by *A. carotae* are mechanical damage at harvest and number of carrot growth days, i.e. number of days from sowing to harvest. By reducing mechanical damage at harvest and by harvesting within 150 days from sowing, the damage can be reduced by 50%. Results from another project by us, focusing on growth days of carrots supported these findings (Haraldsson *et al.*, 2013). Also, the presence of umbelliferous plants (wild carrot, fool's parsley and cow parsley) in the neighbourhood of the crop increased the prevalence of the disease. In another project, we found very high amounts of *Acrothecium*-DNA in leaves of wild carrots (Hökeberg, *et al.*, 2015). Finally, soil type was another important factor affecting the disease, i.e. calcium rich soils had a lower disease incidence.

In trials performed during 2014-2015 at the island Gotland, we noted a significant attack by the carrot psyllid. Where the carrots were grown close to field margin, the carrots had a higher severity of *Acrothecium* rot than further out in the field. Also, the attack by the carrot psyllid was often more severe close to the field margins. In this project we wanted to test the hypothesis that the carrot psyllid and/or other insects can act as vectors and initiate attack by *A. carotae*. We have earlier shown that the severity of *A. carotae* will be significantly higher if there is any mechanical damage on the carrots. *A. carotae* seems to be a wound pathogen which easier attacks if there already is damage on the carrots. Possibly the damage of the carrot psyllid constitutes a point of entry for the pathogen.

The purpose of this project was also to provide farmers with an early detection method for carrot lots that will be attacked by *Acrothecium* rot during storage. The DNA-based method

to detect *A. carotae*, which was developed in the previous SLF project, works great, but the attack of Acrothecium rot has shown to be dependent on a number of factors in addition to the amount of Acrothecium-DNA in the leaves or carrots. The goal was that a sample can be taken out at harvest time, be stored in a controlled manner and give an answer on whether the carrot lot can be stored or not.

MATERIALS AND METHODS

Work package 1. Epidemiology and effect of seed treatments

Subproject 1.1. Analysis of seed samples

Seeds from 19 different commercial seed lots were collected from different carrot growers in 2016. The seeds were ground and sent to the Netherlands for DNA analysis for detection of possible *A. carotae* on the seeds. The different seed lots were then sown on different fields in 2016. Leaf and root samples were collected in four different sampling areas per field every four weeks from sowing until just before harvest. Leaf and root samples were freeze dried, milled in a Cyclotec sample mill, and sent to the Netherlands for DNA analysis. The quantitative Taqman-PCR with specific primers for *A. carotae*, that was earlier developed by our Dutch partners, was used for all these analyses.

Handling of carrot samples and assessment of disease caused by *A. carotae*: A sample of carrots for long-term storage was collected from each field just before harvest in the autumn. The samples were stored in commercial carrot stores at about 1°C for approximately six months and the attack of Acrothecium rot was assessed in spring the next year. Carrots were washed and the infected root area of each carrot was assessed according to Fig. 1. and the percentage of marketable carrots was calculated.



Fig. 1. The infected root area caused by *A. carotae* has been assessed on all carrots in the different trials. Marketable carrots are those which are completely healthy or with one small dark spot as can be seen to the left in the photo. Photo: Mariann Wikström.

Subproject 1.2.1. Development of the LAMP-technology

In this subproject the original plan was to perform seed treatments and to use treated seeds in field trials in order to start with as clean and healthy seeds as possible. Due to various reasons this was delayed one year, and we got permission from SLF to use the funds granted for developing a new method, Loop mediated AMPlification (LAMP) for on-site detection of *A.*

carotae instead. Our Dutch colleagues have experiences in this work, and they started to develop, validate and implement this method. The LAMP technology is based on reproduction of specific DNA or RNA fragments at a constant temperature of 65°C. The advantage of this approach is that the DNA/RNA in the (plant) sample does not need to be purified, as is the case for most molecular assays (PCR/TaqMan). After a short and simple preparation this method enables - within 10 minutes – on-site determination of the presence and/or identity of a specific pathogen (or insect) with a simple instrument (Genie II or III) without special training in molecular biology.

Subproject 1.2.2. Seed treatment

Seeds from a seed lot without known presence of pathogens were used to assess the effect of different seed treatments. The seeds have been treated with chemical fungicides, heat treatment, microbial preparations and combinations of these. Four field trials with different seed treatments were sown in 2017 and 2018. All trials had a randomized design with four replicates. The carrots were harvested by hand in the autumn. Carrot samples were cold stored and Acrothecium rot was assessed.

