Incipient infections caused by *Botrytis cinerea* in carrots entering storage

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**SUMMARY**

Out of 1200 autumn-lifted carrots (*Daucus carota*), stored for 40 days at 4–6 °C in the laboratory, twenty-seven developed lesions caused by *Botrytis cinerea*; of these primary lesions, twenty-three occurred in the crown. Similar lesions were observed on the crowns of field-stored carrots whether or not they were protected from frost damage by strawing over. The fungus was isolated from lesions on the foliage and petiole bases of freshly harvested carrots throughout the storage season. All such isolates proved to be pathogenic to roots at storage temperatures. Carrots may thus enter storage with an incipient infection, which may develop into a crown rot and spread to adjacent healthy roots by hyphal growth.

**INTRODUCTION**

The most widespread and consistently successful technique for the storage of carrots has been the earthing or strawing over of the crop, in rows, in the field. This has two major drawbacks. First, the carrots cannot be harvested in unfavourable weather, particularly if the ground becomes frozen, when freezing injury may also occur. Secondly, carrots are biennial and commence re-growth in early spring when the roots become woody and unpalatable.

In recent years, the cold storage of carrots has become feasible through the development of cheap methods of refrigeration (R. Thompson, private communication). Under ideal conditions carrots can be stored in this way for up to 9 months (Van den Berg & Lentz, 1966).

Infection by either of three psychro-tolerant pathogens, *B. cinerea* Pers. ex Pers., *Sclerotinia sclerotiorum* (Lib.) de Bary and *Centrospora acerina* (Hartig) Newhall, is probably the most important factor limiting the storage life of cold-stored carrots in this country (Derbyshire, 1971). *B. cinerea*, the cause of the ubiquitous grey-mould disease in Britain, has also been reported to be a serious pathogen of stored carrots in North America (Lauritzen, 1932; Virgin, 1942; Rader, 1952), Norway (Årsvoll, 1969) and Finland (Mukula, 1957).

This paper reports our observations, during the storage season 1973–4, of natural infections in carrots, which show that incipient infection by *B. cinerea* can be carried into storage.
MATERIALS AND METHODS

Red-core Chantenay carrots (Suttons Seeds Limited) were used throughout. They were grown at the University of London Botanical Supply Unit, Egham, Surrey, on mineral soil in rows 1 ft apart, thinned c. 6 wk after sowing to a distance of 4 in, and harvested when required. Harvesting was done carefully by hand, the foliage being cut off with a sharp knife, leaving c. 1 in of petiole base attached. The roots were stored in polystyrene boxes in a cold room at 4–6 °C (Lauritzen, 1932).

Isolates of *B. cinerea* were cultured on a medium (GPSA) containing 20 g glucose, 4 g bacteriological peptone (Oxoid), 1.75 g KH₂PO₄, 0.75 g MgSO₄·7H₂O and 20 g agar (Difco Bacto) per l of distilled water (Townsend, 1957). Carrot roots were washed carefully to minimize damage. They were soaked in cold tap water for 15 min, rubbed gently with the fingers to remove as much soil and debris as possible and resoaked in clean tap water for a further 5 min. They were dried between several layers of absorbent paper towelling and then replaced in a clean box.

To test the pathogenicity of fungal isolates, mycelial inoculations were made on washed roots. In each of ten roots, two wells, extending into the secondary phloem parenchyma, were made with a sterile No. 3 cork borer. Into one well was placed a disk of GPSA, cut with the same cork borer, containing mycelium from the growing edge of a 3-day-old culture of the isolate incubated at 23.5 °C in the dark. A disk of GPSA medium from an uninoculated Petri dish was inserted into the second well as a control. Illumination used to stimulate sporulation on the surface of infected tissues for identification purposes was by two 80 W Crompton warm-white fluorescent tubes, one either side of a Philips TL 20W08 black light at a height of 16 in above the irradiated material.

RESULTS

In September 1973 we harvested 1200 carrots, 600 of which were then washed. The roots were placed in cold storage and observed every 4 or 5 days for the first appearance of lesions caused by *B. cinerea*; Table I shows that 2–2.5% of the carrots developed lesions. Where primary lesions occurred on the crown, sporulating mycelium was often observed initially on the bases of the petioles before it appeared on the root. Within 70 days of observation of primary lesions (i.e. after 110 days in store), mycelial growth from two-thirds of the infected roots had spread to adjacent carrots.

Subsequently, six carrots, showing rotting of foliage and petioles, but not of the root, were selected from a batch of freshly harvested carrots.

