Black spot diseases in carrot

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***Abstract* - An important problem in organic carrot production in the Netherlands is the occurrence of black spots during storage. Several different fungal pathogens cause similar symptoms, which are collectively known as black spot diseases. We identified *Alternaria radicina, A. dauci* and *Rhexocercosporidium carotae* (syn. *Acrothecium carotae*) as the most prevalent black spot pathogens. We developed laboratory test methods for resistance to *A. radicina* and *R. carotae*, and assessed resistance in a collection of cultivated varieties and more exotic material.[1]**

Introduction

Carrot is one of the most important crops in organic vegetable production, in terms of both cultivated area and production value. Especially when mature carrot roots are harvested before winter and held in refrigerated storage for several months, returns are profitable. In the last few years however, black spots are observed on stored carrots in the Netherlands. When more than 5% of the carrots are affected, the lot is rejected for the fresh market.

In organic farming, the possibilities to control diseases are limited. The use of varieties resistant to black spot diseases would therefore be useful. Currently however the knowledge on pathogens causing black spot diseases is limited. This also hampers the development of resistance tests and the breeding for enhanced resistance.

In order to address these problems, we first carried out a survey for pathogens involved in the black spot syndrome. Next we developed laboratory test methods for assessing the level of resistance to the more important of these pathogens. These methods were then used to determine the resistance of currently cultivated varieties, and to search for stronger sources of resistance for use in breeding.

Materials & Methods

Survey

An inventory was carried out in 2001/2002 and 2002/2003 including in total 42 carrot lots from 22 organic growers. The farms were located on clay soils in the south-west, the centre and the north of the Netherlands.

Information on acreage, soil conditions and previous history of the parcel, tillage, sowing, crop husbandry and harvesting conditions was obtained by means of questionnaires. Seeds were assessed for pathogen contamination according to rules of the International Seed Testing Association (ISTA). Two weeks before the harvest date of a crop the percentage of foliar necrosis was assessed and the crop was surveyed for occurrence of foliar diseases. At harvest, a 100-root subsample was taken from the storage boxes to assess the pre-storage sanitary conditions and crop damage. Additionally, a sample of the top soil was taken to determine the water content and the harvest weather was described. Another subsample of 100 to 200 roots was crated unwashed, crates were wrapped in perforated plastic foil and stored within 48 h after harvesting in a cold room at 1 to 5 °C and 95% RH for 4 to 5 months. After storage, samples were washed and visually inspected for pests and diseases, which were identified by the appearance of lesions or damage. For each pest or disease the number of affected roots was recorded. Pieces of skin showing a superficial dark brown to blackish lesion were excised from the washed carrot roots and incubated at 15 °C in humid chambers for two weeks under NUV-illumination with a daily photoperiod of 12 h. Incubated lesions were examined for presence of conidia or chlamydospores of fungi known to cause root spotting (Snowdon, 1991).

Resistance tests

Carrots were taken from the cold storage and washed just before each experiment. The top- and bottom end of the carrots were removed, leaving a 10 cm carrot piece. Sixteen carrot pieces were placed in a polycarbonate box on dry filter paper. Under the filter paper, which was supported on a plastic grid, was a layer of water in order to maintain high air humidity.

For the tests with *Alternaria radicina,* the carrot pieces were peeled on one side and inoculated with agar plugs, punched from the edge of four days old colonies on V8 agar plates. Three plugs were placed upside down, 2.5 cm apart on the peeled carrot. The boxes were kept at 25°C in darkness. The diameter of the resulting black spot was measured twice, respectively three and six days after inoculation. The average difference between the two measurements was taken as a measure for the susceptibility of the carrot.

Inoculation with *Rhexocercosporidium carotae* was performed using a toothpick inoculation method adapted from Pryor et al. (2000). Bundles of about 100 dry, autoclaved toothpicks were placed 30 min in 20 ml of a suspension of $3x10^5$ conidia/ml. Three toothpicks were inserted 2.5 cm apart, about 5 mm deep into each carrot. The size of the resulting black spot around the toothpick was measured after about 21 and 35 days of incubation at 20°C in darkness.