Subproject 1.3. Seed production

Three different batches of carrots infested with Acrothecium rot were selected in 2016. The carrots were kept in cold store until May to get as much Acrothecium rot as possible. From each batch both heavily infested carrots and healthy carrots were used. Healthy carrots were planted on six different locations and infested carrots were planted on seven different locations in the middle of large cereal fields in growing areas without carrot cultivation. Sample sites were additionally at least 100 m apart from each other and away from wild host plants. From the carrots that have produced flowers and seeds, the seeds have been collected and where sent to the Netherlands for DNA analysis. Seeds from these plants were sown in two places in 2017 on trial sites where we minimized the risk of contamination of *A. carotae*. Carrots had never been cultivated on these two places earlier and there were no host plants in the neighbourhood. All seeds produced from diseased carrots were sown in one place and seeds from healthy carrots were sown in another field about 500 m away. The carrots produced from these seeds were harvested in autumn 2017 and were cold stored until spring 2018. The attack of *A. carotae* was assessed after storage.

Work package 2. The carrot psyllid and/or other vectors

Three different types of trials to control carrot psyllid and other vectors have been carried out in 2016; 1) To combat all insects, repeated spraying with the insecticide Karate 2,5 WG (a.i. lambda-cyhalothrin) was tested. Treatments have been carried out every seven days from the carrot emergence until harvest. The insects have been monitored by using yellow sticky traps and both carrot psyllids and carrot flies have been observed. The trapped carrot psyllids were assessed for the presence of Acrothecium-DNA by using the developed LAMP-method. 2) In an organic field, cages have been used to prevent insects from approaching the carrots. 3) In the third type of experiment, carrot seeds were treated with different insecticides; Cruiser (a.i. tiametoxam), Force (a.i. teflutrin) and Gaucho (a.i. imidakloprid). The seeds were then sown on two different locations. Carrots in all trials have been harvested by hand and have been cold stored and Acrothecium rot was assessed.

In another series of trials financed by LRF Minor Use project (The Federation of Swedish Farmers), the carrot psyllid has been treated by repeated sprayings with different insecticides. The carrots from these trials have been cold stored and Acrothecium rot was assessed.

Nematodes: In another project financed by SLF “Influence of freeliving nematodes on yield in sugar beets and carrots - PCR, damage thresholds and varieties” O15-20-313, four cultivar trials were performed in carrots in order to investigate the nematode attack on different cultivars. After harvesting these trials, the carrots were cold stored during the winter and we assessed the attack of Acrothecium rot the next spring in order to obtain any possible relationships between nematode attack and Acrothecium rot.

Work package 3. Development of a forecasting method

In 2016, samples of 400 carrots per field from 14 fields in Skåne and on Gotland have been harvested by hand just before the field was harvested by the farmer. The carrots were divided into four different samples. Three of them have been stored in different temperatures. The fourth sample was stored in a commercial carrot store until spring next year. Every two weeks 25 carrots from each field and each temperature were washed and any attacks of Acrothecium were assessed. Similar work was performed with machine harvested carrots from the 14 fields. The results from storage in different temperatures were compared to the attacks of Acrothecium rot after long-term storage. In 2017 and 2018, similar work has been performed in 8 and 9 fields, respectively. The number of degree days (DD) were calculated, and the DD until the carrots had an infection of 0.2% root area, i.e. not marketable because of Acrothecium rot, was calculated.

All the data processing and statistical analysis were performed by using SAS statistical software. Analysis of variance (ANOVA) followed by the Duncan’s Multiple Range Test has been performed.

RESULTS

Work package 1. Epidemiology and effect of seed treatments

Subproject 1.1. Analysis of seed samples

In 9 of the 19 investigated seed samples, there were traces of Acrothecium-DNA (0,006 – 0,149 pg/mg dry weight). All data are shown in Table 1. Average marketable carrots from seeds with no DNA was 76.0% and from seeds with traces of DNA 76.4%, i.e. there was no difference in final disease between the different seed lots. Another important result from all these DNA-tests was that Acrothecium-DNA can be found in both leaves and roots of carrots from a very early developmental stage (BBCH 10.5) until just before harvest. In cases where we started with completely healthy seeds and although found DNA in the carrots very early, we conclude that the infection comes from the soil. In other cases where we started with seeds without any traces of DNA and then did not find any DNA in neither roots nor leaves until just before harvest, we conclude that the infection comes from other infested host plants in the neighbourhood.

