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# 18 Genetics and Genomics of Carrot Biotic Stress

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**Abstract**

Carrot (*Daucus carota* ssp. *sativus*) production can be affected by a wide range of pests and pathogens. At least five diseases of carrot are caused by bacterial pathogens, 36 by fungal and oomycete pathogens, two by phytoplasmas, and 13 by viruses; and seven genera of nematodes and two genera of parasitic plants affect carrot. In addition, numerous insect and mite pests can cause losses. There have been extensive efforts to select carrot cultivars with partial or complete resistance to many of these pathogens and pests, and to identify wild species with resistance to specific biotic stresses for introgression into breeding populations and commercial cultivars. For some pathogens and pests, significant advances have been made at identifying resistance and mapping that resistance to the carrot genome. For others, resistance has been identified, but the genetic basis is yet to be determined. For a majority of these diverse stresses, however, there has been little success at identifying highly effective resistance and understanding the genetic basis of resistance. The diversity of stresses as well as interactions among these pests and pathogens can complicate efforts to develop cultivars with resistance to all key biotic stresses in a region that also meet market and consumer expectations. New approaches to identifying resistant material and speeding traditional breeding are being developed with molecular breeding tools, including simple sequence repeat markers and deep-coverage libraries of the carrot genome. These valuable genomic resources will enhance efforts to identify and breed for resistance to carrot pests and pathogens.

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## 18.1 Introduction

Diseases and insect or mite pests limit carrot production to various degrees in most regions of carrot production in the world (Rubatzky et al. 1999). The foliar diseases of primary concern tend to be Alternaria leaf blight (caused by *Alternaria dauci*), Cercospora leaf spot (*Cercospora carotae*), bacterial blight (*Xanthomonas hortorum* pv. *carotae*), and powdery mildew (*Erysiphe heraclei*) (Davis and Raid 2002). The most widespread soilborne root pathogens of carrot are cavity spot (caused by several species of *Pythium*), white mold (*Sclerotinia sclerotiorum*), and root-knot nematodes (various species of *Meloidogyne*) (Davis and Raid 2002).

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Widespread pests of concern to carrot producers include the carrot rust fly (*Psila rosae*), aphids (e.g., the willow-carrot aphid, *Cavariella aegopodii*), and the two-spotted spider mite (*Tetranychus urticae*) (Simon et al. 2008). Other carrot pathogens and pests cause losses of regional significance (Davis and Raid 2002), such as violet root rot, bacterial soft rots, and Fusarium dry rot. For most of these biotic stresses, breeders have relied on natural infection in areas where the pathogen or pest is well established to facilitate identifying and selecting genetic resistance. Highly susceptible cultivars or breeding lines sometimes are planted at intervals among carrot entries to promote the development of the biotic stress. For some of these pests and pathogen, these screening and breeding efforts include plants inoculated with the pathogen or infested with the pest. Screening for resistance to soilborne pests and diseases can be particularly complex because of the difficulty of establishing uniform soilborne disease pressure, especially for screening large numbers of lines and for stresses caused by multiple species or races of a pathogen (e.g., cavity spot and root-knot nematodes) or a pest for which there could be different types or sources of resistance (e.g., root-knot nematodes and aphids). This chapter describes efforts to identify resistance to specific pests and pathogens of carrot, phenotypic screening methods evaluated, and what is known about the genetic basis of resistance, including inheritance of resistance and annotation of resistance genes on the carrot genome. Unfortunately, for a majority of the diverse biotic stresses of carrot, resistance genes have not been identified and/or little is known about the genetic basis of resistance that has been identified. The numerous gaps in understanding of the genetics of carrot germplasm reactions to these biotic stresses, as detailed in this chapter, highlight the need for additional research.

This chapter is not a comprehensive review of the literature on resistance to all known pathogens and pests of carrot. The chapter focuses on some key pathogens and pests for which there have been efforts to screen for resistance and to evaluate the genetic basis and genomics of resistance. Some pathogen and pest names used in older literature cited in this chapter have changed. The effort was made to use current scientific nomenclature. Synonyms of these pests and pathogens are noted. The carrot diseases reviewed in this chapter are divided into those caused by soilborne pathogens and those caused by foliar pathogens, followed by a section on nematode and insect pests.

## 18.2 Carrot Diseases

### 18.2.1 Soilborne Diseases

#### 18.2.1.1 Cavity Spot (*Pythium* spp.)

Cavity spot has been documented in almost all regions of carrot production in the world (McDonald 2002). The disease is caused by several species of *Pythium*, the most common being *P. violae* and *P. sulcatum*, two slow-growing species that typically are the most virulent on carrot roots (McDonald 2002). Other species associated with cavity spot include *P. ultimum*, *P. intermedium*, *P. irregulare*, and *P. sylvaticum*. The disease rarely causes a reduction in root yield but can have significant economic impact because the shallow, surface lesions render roots unsuitable for fresh and processing markets (McDonald 2002) (Fig. 18.1a). *Pythium* spp. typically infect carrot roots within the first four to six weeks after seeding and probably throughout the growth of the carrot (McDonald 1994b). Cavity spot will continue to develop on roots in storage. Root lesions can be invaded by secondary microorganisms, including bacteria, which can lead to discoloration around the cavities, particularly during heating/blanching (Fig. 18.1b). Severity of cavity spot generally increases the longer the roots are in soil (Montfort and Rouxel 1988; Vivoda et al. 1991).

Resistance screening *Partial resistance to cavity spot*. Differences in susceptibility of carrot cultivars to cavity spot have been identified, but no commercially available carrot cultivars are completely resistant (Groom and Perry 1985; McDonald 1994b; McDonald 2002; Soroker et al. 1984; Sweet et al. 1986; Vivoda et al. 1991;

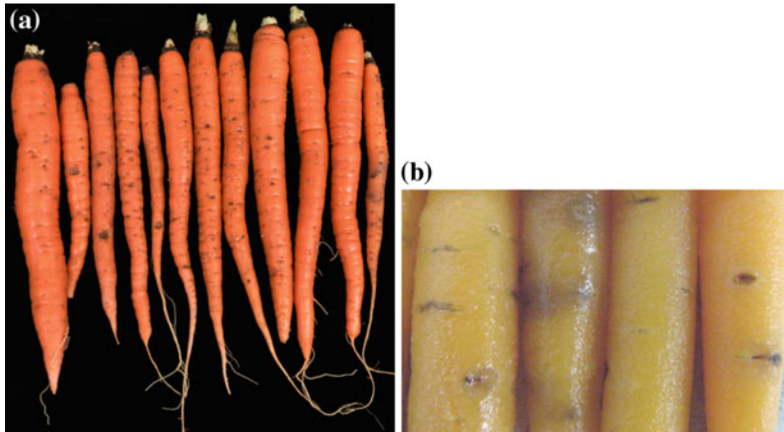


**Fig. 18.1** **a** Severe symptoms of cavity spot on carrot roots caused by *Pythium sulcatum* (Alex Batson, Washington State University). **b** Lesions and discoloration of peeled and blanched carrot roots resulting from cavity spot (Lindsey du Toit, Washington State University)

White 1988). Guba et al. (1961) first reported differences in cultivar susceptibility to cavity spot. 'Hutchinson' roots developed less cavity spot than those of 'Waltham Hicolor,' but the differences were greater among lots of 'Waltham Hicolor' than among cultivars. The National Institute of Agricultural Botany (1991) in the UK reported variation in susceptibility among groups of carrots (Sweet et al. 1989). 'Redca' was a more resistant Chantenay cultivar than 'Supreme,' and 'Nandor' was a more resistant Nantes cultivar than 'Tino.' That study also documented increased severity of cavity spot on later maturing types or when cultivars were harvested later in the autumn; e.g., the Autumn King type Vita Long was more susceptible at late harvest compared to early harvest (National Institute of Agricultural Botany 1991).

In growth chamber trials, six Imperator cultivars commonly grown in California were planted in a potting medium inoculated with *P. violae* and *P. ultimum* and maintained at 20 °C (Vivoda et al. 1991). All six cultivars were susceptible to cavity spot caused by the two species, but *P. violae* isolates were more virulent than *P. ultimum* isolates. 'Topak' was the most susceptible cultivar to both species. The other five cultivars varied in response to *P. violae* but were similarly susceptible to *P. ultimum*. 'Caropak' and 'Pakmor' were the next most susceptible after 'Topak,' followed by 'Sierra' and 'Dominator.' The cultivars originated from a few parental lines which might account for the limited variation in response to *P. violae* and *P. ultimum* (Vivoda et al. 1991).

White et al. (1987) screened 19 carrot cultivars representing five main types of carrots (Amsterdam Forcing, Nantes, Chantenay, Berlicum, and Autumn King) for resistance to cavity spot caused by each of *P. violae*, *P. sulcatum*, and *P. intermedium*. Roots of each cultivar were grown in a greenhouse, washed, and inoculated with colonized agar plugs of each *Pythium* species (10 plugs per root, with 5 roots tested for each of two replicate trays per cultivar). For *P. violae*, there were no significant differences among the five carrot types or the 19 cultivars. For *P. sulcatum*, differences were detected among types of carrots but not among cultivars, and then only for one of the three measures of cavity spot (percentage of agar disks that resulted in lesions on roots two days after inoculation). For *P. intermedium*, White et al. (1987) only detected significant differences among cultivars, not types of carrots, and only for one of the three measures of cavity spot (percentage of disks causing lesions four days after inoculation). They concluded there was no 'useful' genetic resistance to the three *Pythium* spp. among the cultivars tested.

White et al. (1988) found no differences in frequency of recovery of *Pythium* spp. from the periderm of asymptomatic carrots of 'Chantenay New Supreme,' 'Fingo,' and 'Sweetheart.' They also found no useful genetic resistance when mature roots of 19 cultivars representing five main groups of carrots were inoculated with mycelial plugs of *P. violae*, *P. sulcatum*, and *P. intermedium*. However, results of a similar trial by Vivoda et al. (1991) suggested that inoculation of roots with mycelial plugs may not provide accurate determination of differences in cultivar resistance. In contrast, Benard and Punja (1995) indicated that inoculation of 36 carrot cultivars with *P. violae* in a laboratory study demonstrated differences in susceptibility that correlated significantly with field results. Various private breeding programs have made effective progress at developing cultivars with enhanced resistance to cavity spot using combinations of field nurseries and greenhouse screening protocols with inoculated soil or potting medium, as well as laboratory assays that entail root inoculation.

McDonald (1994b) found the partially resistant cultivar 'Six Pak' effectively suppressed cavity spot in field trials in Ontario, Canada. 'SR-481,' 'Eagle,' and 'Red Core Chantenay' had intermediate levels of resistance, and 'Chanton' and 'Huron' were the most susceptible. Similarly, 'Six Pak' was more resistant than 'Cellobunch' and 'Chancellor.' Interestingly, 'Eagle' was as resistant as 'Six Pak' in non-irrigated plots but was more susceptible under irrigated conditions. The cultivars had little effect on early season development of cavity spot, with differences in susceptibility only becoming evident as roots matured. This was the first study to demonstrate that older carrots are not necessarily more susceptible to cavity spot than younger carrots, based on seeding carrots on different dates in the same plots. Also, a marked decrease in cavity spot incidence was observed late in the season (McDonald 1994b).

Using an in vitro mature carrot root inoculation protocol, Benard and Punja (1995) screened 37 carrot cultivars for reaction to cavity spot. 'Panther,' 'E0792,' 'Caropride,' 'Fannia,' and 'Navajo' were the most resistant. 'Six Pak,' 'Imperator,' and 'XPH 3507' also appeared very resistant but were tested only once. There were inconsistencies in results between years among 18 cultivars tested in both years; e.g., 'Eagle' was resistant in 1991 but susceptible in 1992, although ratings of most cultivars were similar between years. They noted that cultivars with discrepancies might have reflected differences in rootage or growing conditions between years.

Cooper et al. (2004) examined morphological and biochemical responses of commercial carrot cultivars Bertan, Narbonne, and Bolero as well as the 'Eastern' carrot genebank variety 'Purple Turkey' to inoculation with *P. violae* in a greenhouse bioassay and field trials. 'Purple Turkey' was less susceptible than all commercial cultivars. The small cell size in the roots and higher constitutive levels of enzymes in the roots of 'Purple Turkey' was hypothesized as the basis for resistance of this line to cavity spot. Of the commercial cultivars tested, 'Bolero' was the least susceptible, 'Narbonne' was intermediate, and 'Bertan' was the most susceptible.

Cooper et al. (2006) screened seed progeny from 19 tissue culture-derived carrot somaclone families for resistance to cavity spot caused by *P. violae* in greenhouse and field trials, along with 'Bertran,' 'Nando,' 'Bolero,' and 'Vita Longa' as commercial control cultivars. There was little relationship between greenhouse and field trial results although the results suggested there might be genetic variation in susceptibility to cavity spot in some of the somaclones.

For several years, McDonald et al. (2017) have screened experimental carrot breeding lines from the United States Department of Agriculture (USDA) carrot breeding program alongside commercial carrot cultivars in a field site at the Muck Crops Research Station of the University of Guelph in the Holland Marsh of Ontario, Canada. The site has a high level of natural infestation with the cavity spot pathogen. Each year, a wide range in incidence and severity of cavity spot has been observed in breeding lines and cultivars. Lines with low cavity spot incidence and severity displayed a consistent response among years, including crosses with these more resistant lines,

e.g., the orange lines CS736 (pedigree 1137A), and CS732 (1137B-$F_2M_5$), and the USDA parent lines 1137, 5367, and 6526. A similar consistent response was observed for lines that had the greatest cavity spot incidence and severity ratings, and many crosses with those lines, e.g., 2205, 5494, and CS 724 (2205B). However, despite the relatively uniform disease pressure in this nursery, some lines did not respond consistently among years, illustrating the difficulty of screening for resistance to cavity spot (McDonald et al. 2017). Forking of carrot roots, which has been attributed in some studies to be caused by the same *Pythium* spp. that cause cavity spot, was not correlated with cavity spot incidence and severity ratings in the muck nursery trials (McDonald et al. 2017).

*Phenotypic screening methods*. Uneven distribution of inoculum in fields and the very sporadic nature of the disease within and among fields make screening for resistance to cavity spot very difficult. Wide variation in responses among roots of the same cultivar necessitates evaluating large numbers of roots of each carrot line in replicated and randomized plots over multiple seasons for robust differentiation of responses among cultivars. The occurrence and severity of cavity spot in phenotypic screening methods can be influenced significantly by soil temperature, soil moisture, other soil properties (including soil microflora), the species of *Pythium*, age of carrot roots, etc. (Benard and Punja 1995; McDonald 1994b, 2002). Higher soil moisture, particularly flooding, and cool soil temperatures (~15 °C) tend to be optimal for cavity spot development.

Severity of cavity spot generally increases with the length of time roots remain in the soil (Montfort and Rouxel 1988; Sweet et al. 1989; Vivoda et al. 1991). This could reflect increased susceptibility of roots as they mature, accumulation of lesions over the season, expansion of lesions as the root diameter increases, or increased change of infection as the root surface increases (Vivoda et al. 1991; Wagenvoort et al. 1989). However, McDonald (1994b) demonstrated in field trials in Ontario, Canada, that older carrots are not necessarily more susceptible to cavity spot than younger carrots when carrots were planted on different dates in the same plots. McDonald (1994b) observed that cultivars had little effect on early season development of cavity spot, but severity and incidence of the disease differed among cultivars as the season progressed, despite similar inoculum levels and environmental conditions. She stated that an increase in severity of cavity spot during the growing season did not indicate roots become more susceptible as they aged, only that the disease continued to develop. Benard and Punja (1995) also found that carrot age (1–3 months) did not affect cavity spot development. Vivoda et al. (1991) found that the incidence of cavity spot did not increase during the season, but the number of lesions per root increased with plant age from three to five months after planting. McDonald (1994b) demonstrated that changes in cavity spot during the season appear more closely related to environmental factors than plant age. These temporal characteristics of cavity spot development illustrate the potential influence of timing of cavity spot evaluations on efforts to screen for resistance to the disease.

A number of breeding programs have used inoculation of mature carrot roots with agar plugs colonized by *Pythium* spp. that cause cavity spot to facilitate testing large numbers of roots per carrot line and large numbers of lines to counter the variability associated with efforts to screen for resistance to cavity spot. However, lesions induced by root inoculation with colonized agar plugs typically are more superficial, discolored, and have indistinct margins compared to cavity spot lesions that develop when roots are grown in infested soil or planting media (Vivoda et al. 1991). Vivoda et al. (1991) suggested that screening for resistance to cavity spot using colonized agar plugs may not reflect accurately the response of cultivars or breeding lines in soil conditions. However, others have demonstrated that inoculating carrot roots with agar plugs colonized by *P. violae* only works if roots are inoculated within 24 h of harvest because rapid suberization of the epidermis following harvest limits infection of the roots by that species. In contrast, inoculation of roots with *P. sulcatum*-inoculated plugs can be done as late as a week

after harvest as long as the roots are stored in cool conditions to limit the extent of root suberization and desiccation. To avoid these issues, some bioassays that entail root inoculations entailed removing the tops after harvest of the roots to prevent excessive dehydration, and submerging the roots in water until they were inoculated to improve the reliability of the bioassays (e.g., Cooper et al. 2004). Other recommendations for increasing consistency in results with root inoculations include not surface-sterilizing the roots prior to inoculation (just washing the roots gently to avoid damaging the periderm), incubating the roots in the dark at cool temperatures (15–20 °C), and incubating the roots at high relative humidity (e.g., with regular misting or in dew chambers) for a 7- to 10-day duration of incubation before rating severity of cavity spot. Variability in lesion size among inoculation sites on the same root and among roots of the same entry necessitates inoculating and rating large numbers of roots (e.g., inoculating and rating 40–50 roots/entry/replication). The tedious nature of root agar plug inoculation protocols has limited the use in carrot breeding programs. Others have dipped roots of entries into a slurry of inoculum prepared by blending colonized agar plates in water, and then incubating the inoculated roots at high relative humidity before rating the roots for severity of cavity spot. Suffert and Montfort (2007) developed a soil infestation method in which an inoculated and infected carrot root was planted in close proximity to healthy roots to induce typical symptoms of cavity spot. Cavity spot lesions were induced more efficiently with this method than inoculating soil with *P. violae*.