Between twelve and thirty small pieces of the rotting foliage and petioles were removed from each plant. Half of the pieces were surface-sterilized in 1% sodium hypochlorite for 2 min followed by a 2 min rinse in sterile distilled water; the other half were untreated. Half of the pieces from each of these treatments were plated on to GPSA and the other half on to GPSA containing 5 x 10⁴ units of penicillin and 10⁵ units of streptomycin per l of medium. The Petri dishes were incubated at laboratory temperature under the illumination system described above. *B. cinerea* was consistently isolated from samples originating from three of the six carrots, irrespective of sample treatment or medium. Further, when leaves and petioles were removed from these
three roots and incubated in sealed plastic bags under the lights, sporulating mycelium of *B. cinerea* often emerged from large areas of either the leaf, petiole or both. Well-inoculation of roots with all of these isolates showed them to be pathogenic at storage temperatures.

Table 1. *Distribution of lesions caused by Botrytis cinerea in washed and unwashed carrots stored at 4–6 °C for 40 days*

<table>
<thead>
<tr>
<th>Treatment</th>
<th>No. carrots stored</th>
<th>No. carrots bearing lesions*</th>
<th>Site of lesions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unwashed</td>
<td>600</td>
<td>15</td>
<td>Crown Root tip</td>
</tr>
<tr>
<td>Washed</td>
<td>600</td>
<td>12</td>
<td>13 2</td>
</tr>
<tr>
<td></td>
<td></td>
<td>10</td>
<td>10 2</td>
</tr>
</tbody>
</table>

* In each case only one lesion was observed per infected carrot.

Carrots were stored in the ground at Egham over the relatively mild winter of 1973–4, with or without strawing over. When these carrots were harvested in March 1974, many had foliage infections of *B. cinerea*. The disease was sometimes present in the crown of the root and here little foliage remained. Isolation from the advancing edge of such root lesions yielded the fungus. Twelve lots, each of fifty field-stored carrots, were harvested without removing the foliage, placed in large plastic bags and returned to the laboratory, where the foliage of each carrot was examined for the presence of sporing *B. cinerea*. An average of 17 % had such foliage infections, the range being from 10 to 38 % in different lots. No significant difference was observed between carrots which had been strawed over and those which had not. In addition, ten carrots showing rotting foliage, without external sporulation, were selected from one batch of fifty roots, placed in sealed plastic bags and irradiated as before. Within 8 days the fungus was sporulating on the foliage of three of these carrots. Subsequent isolation of the fungus from the foliage and well-inoculation again proved the isolates to be pathogenic at storage temperatures.

**DISCUSSION**

The accepted view that *B. cinerea* is brought into store in soil and debris clinging to the carrot root and that primary infection of the roots takes place through wounds by growth of the fungus from this source (Van den Berg & Lentz, 1966) is not unreasonable. The fungus is commonly distributed on dead or dying plant material in the soil (Gilman, 1957). Inoculation experiments have indicated that the intact surface of the carrot root is resistant to penetration by either a mycelial or spore inoculum (Lauritzen, 1932). When carrots are harvested damage always occurs at the crown where the foliage is removed and at the fine tip where the tap-root is broken. It is commonly reported that lesions caused by *B. cinerea* in storage are found most frequently in these two areas.

Reports of *B. cinerea* infecting carrots in the field are scarce. Mukula (1957) found that only frostbitten carrots had become infected before harvesting. Årvoll (1969) isolated the fungus from seedlings and from foliage. Only Rader (1952) reported a high incidence of the fungus in carrots growing under wet conditions, and suggested
that the disease might be carried into store not only in soil but possibly as an incipient infection.

Our observations confirm that the fungus can infect both the leaves and petioles of carrot plants before harvesting. From such infected petioles the fungus can sometimes extend to the crown of the root both in the field and in storage, where spread to neighbouring roots by mycelial contact can occur. The original inoculum for upper foliage infections we presume to be airborne conidia; infection of the petiole bases could arise either (a) from extension of the upper foliage lesions down the petiole towards the crown or (b) from mycelium in the soil lying in the crown of the carrot. Removal of this soil by washing immediately after harvesting had little effect upon the number of crown infections in storage over the comparatively short term of 40 days. The observation by D. M. Derbyshire (personal communication) that disease development in the longer term is associated with the amount of soil and plant debris in the store is explained not only by the fact that such foliage would provide a source of organic material which the fungus present in the soil could colonize, and upon which it would spread, but also that such debris might already contain infections.

Virgin (1942) reports that new foliage, produced by carrots in store at 5 °C, is very susceptible to infection by *B. cinerea* and that from such leaves the organism grows down into the carrot root crown. The source of the inoculum for the original new-leaf infection is unstated in the report, but it may well be that the fungus invades such leaves from outer petiole bases where it becomes localized.

In conclusion, attempts to control *B. cinerea* in storage must take into account not only the amount of soil in the storage lots, the movement of airborne conidia, the damage incurred by roots on harvesting and other factors which may predispose roots to infection, but also the occasional presence of incipient infections of the plant at the time of harvesting.

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**REFERENCES**