Table 1. Acrothecium-DNA in seeds, leaf samples and root samples at different developmental stages and the final damage caused by *A. carotae*

Field	Year	Seed lot No.	DNA in seeds	DNA in leaf samples				DNA in root samples				Damage by <i>Rhexocerosporidium</i>	
				T1	T2	T3	T4	T1	T2	T3	T4	% infected root area	% marketable carrots
So	2017	1	0.034	0.055	0.035	0	0.095	0.018	0.020	0.015	0	0.158	75.5
Ly	2017	1	0.034	0.008	0	0.078	0.208	0.113	0	0	0.153	0.461	47.3
Bo	2016	1	0.034	0	n.t.	0	1.180	0	n.t.	0	0.058	0.154	77.5
Nb	2016	1	0.034	0.030	0	0.013	0.195	0.300	0.030	0.040	0.823	0.082	91.1
So	2017	2	0	0	0	0.018	0.290	0.060	0	0	0	0.587	52.1
WN	2016	2	0	0.145	0	0.018	0.003	0.735	1.548	n.t.	3.338	0.118	84.1
WS	2016	2	0	0.038	0	0	0.063	0.488	0.305	n.t.	8.253	0.093	90.0
Ly	2017	2	0	0	0	0	0.115	0	0.110	0.053	0	0.430	58.1
Co	2016	3	0	0	0	0	0	0.265	0.193	0.013	0.743	0.140	86.9
Bj	2016	3	0	0.017	0	0	n.t.	0.033	0	0	0.045	0.035	95.5
PH	2016	3	0	0	0	0.015	0	0	0.015	0	0	0.255	84.6
At	2016	5	0	0.025	0	0	0.010	0.655	0.265	0.840	8.850	0.888	51.0
PE	2016	6	0.149	0.058	0	0	0	0	0.013	0	0.090	0.145	78.4
Va	2016	7	0.106	0.005	0.005	0.013	0	0.005	0	0.008	0	0.069	96.0
Ba	2016	8	0	0	0.075	0.008	0	n.t.	0	0.010	0.103	0.194	81.3
Ba	2016	9	0.040	0.023	0	0	0.035	n.t.	0	0	0.035	0.192	83.0
Ba	2016	10	0.042	0	0	0	0.025	11.900	0	0	0	0.047	95.4
Ba	2016	11	0	0.123	0	0	0	n.t.	0.013	0	0.030	0.157	79.2
Atl	2016	12	0	0	0	0.020	0.015	0	0	0.008	0	0.045	95.0
MB	2016	14	0.082	0.090	0	0	0.023	0	0.018	0	0	0.223	72.9
WR	2016	15	0.006	0.015	0	0	0.090	0.060	0.148	0.008	0	0.415	42.0
Nb	2016	16	0	n.t.	0.040	0	0	n.t.	0.003	0	0	0.370	59.5
Nb	2016	17	0.059	n.t.	0	0	0	n.t.	0	0	0	0.134	82.7
Nb	2016	18	0.093	n.t.	0	0	0	n.t.	0	0	0	0.203	75.2
Nb	2016	19	0	0	0	0	0.027	n.t.	0	0	0	0.070	91.1
SB	2016	20	0	0	0	0	0	0.040	0	0	0.020	0.233	75.5
Sk	2016	21	0	0	0	0	0	0	0	0.003	0	0.317	56.7

T1 – BBCH 10,5-12; T2 – BBCH 15-16; T3 – BBCH 45; T4 – BBCH 49

Subproject 1.2.1. Development of the LAMP-technology

The LAMP technology was successfully developed for Acrothecium rot by our Dutch partners. They have helped us to implement it in Sweden and analyses can now be done.

Subproject 1.2.2. Seed treatment

The results from four field trials with seed treatments are given in Fig. 2. The best seed treatment in these trials was the biological product Cedress (a.i. *Pseudomonas chlororaphis*, strain MA 342), which resulted in a significantly higher amount of marketable carrots in comparison with all other treatments. The ThermoSeed treatment did not increase the marketable yield, which also indicates that soil infection is the main infection source in these trials.