*Rating carrot roots for cavity spot*. Various ways of assessing cavity spot have been reported. Some have been based on the incidence of roots with lesions, severity of lesions (e.g., number of lesions per root or the size of the lesions using horizontal and/or vertical length of each lesion), combinations of the two lesion dimensions (McDonald 1994b), length of the largest lesion/root, or categorizing lesions as small, medium, and large to facilitate rating large numbers of roots. The use of different assessment methods can make it difficult to compare results among studies. Assessment of cavity spot incidence or severity on a single harvest date can give variable results because cavity spot levels can increase or even decrease during the season. For this reason, McDonald (1994b) found the area under disease progress curve (AUDPC) to be more informative than incidence ratings for differentiating treatment effects in field trials in Canada. However, calculation AUDPC necessitates multiple assessments. Comparing slopes and elevations of disease progress curves also provided useful information for assessing the resistance of cultivars to cavity spot in field trials (McDonald 1994b).

*Genetics of resistance*. Several studies have suggested that cavity spot lesions represent a hypersensitive response of carrot root tissues to abort *Pythium* infections (Endo and Colt 1974; Klisiewicz 1968). Others have demonstrated that resistance generally is quantitative based on relatively minor differences in severity among cultivars (e.g., Johnston and Palmer 1985; White 1991). To date, there appear to be no published (publicly accessible) studies on the genetics of resistance to cavity spot.

Pectate lyase and cellulose produced by *Pythium* spp. are involved in the development of cavity spot lesions (Cooper et al. 2004). Induction of cell wall-degrading enzymes occurs after extensive penetration of root tissue by these pathogens, with enzyme production localized near the area of hyphal penetration (Campion et al. 1988; Guérin et al. 1994). Benard and Punja (1995) showed that highly virulent isolates of *Pythium* spp. produced significantly greater concentrations of pectolytic enzymes compared to moderately or weakly virulent isolates. The pathogens caused disintegration of host cells and the development of hyphae beneath the epidermis, followed by collapse of the infected area to form a cavity. Carrot roots respond to infection with synthesis and deposition of material around the site of infection, including oxidized phenolics and phenylalanine-ammonia lyase. The latter is thought to be associated with deposition of lignin

around the lesion, providing physical protection against the pathogen. Impeding internal spread of *Pythium* has been proposed as one component of resistance to cavity spot (Endo and Colt 1974). Root defense mechanisms are thought to be activated after cell collapse to impede infection, as protease, peroxidase, and polyphenol oxidase activity were elevated in cavity tissue compared to healthy surrounding root tissue (Perry and Harrison 1979; Soroker et al. 1984). The phenol content of cavity tissue increased proportionally to the severity of cavity spot (Soroker et al. 1984). Suberin and lignin were deposited in the cell walls of periderm surrounding the lesions and accumulated in the phloem parenchyma cells near the wound surface (Perry and Harrison 1979). Garrod et al. (1982) indicated the development of these structural barriers was less important than accumulation of antifungal compounds in resistance to *Pythium*. Some antifungal compounds have been detected in non-inoculated root tissue, e.g., falcarindiol (Garrod et al. 1978), and others are elicited in response to injury or infection by pathogens, e.g., the phytoalexin 6-methoxymellein (Kurosaki et al. 1985). This was confirmed by structural analysis by Guérin et al. (1998) of susceptible and partially resistant cultivars who suggested that cell walls of the more resistant cultivars were better preserved, possibly as a result of fungitoxic phenolic compounds synthesized in response to infection. As noted above, Cooper et al. (2004) suggested the small cell size in roots, and higher constitutive levels of enzymes in the roots of 'Purple Turkey' might account for the resistance of this line to cavity spot compared to commercial cultivars they screened for morphological and biochemical responses of roots to *P. violae*.

White et al. (1988) suggested the speed at which a carrot root responds to infection with these defense mechanisms might determine the degree of susceptibility to cavity spot. *Pythium* spp. were recovered more frequently from juvenile tissue approximately eight weeks after seeding compared to isolations as plants matured. Fast-growing *Pythium* spp. were recovered readily from asymptomatic periderm but not from symptomatic tissues, which suggested that carrot defense mechanisms prevent infection by these fast-growing species or the roots do not react to these species (McDonald 1994b). In contrast, the cavity reaction is elicited by slow-growing species such as *P. violae* and *P. sulcatum* (White et al. 1988). This supported observations by Zamski and Peretz (1995) that fast-growing species did not cause lesions, only slow-growing species which penetrated root tissue for 3–4 days, releasing small amounts of wall-degrading enzymes before a host response occurred. They observed a lag of about 5 days before lignin deposition increased linearly.

Severity of cavity spot typically increases while carrot roots are in cold storage (McDonald 1994b). The increase in susceptibility with storage may be associated with changes in the carrot root that occur with the onset of bolting, a vernalization-induced physiological shift from vegetative to reproductive growth. An increase in the number of lesions per root also can occur in storage, which suggests latent infections present on the roots at harvest can progress to active infections in storage. However, wound healing during storage may heal some smaller cavity spot lesions (McDonald 1994b).

#### 18.2.1.2 Phytophthora Root Rot or Rubbery Brown Rot (*Phytophthora* spp.)

Phytophthora root rot of carrot, also called rubbery brown rot, is generally a minor disease but can cause significant losses in waterlogged soils and usually occurs after periods of excessive rain or irrigation (Browne 2002). The disease can be caused by several species of *Phytophthora*, including *P. cactorum*, *P. cryptogea*, *P. megasperma*, and *P. porri*. Phytophthora root rot has been documented in Canada, France, Norway, Australia, and the USA (Browne 2002; Ho 1983; Rader 1952; Saude et al. 2007; Stelfox and Henry 1978; White 1945). Symptoms can develop in the field, usually close to harvest, and in storage, with roots becoming dark brown to black and rubbery (Fig. 18.2). However, often symptoms only become visible after roots have been in storage for some time. The firm, water-soaked lesions usually develop in the

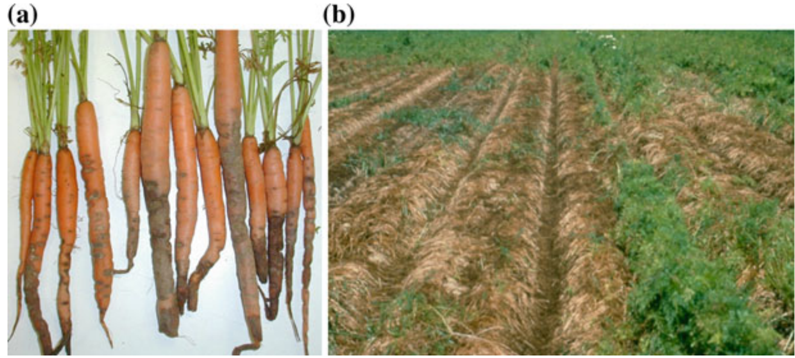


**Fig. 18.2** Phytophthora root rot (rubbery brown rot) symptoms on individual carrot roots (**a**) and in a low-lying, saturated area of a carrot crop (**b**) (R. Michael Davis)

middle and crown areas of roots (Saude et al. 2007). Losses have been documented in fields in France in the winters. The pathogen can produce white mycelium on root lesions. Secondary invasion of the lesions by bacteria and fungi can lead to a soft rot. Periods of prolonged saturation during carrot growth, processing, or storage favor production and release of swimming spores called zoospores. Cool to moderate conditions favor inoculum production and spread of the pathogen. The pathogen spreads readily in storage.

Resistance screening: To date, there appear to be no published studies on screening for resistance to Phytophthora root rot of carrot. Stelfox and Henry (1978) noted the disease was documented in Alberta, Canada, in stored carrots of 'Imperator II' in the winter of 1969–1970. Symptoms were not observed on roots at harvest or during the washing operation. Saude et al. (2007) reported this disease in processing carrot fields in multiple counties in Michigan, but no information was provided on specific cultivars affected or differences in severity of the disease among cultivars. Based on the protocols used for testing pathogenicity of isolates of *Phytophthora* on carrot roots (e.g., Saude et al. 2007; Stelfox and Henry 1978), it should be possible to screen carrot cultivars or breeding lines for resistance to rubbery root rot using a protocol similar to the root agar plug inoculation method described for cavity spot. Colonized agar plugs of the pathogen, taken from 7- to 14-day-old cultures, could be placed on washed carrot roots, the surface of the roots moistened or misted regularly with sterilized water, and the inoculated roots incubated at high relative humidity for up to seven days at cool to moderate temperature. Various studies have incubated inoculated roots at temperatures ranging from 20 to 25 °C, but the optimal temperature might depend on the particular *Phytophthora* species being used to screen for resistance. Symptoms developed within a week when roots were stored at 20 °C, but only after seven weeks when stored at 0 °C (McDonald 1994d). Saude et al. (2007) demonstrated that wounding was not necessary to get typical symptoms of rubbery root rot with this method of inoculation. In fact, wounding resulted in slightly different symptoms. Key features of a phenotypic resistance screening protocol are likely to include the equivalent of saturated soil conditions that are necessary for the development of Phytophthora root rot, and storing carrots at 20 °C with high relative humidity (>95%).

#### 18.2.1.3 Diseases Caused by *Rhizoctonia* spp.

Three species of *Rhizoctonia* have been demonstrated to cause diseases of carrot: *R. carotae* (= *Fibularhizoctonia carotae*, sexual stage *Athelia arachnoidea*), *R. crocorum* (sexual stage *Helicobasidium brebissonii* = *H. purpureum*), and *R. solani* (sexual stage *Thanatephorus cucumeris*) (Davis and Raid 2002). All three species are soilborne. *R. solani* is found in most soils.

*Rhizoctonia solani* is one of the multiple soilborne pathogens that can cause damping-off of carrot seedlings (Nuñez and Westphal 2002), and the fungus also causes crown rot of mature carrots (Punja 2002b) (Fig. 18.3). Isolates of this

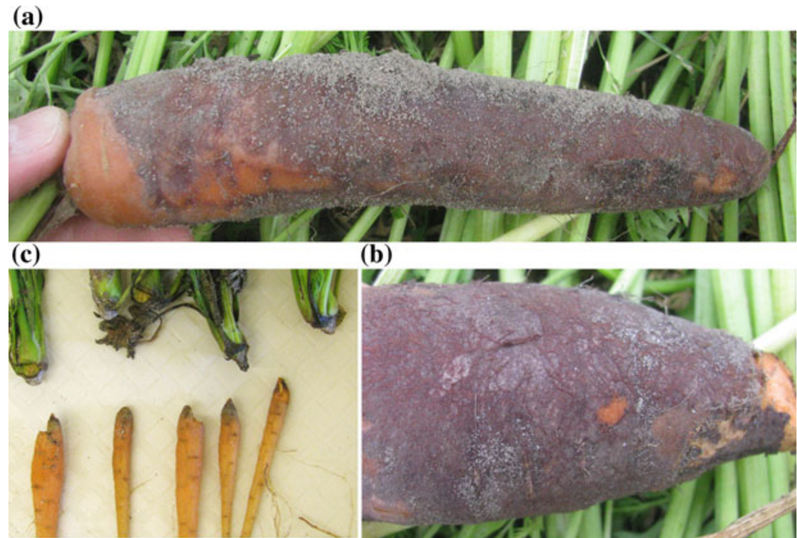


**Fig. 18.3** Severe symptoms of violet root rot of carrot (**a** and **b**) caused by *Rhizoctonia crocorum*, and detached crowns (**c**) as a result of crown rot caused by *Rhizoctonia solani* (Lindsey du Toit, Washington State University). *Note* the web-like growth of purple-brown hyphae on the surface of the root infected with violet root rot (**b**)

pathogen that cause damping-off of carrot tend to belong to one of three anastomosis groups (AGs), AG-2 (primarily) and, to a lesser extent, AG-1 and AG-4 (Grisham and Anderson 1983; Nuñez and Westphal 2002). Conditions that delay seed germination and emergence, e.g., cool, and wet soils, favor damping-off. Damping-off results in poor seed germination (seed rot), root dieback as a result of loss of the apical meristem, death of seedlings pre-emergence or post-emergence, and seedlings with extremely poor vigor and stunting (Nuñez and Westphal 2002). Crown rot tends to be problematic in muck soils with high levels of organic matter, particularly in regions with warm, wet conditions close to harvest (Howard and Williams 1976; Punja 2002b). The disease may only become evident late in the season when the foliage senesces prematurely, sometimes in patches. The petioles and crowns rot, and dark brown, sunken lesions develop near the crown and sometimes further down the root (Punja 2002b). Crown rot lesions can be similar to those associated with cavity spot. Lesions on the crown or taproot render roots unmarketable, and secondary invasion of lesions by bacteria can initiate soft rot. Web-like mycelium can develop in lesions under very moist conditions. Lesions continue to expand when roots are placed in storage. Although empirical observations of carrot cultivars in growers' fields indicated all cultivars were susceptible to crown rot, partial resistance to crown rot has been suggested (Howard and Williams 1976) based on cultivar responses in fields with different amounts of inoculum and favorability of conditions for this disease.

*R. crocorum* causes violet root rot of carrot, celery, fennel, parsley, and parsnip as well as many other vegetable crops (e.g., table beets and potato) and weeds (Cheah and Page 1999; McDonald 1994e; Punja and McDonald 2002). Violet root rot of carrot occurs in many regions of carrot production but has caused greater losses in Europe, New Zealand, and Australia than in North America. Patches of dead or dying plants are usually the first evidence of this disease, with soil adhering to roots pulled out of the ground. Dark purple-brown, firm lesions develop on roots, on the surface of which a dense mat of mycelium of the fungus forms that can become violet to dark brown and leathery. The fungus can grow between plants as a thick, brown, mycelial mat on the soil surface (McDonald 1994b). Soft rotting of the root develops beneath the lesions. Symptoms tend to appear later in the season, and violet root rot can continue to develop in storage. The pathogen can infect carrot roots at soil temperatures ranging from 5 to 30 °C, with an optimum of 20 °C. This disease

can occur in all soil types but tends to be more severe at high soil moisture, low soil pH, and low soil nitrogen (Cheah and Page 1999; Garrett 1949). An effort to screen 54 carrot cultivars for resistance to violet root rot in each of three sites naturally infested with *R. crocorum* in the UK did not reveal differences in susceptibility among the cultivars (Dalton et al. 1981). Similarly, field trials in New Zealand revealed all commercial carrot cultivars tested to be susceptible to violet root rot (Cheah and Page 1999).

*R. carotae* causes crater rot of carrot, a postharvest disease observed on carrots placed in longer-term storage (Punja 2002a). The fungus is not known to cause disease on any other plant species. Crater rot occurs in North America and Northern Europe, with losses as great as 50–70% recorded in Denmark (McDonald 1994a). Dry, sunken craters or pits form on the surface of roots under very humid, cool conditions in storage, with white mycelium lining the lesions and appressed to the root surface, and in which dark brown sclerotia may form (McDonald 1994c; Punja 2002a). The fungus spreads readily in storage. Crater rot is a dry rot, but infected roots can become colonized by bacteria, leading to soft rot. Latent infections of roots occur in the field, and roots with senescent foliage attached to the crown may harbor greater inoculum. The fungus can develop on roots at temperatures as low as 2–3 °C, with infection favored when roots are held at high relative humidity or a film of water develops on the roots (Punja 2002a). *R. carotae* can even grow at −1 °C (Punja 1987). Delayed harvest of carrots to late autumn appears to exacerbate disease pressure.

Resistance screening: Damping-off: Since cool, wet soil conditions delay seed germination and seedling emergence, and favor damping-off, planting carrot seed into cool, wet, poorly drained soils or providing excessive irrigation after seeding can favor damping-off and enhance efforts to screen for resistance to damping-off (Nuñez and Westphal 2002). Raised beds increase soil draining, so planting into flat fields can enhance damping-off in screening trials. However, these conditions favor all damping-off pathogens, not just *R. solani*, so it may be difficult to separate responses of carrot germplasm to different causal agents of damping-off, including *Pythium* spp., unless carrots are screened in sterilized or pasteurized soil or other planting media to which the target pathogen has been added, or seed is treated with a fungicide such as mefenoxam which can control *Pythium* spp. without affecting *Rhizoctonia* spp.

