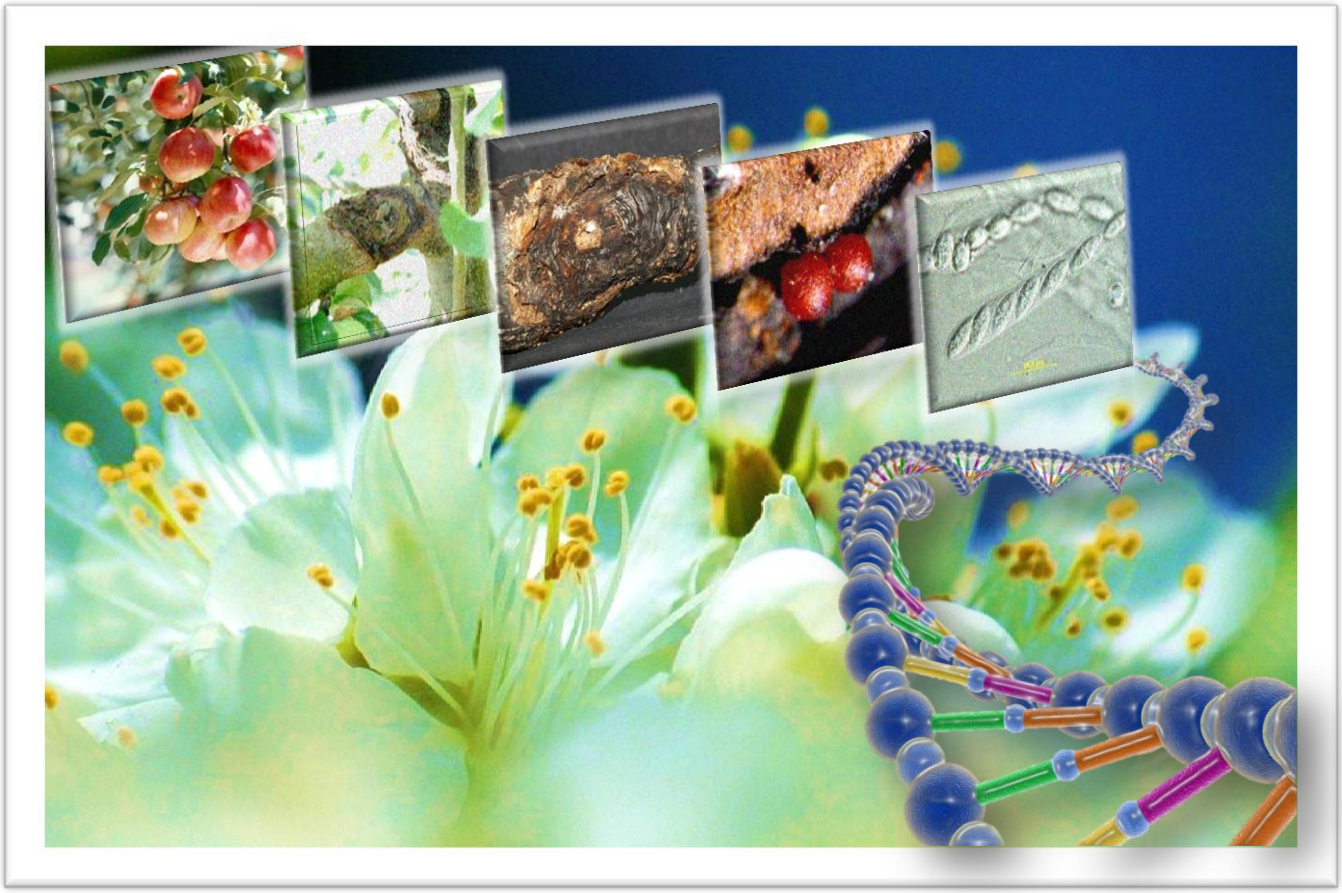
Genetic Basis for Resistance Against Fruit Tree Canker in Apple