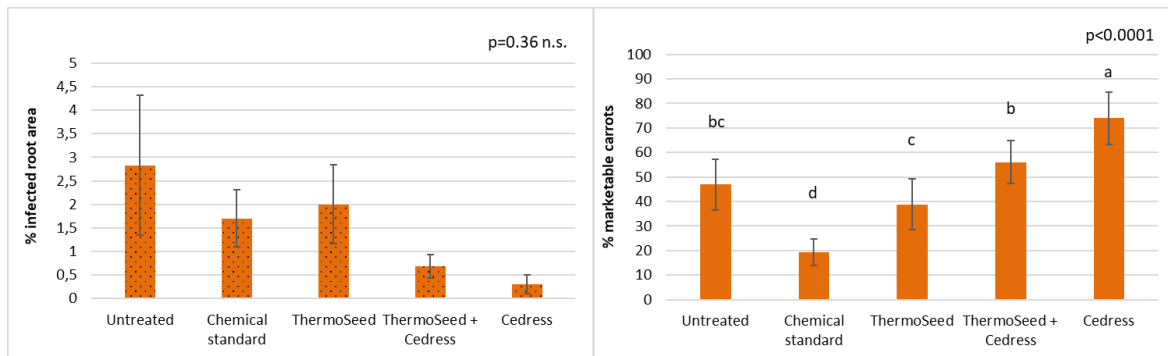


Fig. 2. Average % infected root area and % marketable carrots from four field trials with seed treatment. If the p-value >0.05 the difference between the treatments is considered as non-significant. Small bars represent standard error of the mean and the letters above each bar the Duncan grouping for each treatment. Mean values with the same letter are not significantly different.

Subproject 1.3. Seed production

Our own seed production when starting with completely healthy carrots, which were grown in a field where carrots had never been grown before and without host plants for *A. carotae* in the neighbourhood, resulted in 97% marketable carrots. Corresponding seed production when we started with carrots with a severe infestation of *Acrothecium* rot, which were grown in a similar way as the healthy carrots, resulted in on average 44% marketable carrots. DNA samples from the different seed lots showed that there was no *Acrothecium*-DNA in the seeds produced from healthy carrots, while the DNA content of seeds produced from severely infected carrots was 59 and 1454 pg/mg dry weight. The amount of *Acrothecium*-DNA in these seeds were comparatively very high, i.e. about 400-10000 times higher than in commercial seeds (Table 1). These findings suggest that it is possible for *Acrothecium* to be spread by seeds, if the amounts are high.

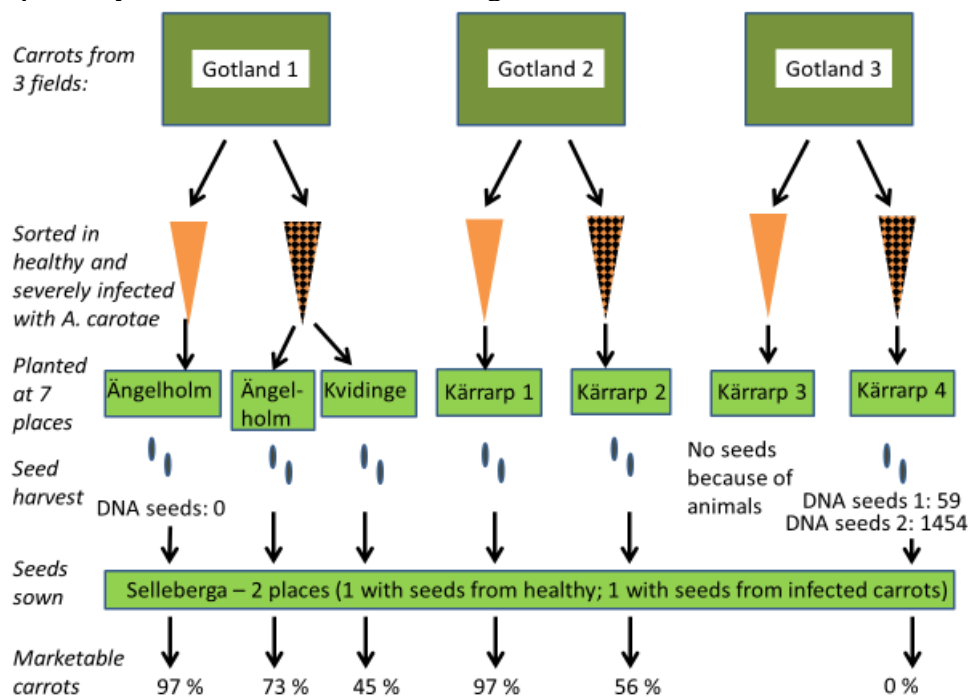


Fig. 3. Sketch showing our own seed production.