Crown rot: Howard and Williams (1976) attempted to screen carrot lines for resistance to Rhizoctonia crown rot by planting carrot seed in steamed muck soil, inoculating the carrots after 4 weeks with *R. solani* infested corn kernels, and assessing the number of normal and abnormal roots when the roots were harvested 16–20 weeks after planting. Seven-day-old agar plugs of a highly virulent isolate of *R. solani* grown on cornmeal agar were added to flasks containing autoclaved corn kernels and incubated at 20–24 °C for two weeks with the flasks shaken every 2–3 days to facilitate uniform colonization of the corn kernels by the fungus. Inoculum age (2–16 weeks after preparation) did not affect the reaction of 'Royal Chantenay' and 'Scarlet Nantes,' but they recommended using 'fresh' inoculum for each test. They observed no difference in disease incidence/severity when inoculum was added to the soil 2, 3, 4, or 6 weeks after planting, but found it most convenient to thin carrots three weeks after seeding and add inoculum a week later, similar to the protocol used by Mildenhall and Williams (1970). Howard and Williams (1976) also recommended maintaining soil moisture at approximately −0.1 bars and growing carrots at 20, 24, or 28 °C for optimal carrot plant and crown rot development. Crown rot pressure can be enhanced by placing infested soil or infested carrot debris in contact with the crown and petioles, by close spacing of carrots to promote a humid microclimate once the canopy closes, and if temperatures are warm (>18 °C) (Gurkin and Jenkins 1985; Punja 2002b). Planting carrot crops into infested debris, following perennial crops such as alfalfa, and adding inoculum (e.g., colonized grain kernels) to soil or other potting media can increase disease pressure in resistance screening trials (e.g., Breton et al. 2003).

Violet root rot: Since violet root rot is favored by high soil moisture, low soil pH, and low soil nitrogen levels, screening for resistance could be enhanced by using field sites with acid soils or using acid planting media, maintaining high soil moisture, and keeping roots in infested soil or planting medium as long as possible as disease incidence and severity increase the longer roots are in infested soil (Cheah and Page 1999; Garrett 1949; McDonald 1994e; Punja and McDonald 2002). However, Dalton et al. (1981) were not able to detect any differences in susceptibility among 54 commercial carrot cultivars (9 Amsterdam Forcing selections, 11 Nantes, 10 Chantenay, 9 Autumn King, 5 Danvers, 3 Berlicum, 6 Feonia or Imperator, and 1 unknown type) tested in three field sites in the UK that were naturally infested with *R. crocorum*. Similarly, Cheah and Page (1999) did not observe differences in susceptibility among commercial carrot cultivars. Slight differences in severity of violet root rot at one site were not significant statistically because of inadequate disease pressure, and severe disease pressure at another site still did not enable differentiation of cultivar reactions to violet root rot. Dalton et al. (1981) hypothesized the lack of differences may reflect the fact that western carrots have been developed from closely related types—Late Half Long, Early Half Long, and Early Scarlet Horn, all of which were derived from Long Orange by selection or intercrossing. They recommended searching for resistance in pre-cursor types to western types, namely anthocyanin and yellow types. There remains a need for an effective and efficient protocol to screen for resistance to violet root rot.

Crater rot: Hyphae of *R. carotae* can grow over a carrot root within a few days, penetrating the root surface without forming appressoria or other infection structures, and killing root cells in advance of hyphal penetration (McDonald 1994a). Roots placed in storage can be rendered unmarketable within three weeks of infection. There does not appear to have been any effort to screen for resistance to crater rot of carrot, but a root screening protocol should be feasible given crater rot is a postharvest disease and the pathogen is highly virulent in cool, moist storage conditions. Wounding of roots increases the severity of crater rot, so wounding could be incorporated into a screening protocol. It may be difficult to develop a soil inoculation protocol that mimics field infection, given the latent nature of field infections.

#### 18.2.1.4 Bacterial Soft Rots

Several bacteria can cause soft rots of carrot, including *Pectobacterium carotovorum* subsp. *carotovorum* (formerly *Erwinia carotovora* subsp. *carotovora*), *Dickeya dadantii* (formerly *D. chrysanthemi* = *Pectobacterium chrysanthemi* = *Erwinia chrysanthemi*), and *Pectobacterium atrosepticum* (formerly *P. carotovorum* subsp. *atrosepticum* = *E. carotovora* subsp. *atroseptica*) (Farrar 2002; McDonald 1994a; Nuñez and Davis 2016). These bacteria tend to be ubiquitous in soils and can infect a wide range of plant species, including most vegetables. Bacterial soft rot of carrot is a problem primarily in storage, where the pathogens can cause major losses as secondary invaders of roots that were wounded or infected with other pathogens. Soft rot symptoms usually only develop in the field in low-lying areas or other areas that become saturated (e.g., near broken irrigation pipes). Sporadic reports of severe outbreaks in fields are associated with extended periods of saturated soil conditions and warm temperatures as these bacteria are thermophilic, facultative anaerobes (Farrar 2002). The pathogens also can be found in sources of water used for irrigation or water used to wash carrot roots after harvest (Segall and Dow 1973), and can be disseminated by insects (Phillips and Kelman 1982). The bacteria infect carrot roots through wounds or natural openings, causing small, water-soaked lesions that enlarge rapidly (Fig. 18.4). The pathogens degrade roots most rapidly under warm conditions (20–25 °C for *P. carotovorum* subsp. *carotovorum* and 30–35 °C for *D. dadantii*), and infected roots become mushy and soft. The outer surface of infected roots may remain intact over a softened interior, and macerated interior tissue may ooze through cracks that form on the root surface (McDonald 1994a).

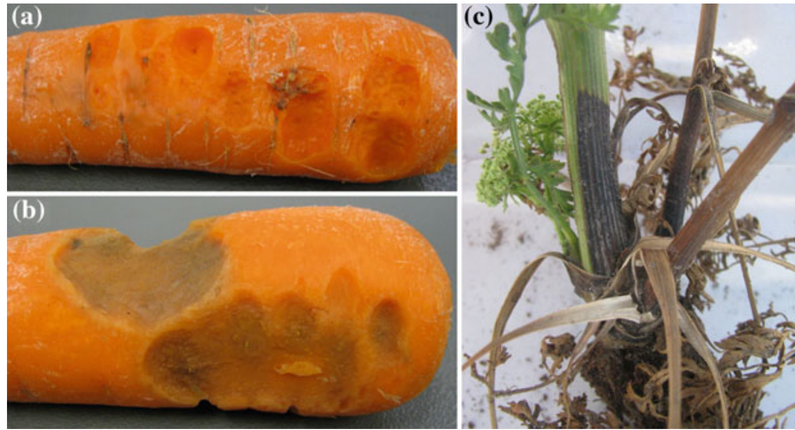


**Fig. 18.4** Severe root pitting symptoms caused by bacterial soft rot (**a** and **b**), and infection of the base of seed stalks of a bolted carrot plant by *Pectobacterium carotovorum* subsp. *carotovorum* (**c**) (Lindsey du Toit, Washington State University)

Resistance screening: There have been various attempts to screen for resistance to soft rot of carrot (Bedlan 1984; Lebeda 1985; Michalik et al. 1992; Michalik and Sleczek 1997; Skadow 1978). Although Segall and Dow (1973) did not focus on screening for resistance to soft rot, they demonstrated that holding carrot roots naturally infected with *P. carotovorum* subsp. *carotovorum* at 21 °C for four days resulted in more severe soft rot than holding roots at 2 °C for three days and then at 21 °C for four days. They suggested that less severe soft rot associated with refrigerated storage before and after inoculation may reflect the development of phenolic or related compounds in refrigerated carrots. For example, the antimicrobial compound 3-methyl-6-methoxy-8-hyrdoxy-3, 4-dihydroxoisocoumarin was found in carrot roots stored for 4–8 weeks at 0 °C but not in freshly harvested carrot roots. Segall and Dow (1973) suggested this may contribute to resistance to bacterial soft rot after cold storage of carrots.

In an effort to develop an efficient, reliable method of screening carrot breeding material for resistance to soft rot caused by *P. carotovorum* subsp. *carotovorum* and *P. atrosepticum*, Michalik et al. (1992) evaluated four methods of inoculation of roots. They used roots harvested from an organic soil in Wisconsin that had been stored for 1–3 weeks at 0–4 °C, washed with tap water, surface-sterilized with 0.05% NaOCl for 40 min and 70% ethanol for 5 min, rinsed in sterilized water, and air-dried. The inoculation methods included: (1) injecting a 10 µl aliquot of bacterial suspension into each of two holes (1 mm diameter × 2 cm deep) in the cambium region through the cut surface of the crown portion of each root (top third of the root, including 2 cm of trimmed petioles attached); (2) the same injection method using the middle third of the root; (3) 5-mm-thick, cross-sectional slices of the root on each of which a 5-mm-diameter filter paper disk was placed on the cambial region of the proximal cut surface after the disk had been soaked in a bacterial suspension for 30 min; and (4) root slices each inoculated with a 10 µl aliquot of bacterial suspension placed on the cambial region of the freshly cut surface without additional wounding (Michalik et al. 1992). Inoculated root samples were incubated at 22 °C for 48–96 h in boxes lined with wet paper towel, sealed with plastic wrap, and enclosed in plastic bags. They observed differences in responses of inbred lines and open-pollinated cultivars to the two pathogens, with *P. carotovorum* subsp. *carotovorum* causing more severe soft rot than the isolate of *P. atrosepticum*, although the severity of soft rot increased with increasing inoculum concentration for both bacteria. They did not detect bacterial strain × carrot line or strain × inoculum concentration interactions. The two root cross-section inoculation methods resulted in more severe soft rot and less variability in reactions than methods using larger root sections. The most consistent responses were achieved

with bacterial-soaked filter disks as the disks reduced evaporative drying of the inoculum. The use of root slices also enabled replicated screening from individual roots, and the crown of the root could be maintained for seed production. The duration of postharvest storage of carrot roots (2, 6, or 12 weeks) did not influence the severity of soft rot. Results were similar for stored intact versus cut roots, although the root tip tended to be more susceptible than the crown or middle of the root (Michalik et al. 1992). The variation observed among carrot lines suggested advances could be made in breeding for resistance to soft rot, as observed by others.

In a follow-up study, Michalik and Ślęczek (1997) evaluated progenies from crosses of orange carrot cultivars with five wild *D. carota* subspecies and four local Mirzoe cultivars from Uzbekistan to identify a source of resistance to soft rot caused by *P. carotovorum* subsp. *carotovorum*. Although genetic variation in sensitivity to soft rot had been observed in orange carrot cultivars, they considered the variation too limited for breeding purposes. They inoculated carrot root disks with filter disks soaked in a bacterial suspension ($5 \times 10^6$ CFU/ml) for 30 min, placed on the cambial region of each disk as described above. Inoculations of roots from the progeny of crosses of *D. carota* subsp. *sativus* with *D. carota* subsp. *commutatus*, *D. c. gummifer*, *D. c. drepanensis*, *D. c. maritimus*, and *D. c. gadecaei* did not indicate the presence of resistance genes to soft rot in these wild species since all the F2 and BC1 generations had more severe soft rot than the original orange cultivars. However, one of the four local Mirzoe cultivars showed some promise as a source of partial resistance to soft rot, although soft rot severity increased in the F2 generation. Stein and Nothnagel (1995) noted that pronounced differences in susceptibility to bacterial soft rot have been detected among lines, F1 hybrids, and open-pollinated carrot lines in Germany. For any laboratory screening method, results need to be correlated with storage and field evaluations, and it is important to use roots grown, harvested, and stored together to avoid confounding the various factors that can influence severity of soft rot (Lebeda 1985; Michalik et al. 1992; Michalik and Sleczek 1997; Skadow 1978).

The genetic basis of resistance of carrot lines to bacterial soft rot pathogens has not been determined, but variation in responses to soft rot bacteria among carrot lines suggests the potential for molecular screening methods to enhance the identification of QTLs associated with resistance to these pathogens.

#### 18.2.1.5 Black Rot (*Alternaria radicina*)

Black rot of carrot is caused by *Alternaria radicina* (formerly *Stemphylium radicinum*). Black rot has been documented mainly as a postharvest disease during root storage, as a disease affecting seedlings as a result of planting infected seed, and as a disease affecting carrot seed crops. In the field, *A. radicina* can cause a black decay on the foliage, petioles, and umbels (Meier et al. 1922). First described in New York, black rot has now been reported all over the world. The pathogen is seedborne and seed transmitted, causing seed rot, poor seedling establishment, and/or damping-off. *A. radicina* can persist in the soil for long periods (as long as eight years) and cause disease in subsequent carrot crops (Farrar et al. 2004; Maude 1966; Pryor et al. 1998; Scott and Wenham 1972). Black rot is characterized by black, sunken necrotic lesions on the taproots and crowns (Fig. 18.5). Under wet conditions, infection of the crown can result in rotting of petioles and leaf blight symptoms similar to those caused by *Alternaria dauci*, leading to significant crop losses because of the tops breaking during mechanical harvest with harvesters that pull roots out of the ground by their tops (Farrar et al. 2004; Grogan and Snyder 1952; Pryor et al. 1998). Once roots are infected, the pathogen spreads readily between roots in storage. Infection on umbels can reduce seed yield and seed germination.

Seed treatment with fungicides like azoxystrobin, fludioxonil, iprodione, or thiram, or with hot water or disinfectants like sodium

**Fig. 18.5** Severe symptoms of black rot caused by *Alternaria radicina* on carrot stecklings (vernalized roots used for a carrot seed crop)

hypochlorite can be effective methods to control seedborne inoculum of this pathogen (Biniek and Tylkowska 1987; Maude 1966; Pryor et al. 1994; Soteros 1979). Chen and Wu (1999) documented significant effects of two biological control agents, *Burkholderia cepacia* No. 229 and *Bacillus amyloliquefasciens* No. 224 against *A. radicina*. Kordowska-Wiater et al. (2012) showed that application of the yeast *Candida melibiosica* to carrot roots before inoculation with *A. radicina* partially reduced progress in the development of black rot.

Resistance screening: Pryor et al. (2000) evaluated 46 carrot cultivars under field conditions using a toothpick inoculation method and observed significant differences in lesion size among cultivars. Relatively resistant cultivars included 'Panther' and 'Caropak,' and susceptible cultivars included 'Royal Chantenay' and 'Nogales.' Lesion development was greater in cold storage conditions than in the field, but the relative ranking of cultivars in terms of resistance to *A. radicina* was similar. In 2008–2009, Karkleliene et al. (2012) completed a black rot field experiment with organic production conditions under which they also observed varietal differences. The cultivar Magi was the most susceptible to *A. radicina* among 13 cultivars screened. Cwalina-Ambroziak et al. (2014) reported more severe symptoms on 'Koral' than on 'Bolero.'

*Phenotypic screening methods*: Pryor et al. (2000) developed a toothpick inoculation method for black rot resistance evaluation. After sterilization, toothpicks were incubated with 2 ml of a suspension of *A. radicina* conidia ($1 \times 10^4$ conidia/ml) and incubated for five days at 28 °C in the dark. The colonized end of each toothpick was inserted into the shoulder of a 10- to 12-week-old carrot root, and the area of the lesion measured 9–10 weeks later. Grzebelus et al. (2013) set up an in vitro selection protocol for plants with superior phenotypic performance against *A. radicina*. They isolated somaclonal variants within protoplast cultures that were challenged by fungal culture filtrates and obtained regenerated plants with greater tolerance to the pathogen. Cwalina-Ambroziak et al. (2014) inoculated petioles and seedlings directly with agar disks (each 5 mm in diameter) taken from 5-day-old cultures of *A. radicina*.

*Genetics of resistance*: As detailed for gray mold caused by *Botrytis cinerea*, Baranski et al. (2008) confirmed the positive impact of chitinase on *A. radicina* when using transgenic plants expressing CHIT36, one of the chitinase lytic enzymes secreted by *T. harzianum* that exhibits antifungal activity in vitro. Infection by *A. radicina* was reduced by 50%. When looking for a modification in systemic acquired resistance (SAR), Wally et al. (2009a) achieved a

significant reduction in the severity of taproot symptoms caused by *A. radicina* (diameter of lesions reduced by 50%) and in the number of foliar necrotic spots (greater than 33% reduction in foliar disease severity index) by inoculating transgenic plants expressing the *NPR1* gene of *Arabidopsis*. Wally and Punja (2010) examined the mechanisms of resistance in a transgenic carrot line, P23, which constitutively overexpressed the rice cationic peroxidase OsPrx114 and which exhibited enhanced resistance to necrotrophic foliar pathogens. OsPrx114 overexpression led to a slight enhancement of constitutive transcript levels of pathogenesis-related (PR) genes, and taproots had increased lignin formation in the outer periderm tissues, particularly after inoculation with *A. radicina*.

#### 18.2.1.6 Fusarium Dry Rot (*Fusarium* spp.)

Fusarium dry rot has been reported in the USA, Canada, France, Japan, and China (Rubatzky et al. 1999; Sherf and MacNab 1986; Villeneuve 2014; Zhang et al. 2014) and can be of great economic importance in some regions. Zhang et al. (2014) reported losses of up to 80% in Tuo Ke Tuo County, China. Symptoms include round, 3–4 cm in diameter, black lesions on root surfaces. The lesions evolve into a soft rot or brown canker, resulting in unmarketable roots. Symptoms may also include black spots on the crown that reduce nutrient translocation between the root and foliage and, therefore, impact root quality and yield. The disease also can cause significant losses during storage. Four species have been described as causing this disease, *Fusarium solani*, *F. avenaceum*, *F. culmorum* and, more recently, *F. caeruleum*. Zhang et al. (2014) described two ways to reproduce typical symptoms that could be used as a screening tool for breeders to evaluate varietal differences. The first was with mature carrot roots inoculated with colonized agar plugs (5 mm in diameter) cut from the margin of actively growing colonies on potato dextrose agar plates. One mycelial plug was placed on each carrot root, with the mycelial side facing the root. The inoculated roots were then incubated in a humid chamber (90% relative humidity) at 25 °C. Four days after incubation, mycelium had covered most of the surface of the root, and brown lesions were observed on the root. The second protocol entailed a potting trial in which carrot seeds were sown in sterilized soil in pots (30 cm × 25 cm) with 15 seeds per pot. The soil was infested by adding a spore suspension at a final concentration of $1 \times 10^4$ CFU/g soil. Plants grown in non-infested soil served as the control treatment. All the plants in inoculated pots were placed in a field. After 13 weeks, symptoms of dry rot were evident. Even though there are no known sources of resistance or published varietal screening trials, genetic transformation has been reported by Sidorova and Miroshnichenko (2013). They reported that ‘Nantskaya 4’ transgenic carrot plants overexpressed a single-gene coding for a thaumatin II protein and showed enhanced tolerance to infection by *F. avenaceum*.