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Summary

Neonectria ditissima (formerly *Neonectria galligena,* anamorph *Cylindrocarpon heteronema*) is the causal agent of fruit tree canker which is regarded as a serious economic problem in horticulture. This fungus causes notable damage to apple trees and it is very important in some regions, especially North western Europe, where it can result in death of spur shoots and branches. Although it occurs in a wide range of temperatures, it is associated with wet weather and climate has an important effect on the geographic distribution. The fungus produces conidia and ascospores, both of which are dispersed and cause infection during prolonged periods of rainy weather. Also, spores produced on the infected wood can act as an infection source in the orchards. The fungus can therefore be introduced into new orchards with infected planting material from other orchards or tree nurseries. Chemical and mechanical control like spraying of fungicides, covering wounds with paint, and cutting out infected branches, do not prevent the occurrence of epidemics. Breeding cultivars with a high level of resistance towards canker would be of great help towards the avoidance of this disease. Apple cultivars show variable levels of partial resistance to the fungus, whereas complete resistance has not yet been reported and no major genes have been identified. Therefore, apple genotypes with comparatively high levels of genetically determined partial resistance should be identified for use in apple breeding.

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1. Introduction to apple

Apple is a temperate fruit crop and the fourth most widely grown fruit in the world after citrus, grapes and banana (Khachatourians 2002; O'Rourke 2003). In addition, it can be ranked as the most important among deciduous fruits based on trade, production, and consumption (Maric et al. 2010).

Apple can grow in all temperate and subtropical areas of the world because of the broad genetic variation in this crop, although production is quite low when grown at high altitudes of tropical countries. Also, production of apple occurs in some orchards in Siberia and northern China where temperatures fall to –40°C and also in Colombia and Indonesia with very high temperatures.

The obtained evidence of prehistoric remains and historical records has shown the existence of cultivation and dispersal of apple in Asia and Europe more than several thousand years ago. The origin of apple may be referred to the discovered archaeological remains of apple almost 6500 BC in Anatolia. By 500 BC, apple was certainly cultivated extensively in the whole of the Persian Empire. Then, the cultivation of apple was spread through Greece and the Roman Empire to Europe, where its cultivation was well known by the Ancient Greeks and Romans. Apple was planted in the whole of Europe so that a large number of cultivars were identified by the end of the 18th century. The highest diversity in apple production was observed in the 19th and 20th centuries, when *Malus* × *domestica* cultivars were found in Europe, North America, Russia, New Zealand, Australia and Japan (Luby 2003). For over 2000 years, *Malus asiatica* Nakai was cultivated in southern and eastern Asia, China and surrounding areas, but was replaced by *M.* × *domestica* during the late 19th and early 20th centuries (Zhou 1999).

Nowadays, almost all commonly grown apple cultivars belong to the species *M.* × *domestica* and the world production of apples is close to 71 million tons annually (FAO 2009; Folta and Gardiner 2009) with China being the largest apple producer in the world (Folta and Gardiner 2009).

2. Taxonomy of apple

Apple belongs to the family Rosaceae, which is the 19th largest family of plants (AWP 2007) and contains a lot of beloved species of edible temperate zone fruits (Janick 2005), ornamentals and some medicinal and timber crops. It is subdivided into several subfamilies including *Maloideae.* The subfamily *Maloideae* contains edible temperate fruit species and a large number of landscape plants (Hummer and Janick 2009), almost 20–30 genera and 1000 species, that are characterized by a synapomorphic pome fruit and a basic chromosome number of x=17 (Phipps et al. 1991; Evans and Campbell 2002; Folta and Gardiner 2009). The most important members are apple (*Malus*) and pear (*Pyrus*). Determination of species in the genus *Malus* has differed widely between different taxonomic treatises with as few as eight up to as many as 78 different wild and domestic apple species being described. The domesticated apple is a complex interspecific hybrid (Phipps et al. 1990; Robinson et al. 2001; Khachatourians 2002), with *M.* × *domestica* generally regarded as the most appropriate scientific name, replacing the previously common usage of *M. pumila* (Korban and Skirvin 1984). According to new research relied on multilocus concatenated sequence alignment, *M. domestica* and *M. sieversii* were categorized in the same cluster, and it therefore supports the proposal that they are probably the same and *M. pumila* could be accepted as an appropriate nomenclature for this cluster (Velasco et al. 2010).