Work package 2. The carrot psyllid and/or other vectors

There was no effect on the attack of Acrothecium rot in the field trials where carrot psyllids and other insects were controlled through repeated sprayings with the insecticide Karate or by seed treatment with insecticides in 2016. However, the effect on the damage caused by the carrot psyllids was also very limited. In the trial where cages were used to prevent insects from approaching the carrots, the attacks of both insects and Acrothecium rot were lower on the carrots grown inside than outside the cages. The trial was repeated in 2017, but it was difficult to prevent the insects from coming into the cages and no clear conclusions can be drawn from these experiments.

The trapped carrot psyllids were assessed for the presence of Acrothecium-DNA by using the developed LAMP-method, but no DNA could be found in the psyllids by using this method.

In the other series of trials financed by LRF Minor Use project, there was a tendency to relationship between the attack of carrot psyllids and the percentage of marketable carrots (after sorting out carrots with spots caused by *A. carotae*) (Fig. 4). Further, in the trial series in our SLF project “Influence of freeliving nematodes on yield in sugar beets and carrots - PCR, damage thresholds and varieties” O15-20-313, we could see a similar relationship between nematode index and % marketable carrots (Fig. 4). These results strengthen the hypothesis that the wounds caused by the carrot psyllid or nematodes, or maybe even other organisms that are able to wound the carrots, can be a point of entry for the pathogen.

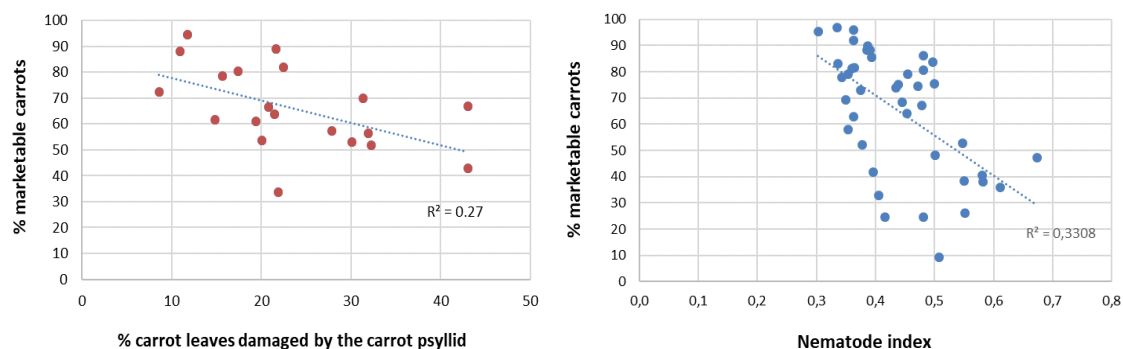


Fig. 4. The relationship between damage caused by the carrot psyllids (to the left) or nematodes (to the right) and % marketable carrots. A nematode index of 0.5 means forking of 50% of the roots.

Work package 3. Development of a forecasting method

In all fields where carrot samples have been taken by harvesting by hand or by machine, the symptoms of Acrothecium rot are visible earlier in carrots harvested by machine. In seven of the investigated fields, the harvest with both methods were performed on the same day, and the average percentage of infected root area was 0.32% on carrots harvested by hand and 1.27% on carrots harvested by machine. It is very clear that the symptoms start in wounds on the carrots.

One example of a field where carrots have been harvested by hand or by machine for short storage for developing the forecasting method is shown in Fig. 5. The carrots are considered to be unmarketable if the infected root area is 0.2% or more. In this case the number of degree days when the carrots harvested by hand were unmarketable was about 1100 DD. The corresponding for carrots harvested by machine was about 350 DD.