#### 18.2.1.7 Gray Mold (*Botrytis cinerea*)

Gray mold is caused by the fungus *Botrytis cinerea* and can result in considerable losses in temperate regions of Europe, North America, and Asia (Rubatzky et al. 1999). Primary infections occur in fields, principally from airborne spores. The development of symptoms mainly occurs in cold storage. The fungus generally spreads into carrot roots at the base of petioles or on the crown. Watery brown lesions expand rapidly to become water-soaked, dark brown lesions covered with gray mycelium and, as the lesions age, small sclerotia. Resistance tests based on root inoculation were developed for screening the susceptibility of carrot cultivars to *B. cinerea* in storage and to study the process of induced resistance (Bowen and Heale 1987; Goodliffe and Heale 1975). Baranski et al. (2006) set up a leaf assay using colonized agar plugs to get rapid assessment of carrot leaf susceptibility to gray mold for a non-destructive, preliminary evaluation of precious and limited carrot source materials. Mercier et al. (2000) reported that heat-killed conidia of *B. cinerea* induced systemic resistance to *B. cinerea* in carrot slices through enhanced suberization and local accumulation of the phytoalexin 6-methoxymellein.

They questioned the role of a 24-kDa chitinase in the induced resistance response. Baranski et al. (2008) confirmed the impact of chitinase on gray mold when using transgenic plants expressing CHIT36, one of the chitinase lytic enzymes secreted by the biological control fungus *Trichoderma harzianum* that exhibits antifungal activity in vitro. Transgenic plants reduced *B. cinerea* attack by as much as 50%.

#### 18.2.1.8 Sclerotinia Soft Rot or White Mold (*Sclerotinia sclerotiorum*)

Carrot foliage and roots in fields may be destroyed by Sclerotinia soft rot or white mold, but it is mainly in cold storage conditions and long distance transportation that damage to this disease is significant. Small translucent spots on roots are covered rapidly by white, flocculent mycelium, which develops into melanized, black structures called sclerotia (Fig. 18.6). Sclerotia can survive up to 10 years in soils. Three species are cited as causal agents: *Sclerotinia sclerotiorum*, *S. minor*, and *S. subarctica* (Leyronas et al. 2018). *Sclerotium rolfsii*, which causes southern blight of carrot, is a basidiomycete that is not related to the white mold fungi. The white mold pathogens are ascomycetes. White mold is distributed worldwide (Kora et al. 2003; Rubatzky et al. 1999) and has a host range of more than 500 species, including weeds.

No resistance sources have been identified in carrot germplasm, but a phenotyping test with *S. sclerotiorum* was described by Ojaghian et al. (2016). Freshly harvested carrot roots were disinfected with 2% sodium hypochlorite for three minutes, and then washed with sterilized tap water and dried on sterilized filter paper. Roots were inoculated using fungal isolates grown on carrot dextrose agar. A 5-mm diameter colonized agar plug taken from the leading edge of a 3-day-old culture was then placed centrally on the root with the colonized agar surface facing the root. The carrots were placed in plastic boxes (12 per box) and covered with three thin layers of plastic to provide a moist chamber. To increase humidity, moist cottonwool pieces were placed in the boxes, and the roots were stored at 21–23 °C. Disease severity was determined six days after inoculation on a scale of y1 to y4, where: y1 = no lesion,

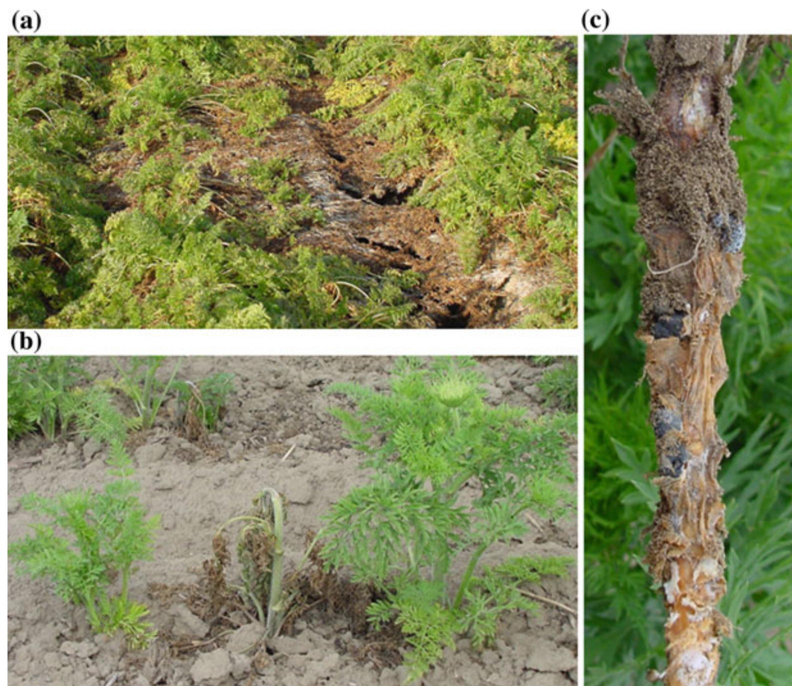


**Fig. 18.6** Matted foliage in a carrot crop as a result of white mold caused by *Sclerotinia sclerotiorum* (**a**), dieback of bolted carrot plants in a seed crop following root infection by *S. sclerotiorum* (**b**), and severe rot of a carrot root on which black sclerotia of *S. sclerotiorum* had formed (**c**) (Lindsey du Toit, Washington State University)

y2 = 1–4 cm long lesions on roots without sclerotium formation, y3 = 4–8 cm long lesions with 1–4 mature or immature sclerotia, y4 = $\geq 8$ cm long lesions on the roots with more than 4 mature or immature sclerotia. A disease index was then calculated using the formula: Disease index = $[(1.25 \times y2) + (2.5 \times y3) + (3.75 \times y4)]/\text{total number of carrots} \times 1/0.05$. In this formula, 0.05 is a constant coefficient (Ojaghian et al. 2016). Punja and Chen (2004) reported that transgenic carrot plants expressing a thaumatin-like protein from rice showed significantly enhanced tolerance to *S. sclerotiorum* when detached petioles and leaflets were inoculated under controlled environmental conditions. Wally et al. (2009b) showed that carrot lines overexpressing OsPrx114 peroxidase were highly resistant to *S. sclerotiorum* without showing any visible phenotypic abnormalities of the roots. The resistance was associated with increased transcript levels of pathogenesis-related (PR) genes when tissues were treated with cell wall fragments of *S. sclerotiorum* (Wally and Punja 2010).

#### 18.2.1.9 Common Scab (*Streptomyces scabies*)

Common scab of carrot, caused by *Streptomyces scabies*, occurs in many areas of carrot production but is particularly problematic in Canada and Europe, especially France and the Netherlands (Janse 1988; Villeneuve 2014). Infections occur through wounds or lateral secondary roots, and death of infected epidermal cells occurs during dry periods. After a few months, a corky bulge expands horizontally through the root surface, particularly toward the top of the root (Fig. 18.7). *Streptomyces scabies* can survive in soils for several years as a saprophyte. Schoneveld (1994) demonstrated that 4–5 weeks after spring sowing was the most susceptible stage of growth for infection by *S. scabies*. A phenotyping test was described by Janse (1988). A 60-ml aliquot of a bacterial suspension ($10^7$ spores/ml) prepared from a 4-week-old culture on yeast malt agar was added to 20 L of a steam-sterilized loamy soil at pH 5.9 prior to sowing carrot seed. Plants were grown at 18 °C and 80% RH with 10 000 lx of light and at 50% soil saturation. Plant roots were harvested four months after sowing and examined for symptoms. There are no reports of resistance to common scab in carrot germplasm.

### 18.2.2 Foliar Diseases

#### 18.2.2.1 Alternaria Leaf Blight (*Alternaria dauci*), Cercospora Leaf Spot (*Cercospora carotae*), and Bacterial Leaf Blight (*Xanthomonas hortorum* pv. *carotae*)

Carrot leaf blights can be caused by two fungal pathogens, *Alternaria dauci* and *Cercospora carotae*, and the bacterial pathogen *Xanthomonas hortorum* pv. *carotae* (formerly *Xanthomonas campestris* pv. *carotae*) (Fig. 18.8). Alternaria leaf blight caused by *A. dauci* is the

**Fig. 18.7** Common scab on carrot roots caused by *Streptomyces scabies* (Lindsey du Toit, Washington State University)

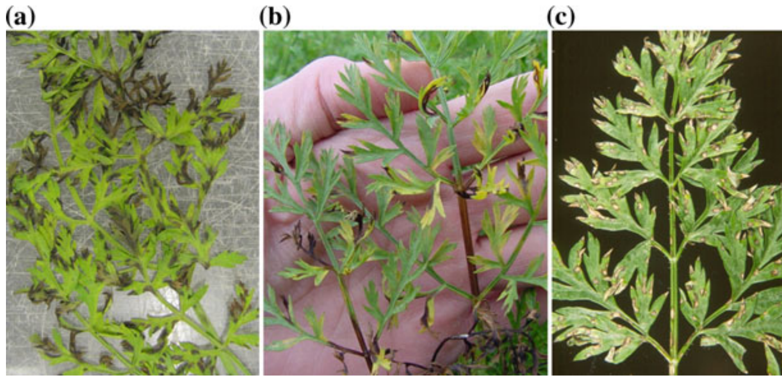


**Fig. 18.8** Symptoms of Alternaria leaf blight caused by *Alternaria dauci* (**a**), bacterial leaf blight caused by *Xanthomonas hortorum* pv. *carotae* (**b**), and Cercospora leaf spot caused by *Cercospora carotae* (**c**) (Lindsey du Toit, Washington State University)

major foliage disease of carrots in most areas of production. First described in 1855 in Germany and 1890 in the USA, *A. dauci* causes severe defoliation in carrot crops all over the world where there are conditions of high moisture and temperature (Farrar et al. 2004). The relatively large, dematiaceous spores (Maude 1966) can be produced and dispersed aerially over a wide range of temperatures (8–28 °C) and moisture conditions throughout the growing season (Strandberg 1977), although periods of dark and leaf wetness durations of at least 10 h also favor sporulation (Langenberg et al. 1977). Foliar symptoms appear 8–10 days after infection as small, green-brown lesions. The lesions enlarge and infected tissue becomes dark brown to black, sometimes surrounded by a chlorotic halo (Farrar et al. 2004). While foliar symptoms are the most common, *A. dauci* can also infect the inflorescences and seeds developing in umbels. Seedborne inoculum can lead to seed transmission and infection of seedlings, resulting in damping-off (Farrar et al. 2004; Maude 1966).

Cercospora leaf spot, caused by *C. carotae*, can result in similar symptoms to Alternaria leaf blight, although lesions on leaves and petioles tend to be more circular, and each lesion may develop a distinct, dark margin with a lighter brown center (Bourgeois et al. 1998; Carisse and Kushalappa 1990; Gugino et al. 2007; Milosavljević et al. 2014; Raid 2002). The fungus only infects aerial parts of carrot plants. The optimum temperature range for infection is 20–28 °C with short periods of leaf wetness (<6 h) followed by high relative humidity sufficient to result in infection (Carisse and Kushalappa 1992).

As highlighted by Pfleger et al. (1974), bacterial leaf blight is caused by the seedborne pathogen *X. hortorum* pv. *carotae*. This disease can be indistinguishable from the fungal blights caused by *A. dauci* and *C. carotae* based on foliar symptoms alone. However, bacterial leaf blight lesions sometimes are accompanied by production of a gummy bacterial exudate, particularly lesions on petioles, umbels, and seed stalks (du Toit et al. 2005). Bacterial leaf blight was first reported in California in 1934. The disease can be found worldwide wherever carrots are grown. The pathogen can infect the foliage, stems, umbels, and seed (Pfleger et al. 1974; du Toit et al. 2005). Some studies have suggested roots can become infected, but these probably reflect infection limited to the crown where petioles attach to the root. Seeds may be infected internally or contaminated on the surface. Infection of seed by *X. hortorum* pv. *carotae* does not necessarily reduce seed germination or vigor, but the need to use hot water seed treatment to eradicate the pathogen or to reduce seed infection levels can impact seed germination or vigor.

Where severe outbreaks of foliar diseases of carrot occur, regardless of the causal agent, harvest operations can be hindered as lesions

coalesce and weaken the tops which may break during mechanical harvest. A reduction in green leaf area for photosynthesis may also reduce yields. Planting seed lots that have been tested for the pathogen, or infected seed lots that have been treated (e.g., with hot water for the bacterial blight pathogen or with fungicides for the fungal pathogens), and the use of cultural and chemical control methods in carrot fields are important management strategies for these foliar diseases. Using healthy seed lots or treated seed lots remains particularly important for bacterial leaf blight as copper bactericides or antibiotics are not permitted in some countries, and copper bactericides are purely protectant, so the disease cannot be controlled once infection is established in a crop. *A. dauci* and *C. carotae* can be managed with timely applications of relevant fungicides to crops during the growing season. Although chemical control is a primary means used by some growers for management of the fungal foliar diseases, the use of resistant cultivars, when available, together with chemical and cultural control measures, remains the most effective integrated approach to managing these diseases.

Resistance screening: Traditional carrot breeding methods have relied on phenotypic evaluations of large numbers of carrot accessions. Most of the literature on phenotypic evaluations have been on characterizing the resistance mechanisms of carrot against *A. dauci*, with very few published studies on *C. carotae* and *X. hortorum* pv. *carotae*.

Numerous carrot cultivars have been evaluated for resistance to *A. dauci*, and several sources have been identified. However, none of the cultivars evaluated to date has been completely resistant to Alternaria leaf blight. Strandberg et al. (1972) screened 90 breeding lines and 241 Plant Introduction (PI) lines from 31 countries. They identified nine lines with high levels of resistance. Significant differences in resistance to *A. dauci* were observed among four cultivars evaluated under natural infection in Brazil, with 'Brasilia' being the most resistant (Boiteux et al. 1993). Field evaluations of tropical germplasm were also reported by Pereira et al. (2012), Silva et al. (2009), and Carvalho et al. (2015). Amirov et al. (2014) evaluated 86 accessions in Kazakhstan. Information on the stability of resistance evaluated in different environments has been important for breeders to utilize the documented resistance. Using 21 isolates of *A. dauci* collected from commercial carrot fields in northeastern North America, Rogers and Stevenson (2010) detected variety-by-isolate interactions with three commercial carrot cultivars. Conversely, testing 11 *A. dauci* isolates from different parts of the world on eight varieties or inbred lines and one segregating population for *A. dauci* resistance, Le Clerc et al. (2015a) did not find a significant interaction between isolates and varieties. The different conclusions may reflect differences in carrot varieties, fungal isolates, and environmental conditions in the two studies. As suggested by Le Clerc et al. (2015b), it is possible that some isolate-specific resistance factors in some varieties may confer varying degrees of resistance in different environments. The disease phenotyping by Rogers and Stevenson (2010) was done 8 and 16 days after inoculation, while this was done 20 and 35 days after inoculation in greenhouse trials and 30 days after inoculation (and every 15 days thereafter) in tunnel trials by Le Clerc et al. (2015b). For a given isolate, disease development varies among carrot genotypes, which may reflect activation of different resistance mechanisms at different time periods after inoculation.

Simon and Strandberg (1998) confirmed that evaluations for resistance to *A. dauci* in field conditions generally correlate well with resistance ratings in greenhouse trials. Although widely used, field testing can be time-consuming, expensive, potentially affected by uncontrollable environmental conditions, and generally can only be done once a year. To address these issues, tests have been developed under controlled conditions such as greenhouses, tunnels, or growth chambers. While field evaluations generally can include assessment of a large number of plants and entries, assays in controlled conditions tend to necessitate using fewer plants, even one plant per variety or detached parts of

plants. Baranski et al. (2007) evaluated the resistance of transgenic plants using a laboratory-based assay with detached leaflets and petioles that were inoculated with fungal pathogens. Pawelec et al. (2006) reported effective ranking of carrot cultivars using plants grown in pots in a greenhouse and inoculated with the pathogens, whereas detached leaf and hypocotyl tests failed to discriminate responses among cultivars. For detached leaf and petiole tests, a drop inoculation method was developed to facilitate more rapid screening than field evaluations and to use less plant material (Boedo et al. 2010). The responses of carrot lines to *A. dauci* have also been evaluated in vitro (Dugdale et al. 2000). Regenerant somaclone plants initiated from seedling hypocotyls were evaluated for susceptibility to a pathogen by measuring the loss of chlorophyll of infected, detached leaves. Lecomte et al. (2014) and Courtial et al. (2018) challenged embryogenic cell cultures with fungal extracts to evaluate the resistance of carrot genotypes to *A. dauci*. However, these tests were mainly dedicated to comprehension of resistance mechanisms than to high-throughput phenotyping and would need to be automated to be of value for more extensive phenotyping.