3. Apple cultivars

Apple is a diverse fruit crop, with many thousands of cultivars from different countries around the world. Apple cultivars have been derived from modern apple breeding programs or, traditionally, from selection among spontaneously occurring seedlings (Brown and Maloney 2005). ‘Golden Delicious’ is the most widely grown apple cultivar (Scalzo et al. 2005) and has also been much used in breeding (Troggio et al. 2012). Other important cultivars used for developing modern apples are ‘McIntosh’, ‘Jonathan’, ‘Cox’s Orange Pippin’, and ‘Red Delicious’ (Pereira-Lorenzo et al. 2009).

4. Apple diseases

Various diseases on apple trees, caused by fungi, viruses, mycoplasmas, bacteria, and nematodes, reduce the yield and growth of individual trees and may lead to their death. Most apple cultivars are susceptible to such diseases, and chemical control may not be sufficient for protecting the trees and the fruit. Hence apple genotypes with high levels of genetically determined resistance are very valuable for successful breeding of resistant cultivars.

Apple scab, caused by the fungus *Venturia inaequalis,* is the most economically important disease in temperate and humid regions but has less effect in semi-arid regions. It is known as black spot in Australia, England, and South Africa. Scab infects the entire apple tree, i.e., leaves, petioles, blossoms, fruit, pedicels, buds, and shoots (Sandskär 2003), and yield is reduced through direct infection of fruit and pedicel (Naqvi 2004). Scab can be managed through the application of fungicides but at significant expense and difficulty. Breeding programs have been carried out to find sources of resistance and develop resistant cultivars. Both polygenic and monogenic types of resistance occur, and a combination of both types is highly desirable. 'Antonovka Poltobutanaja' was introduced as a cultivar with polygenic resistance to all known races of apple scab (Shay et al. 1962) but now some races attack it. This kind of polygenic resistance has also been identified in some species; *M. sieboldii, M. × zumi calocarpa, M. sargentii,* and *M. baccata*, and these have therefore been used in breeding programs, especially in Europe (Janick et al. 1996). Several modern cultivars carry resistance to apple scab, e.g., 'Goldstar', 'Florina', 'Goldrush', 'Rubinola', 'Topaz', 'Golden Orange', 'Prime Red', 'Prima', 'Erwin Baur', and 'Discovery', whereas e.g., 'Golden Delicious', 'Jonagold', 'Braeburn', and 'Elstar' are known as susceptible cultivars (Quamme et al. 2003; Sansavini 2003; Petkovsek et al. 2007; Borovinova 2011; Ignatov and Bodishevskaya 2011).

Apple powdery mildew caused by *Podosphaera leucotricha* is the second most important disease of apple after scab. Although it does not attack the fruit, it can kill seedlings and cause weakening of adult trees. The foliage and young shoots are attacked by the pathogen, reducing both the quality and the quantity of fruit (Ignatov

and Bodishevskaya 2011). Most of the economically important apple cultivars are susceptible. 'McIntosh', 'Delicious', and 'Geneva 65' rootstock are field resistant, and rarely produce infected shoots, whereas e.g., 'Jonathan', 'Idared' and Malling-Merton rootstocks are susceptible (Janick et al. 1996). Recently, some QTLs close to identified resistant genes have been detected in a F_1 apple progeny derived from a cross between 'Discovery' and 'TN10-8' (apple hybrid) that can be useful especially combined with other major resistance genes for breeding purposes (Calenge and Durel 2006).