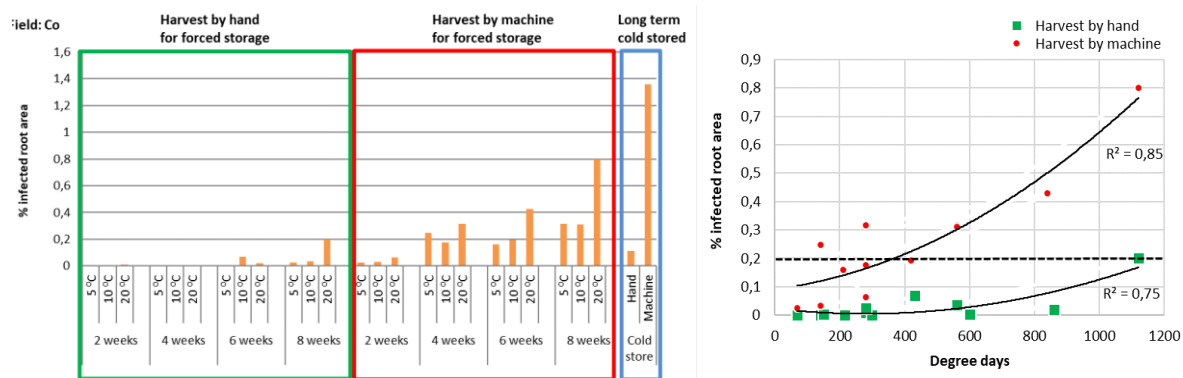


Fig. 5. One example of a field where carrots have been harvested by hand or by machine for short, forced storage for developing the forecasting method. The final infected root area after long term cold storage in this field could be seen in the blue frame. The same figures recalculated to degree days are shown in the graph to the right.

After analysing all data from all investigated fields, the following results were obtained: If carrots were harvested by hand: After on average 900 DD, 86% of the fields that have got the most severe symptoms (more than 1% infected root area) in the spring after cold storage were detected. Also 33% of the fields yielding 0.5-1% infected root area were detected. After 1200 DD 14% of the fields with less symptoms (0.2-0.5% root area) were detected. There were no false positive fields. If the carrots were harvested by machine: After on average 400 DD, 100% of the fields that have got the most severe symptoms (more than 1% infected root area) after cold storage were detected. After 900 DD also 100% of the fields yielding 0.2-1% infected root area were detected. In 12% of the fields the forecasting method resulted in false positive results (Table 2).

Table 2. Results from the trials with forced storage compared to final results after cold storage

% infected root area after long term cold storage (carrots harvested by machine by the growers)	Percentage of the fields detected by forced storage ($\geq 0.2\%$ infected root area)	
	Harvest by hand	Harvest by machine
> 1 % (not marketable)	86%	100%
0.5-1% (not marketable)	33%	100%
0.2-0.5% (not marketable)	14%	100%
0-0.2% (marketable)	0%	12%

DISCUSSION

The main source of infection of *A. carotae* in the investigated fields in Sweden seems to be inoculum in soil and on other infested host plants. The very early detection of Acrothecium-DNA in especially roots indicates soilborne infection (Table 1). This is in accordance with earlier findings in Norway (e.g. Årsvoll, 1965). However, our investigations show that there might be a very high amount of DNA on seeds if they are produced from carrots with severe symptoms (Fig. 3). Such seeds also produced carrots with severe Acrothecium rot. In none of the investigated commercial seed lots, we found more than very small amounts (traces) of DNA. There was no correlation between seeds with traces or seeds with no DNA and final damage. In an earlier project, we found Acrothecium-DNA in very high amounts on wild

carrots (213 pg/mg dry weight) close to carrot fields at Gotland (Hökeberg *et al.*, 2015). Our hypothesis is that the disease was spread by infected seeds a long time ago, but nowadays the pathogen is widely spread and occurs in soil and on other umbelliferous plants in or near carrot fields everywhere in carrot growing areas in Sweden.

Our detection of *Acrothecium*-DNA in both leaves and roots from a very early developmental stage until harvest shows that the pathogen occurs in the carrots during the whole season, which makes it difficult to control by spraying. Other results from a project financed by LRF Minor Use confirm this. Repeated sprayings five times per season have resulted in significantly lower disease severity (Wikström, 2017). The best way to treat the seeds in order to reduce the infection later is by using the biological seed dressing Cedress (Fig. 2), which has also been shown in several GEP registration trials for this product (M. Wikström pers. com).

Mechanical damage and wounds are shown to be very important for the development of symptoms. The damage when carrots were harvested by hand was on average 0.3% infected root area, while 1.3% infected root area was obtained after machine harvest, which is a very significant difference that means a lot in practice. These results are also in accordance with findings by us in an earlier SLF-project (Wikström *et al.*, 2009). Now we have also indications that wounds caused by the carrot psyllid or nematodes, or maybe even other organisms that are able to wound the carrots, can be a point of entry for the pathogen (Fig. 4). Earlier findings indicated that the mechanical damage together with the number of growing days (days from sowing until harvest) were the most important factors for the disease development.