There is very little information on screening for resistance of carrot cultivars to *C. carotae*. Lebeda et al. (1988) evaluated a worldwide collection of 142 carrot cultivars for resistance to *C. carotae*. A large proportion of the cultivars was highly susceptible, with only ~30% expressing resistance under field conditions. Field trials also were done by Gugino et al. (2007). None of the cultivars displayed complete resistance although there was variability in reaction among cultivars. Data on the genetics of resistance to Cercospora leaf spot are incomplete, and there do not appear to have been any public efforts to breed for resistance to Cercospora leaf spot.

Similarly, genetic resistance to *X. hortorum* pv. *carotae* is not well documented and there has been very little public research on screening for resistance. No commercial cultivars currently are marketed as resistant to bacterial blight (Christianson et al. 2015). Pfleger et al. (1974) indicated varietal differences in response to bacterial blight among six cultivars and breeding lines. Christianson et al. (2015) screened 66 PI lines, two public inbred lines, and 17 commercial carrot cultivars and carrot wild relatives for response to *X. hortorum* pv. *carotae* in a greenhouse by rating severity of bacterial blight and quantifying the amount of *X. hortorum* pv. *carotae* that developed on the leaves of each inoculated line. Eight putative resistant PI lines and five highly susceptible PI lines identified in the first screening were tested again with an additional two PI lines, 12 cultivars, two inbred lines, and 12 carrot wild relatives. PI lines 418967, 432905, and 432906 were identified as partially resistant to bacterial blight, with potential value in breeding more resistant cultivars. None of the accessions had complete resistance. Of the 12 carrot wild relatives, only Ames 7674 and SS10 OR had relatively limited bacterial blight. Overall, the severity of symptoms and the amount of *X. hortorum* pv. *carotae* recovered from the foliage differed significantly among the accessions tested. Christianson et al. (2015) showed that using visual foliar disease severity ratings instead of the highly resource- and labor-intensive *X. hortorum* pv. *carotae* quantification protocol was effective, subject to using adequate number of replications for accurate assessment, as foliar severity ratings were positively correlated with *X. hortorum* pv. *carotae* quantification in both trials ($r = 0.52–0.62$ at $P < 0.0001$). This study illustrated that the *Daucus* germplasm in the United States Department of Agriculture's National Plant Germplasm System represents a valuable public source of potential resistance for breeders. As suggested by Christianson et al. (2015), the susceptible and resistant PI lines identified in that study could be used to study the inheritance of *X. hortorum* pv. *carotae* resistance in carrot.

*Genetics of resistance*: In order to develop hybrid carrot cultivars with high level of resistance, knowledge of the heritability and combining abilities of sources of resistance is needed in breeding programs. Studying resistance to *A. dauci*, 40% narrow-sense heritability ($h^2$) was calculated for the open-pollinated cultivar Brasilia (Boiteux et al. 1993). Vieira et al. (1991)

found broad-sense heritabilities ranging from 45 to 82% when evaluating foliar leaf blight resistance (without distinguishing the potential causal agent(s) as *A. dauci*, *C. carotae*, or *X. hortorum* pv. *carotae*), with the higher heritability observed for a hybrid population of Kuroda and Nantes cultivars. Evaluating different hybrid combinations, Simon and Strandberg (1998) suggested that a preponderance of additive variation with some dominant gene action and epistasis may contribute to resistance to Alternaria leaf blight. In 2009, Le Clerc et al. (2009) confirmed the polygenic nature of resistance to this disease, with identification of three quantitative trait loci (QTL) regions in a population of $F_{2:3}$ progeny. The phenotypic variation explained by each QTL ranged from 10 to 23%. Some QTLs were only detected in the tunnel trial or field trial, and only at one screening date, suggesting that expression of these QTLs might be influenced by the environment, with a delay in expression after infection. Two other populations with different genetic backgrounds were evaluated under field conditions over two years, from which 11 QTLs were identified (Le Clerc et al. 2015b). Complementarity between the parental origins of the favorable alleles at each QTL provides potential opportunities for breeders to combine resistance in one genotype in an effort to achieve higher levels of resistance. It would be valuable to understand the mechanisms underlying these QTLs in order to select those with complementary actions; e.g., some QTLs may delay penetration of carrot foliar tissue by the pathogen, while some QTLs may confine the pathogen after penetration into the leaf.

Little information is available on the genetic nature of resistance in carrot lines to *C. carotae* and *X. hortorum* pv. *carotae*. Lebeda et al. (1988) suggested that heredity of resistance to *C. carotae* could be oligogenic, with different degrees of phenotypic expression. Using glasshouse experiments, Angel and Gabelman (1968) found that a single dominant gene determined resistance of inbred line WCR 1.

Comprehension of the mechanisms underlying resistance to foliar diseases of carrot is important to develop durable and highly resistant cultivars, i.e., by combining resistance mechanisms. Boedo et al. (2008) characterized the different stages of fungal infection and development in carrot leaves using a resistant and a susceptible cultivar to *A. dauci*. Based on scanning electron microscopy, differences in *A. dauci* development between the two cultivars were only obvious 21 days post-inoculation (dpi). In contrast, the fungus was able to invade the leaf tissues of the susceptible cultivar rapidly. This was supported by significantly greater fungal biomass detected in leaves of the susceptible cultivar than leaves of the resistant cultivar, measured by quantitative, real-time PCR assay. The result was confirmed by Boedo et al. (2010) with two partially resistant cultivars in which significantly less fungal biomass was detected 15 dpi than in a susceptible cultivar. Investigating the germination of *A. dauci* conidia on carrot leaves *in vivo*, they also highlighted that a greater mean number of germ tubes per conidium (up to $3.42 \pm 0.35$) was observed on the two partially resistant cultivars than on the susceptible cultivar ($1.26 \pm 0.18$). Penetration of the fungus into the epidermis of the susceptible cultivar was rapid. Several germ tubes per conidium were produced by spores infecting the resistant cultivar, which might reflect multiple attempts by the fungus to penetrate the epidermis.

Since plant resistance to pathogens can be due, in part, to production of secondary metabolites, Lecomte et al. (2012) investigated the role of 6-methoxymellein (6-MM) and falcarindiol in the resistance of carrot lines to *A. dauci*. After inoculation with *A. dauci*, significantly faster production of 6-MM in the resistant cultivar Bolero than the susceptible cultivar Presto suggested that this phytoalexin was involved in the resistance reaction by slowing pathogen spread, but with relatively limited effect. Falcarindiol was more active than 6-MM at reducing in vitro development of the fungus and was able to induce permeabilization of the *A. dauci* plasma membrane. Greater accumulation of this metabolite in leaves of Bolero than Presto suggested this metabolite contributes to resistance to the fungus. Lecomte et al. (2014) demonstrated that carrot tolerance to toxins

produced by *A. dauci* could be another component of partial resistance. Embryogenic cellular cultures from carrot genotypes with different level of resistance were challenged with fungal extracts. A differential response between resistant and susceptible cultivars was demonstrated with a positive correlation between resistance to the fungus on the whole plant and resistance at the cellular level to fungal exudates. This suggested that phytotoxic compounds were present in the exudates and should be characterized. In 2018, Courtial et al. identified a new phytotoxin named aldaulactone that was shown to mimic the effect of fungal extracts on carrot embryogenic cell cultures, but at lower toxicity. Aldaulactone could be a major toxin produced by the fungus. Cellular targets of aldaulactone should be investigated. In order to identify compounds potentially associated with resistance to *A. dauci*, Koutouan et al. (2018) compared the leaf metabolome of four carrot genotypes with different levels of resistance, and the metabolomes of a set of resistant and susceptible progenies, by bulk segregant analysis. Based on results for the two parental lines, luteolin 7-*O*-glucuronide and feruloylquinic acid were expressed and accumulated differentially to camphene, α-pinene, apigenin 4′-*O*-glucoside, luteolin 4′-*O*-glucoside, caryophyllene, and β-bisabolene in the susceptible and resistant bulk populations. Analyses are in progress to characterize the involvement and role of those secondary metabolites in resistance to *A. dauci*, and their association with QTLs previously identified by developing a metabolite QTL approach as well as microarray analysis to characterize gene expression in the metabolic pathways.

Mercier and Kuć (1996) studied the induction of systemic resistance in carrot to Cercospora leaf spot by inoculation with *C. carotae*. Interestingly, newly emerged leaves of carrot plants previously inoculated with *C. carotae* developed significantly fewer lesions than comparable leaves of the control plants after challenge with the pathogen, suggesting that the foliar pathogen induced defense mechanisms in carrot leaves.

Instead of classical screening of wild and cultivated carrot accessions to identify new sources of resistance, Arbizu et al. (2017) suggested relying on prediction approaches by investigating the association between *Daucus* clades and severity ratings for Alternaria leaf blight. Using a phylogenetic linear regression model, they identified plant height as the best explanatory variable to predict resistance to this disease based on a study of 106 accessions of wild and cultivated *Daucus* spp. and related genera. The authors concluded that *D. carota* subsp. *capillifolius* and *D. c.* subsp. *maximus*, as well as *D. crinitus*, may provide new sources of resistance. They established the feasibility of hybridization of these species and subspecies with the cultivated carrot.

Strategies to develop transgenic plants exhibiting enhanced resistance to foliar fungal and bacterial pathogens of carrot have been evaluated. Lysozymes of plant origin have been suggested to be protective and defensive against bacterial or fungal pathogens. The human lysozyme cleaves peptidoglycan in the bacterial cell wall or chitin in the fungal cell wall. The human lysozyme gene was used to transform carrot via *Agrobacterium tumefaciens*, resulting in transgenic plants with enhanced resistance to *A. dauci* (Takaichi and Oeda 2000). Punja (2005) obtained two transgenic carrot lines via transformation with *A. tumefaciens* to express a thaumatin-like protein. Both lines had significantly less severe disease caused by *A. dauci*, *A. radicina*, *B. cinerea*, and *S. sclerotiorum*. Carrot transgenic plants expressing a microbial factor 3 (MF3) gene from a plant-growth promoting rhizobacterium, *Pseudomonas fluorescence*, were evaluated for resistance to *A. dauci*, *A. radicina*, and *B. cinerea* (Baranski et al. 2007). The homology of MF3 to FKB proteins suggested that MF3 is involved in the signaling pathway affecting induced systemic resistance. The transgenic plants had significantly enhanced resistance to the three pathogens (by 20–40%) in comparison to non-transformed plants. Less convincing results were obtained from the transformation of polyethylene glycol

carrot protoplast with a chitinase gene. While slower disease progress caused by *A. dauci* was observed for two of the clones, a third clone was more susceptible than the control plants (Baranski et al. 2008). Wally et al. (2009a) considered it more efficient to manipulate the plant innate defense signaling pathways by controlling a large number of induced genes instead of using traditional gene expression strategies (i.e., relying on the expression of a single pathogenesis-related protein) to develop transgenic plants highly resistant to a range of pathogens. Modification of systemic acquired resistance was obtained through overexpression of the controlling gene NPR1 introduced into a carrot cultivar. The transgenic lines challenged with *X. hortorum* pv. *carotae* exhibited an 80% reduction in disease severity and 35–50% reduction in disease severity when challenged with *B. cinerea*, *A. radicina,* and *S. sclerotiorum*, confirming that this strategy offered the ability to control a range of pathogens. Klimek-Chodacka et al. (2018) reported the first use of the CRISPR/Cas9 system for efficient, site-targeted mutagenesis of the carrot genome, which offers new perspectives for improving carrot resistance to diseases such as those caused by foliar fungal and bacterial pathogens.

#### 18.2.2.2 Powdery Mildew (*Erysiphe heraclei, Leveillula lanuginosa, L. taurica*)

Two types of powdery mildew can occur on carrot, the common *Oidium* type caused by *Erysiphe heraclei* (synonyms *E. polygoni* and *E. umbelliferarum*), and the less common *Oidiopsis* type caused by *Leveillula lanuginosa* and *L. taurica* (Aegerter 2002). *Erysiphe heraclei* has been documented wherever carrots are grown, but tends to be most severe in warm, semiarid regions or seasons. The severity of powdery mildew is influenced by weather conditions, growth stage of the crop at the onset of disease, production practices, and cultivar (Abercrombie and Finch 1976; Aegerter 2002; du Toit and Derie 2008; Palti 1975). Powdery mildew tends to be most severe on susceptible cultivars or parent lines grown with drip or furrow irrigation in warm and semiarid regions. Severe leaf infections can impede mechanical harvesters that pull roots out of the ground by the tops. The disease can be severe in greenhouses (Geary and Wall 1976). *Leveillula* spp. are favored by very warm, very dry climates and have been documented infecting carrot primarily in the Middle East, Armenia, India, Kazakhstan, and other countries in central Asia and Mediterranean regions of Europe and Africa. In Israel, these species only occur in the driest parts of that country (Palti 1975).

*Erysiphe heraclei* is ectotypic on carrot; i.e., the fungus grows externally on the plant surface and produces haustoria that penetrate the host epidermal cells (Aegerter 2002). The fungus produces white mycelium and sporulation on any aboveground part of carrot plants, including leaves, petioles, flower stalks, bracts, and umbels (Aegerter 2002) (Fig. 18.9). Severely infected foliage can become chlorotic and leaves may senesce prematurely. In contrast, *L. lanuginosa* and *L. taurica* produce mycelium that is both endophytic and ectotopic. These two fungi produce conidia at the ends of long conidiophores that protrude through stomata. Powdery mildew caused by *Leveillula* spp. usually appears as pale yellow lesions on the upper leaf surface, with white sporulation on the lower leaf surface (Aegerter 2002). Lesions can appear angular because the leaf veins limit expansion of infections. As the disease progresses, sporulation can develop on the upper leaf surface and chlorotic areas become necrotic. The white fungal growth is not nearly as conspicuous as that caused by *E. heraclei*.

Conidia produced by powdery mildew fungi are carried long distances by air movement (Aegerter 2002). Unlike most fungal plant pathogens, the spores do not require free water for germination and infection of plants, but require high humidity and moderate temperatures. Powdery mildews are most severe in shaded areas as sunlight damages conidia and mycelium. Therefore, powdery mildew tends to start on the older foliage and spread to newer leaves, reflecting greater humidity and shading lower in the canopy. Carrot plants become more susceptible to powdery mildew as they age

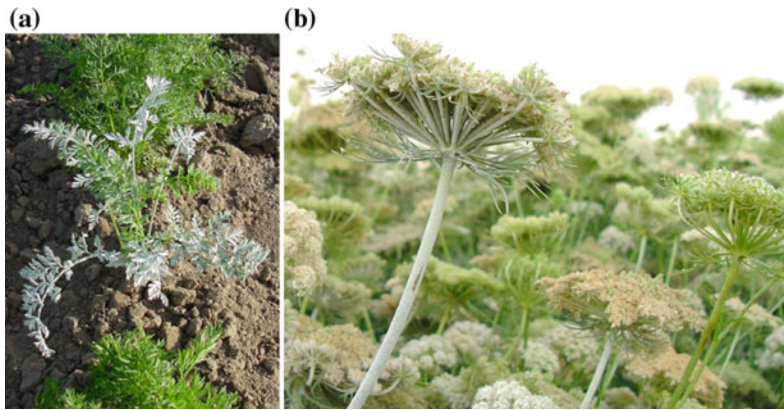


**Fig. 18.9** Severe powdery mildew on a carrot plant infected with *Erysiphe heraclei* and transplanted into a field trial to increase disease pressure (**a**) and colonization of an umbel by *E. heraclei* in a carrot seed crop (**b**) (Lindsey du Toit, Washington State University)

(Aegerter 2002). Sporulation can occur within 7–14 days of infection, with symptoms developing rapidly under favorable conditions for this highly polycyclic disease. The disease can be particularly severe in carrot seed crops because the tall, dense canopy results in highly favorable conditions after canopy closure (du Toit and Derie 2008; du Toit et al. 2009). There is no evidence the powdery mildew pathogens infect carrot seed, but seed lots can be contaminated with cleistothecia (sexual fruiting bodies) (Boerema et al. 1963; Vasudeva 1963).

*Erysiphe heraclei* can infect at least 86 plant species in Apiaceae, although cross-inoculation studies suggest a degree of host specialization (Aegerter 2002; Braun 1987; Cunnington et al. 2008; Glawe et al. 2005; Hammarlund 1925; Marras 1962). Therefore, a powdery mildew on one host species might not serve as a source of inoculum for another plant species. Nonetheless, some isolates have been demonstrated to infect multiple plant species and genera, including differences in degree of virulence on different Apiaceae genera and species (e.g., Cunnington et al. 2008; Koike and Saenz 1994, 1997). Similarly, *L. lanuginosa* can infect different Apiaceae genera and species, with evidence of host specialization among isolates (e.g., Cirulli 1975). *Leveillula taurica* has an even broader host range, infecting many plant families with varying degrees of host specialization having been reported (Aegerter 2002; Braun 1987; Palti 1975).