Fire blight, caused by *Erwinia amylovora*, is a destructive bacterial disease of apple, pear, quince, hawthorn, firethorn, cotoneaster, and many other members of the family Rosaceae. This pathogen can infect fruit, shoots, flowers, and branches, and can kill the whole tree and destroy an entire orchard (Naqvi 2004). Several factors can affect the levels of resistance of a cultivar to fire blight e.g., environmental and growth conditions. In addition, physiological races of this bacterium can differ in pathogenicity. Pathogens infect also so-called resistant cultivars under notable selection pressure. Differences in pathogenicity would therefore be expected to develop gradually, and these differences can cause loss of resistance (Janick et al. 1996; Beckerman et al. 2009). 'Winesap', 'Enterprise' and 'Delicious' are considered as resistant cultivars, while 'Rome Beauty', 'Jonathan', 'Idared' and 'York Imperial' are highly susceptible (Janick et al. 1996; Nybom et al. 2012). A major QTL has been detected on linkage group 7 of the apple cultivar 'Fiesta', explaining 34–47% of the total phenotypic variation (Khan et al. 2006; Khan et al. 2007). Le Roux et al. (2010) have identified two QTLs on linkage group 5 and 10 in 'Florina' explaining 10% and 15% of the phenotypic variation, respectively. Possibly, relatively resistant cultivars can be obtained through QTL pyramiding of this region together with other identified regions.

Crown rot is caused by the fungus *Phytophthora cactorum*. The pathogen is more active in soil, infecting the bark of apple trees and sometimes killing the entire tree by girdling the main stem at ground level, especially in high moisture areas. *Phytophthora* can become a very destructive disease in nurseries and apple orchards (Nakova 2010). Both rootstocks and scion cultivars are attacked by this fungus, so it seems that resistance of both rootstocks and cultivars are necessary. It has been suggested that resistance is partially or completely dominant (McIntosh and Mellor 1954). Based on

the research, 'SJM189' rootstock showed resistance to *P. cactorum* while 'SJM15' and 'SJP84-5162' rootstocks were susceptible (Carisse and Khanizadeh 2006).

Cedar Apple rust is an important fungal disease, caused by *Gymnosporangium juniperi-virginianae.* It attacks fruits and leaves of apple and requires red cedar (*Juniperus virginiana* L.) as alternate host to survive each year. It is a serious problem in the eastern part of North America. It has been reported that resistance may be controlled by a single dominant gene or by two dominant genes; 'Jonathan' and 'Rome Beauty' are considered as fully susceptible (Shay and Hough 1952) and 'Delicious' is susceptible (Chen and Korban 1987). Some cultivars, e.g., 'Enterprise', 'NY 65707-19', 'NY 79507-72', 'NY 75414-1', and 'NY 79507-49' rootstocks were considered as resistant while 'Crimson Crisp', 'Princess', 'Scarlet O'Hara', and 'Pristine' were highly susceptible (Biggs et al. 2009).

5. History of fruit tree canker

There are several fungi that cause cankers on apple trees and reduce the growth and yield, and may lead to their death. Fruit tree canker, caused by the fungus *Neonectria ditissima* (*Neonectria galligena* Bres., formerly known as *Nectria galligena*) is one of the most important diseases of apple and it has a serious impact on the quality and quantity of fruits. *Neonectria* is the only identified and proven teleomorph of *Cylindrocarpon heteronema* (Rossman et al. 1999; Rossman and Palm-Hernandez 2008).

The first reports on *Neonectria ditissima* probably date from approximately 1880 when Goethe published a study of fruit tree canker (Goethe 1880) and Hartig published a study of a similar disease on a selection of broad-leaved trees, especially copper beach (Hartig 1880). Wiltshire (1921) described the early stages of canker formation on apple stems, and reported that the fungus penetrates into the tissue through small cracks, which occur after leaf-fall or in the spring when the neighboring buds are swelling. In addition, the fungus can enter through lesions produced by the scab fungus (*Venturia inaequalis*). Zeller (1926) has published detailed descriptions of the disease and the anatomy of the lesions. The life-history of *N. ditissima* has also been

published by Cayley (1921) to enable application of fungicides during the spore-producing periods.