A lot of effort has been made to develop a forecasting method for this very severe disease. The best results are given when we take a sample of carrots harvested by machine and store them in about 20 °C. The problem with storing carrots in a high temperature could be that they will rot because of white mold or other diseases. Another problem is that if we use carrots harvested by machine by the growers, there will be no time for forced storing. Therefore, it is better to harvest the carrots by hand some weeks before the field is harvested. Then there is some time for forced storage. We have found that it takes about 900 degree days in order to find most of the fields where there is a high risk of *Acrothecium* rot if we use carrots harvested by hand. If we use carrots harvested by machine it takes less than half the time for the symptoms to develop. A suggestion is that carrots should be harvested by hand about four weeks before the farmer will harvest the field. The carrots should be damaged and stored at a high temperature for about four weeks, then they should be washed, assessed for symptoms and it is also recommended to use the developed LAMP-method for confirming the symptoms. This method has been used in practice the last years and seems to work quite well.

CONCLUSIONS - including benefits for growers and recommendations

- In commercial carrot seeds, there were sometimes traces of *Acrothecium*-DNA. However, the amounts were very low and there was no correlation between seeds with traces or seeds with no DNA and final damage.
- The pathogen seems to be spread everywhere in carrot growing areas in Sweden and infection occurs from soil and infested umbelliferous host plants close to the carrot fields. Wild carrots and other hosts should be controlled in the neighbourhood of carrot fields.
- The pathogen occurs in the whole plant from a very early developmental stage (only one pair of true leaves) until harvest, which makes it difficult to control by fungicides.

- The best way we know so far, for reducing the disease, is to treat the seeds with the biological seed dressing product Cedress.
- Mechanical damage of the carrots is one of the most important factors for development of the disease. There are also indications that damage by the carrot psyllid or nematodes might increase the risk. Therefore, all possible ways for reducing wounds should be practiced and the carrots should be harvested and handled as carefully as possible and they should also not be harvested too late.
- A DNA-based method, LAMP, is developed for rapid analysis of carrots with symptoms. Carrot producers and advisors can send in samples for detection.
- A forecasting method based on forced storage in higher temperature is developed and can be used for detection of infected carrot lots.

REFERENCES

- Ewaldz, T. 1992. Lagringssjukdomar i morötter. SLU Info rapporter. Inst. f. växtskyddsvetenskap, Alnarp. Trädgård nr. 374.
- Haraldsson, T., Wikström, M. and Ragnarsson, S. 2013. Lagringssjukdomar på morötter – Inverkan av tillväxtperiod och sort. Jordbruksverket Dnr 21-11886/11.
- Hay, F.S., Vaghefi, N., Ivy, A., and Pethybridge S.J. 2017. First report of carrot root rot caused by *Rhexocercosporidium carotae* in the United States. Plant Disease 101(1):248.
- Hobolth, L.A. 1983. Ny sygdom i gulerødder – gulerodsrotplet. Gartner Tidende 83: 205
- Hökeberg, M., Wikström, M., och Köhl, J. 2015. Biologisk bekämpning av Acrothecium-röta i morötter. FoU projekt Jordbruksverket 2012-2014.
- Jeske, M., Lukanowsky, A., Panka, D. and Zary-Sikorska, E. 2014. *Rhexocercosporidium carotae* as a new causal agent of carrot disease. Conference abstract, 11th EFPP conference, Krakow, Poland, 8-13 September 2013.
- Kastelein, P., Stilma, E., Elderson, J., and Köhl, J. 2003. Occurrence of *Rhexocercosporidium carotae* on cold stored carrot roots in the Netherlands. European Journal of Plant Pathology.
- Petersson M-L (1992) Växtskyddsåret 1991- Trädgård [Horticultural pests and diseases in 1991]. Växtskyddsnotiser 56(1): 2-6.
- Shoemaker RA, Hambleton S., Lacroix M, Tesolin M and Coulombe J. 2002. Fungi Canadenses No. 344. Canadian Journal of Plant Pathology 24: 359-362.
- Tailleur, A. and Fredon, A. 2014. Bulletin de Santé du Végétal Aquitaine - Légumes de plein champ N°8 2014:3.
- Wikström, 2017. Effekt av fungicidbehandling på Acrothecium-röta i morötter. Minor Use Projektnummer 17.29.
- Wikström, M., Ragnarsson, S., and Jönsson, B. 2009. Svarta fläckar på lagrade morötter – ny sjukdom i Sverige orsakad av *Acrothecium carotae*. Slutrapport Stiftelsen Lantbruksforskning. H0556131; H0656455.
- Wikström, M., Ragnarsson, S., Jönsson, B., Köhl, J., Arvidsson, A.-K., Burgers, S.L.G.E., Groenenboom-de Haas, B.H., Haraldsson, T., and Persson, L. 2007. Black spots caused by *Rhexocercosporidium carotae* (syn. *Acrothecium carotae*) on cold stored carrots in Sweden. Poster. 32nd International Carrot Conference, Arcachon (Bordeaux), France.
- Wikström, M., Ragnarsson, S., Jönsson, B., Köhl, J., Arvidsson, A.-K., Burgers, S.L.G.E., Groenenboom-de Haas, B.H., and Lombaers-van der Plas, C.H. 2009. Black spots caused by *Rhexocercosporidium carotae* (syn. *Acrothecium carotae*)—a new threat against cold stored carrots. Poster. 33rd International Carrot Conference, Anaheim, California.
- Årsvoll, K. 1965. *Acrothecium carotae* n.sp., a new pathogen on *Daucus carotae* L. Acta Agriculturae Scandinavica 15: 101-114.