Resistance screening: Partial resistance to powdery mildew was identified in four subspecies of *Daucus* (Bonnet 1977; Umiel et al. 1975) used to initiate breeding for resistance. Single-gene, dominant resistance to powdery mildew was identified by Bonnet (1983) in *D. c.* subsp. *dentatus*. A backcross study with the susceptible cultivar Touchon demonstrated that resistance was controlled by a single dominant gene, *Eh*. Resistant lines were selected based on an orange root color. Bonnet (1983) also documented *D. siculus* as well as the *D. carota* cultivar Bauers Kieler Rote as potential sources of resistance to powdery mildew. Lebeda and Coufal (1987) screened 111 cultivars of *D. c.* subsp. *sativus* under field conditions in Czechoslovakia for resistance to natural infection by *E. heraclei*. One cultivar, 'Gavrilovskaya' from the former USSR, remained asymptomatic, and 13 developed very limited powdery mildew. They categorized about half of the 111 cultivars as 'moderately vulnerable' and interpreted the results to indicate possible incomplete dominance and quantitative resistance to powdery mildew. Takaichi and Oeda (2000) developed transgenic versions of the carrot cultivars Kurodagosun and Nantes Scarlet using *A. tumefaciens* to transfer a plasmid containing the human lysozyme under control of the constitutive

CaMV 35S promoter. As detailed above, the enzyme has lytic activity against plant pathogenic fungi and bacteria. Two of the transgenic plants of 'Nantes Scarlet' displayed partial resistance to powdery mildew, and one was also partially resistant to Alternaria leaf blight. The increase in resistance in these lines was correlated with an increase in the production of the human lysozyme. Wally et al. (2009a) developed transgenic lines of the carrot cultivar Nantes Coreless containing the *Arabidopsis* (*At*) *NPR1* gene (non-expressor of PR genes) for enhancing the plant innate defense system. Two transgenic lines, NPR1-I and NPR1-XI, displayed increased duration and intensity of expression of DcPR-1, -2, and -5 genes when the lines were treated with purified cell wall fragments of the white mold pathogen, *S. sclerotiorum* or with 2,6-dichloroisonicotinic acid. Leaves of these lines had 90% less severe powdery mildew when inoculated with *E. heraclei* compared to non-transgenic lines of the cultivar. Overexpression of the systemic acquired resistance (SAR) master switch, *NPR1*, resulted in increased resistance to powdery mildew as well as necrotrophic pathogens such as *B. cinerea*, *A. radicina*, *S. sclerotiorum*, and *X. hortorum* pv. *carotae* (Wally et al. 2009a).

Although Lebeda and Coufal (1987) relied on natural infection to screen cultivars for resistance in field trials in Czechoslovakia, adequate disease pressure only occurred in one of three field seasons to assess cultivars for differences in susceptibility to powdery mildew. If conditions remain relatively warm and dry, fairly uniform powdery mildew pressure can be generated readily under field conditions or in greenhouses using plants of highly susceptible cultivars infected with powdery mildew as 'spreader' plants. Inoculum can be maintained by growing infected plants in a greenhouse and adding new plants alongside infected plants at regular intervals to keep propagating the pathogen. Placing plants close together increases relative humidity for promoting powdery mildew. Infected plants can be transplanted into field sites to establish powdery mildew nurseries. Using this protocol, du Toit and Derie (2008) and du Toit et al. (2009) were able to get very severe powdery mildew pressure in carrot seed crops to assess the impact of this disease on carrot seed yields.

#### 18.2.2.3 Virus Diseases

Approximately 14 virus diseases of carrot are recognized (Moran et al. 2002; Nuñez and Davis

**Table 18.1** Carrot diseases caused by viruses (Nuñez and Davis 2016)

| Disease | Virus name | Virus genus |
|---|---|---|
| Alfalfa mosaic or calico | *Alfalfa mosaic virus* (AMV) | Alfamovirus |
| Carrot latent | *Carrot latent virus* (CLtV) | Nucleorhabdovirus |
| Carrot mottle | *Carrot mottle virus* (CMoV) | Umbravirus |
| Carrot red leaf | *Carrot red leaf virus* (CRLV) | Polerovirus |
| Carrot thin leaf | *Carrot thin leaf virus* (CTLV) | Potyvirus |
| Carrot yellow leaf | Coinfection with *Carrot yellow leaf* (CYLV) and | Closterovirus |
| | *Carrot virus Y* (CarVY) | Potyvirus |
| Celery mosaic | *Celery mosaic virus* (CeMV) | Potyvirus |
| Cucumber mosaic | *Cucumber mosaic virus* (CMV) | Cucumovirus |
| Curly top | *Beet curly top virus* (BCTV) | Curtovirus |
| Parsnip yellow fleck | *Parsnip yellow fleck virus* (PYFV) | Sequivirus |
| Tobacco necrosis | *Tobacco necrosis virus* (TNV) | Necrovirus |
| Tomato spotted wilt | *Tomato spotted wilt virus* (TSWV) | Tospovirus |
| Carrot motley dwarf | Coinfection with CRLV and CMoV | Polerovirus and Umbravirus |

2016) (Table 18.1). Some of these diseases are of minor importance economically (e.g., AMV, CTLV, and TSWV), while others can cause significant decreases in root yields or seed production, including total loss (Lebeda and Coufal 1985; Nuñez and Davis 2016; Stein and Nothnagel 1995). Motley dwarf (CRLV and CMoV) is probably the most important and persistent virus disease of carrot (Waterhouse 1985; Watson and Sarjeant 1964). The majority of viruses infecting carrot are transmitted by aphids, and early efforts to screen for resistance to viruses and/or the vectors indicated a lack of highly effective resistance (Van Dijk and Bos 1985; Elnagar and Murant 1978). However, efforts to screen for variation in susceptibility to some of these viruses have demonstrated differences among carrot breeding lines, with the potential to improve the resistance of commercial cultivars.

Motley dwarf: Motley dwarf was first detected in Australia in 1948, and now occurs in most regions of carrot production in the world where the climate is relatively cool (Koike et al. 2002). The two viruses that cause motley dwarf also infect cilantro, dill, and parsley. Each virus can infect carrot plants alone, but motley dwarf only occurs when plants are infected with both CRLV and CMoV. The viruses are both transmitted by the willow-carrot aphid, *Cavariella aegopodii*, which transmits the viruses in a circulative, non-propagative manner. If plants are infected with CRLV alone, CRLV can be transmitted by aphids but not transmitted mechanically. The opposite is true for carrots infected only with CMoV, which can be transmitted mechanically but not by aphids in the absence of CRLV (Koike et al. 2002; Waterhouse and Murant 1983). A third virus-like RNA was identified in motley dwarf-infected carrots in California (Watson et al. 1998). This small, CRLV-associated RNA (CRLVaRNA) is transmitted by the carrot-willow aphid with CRLV and CMoV, although it is not known if this RNA affects symptom expression. The viruses have a relatively narrow host range within Apiaceae, as does the aphid vector, which preferentially feeds on carrot. As a result, older carrot plantings or overwintered carrots infected with motley dwarf are the most common inoculum sources (Howell and Mink 1977; Watson and Falk 1994). Losses to motley dwarf can be severe if infection occurs early in the season and carrots are grown when conditions are relatively cool (15–20 °C) with low light conditions (e.g., late winter and early spring plantings in California, and plantings in the mild, maritime region of the Pacific Northwest USA). Symptoms vary depending on the age of plants at the time of infection, but typically include reddening and yellowing of leaves along with stunting if plants are infected at an early growth stage. Symptoms can resemble those caused by nutrient deficiency. Roots can be affected severely, and plants may die. Symptoms on older plants tend to be less severe, and plants may be symptomless at temperatures >24 °C.

CarVY: CarVY was first described on carrot in Australia in 2002 (Moran et al. 2002) and is now well established in that country (Jones 2005). The virus has not been reported in other countries. CarVY is transmitted non-persistently by at least 14 aphid species, with infected carrot crops and volunteer carrot plants serving as the main reservoir for newly planted crops. This is particularly problematic in Australia where carrots can be grown year-round, typically using sequential plantings. CarVY causes chlorotic mottle of the foliage, a feathery appearance of foliage from increased subdivision of leaflets, marginal necrosis or reddening of leaves, and stunting (Latham and Jones 2004). Roots become stubby and knobby if plants are infected at an early growth stage, and severely symptomatic roots are unmarketable. There is no evidence of CarVY being seedborne or seed transmitted in carrot (Jones 2005). Carrot was thought to be the only known host until Jones (2005) demonstrated that CarVY can infect *Chenopodium amaranticolor* and *C. quinoa*, although neither became infected systemically. Carrot appears to be the primary source of inoculum for spread of CarVY in Australia.

PYFV: PYFV has caused significant economic losses in carrot root production in the UK and Germany, and carrot seed production in the Netherlands (Murant and Spence 2002). The virus infects carrot, celery, chervil, coriander, dill, and parsnip, as well as wild chervil

(*Anthriscus sylvestris*) and cow parsley (*Heracleum sphondylium*). There are two serotypes of PYRV, the parsnip serotype which infects celery, cow parsley, and parsnip; and the anthriscus serotype which infects carrot, chervil, coriander, cow parsley, and dill. Infection of young carrot plants by the anthriscus serotype can cause severe stunting, necrosis, and death of plants. Infection of older plants leads to mottled foliage with yellow flecks. Infected plants can develop misshapen rots and/or secondary roots. PYFV causes premature dieback in carrot seed crops in the Netherlands (Van Dijk and Bos 1985). The virus is transmitted semi-persistently by the carrot-willow aphid, *C. aegopodii*, as well as *C. pastinacae* but not by *C. theobaldi* or the green peach aphid, *Myzus persicae*. A helper virus, *Anthriscus yellows virus* (AYV), must be present in source plants of anthriscus for transmission of PYFV. AYV is phloem limited, unlike PYFV, is not transmissible mechanically, and is in the family Sequiviridae. PYFV is not seed transmitted. Aphids fed on plants infected with AYV alone, and then on plants infected with PYFV alone, were able to transmit both viruses, but not if fed in the reverse order. Infective aphid adults can transmit the viruses for up to four days, but nymphs cease to transmit the viruses after molting. AYV and PYFV host ranges overlap, and only plant species that are a host to both serve as a source for aphid transmission (Murant and Spence 2002).

Resistance screening: Motley dwarf: A wide range in responses of carrot cultivars to motley dwarf has been documented (Fig. 18.10), and there is genetic resistance to motley dwarf (Koike et al. 2002). In California, the cultivar Danvers was very susceptible while CVC-14 was partially resistant (Watson and Falk 1994). Differentiating resistance of cultivars to motley dwarf versus resistance or tolerance to the carrot-willow aphid vector complicates efforts to screen for resistance (Dunn 1970). Dunn (1970) noted that the cultivar Autumn was highly susceptible to the aphid but

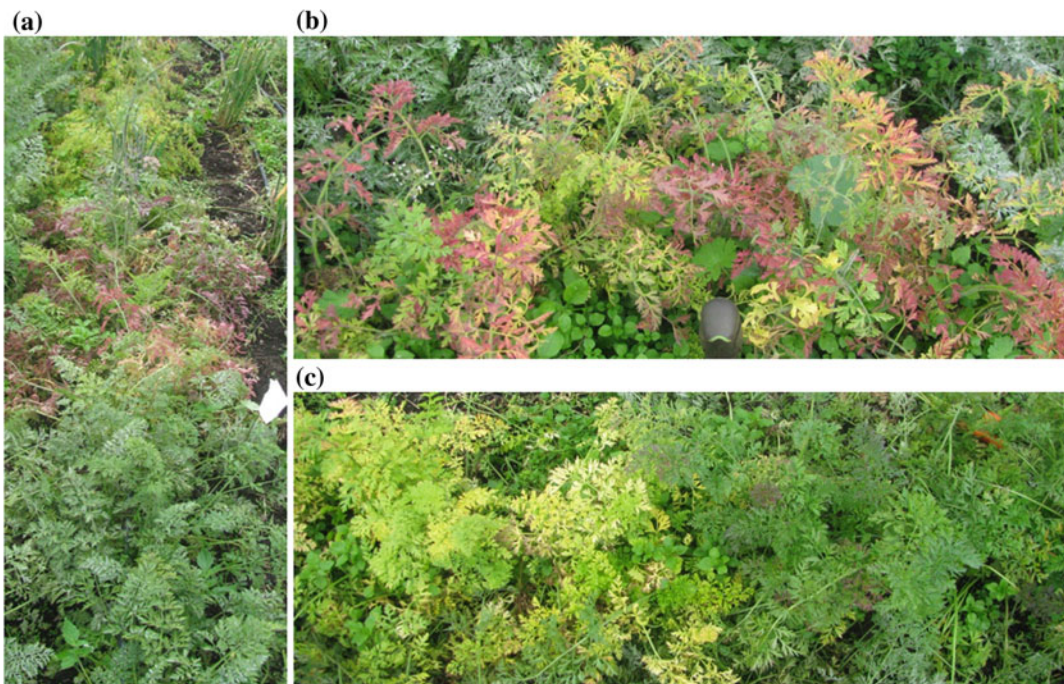


**Fig. 18.10** Carrot cultivars can vary widely response to motley dwarf, caused by coinfection with *Carrot red leaf virus* (CRLV) and *Carrot mottle virus* (CMoV), as illustrated in these small breeding plots in western Washington State in 2017 and 2018, with dramatic differences observed in severity of symptoms of breeding lines in adjacent plots (Lindsey du Toit, Washington State University)

displayed partial resistance to motley dwarf in multiple trials, whereas Nantes was not as tolerant of the aphid as the other cultivars screened and was highly susceptible to motley dwarf. Tomlinson (1965) reported 'Kurnella Strongtop' and 'Western Red' to be tolerant of motley dwarf, and Kinsella (1966) reported similar results for 'Western Red,' despite these cultivars being well colonized by the vector *C. aegopodii*. Tomlinson (1965) noted severe motley dwarf symptoms on 'Nantes' and the Chantenay cultivars Early Market and Cluseed Stumprooted. Dunn (1970) also demonstrated both to be highly intolerant of *C. aegopodii*, whereas 'Berlikum' appeared the least susceptible to the aphid of seven cultivars evaluated.

CarVY: All carrot cultivars commonly grown in Australia were reported to be susceptible to CarVY, but symptoms varied in severity among cultivars (Latham and Jones 2004). Jones (2005) inoculated plants of 22 Apiaceae species in a glasshouse using viruliferous green peach aphids (*M. persicae*). Aphids were maintained on canola inside cages at 15–20 °C. For inoculation, the aphids were starved for two hours and then fed on infected leaves of the carrot cultivar Stefano for 10 min and transferred to healthy plants of the target species using a paint brush. The aphids fed for one hour before they were killed with insecticide. Jones (2005) observed systemic infection by CarVY on carrot, four other *Daucus* species (*D. bicolor*, *D. hispidifolius*, *D. muricatus*, and *D. littoralis*), five Apiaceae herbs (anise, chervil, coriander, cumin, and dill), a naturalized weed (Bishop's weed, *Ammi majus*), and two Australian native plants in Apiaceae (Australian carrot, *D. glochidiatus*, and native parsnip, *Trachymene pilosa*). Seven of the 22 host species became infected in field sites, with wide variation in type and severity of symptoms among host plants. An additional screen of 34 wild carrot germplasm accessions and 16 other *Daucus* spp. using viruliferous green peach aphids in a greenhouse again revealed wide variation in severity of symptoms and no complete or extreme resistance. Systemic CarVY infection was observed in plants of all of the following wild germplasm accessions: 21 accessions from the Polish germplasm collection, including 7 wild carrot accessions, 6 of *D. muricatus*, 2 of *D. bicolor*, and 6 of an unidentified *Daucus* sp.; and 29 from a UK collection, including 27 wild carrot accessions and 1 each of *D. hispidifolius* and *D. littoralis*. Some accessions had to be inoculated several times to establish infection, and some did not develop infection, suggesting these lines may have resistance to CarVY. The accessions also were screened in field trials in Australia, where symptoms were less diverse compared to the greenhouse tests.

PYFV: There do not appear to be any reports on screening for resistance to PYFV in carrot.

The genetic nature of resistance of carrot lines to virus diseases such as motley dwarf, CarVY, and other viruses remain to be determined. The variation in severity of symptoms observed among carrot lines infected with specific viruses suggests much potential for using molecular screening methods to identify resistance genes, including QTLs, associated with resistance to these viruses.

#### 18.2.2.4 Diseases Caused by Phytoplasmas and Spiroplasmas

A number of phloem-limited mollicutes (phytoplasmas and spiroplasmas) can cause diseases of carrot. Aster yellows and beet leafhopper-transmitted virescence agent (BLTVA) yellows are phytoplasma diseases that affect a wide variety of wild and cultivated plant species, including carrot and more than 300 other vegetables, weeds, and ornamentals (Blomquist 2002). They are both vectored by leafhoppers. Although losses to these phytoplasmas tend to be sporadic in carrot, aster yellows has been found in carrot production regions worldwide, whereas BLTVA yellows occurs only in the western USA. The aster yellows phytoplasma is in the aster yellows group, 16SrI, and the BLTVA yellows phytoplasma is in subgroup A of the clover proliferation group, 16SrVI (Lee et al. 2006). Symptoms caused by these phytoplasmas are similar. Aster yellows symptoms start with chlorosis of leaf veins and progress to chlorosis of entire leaves. Infected leaves may be narrower than leaves of healthy plants. Dormant buds in the crown sprout, leading to upright, adventitious shoots. Older leaves become bronze,

red, or purple and break readily, interfering with mechanical harvest using the tops (Blomquist 2002). The main root tends to stay small if plants are infected at an early stage, and the taproot of infected plants may develop prolific secondary roots (Fig. 18.11). In carrot seed crops, phyllody (development of leaf-like petals on the flowers) and virescence (greening of flowers) occur after bolting (Fig. 18.11). Similar symptoms develop on plants infected with the BLTVA yellows phytoplasma, but plants infected with this phytoplasma also tend to bolt (flower) prematurely, unlike plants infected with aster yellows, and the taproot may be thin, woody, and develop a proliferation of secondary roots (Fig. 18.11). Seed is not viable in umbels that develop virescence and phyllody.