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The average difference between the two measurements was taken as a measure for the susceptibility of the carrot.

In each test, for both pathogens, all tested accessions were represented by eight carrots, which were randomized over the boxes. All accessions were tested twice during storage. Tests with *A. radicina* and *R. carotae* were performed during two and one storage seasons respectively.

RESULTS

Survey

In 2001 no black spots were found on carrots at harvest, whereas in 2002 black spots were found in 26% of the lots already at harvest. Such lesions were mainly caused by *A. radicina* or *Chalaropsis* spp. After storage, all lots harvested in 2001 showed black spots. On average, 2 to 21% of the carrots of the different lots were diseased. Eighty three percent of the lots harvested in 2002 showed black spots, but only 1 to 10% of the carrots of individual plots were diseased. *R. carotae* (syn. *Acrothecium carotae*; Årsvoll 1965) was the dominating pathogen in black spots of carrots harvested in 2001. From carrots harvested in 2002, *Alternaria radicina* was isolated most frequently, followed by *A. dauci* and *R. carotae*. *Mycocentrospora acerina* and *Chalaropsis thielavioides* were found only occasionally. Attempts were made to distinguish between several types of black spots according to size, shape or colour. No relationships were found between lesion type and pathogen species isolated from such lesions.

The occurrence of *R. carotae* in a huge number of organically produced carrot lots was unexpected. Damage of stored carrots caused by *R. carotae* has been found occasionally by Årsvoll (1965; Köhl and Kastelein, 2003) in Norway, Edwaldz (1997) in Sweden and more recently by Shoemaker (2002) in Canada. The broad occurrence of *R. carotae* had not been reported for the Netherlands before.

Resistance tests

Since resistance to *Alternaria dauci* is already known (e.g. Boiteux et al., 1993) and being used by breeders, we focussed on, *A. radicina* and *R. carotae*.

Although we observed considerable variation in the average lesion growth between carrots of the same accession, differences between accessions were reproducible between tests, and for *A. radicina* also between the two years.

Six varieties were tested that are currently used by organic farmers in The Netherlands (Fig. 1, top six varieties). Among these six, variety "Natalja" was considerably more resistant than the other five against *R. carotae*, and also, with a smaller difference, against *A. radicina*. However, resistance of a higher level to both pathogens was found in PRI 04001, a semi-wild carrot from Lebanon. In variety "Lobberychska", an old Polish variety used for fodder, we also found a strong resistance to *R. carotae*, but resistance to *A. radicina* was comparable to that of "Natalja".

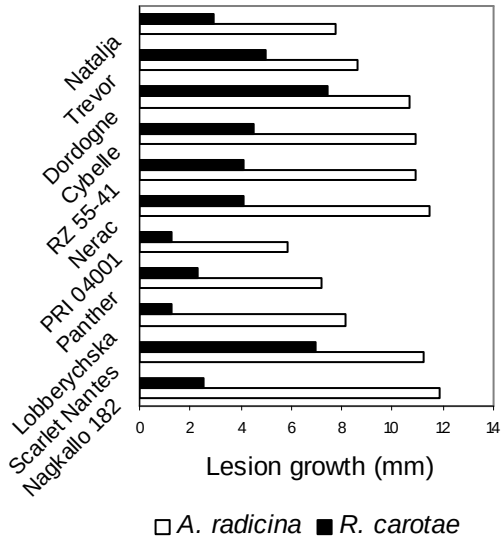

Figure 1. Lesion growth on six carrot varieties commonly used in Dutch organic farming (top) and reference material, inoculated with two black spot pathogens.

Currently resistance tests are under way in fields that have been artificially inoculated with one of the pathogens. These will be used to validate the laboratory test results. Further, the resistant accessions PRI 01004 and "Lobberychska" have been crossed with susceptible varieties in order to study the inheritance of resistance.

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