6. Geographic distribution

Fruit tree canker is also known as apple canker, Neonectria canker, and European canker. Despite its name suggesting that the fungus originated in Europe, this pathogen is indigenous to North America according to a study published by Plante et al. (2002). In North America, it occurs in southeastern Canada and the northeastern United States and westward to the Pacific Coast. It also occurs in Australia, Chile, Northern Continental Europe, New Zealand, South Africa, the United Kingdom, and Japan (Grove 1990; Xu and Robinson 2010). Local climatic factors have a profound effect on its distribution. Heavy buildup of fruit tree canker can occur on exposed slopes with shallow and infertile soils at high altitudes, and poorly drained soils or those that have pockets that are poorly drained at lower altitude (Brandt 1964).

Neonectria ditissima infects apple, pear (usually less severe than in apple), and many species of hardwood forest trees such as maple, quince, aspen, beech, birch, and hickory in most parts of the world (Grove 1990).

7. Classification of *N. ditissima*

The classification of the genus *Neonectria* has been investigated by Rossman et al. (1999) who studied the fungal order of the *Hypocreales* and the family of *Nectriaceae,* and based the taxonomy on morphological and biological characters.

Neonectria ditissima belongs to the phylum *Ascomycota*, class *Sordariomycetes*, subclass *Hypocreomycetidae*, order *Hypocreales*, and family *Nectriaceae*. The *Ascomycota* is a division of the kingdom Fungi, and subkingdom Dikarya. Its members are known as the Sac fungi, and they constitute the largest phylum of Fungi, with over 64,000 species.

8. Morphology of *N. ditissima*

Every species of fungus has specific morphological characters by which it can be readily recognized from other species. Morphological characteristics of *N. ditissima* are: sporodochia, conidiophore, conidia, perithecia, asci, and ascospore.

8.1. Sporodochia

Sporodochia of *N. ditissima* consist of creamy-white pustules of conidiophores. Conidiophores arise from the white mycelium which occurs on the surface of the bark. Sometimes hemispherical sporodochia form because the conidiophores break through epidermis.

8.2. Conidiophore

The conidiophores are simple, usually branched and divaricated (Fig. 1) (Agrios 2005).

Photo: M. Ghasemkhani

Figure 1. Part of a conidiophore from a sporodochium produced on apple bark

8.3. Conidia

Cylindrocarpon heteronema, the asexual stage, belongs to the imperfect fungi, the class *Hyphomycetes* and the order *Hyphales* (Agrios 2005). *Cylindrocarpon heteronema* produces conidia of two types; macroconidia and microconidia. The conidia are formed through an asexual process from phialides in a basipetal succession but they do not

form chains. Macroconidia are produced on the end of conidiophores of the sporodochia.

Macroconidia are straight or curved, and cylindrical to fucoid but always with rounded ends and without septa. They are two- to four-celled and develop on white, yellowish or orange–pink sporodochia, which are found on the surface of the infected and dead bark (Agrios 2005). The macroconidia are creamy yellow but become chalky white when dried out. The full-grown spore is hyaline and 5–7 septate (Fig. 2).

The macroconidia are found on the mycelium when they are fully physically developed, and then they instantly surround the sporodochia. Under natural conditions, the conidia on the bark of apple trees may link to each other when they occur close together (Cayley 1921). One cell of a spore may connect with a cell of a neighboring spore or several cells of one spore by connecting hyphae (Zeller 1926; Hanlin 1971).