Lee et al. (2006) documented natural infection of carrot plants in Washington State, USA by *Spiroplasma citri*. The symptomatic plants displayed chlorosis, purpling, and bronzing of foliage, rosette formation of the crown, stunting of shoots and roots, proliferation of fibrous secondary roots, and even development of multiple taproots. Infected plants were found in multiple processing carrot crops in central Washington. Some plants were co-infected with *S. citri* and the aster yellows phytoplasma or BLTVA yellows phytoplasma. *S. citri* also causes citrus greening of citrus in Florida and California.

Phytoplasmas and spiroplasmas are prokaryotes that infect the phloem sieve cells of host plants where they reproduce by budding or division (Blomquist 2002). They also reproduce in the bodies of their leafhopper vectors. These obligate pathogens cannot be cultured on agar media, so infection is confirmed using enzyme-linked immunosorbent assays (ELISAs) or polymerase chain reaction (PCR) assays with primers specific to the group of phytoplasma or spiroplasma. Aster yellows can be transmitted by many species of leafhoppers, but the aster leafhopper, *Macrosteles fascifrons*, is the most important vector (Blomquist 2002; Boivon 1994). In contrast, the BLTVA yellows phytoplasma and *S. citri* are acquired and transmitted by the beet leafhopper, *Circulifer tennellus*. Once infected, leafhoppers transmit these phytoplasmas and spiroplasma persistently and remain infective until they die.

Significant losses to aster yellows in carrot production occur periodically in the Midwestern USA, where aster leafhoppers migrate from southern states each spring, and where they acquire the aster yellows phytoplasma from

**Fig. 18.11** Carrot plants infected with aster yellows or beet leafhopper-transmitted virescence agent (BLTVA) yellows phytoplasmas. Symptoms include yellow, purple, and/or bronze foliage (**a**), excessive secondary roots (**a**), stunting, and virescence and phyllody of umbels (**b**, healthy umbels on the left and infected umbels on the right) (Lindsey du Toit, Washington State University)

infected weeds and other crops. In the western and eastern USA, aster leafhoppers do not go through an annual migration, but local populations that survive the winter acquire phytoplasmas from infected crops and weeds. In the western USA, beet leafhoppers acquire the BLTVA yellows phytoplasma from infected wild vegetation in hills bordering cropland, and the vectors migrate into irrigated fields when wild vegetation dries during the dry season each summer. Neither of the phytoplasmas nor *S. citri* is seed transmitted in carrot. Female leafhoppers cannot transmit the pathogens to their offspring (Blomquist 2002).

Resistance screening and breeding: Gabelman et al. (1994) initiated a breeding program in 1982 to develop aster yellows resistance in carrot. They developed an aster yellows synthetic (AYSYN) population comprising four open-pollinated cultivars and five inbred lines by screening 200 carrot entries (open-pollinated cultivars and inbred lines) in the field. Since aster leafhoppers feed preferentially on lettuce, each 4-row bed of carrot lines was bordered by rows of lettuce to ensure adequate leafhopper pressure. Leafhoppers infected with phytoplasmas were reared in cages in a greenhouse and distributed evenly throughout the field in June and July. They rated plots visually in October for symptoms of aster yellows to calculate the incidence (percentage) of infection. The top 10% of the 200 lines was selected, and 189 roots from the lines were planted in a greenhouse after vernalization for pollination. Roots of 20 plants that flowered were selected, including five inbred lines (derived from crosses with W33, W263, W266, and W93), six inbreds from a double-cross of OSU260 with an F1 of an unnamed Russian line and W33, and four open-pollinated cultivars (Royal Chantenay, Scarlet Nantes, Gold King, and Nanco). Seed harvested from the crosses formed the AYSYN population, from which inbred lines were extracted using several schemes. Inbred lines also were developed from the Wisconsin carrot breeding program (WBP) using a third method detailed by Gabelman et al. (1994). The inbred W1-1 was developed by selecting four roots from inbred lines in the WBP that had good resistance to aster yellows, and intermating and inbreeding these lines for eight generations. Inbred lines derived from the AYSYN population were developed with three methods, from inbred progeny of the population, from AYSYN-derived inbred lines intermated with inbred selections from that population, and from intermating the AYSYN population with high-color inbred lines. AYSYN lines were inbred for at least five generations and then used to produce AYSYN hybrids. Field trials in 1990, 1991, and 1993 were used to assess the relative resistance to aster yellows of 26 selected lines compared to six commercial carrot cultivars, based on the visual rating of symptoms. Gabelman et al. (1994) detected significant differences among carrot genotypes with the resistant lines ranging from 2.5 to 35.3% infection/plot versus 12 to 42% for the standard cultivars. Many of the selected resistant lines displayed significantly less incidence of aster yellows. ‘Scarlet Nantes,’ ‘Royal Chantenay,’ and ‘Gold King’ exhibited the least aster yellows (average 15.3% infection), whereas ‘Danvers 126,’ ‘Py-60,’ and ‘Spartan Bonus 60’ had greater incidences of infected plants (average 33.3%). Leafhopper populations were comparable across the diverse genotypes, suggesting that resistance was not associated with reduced feeding of the vector. Feeding preferences on specific carrot genotypes were not observed. The most successful breeding scheme for resistance was using the AYSYN population as a source of inbreds, suggesting that combining a synthetic population with established inbred lines was highly effective.

The genetic basis of aster yellows resistance selected by Gabelman et al. (1994) has not been determined, but the inbreeding in that study may have revealed recessive alleles that contribute to resistance. Selection in a naturally infested and inoculated field site increased the ability to screen for resistance by maintaining strong selection pressure. The results suggest resistance to aster yellows is quantitative based on the phenotypic distribution and the influence of environmental conditions on the disease reactions observed.

## 18.3 Carrot Nematode and Insect Pests

### 18.3.1 Root-Knot Nematodes (*Meloidogyne* spp.)

Among the root-knot nematode (RKN) species (*Meloidogyne* spp.) attacking carrots, *Meloidogyne hapla* Chitwood, *M. javanica* (Treub) Chitwood, and *M. incognita* (Kofoid and White) Chitwood are of worldwide economic importance for carrot cultivation. Losses up to 100% may occur with yield reduction and shape deformation, i.e., taproot forking and galling that render carrots unmarketable (Roberts and Mullens 2002) (Fig. 18.12). *M. hapla* is the predominant species in fields in cooler production areas (temperate areas or at higher altitudes in subtropical and tropical areas), while *M. javanica* and *M. incognita* are major pests in warmer areas (Bridge and Starr 2007; Parsons et al. 2015). Even less widespread are *M. chitwoodi* and *M. fallax*, which can cause considerable losses by deforming the carrot taproot. *M. chitwoodi* causes severe galling near the lenticels, resulting in a rough surface to the carrot taproot (Wesemael and Moens 2008). Control of RKN is dependent on various methods, including soil applications of nematicides, crop rotation to non-host species, and flooding. However, genetic resistance appears to be the most effective and environmentally sound method to reduce damage caused by RKN.

Significant genetic variability for resistance to nematodes has been identified in carrot germplasm (Fig. 18.12). Twenty-one cultivars and breeding lines with various root types were evaluated for their responses to *M. hapla* under controlled greenhouse conditions or in field conditions by Yarger and Baker (1981). Gall formation and nematode tolerance appeared to be associated with root type; e.g., Nantes and Long Chantenay root types exhibited tolerance in general while Danvers root types were mostly susceptible. While some cultivars are tolerant or partially resistant to *M. hapla*, based on the taproots being less susceptible to deformation, others exhibit tolerance because roots become parasitized but reproduction of the nematode is inhibited (Wang and Goldman 1996).

Huang et al. (1986) established a reliable method for evaluating varietal responses to *M. javanica* in the greenhouse, highlighting that primary root galling in the carrot seedling stage was an efficient parameter for resistance evaluation to this nematode species. While it appeared that ‘Brasilia’ and other tropical carrot cultivars had the same resistance level to *M. incognita* and *M. javanica*, Nantes and Kuroda groups showed more severe symptoms, suggesting different resistance mechanisms to these two nematode species compared with *M. hapla*. The resistance

**Fig. 18.12** Symptoms on carrot roots infected with the root-knot nematode, *Meloidogyne incognita* (**a**), and comparison of reactions of a resistant breeding line (left) versus a highly susceptible carrot line (right) (**b**) (Lindsey du Toit, Washington State University)

in the cultivar Brasilia was associated with retarded nematode penetration, development, and egg production, and fast plant growth that culminated in a low nematode population density (Huang 1986). Yunhee et al. (2014) screened 170 Korean carrot lines for resistance to *M. incognita* race 1. Among them, 61 resistant lines represented potential genetic resources for breeders. While susceptible root tissues infected with *M. incognita* seven weeks after inoculation showed the formation of well-developed giant cells surrounding the nematodes, root tissues resistant to this RKN did not show giant cells but, instead, relatively small modified cells around the infecting nematode (Yunhee et al. 2014). The expression of resistance genes against RKN could be responsible for the formation of necrotic layers around the modified cells formed in resistant carrot root tissues. 'DR-333', a cultivar commonly grown in north India, was found to be tolerant to *M. incognita* (Siddiqui et al. 2011).

The diversity of nematodes should be considered in screening for resistance, as this can affect responses of cultivars. This is especially true for *M. chitwoodi* as three races have been identified in the USA (Wesemael and Moens 2008). Fifteen carrot cultivars, including Imperator, Nantes, Chantenay and hybrid carrot types, were identified as good hosts for *M. hapla* while their reaction to *M. chitwoodi* varied depending on the inoculated race (Santo et al. 1988). Among the 15 cultivars, 13 were moderate to good hosts of *M. chitwoodi* race 1. On the contrary, all of the cultivars, except Orlando Gold (moderate host), were non-hosts or poor hosts of *M. chitwoodi* race 2. Wesemael and Moens (2008) identified great variation in host status of 19 carrot cultivars to *M. chitwoodi* in glasshouse conditions, with some cultivars (Berlanda, Bolero, Chantenay, Nantucket, and Parmex) observed not to have egg masses, which indicated potential resistance. In 2009, Charchar et al. (2009) identified *M. polycephannulata*, a new species of RKN able to parasitize two important vegetables crops (i.e., carrot and tomato) that are cultivated extensively in Brazil. The identification of resistant cultivars of carrot for use in crop rotations is becoming a necessity for control of this RKN.

Knowledge of the genetic nature of resistance is essential to develop RKN-resistant carrot cultivars. A major part of this type of work has been done with *M. javanica* and *M. incognita*. Huang et al. (1986) found relatively low narrow-sense heritabilities of resistance to *M. javanica*, from 0.16 to 0.48 for root galling and from 0.31 to 0.35 for egg mass production, depending on the cultivar. Simon et al. (2000) identified simply inherited dominant resistance to *M. javanica* (one or two duplicated dominant genes at a single locus, *Mj-1*) in a selection of the carrot variety Brasilia, which was also effective against *M. incognita* based on field tests. Using bulk segregant analysis, Boiteux et al. (2004) identified RAPD markers linked to the *Mj-1* locus that are, therefore, useful for marker-assisted selection to develop hybrids resistant to *M. javanica*. Investigating the association between expression of resistance to this nematode and locus dosage, Boiteux et al. (2004) suggested that phenotypic resistance is affected by *Mj-1* locus dosage, and that the *Mj-1* region may contain a quantitative resistance locus. Screening for additional sources of resistance, Ali et al. (2014) identified a new source of resistance to *M. javanica* in a segregating population bred for resistance to both *M. javanica* and *M. incognita*. A single major gene, designated *Mj-2* and identified on the same chromosome as *Mj-1* but not at the same locus, contributes to this resistance. Working with resistance to *M. incognita*, Parsons et al. (2015) identified five QTLs from the analysis of three segregating populations, with the QTLs located on carrot chromosomes 1, 2, 4, 8, and 9. One QTL, common to all three populations, is on chromosome 8 and co-localized with *Mj-1*, which confers resistance to *M. javanica*. Broad-sense heritability for resistance to *M. incognita* was calculated to be 0.33 and 0.25 in two carrot populations derived from a cross among three sources of resistance from Syria, South America, and Europe (Parsons et al. 2015).

Wang and Goldman (1996) identified two homozygous recessive genes with epistatic control of *M. hapla* resistance, suggesting that this resistance may be relatively simply introgressed into inbred lines via backcrossing. However,

previous studies have reported both quantitative and qualitative resistance to nematode infection. More recently, Yunhee et al. (2014) reported that resistance to *M. incognita* may be governed by one or a few genes.

Commercial cultivars have been released with resistance genes to *Meloidogyne* species from germplasm of *Daucus* spp. and lines derived from the cross 'Brasília 1252' × 'B6274' (Simon et al. 2000), or a population derived from the resistant cultivar Brasília (Vieira et al. 2003) with resistance to both *M. javanica* and *M. incognita*. While 'Brasilia' was developed in 1981, Brasília-derived germplasm is still considered one of the most promising sources of stable, wide-spectrum field resistance to RKN species in carrot (Vieira et al. 2003). In 2009, Embrapa Vegetables in Brazil (Pinheiro et al. 2011) released the cultivar BRS Planalto, which has a high level of tolerance to RKN.

As highlighted by Ali et al. (2014), conventional breeding protocols for developing RKN resistance often include greenhouse and extensive field evaluations for phenotyping, which are time-consuming and labor-intensive. A promising strategy could be the application of RNA interference (RNAi) to confer resistance to host plants engineered to express dsRNA and small interfering RNAs (siRNA) to target and silence specific nematode genes (Roderick et al. 2018). In this manner, Tan et al. (2013) were able to reduce the expression level of two genes involved in structural integrity and proper muscle contraction in *Pratylenchus thornei* and *P. zeae*, by soaking nematodes in dsRNA.

### 18.3.2 Carrot Fly or Carrot Rust Fly (*Psila rosae*)

The carrot fly, *Psila rosae* (F.), is one of the most significant insect pests of carrot and other Apiaceae crops, with the host range restricted to Apiaceae species (Hardman and Ellis 1982). Females search out carrot plants to lay eggs around the crown. The larvae migrate down to feed on carrot roots, making the root unmarketable due to larval damage (Ellis 1999) (Fig. 18.13). Losses are mostly due to a reduction in quality rather than yield (Dufault and Coaker 1987). While antixenosis reduces the initial infestation of flies and contributes more to resistance than antibiosis against larvae in some Apiaceae species, it was shown to be the opposite for carrot varieties (Degen et al. 1999a, b, c).

Carrot lines have been screened for susceptibility to carrot fly. Ellis et al. (1978) reported that many evaluations of carrot cultivars for resistance to carrot fly have not led to consistent results among experiments. When comparing 11 cultivars for the effects of different rooting systems on insecticide efficiency against the carroty fly, the authors identified 'Royal Chantenay' and Speed's 'Norfolk Giant' as having the extremes of a range of resistance to susceptibility among the 11 cultivars. Compared to other methods of assessment, the damage index based on recording weights and numbers of roots in four damage categories discriminated among the cultivars efficiently, especially when carrot fly attack was severe. Michalik and Wiech (2000) divided carrot genotypes into four groups according to the percentage of damaged roots, and identified five breeding lines as resistant to this pest. The greatest resistance found in cultivated carrot resulted in a 50% reduction in damage by *P. rosae*. Several *Daucus* spp. evaluated for reaction to carrot fly also had potential for hybridizing with cultivated carrots to develop cultivars with greater level of resistance (Ellis 1999). Since the first substantial source of resistance discovered in the Nantes cultivar Sytan (partial resistance based on antibiosis) by Ellis and Hardman (1981), breeding programs have been initiated and resistant F3 and F4 lines resulting from the crosses between carrot cultivars and *D. capillifolius* have been released. Nine inbred lines derived from a cross between two carrot cultivars, Sytan and Long Chantenay, with moderate level of resistance to the carrot fly were also released in 1991 with the aim of developing new cultivars (Ellis et al. 1991). Cultivars and wild accessions have been used in breeding programs as source of resistance, resulting in release of the partially resistant cultivar Flyaway

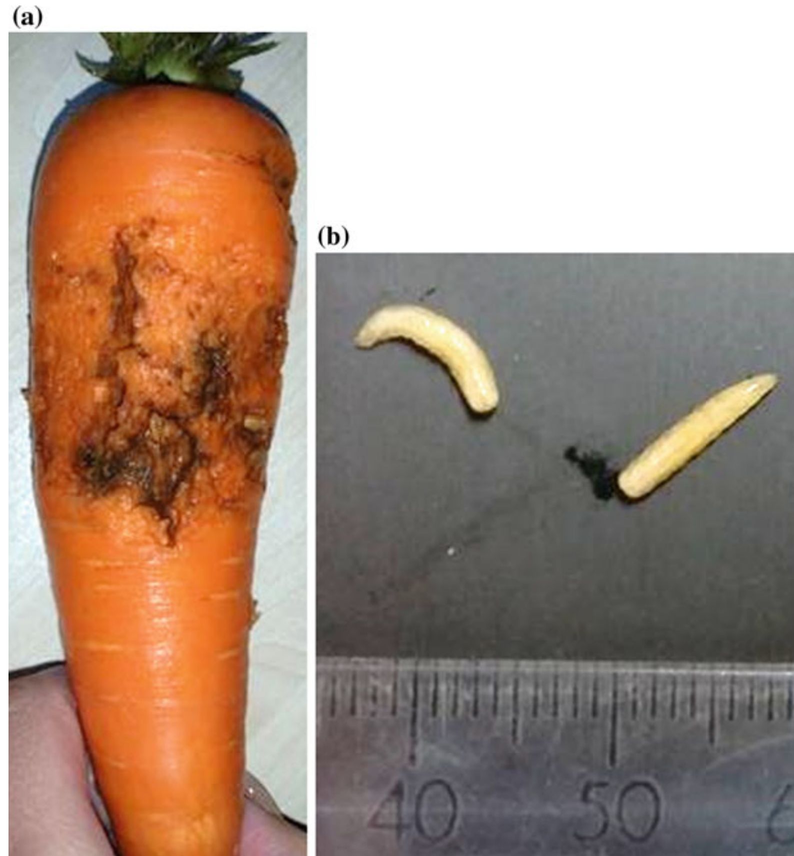


**Fig. 18.13** Damage to a carrot root by the rust fly, *Psila rosae* (**a**), and larvae (maggots) of the rust fly (**b**) (Anita Sequeira, Greenway Farms, South Africa—the ruler is demarcated in millimeters [short lines] and centimeters [numbered lines])

and lines with significantly greater resistance than Sytan (Ellis 1999; Simlat et al. 2013).