Del 3: Resultatförmedling

Vetenskapliga publiceringar	Effect of cropping and harvesting conditions on black spots caused by <i>Rhexocercosporidium carotae</i> : More than one decade of research on the major disease in Swedish cold-stored carrots. M. Wikström, S. Ragnarsson, J. Köhl, S. Burgers, C. Zijlstra, M. De Weerd, B. H. Groenenboom-de Haas, B. Jönsson, and T. Haraldsson. Manuskript för Plant Disease 2020.
Övriga publiceringar	
Muntlig kommunikation	<p>Acrothecium – Nya forskningsresultat. Grönsaksdag - Odlarmöte anordnat av HIR Skåne i Borgeby, 2016-03-03. Mariann Wikström.</p> <p>Acrothecium-röta i morötter. Odlarmöte anordnat av Lantmännen i Klintehamn, Gotland, 2016-03-17. Mariann Wikström.</p> <p>Nya produkter för biologisk bekämpning - pågående forsknings- och utvecklingsarbete. Seminarium ”Biologisk bekämpning och framtidens matproduktion” på Margretetorp, Ängelholm, arrangerat av Kompetenscentrum för biologisk bekämpning (CBC), SLU, Uppsala, Jordbruksverket, och SLU Partnerskap Alnarp, 2016-11-16. Mariann Wikström.</p> <p>Acrothecium-rot in carrots, Meeting at the Dutch Board for the Authorisation of Plant Protection Products and Biocides (Ctgb), Ede, The Netherlands, 2016-12-01. Mariann Wikström.</p> <p>Acrothecium-röta i morötter – Nya forskningsresultat. Grönsaksdag - Odlarmöte anordnat av HIR Skåne i Borgeby, 2018-03-02. Sara Ragnarsson.</p> <p>Acrothecium-röta i morötter – Nya forskningsresultat, Möte för morotsodlare vid Findus R&D Bjuv, 2018-04-09. Mariann Wikström.</p> <p>Acrothecium-rot of carrots, Carrot Advisory Experience Exchange Network Sweden 21-22 nov, Bäckaskogs slott, HIR Skåne, 2018-11-22. Mariann Wikström och Sara Ragnarsson.</p>

	Acrothecium-röta i lagrade morötter – Nya forskningsresultat, Temadag om växtskydd i grönsaker anordnad av HIR Skåne och Jordbruksverket, 2019-02-28, Mariann Wikström.
	Nytt om Acrothecium, Odlarmöte anordnat av Lantmännen i Norrköping, Östergötland, 2019-03-20. Sara Ragnarsson.
	Nytt om Acrothecium, Odlarmöte anordnat av Lantmännen i Klintehamn, Gotland, 2019-03-21. Sara Ragnarsson.
Studentarbete	
Övrigt	Växtdoktorer i Åstorp jobbar för friskare grödor. Tidningsartikel i Helsingborgs Dagblad, 2018-12-02. Mariann Wikström. https://www.hd.se/2018-12-02/vaxtdoktorer-i-astorp-jobbar-for-friskare-grodor?redirected=1
	Deltagande i 38th International Carrot Conference, Bakersfield, Kalifornien, 2017-03-27—29. Sara Ragnarsson.
	Deltagande i IOBC-WPRS Working Group "Integrated Protection Of Field Vegetables", Wädenswil, Schweiz, 2017-10-02—05. Sara Ragnarsson.