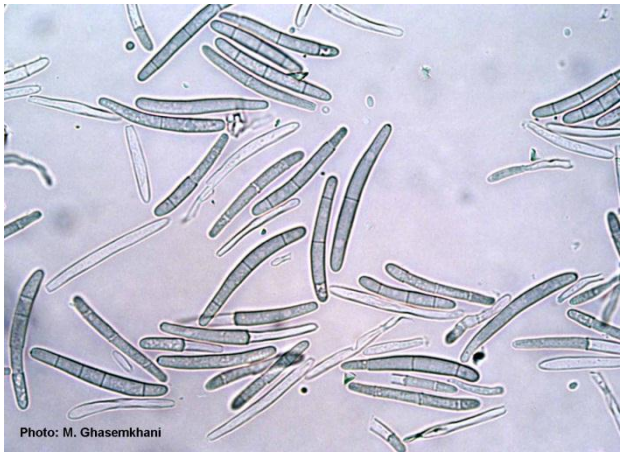


Photo: M. Ghasemkhani

Figure 2. Macroconidia of *N. ditissima*

It has been reported that linking of conidia of *N. ditissima* results in the formation of a palisade pseudo-tissue that helps to increase the mass of the sporodochium (Cayley 1921). At this stage, the nuclei of the cells have a strange behavior. The actual movement of a nucleus from the cell of one spore to the cell of a neighboring spore

through linking hyphae has never been observed, but cells with two nuclei are often seen (Fig. 3).

Microconidia may be produced in large quantities by abruption from hyphal branches (Ogawa and English 1991). They are very variable in size, shape, and septation because of variation in moisture, nutrition, temperature, and other factors. Microconidia are single-celled, hyaline, and ellipsoid oval (Fig. 4). These conidia are about 4–7×1–2 microns (Zeller 1926; Hanlin 1971).

8.4. Perithecia

Perithecia of *N. ditissima* can be found around the edges of the canker. They are ovate to globose, bright red and contain a tangled mass of vegetative hyphae surrounding the perithecia (Ainsworth and Bisby 2011) that become darker when they get older. The perithecia are found in sporodochia on the host (Fig. 5) when they are in early stages of development (Lortie 1964).

Variation in size of the perithecia is dependent on their position on the bark and factors like temperature and moisture (Fig. 6).

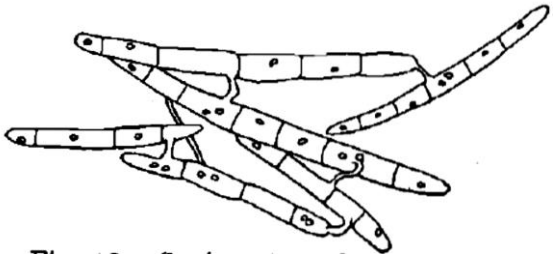


Figure 3. Conjugation of macroconidia

Figure 4. Microconidia of *N. ditissima*

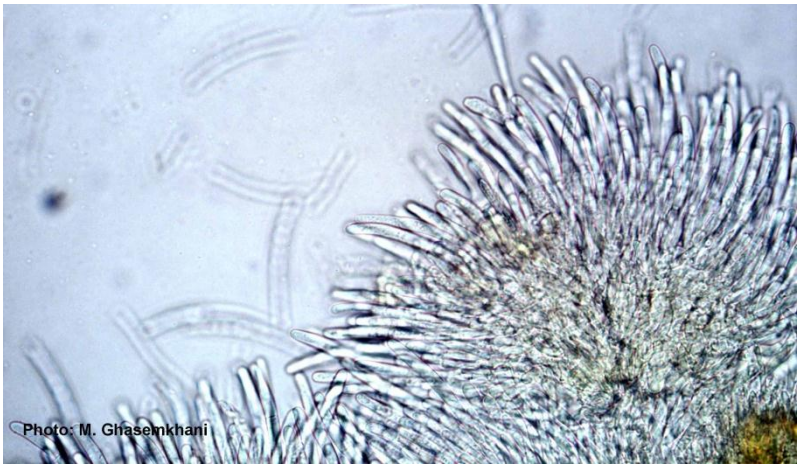


Photo: M. Ghasemkhani

Figure 5. Sporodochium with not fully developed perithecia

Photo: Bruce A. Watt

Figure 6. Perithecia of *N. ditissima*. Fruit tree canker produces bright orange fruiting bodies during winter

8.5. Asci

The asci are hyaline yellow to pale brown, cylindric to club-shaped, ovoid, thin-walled, with a pore in the top, and 8 two-celled ascospores (Fig. 7), 90–125 × 8–15 microns (Hanlin 1971; Ainsworth and Bisby 2011).