Understanding the biological, biochemical, and genetic mechanisms of resistance to the carrot fly could help breeders identify appropriate crosses to make between valuable genotypes. The chemical signature of the leaf surface is probably a complex mixture of more than one key compound (Guerin et al. 1983; Städler and Buser 1984). However, many volatile oviposition stimulants for the carrot fly have been identified from carrot leaves (Guerin and Visser 1980; Städler and Buser 1984). Most of these compounds (propenylbenzenes, coumarins, and polacetylene) are powerful defensive compounds against bacteria, fungi, plants, and herbivores (Städler and Buser 1984). Some studies have attempted to characterize the underlying mechanisms involved in resistance to the carrot fly. 'Oviposition non-preference' has been suggested as one mechanism. Guerin and Stadler (1984) evaluated the impact of the quantity of foliar chemostimulants produced by four cultivars on this parameter. The results suggested olfactory and contact chemostimuli may be involved in preference shown by the carrot fly for specific cultivars, but other factors such as leaf color and morphological characteristics were also involved in host selection and oviposition. While some varieties had antixenosis resistance, resulting in fewer eggs being laid, the principal basis of resistance was found in the root of plants (Guerin and Ryan 1983). The concentration of chlorogenic acid in carrot roots was also correlated with susceptibility to damage by carrot fly larvae (Cole 1985). However, when selected lines of 'Sytan' were screened for levels of this compound, discrimination among the lines was not a reliable indicator of resistance, suggesting that this compound was not the chemical basis of

resistance (Ellis 1999). More recently, Simlat et al. (2013) demonstrated a positive correlation between the ratio of certain phenolic compounds and resistant carrot phenotypes. Greater levels of expression of *PAL1* and *PAL3* were also observed in the most resistant carrot lines.

In conclusion, many sources of resistance to the carrot fly have been identified in wild and cultivated materials, and potentially could be combined to develop even better resistance. Ellis (1999) suggested that resistance to this pest is inherited polygenically, but few studies have investigated the genetic basis of resistance to the carrot fly. This information would be of great help in developing cultivars with greater resistant to this important pest of carrot.

### 18.3.3 Aphids

Numerous aphid species can feed on carrot, with plants damaged by the mechanical feeding action as well as toxic saliva from the aphids (Rubatzky et al. 1999). Aphid feeding weakens plants, and the aphids deposit a sticky, sugary waste product on the foliage called honeydew. Honeydew is a highly suitable medium for growth of saprophytic fungi, which can cover the photosynthetic surface area of foliage. In addition, some aphid species are vectors of viruses that infect carrot, e.g., the carrot-willow aphid, *C. aegopodii*, vectors CMoV and CRLV, which together cause motley dwarf (see virus diseases above). The green peach aphid, *M. persicae*, is considered the most widespread and damaging aphid species on carrot and is a vector of several viruses of carrot, such as CarVY and others. Other aphids that feed on carrot include the carrot-willow aphid, *C. aegopodii*, violet aphid (*Myzus ornatus*), pea aphid (*Acyrothosiphon pisum*), bean aphid (*Aphis fabae*), melon aphid (*Aphis gossypii*), hawthorn-carrot aphid (*Dysaphis crataegi*), honeysuckle aphids (*Rhopalosiphum conii*, *Hyadaphis foeniculi*, and *H. coriandri*), and potato aphid (*Macrosteles fascifrons*), among others.

Resistance screening: Lamb (1953) noted that the Australian carrot cultivar Osborne Park might be partially resistant to the carrot-willow aphid, partly as a result of the strong vigor of this cultivar. In Britain, the vigorous cultivar Autumn King was thought to be resistant to this aphid compared to cultivars with smaller tops, as this cultivar showed less severe symptoms of motley dwarf. Therefore, Dunn (1970) investigated whether this was related to Autumn King being partially resistant to motley dwarf or partially resistant to the aphid. Cultivars representing Chantenay, Nantes, Berlikum, and Autumn King, as well as three Australian cultivars were tested for susceptibility to the aphid at different temperatures in cages, and by evaluating the entries in field trials over three years. Dunn (1970) noted differences in susceptibility to aphid attack but large numbers of aphids were counted on all the cultivars and the differences were too minor to be of practical value in breeding for resistance. In contrast to the observation by Lamb (1953), 'Osborne Park' had intermediate susceptibility to the carrot-willow aphid. In addition, 'Autumn King' was the most susceptible variety but was identified as 'tolerant' to motley dwarf. The Nantes cultivar was rated as susceptible to both the aphid and virus disease, whereas the Berlikum cultivar seemed the least susceptible to the aphid. Dunn (1970) noted that temperature had less effect on cultivar susceptibility than it did on aphid fecundity. Painter (1951) suggested three components of resistance to aphids feeding on plants: preference or non-preference, antibiosis, and tolerance. In the field trials, Dunn (1970) noticed that 20–30% fewer immigrant alate (winged) aphids settled on 'Berlikum', which might infer host non-preference, even though there was no evidence of preference among cultivars in the cage trials. However, 'Berlikum' was the shortest cultivar in these trials, so the fewer number of incoming alates observed on this cultivar may have reflected a form of escape rather than resistance.

Painter (1951) categorized plant resistance to aphids and aphid feeding into three categories: antibiosis, antixenosis, and tolerance. The terminology was used for many years, with modifications, until the advent of plant and arthropod genomics. Smith and Chuang (2014) reviewed the extensive literature on plant resistance to

aphid feeding. They summarized the literature related to advances in understanding of behavioral, biochemical, and physiological aspects of aphid selection of plant hosts for feeding, putative biophysical and chemical resistance factors involved in plant defenses to aphid herbivory, and the genetics and genomics of aphid-resistant cultivars that have been developed for various plant species. They described the relationship between constitutively expressed and aphid-induced plant resistance genes, as well as aphid virulence and the deployment of aphid resistance genes into crops for management of these pests. They noted that aphid resistance often is inherited as a dominant trait but can be polygenic and inherited as recessive or incompletely dominant traits. However, virulence to aphid resistance genes in plants has been documented in at least 17 aphid species, illustrating the need to identify new and diverse sources of resistance. For some plant species, genetic linkage maps and plant microarrays have provided invaluable tools for understanding the identity and location of aphid and aphid-vectored virus resistance genes in plants. However, currently there do not appear to be published studies documenting resistance of carrot cultivars to aphid feeding.

### 18.3.4 Thrips

Thrips are not typically considered a major pest of carrot, but feeding with their rasping mouthparts leads to scarring or silvering of carrot leaves and petioles (Rubatzky et al. 1999). Species that can cause some losses to carrot include the western flower thrips, *Frankliniella occidentalis*, flower thrips, *F. tritici*, and onion thrips, *Thrips tabaci*. In addition to causing damage to carrot by feeding, the western flower thrips also vectors a virus that can infect carrot, *Tomato spotted wilt virus* (TSWV), although this is not an important virus disease of carrot.

Leiss et al. (2013) investigated quantitative resistance to the western flower thrips, *F. occidentalis*, using cultivated carrots, wild carrots, and biofortified carrots with increased concentrations of the antioxidant chlorogenic acid. They evaluated six commercial cultivars (Chantenay, Sugarsnax, Paris Market, Ingot, and Nantes), four wild carrots (S1, D2, D2, and D3 derived from individual plants collected in the Netherlands), and four biofortified accessions (two accessions with high chlorogenic acid levels, purple-yellow 309-2 and purple-orange B7262 from the University of Wisconsin carrot breeding program, and a purple and an orange accession from a seed company). Carrot leaves varied in thrips resistance, with a ten-fold difference observed in the degree of silvering (feeding damage) between the most resistant and most susceptible carrot lines. Comparison of the carrot morphological traits and leaf metabolic profiles of the three most resistant and susceptible carrots using nuclear magnetic resonance microscopy (NMR) revealed the wild carrots were not more resistant to thrips than the cultivated carrots. The most resistant cultivar was Ingot, which is also resistant to the carrot fly, *P. rosae*. The biofortified carrots were not resistant to thrips. In fact, three of the four biofortified carrots were the most susceptible to thrips feeding. There were no significant differences in plant size, leaf area, and number of leaf hairs between the thrips resistant and susceptible carrots, but the metabolic profiles of the leaves of the resistant cultivars differed from that of leaves of the susceptible cultivars. The flavanoid luteolin, the phenylpropanoid sinapic acid, and the amino acid β-alanine were present in greater amounts in leaves of resistant cultivars than those of susceptible cultivars. In vitro bioassays confirmed the inhibitory effects of these compounds on thrips. Leiss et al. (2013) suggested the results could be utilized to improve resistance to thrips based on the natural variation in these metabolites in cultivated carrots. The compounds also function as antioxidants, providing an additional benefit to efforts at breeding for resistance to thrips. They also suggested more sensitive metabolomics might detect additional metabolites associated with host resistance.

### 18.3.5 Other Insect and Mite Pests

Many other insect and mite pests can feed on carrot roots and/or foliage, causing losses in root and/or seed production (Rubatzky et al. 1999). This includes the carrot psyllid (*Trioza apicalis*), leafhoppers (of which the aster leafhopper and beet leafhopper are important vectors of phytoplasmas—see the section on phytoplasmas and spiroplasmas above), lygus bugs (several *Lygus* species), carrot weevils (*Lisonotus oregonensis* and *L. latiusculas*), spider mites (particularly the two-spotted spider mite, *Tetranychus urticae*), carrot leafminer (*Napomyza carotae*), whiteflies (*Bemisia* spp. and *Trialeurodes* spp.), a diversity of lepidopteran pests such as cutworms (e.g., the common cutworm, *Agrostis segetum*, black cutworm, *A. ipsilon*, variegated cutworm, *Peridroma saucia*, and others), armyworm (*Spodoptera exigua*), cabbage looper (*Trichoplusia ni*), webworm (*Depressaria heracliana*), loopers (e.g., *Autographa californica* and *A. falcifera*), corn earworm (*Helicoverpa zea*), and European corn borer (*Ostinia nubilalis*), and other types of insect pests such as symphylans (*Scutigerella immaculata*), wireworms (*Limonius* and other genera), springtails (Collembola family), carrot beetle (*bothynus biggosus*), ground beetles (Tenebrionidae), flea beetles (e.g., the potato flea beetle, *Epitric cucumeris*, and the palestriped flea beetle, *Systena blanda*), and other beetles. For most of these pests, very little is known about variation in susceptibility among carrot cultivars or accessions aside from anecdotal evidence from breeders and growers, and even less is known about potential sources of resistance and genetic mechanisms of resistance. For those pests that also serve as vectors of viruses, phytoplasmas, and spiroplasmas of carrot, differentiating resistance to the vector versus the pathogen further complicates efforts to screen for resistance and develop an understanding of resistance to the insect pest versus the pathogen.

Although not considered a major pest of carrot root crops, lygus bugs can cause significant losses in carrot seed production. The insects feed preferentially on flowers and developing seed, destroying the embryos of carrot seed and resulting in non-viable seed. Scott (1970) reported observing differences in resistance of 'Nantes,' 'Imperator' (which he named 'Imperida'), and 'Royal Chantenay' to feeding by two species of lygus bugs, *L. hesperus* and *L. elisus*. He caged lygus bugs on the umbels of carrot plants in flower, counted the percent mortality of the insects in the cages, and considered percent mortality of the insects on the umbels to represent resistance of the cultivar to feeding by this pest. Scott (1977) used the same approach to attempt to select for resistance to lygus bugs in field trials in Idaho. There was no evidence in either study that Scott (1970, 1977) assessed the umbels for severity of damage to the developing seed by lygus bugs. The insects could have died from a diversity of causes not related to feeding on carrot umbels or seed, and not related to potential differences in resistance of the cultivars to this pest. There were many factors confounding these attempt to compare cultivars for reaction to lygus bugs (e.g., each cultivar was evaluated in a different season). Some umbels may not have provided adequate nutrition for the lygus bugs to persist, which also could have impacted the development of the seeds. He recorded as much variation in lygus bug mortality among plants of the same cultivar as he did among cultivars. The very poor rigor of those studies and the fact that losses to lygus bugs continue to be a major constraint in some areas of carrot seed production, raise doubt about the conclusions reached in those studies.

Kainulainen et al. (2002) examined the essential oil composition in the leaves of seven carrot cultivars in relation to oviposition acceptance by sucking insects with different feeding strategies. They evaluated the carrot psyllid, *T. apicalis*, as a carrot specialist, *T. anthrisci* as an Apiaceae specialist psyllid, and the lygus bug, *L. rugulipennis*, as a generalist in growth chamber, greenhouse, and field trials. The carrot psyllid is a significant pest of carrot in northern Europe,

where feeding by nymphs reduces root growth, and saliva injected as the adults feed causes curling of the leaves. As noted above, lygus bugs can be particularly damaging in carrot seed production as they puncture seed to feed on the developing embryo, resulting in non-viable seed (Scott 1977). Kainulainen et al. (2002) observed that oil composition differed significantly among leaves of the cultivars Flakkeer 2, Nantura, Parano, Napoli, Panther, Splendid, and Nantes 3 Express. The mean number of eggs laid by the carrot psyllid and *T. anthrisci* did not differ significantly among cultivars. However, lygus bug females laid more eggs on Nantes Express 3 than on Panther, even though this preference was not observed in an olfactometer test, which suggests that physical contact with the plant is more important in host selection for this generalist insect than olfactory orientation. There was no evidence of a correlation between the composition of essential oils and egg-laying preference of the lygus bug. A negative linear relationship was detected between concentration of the oil limonene and the number of eggs laid by the carrot psyllid, indicating that this oil is a repellant to the carrot psyllid. The carrot psyllid was attracted to high concentrations of sabinene. Other studies have also demonstrated attraction of the carrot psyllid to Apiaceae species that produce large amounts of α-pinene and sabinene (Nehlin et al. 1996; Valterova et al. 1997). A positive correlation was detected between myrcene concentration and number of eggs laid by the Apiaceae psyllid, *T. anthrisci*. Interestingly, this is the main compound in leaves of wild chervil (*Anthriscus sylvestris*), the main host of *T. anthrisci*. The study illustrated that some compounds influence the egg-laying preference of this psyllid, but these may not be the primary components of essential oils in carrot leaves. Overall, this study indicated that the host preference of the Apiaceae specialized pest, *T. anthrisci*, was affected more by the essential oil composition of carrot cultivars than lygus bugs, a generalist pest. Carrot cultivars with high concentrations of limonene might be more resistant to the carrot psyllid.

## 18.4 Conclusions

As detailed in this chapter, there is wide variation in the degree of understanding of carrot germplasm reactions to the numerous pathogens and pests of carrot. For a few of these biotic stresses, significant phenotypic resistance screening has been completed, candidate genes have been identified, and the resistance incorporated into commercially acceptable cultivars. For others, almost nothing is known about potential sources of resistance, and phenotypic screening methods have not yet been developed. Significant resources are needed to extend this effort to many more carrot pathogens and pests of concern regionally and internationally. Resistance to biotic stresses, combined with chemical (foliar fungicide sprays and seed treatments) and cultural control measures, has contributed significantly to disease and pest suppression compared with individual control measures (Ben-Noon et al. 2003), but far more can be achieved in screening for resistance and incorporating resistance into breeding programs. The absence of crossing barriers between wild carrot species and cultivated carrot greatly facilitates the introduction of resistance genes into cultivated carrot. Furthermore, the development of molecular markers and related tools has facilitated much more rapid progress at identifying resistance genes and developing cultivars with improved resistance (Stein and Nothnagel 1995). A novel set of 300 simple sequence repeat markers, combined with a deep-coverage, highly redundant carrot genome library with >17X coverage (Cavagnaro et al. 2009, 2011) have provided invaluable genomic resources for carrot breeding and genetics. Recent analysis of ~1.74 Mb of BAC-end sequences gave the first overview of the composition and organization of the carrot nuclear genome. Iorizzo et al. (2016) reported a high-quality genome assembly accounting for ~90% of the estimated carrot genome. They predicted 634 putative pest and disease resistance genes that will be very helpful for identifying candidate genes underlying biotic and abiotic stresses, and other important traits. More recently, Wang et al. (2018) sequenced the

genome of 'Kurodagosun,' a major carrot variety in Japan and China, accounting for 78.5% of the estimated genome (473 Mb). These genomic resources enhance fundamental and applied research on carrot, including breeding for resistance to pests and diseases such as leaf blights. Klimek-Chodacka et al. (2018) reported the first use of the CRISPR/Cas9 system for efficient, site-targeted mutagenesis of the carrot genome, which offers new prospectives for improving carrot resistance to diseases and pests. The ultimate challenge is to combine resistance to multiple pests and pathogens with other traits important for carrot root production, seed production, storage, flavor, nutritional qualities, and processing.

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