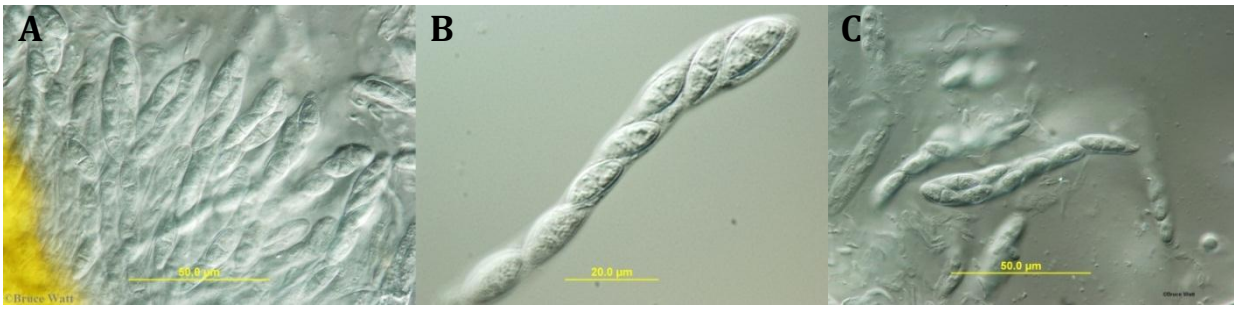


Photo: Bruce A. Watt

Figure 7. *N. ditissima* produces asci in brightly colored perithecia, A: two-celled ascospores, B and C: 8 two-celled ascospores

8.6. Ascospores

These spores are two-celled; they are hyaline to slightly brown, smooth, and striped with parallel longitudinal lines. The ascospores are produced in brightly colored subglobose to globose perithecia on the surface of a cushion-shaped stroma (Agrios 2005), solitary or scattered in or around canker wounds. They vary moderately in shape and size. They can be oval, spindle-shaped, unequal-celled, and slightly constricted at the medial septum in a single ascus (Fig. 8) (Hanlin 1990).

Figure 8. Ascospores of *N. ditissima*

The development of the perithecia proceeds rather quickly during months of continuous rainfall but is slower during drier periods. When the perithecia have reached sufficient size, discharge of the ascospores can be observed (Ingold 1971). The ascospores are mostly released in the spring and early summer but there is also a short period of discharge in the autumn. Few ascospores are spread in late summer or in winter. The ascospores are responsible for long-distance spread of the disease (Swinburn 1971b).

Variation in humidity influences ascospore discharge, which is delayed by periods of low moisture. It has been reported that ascospore discharge does not occur unless the leaves are wet. The most beneficial weather conditions for the release of ascospores from perithecia, is after a rain when the atmosphere remains sufficiently humid. In addition, light may also be needed for the release of large numbers of ascospores. The quantity of released ascospores decreases strongly during night and early morning (Wiltshire 1921; Lortie and Kuntz 1963).

9. Dispersal of spores

Spore dissemination depends on wind, the activity of insects, and rain splash, all of which are considered as carriers.

9.1. Wind

The forcible ejection of the ascospores helps to disseminate the spores, and air currents or wind are considered to act as carriers. These contributing factors are dependent on moist conditions. Many infections are observed in the upper parts of trees, perhaps the spores are carried there by the air currents (Gupta 2004).

9.2. Insects

Woolly apple aphids (*Eriosoma lanigera*) attack the apple and can carry *N. ditissima* spores in their woolly covering some distance, from infected trees to healthy trees (Reding et al. 1997). Ants also carry these ascospores on their bodies and transport them up and down the branches of the tree (Glime 2007).

Although spores can thus be found on the bodies of insects, surely their role of making entry wounds in the bark is more important than transporting the spores.

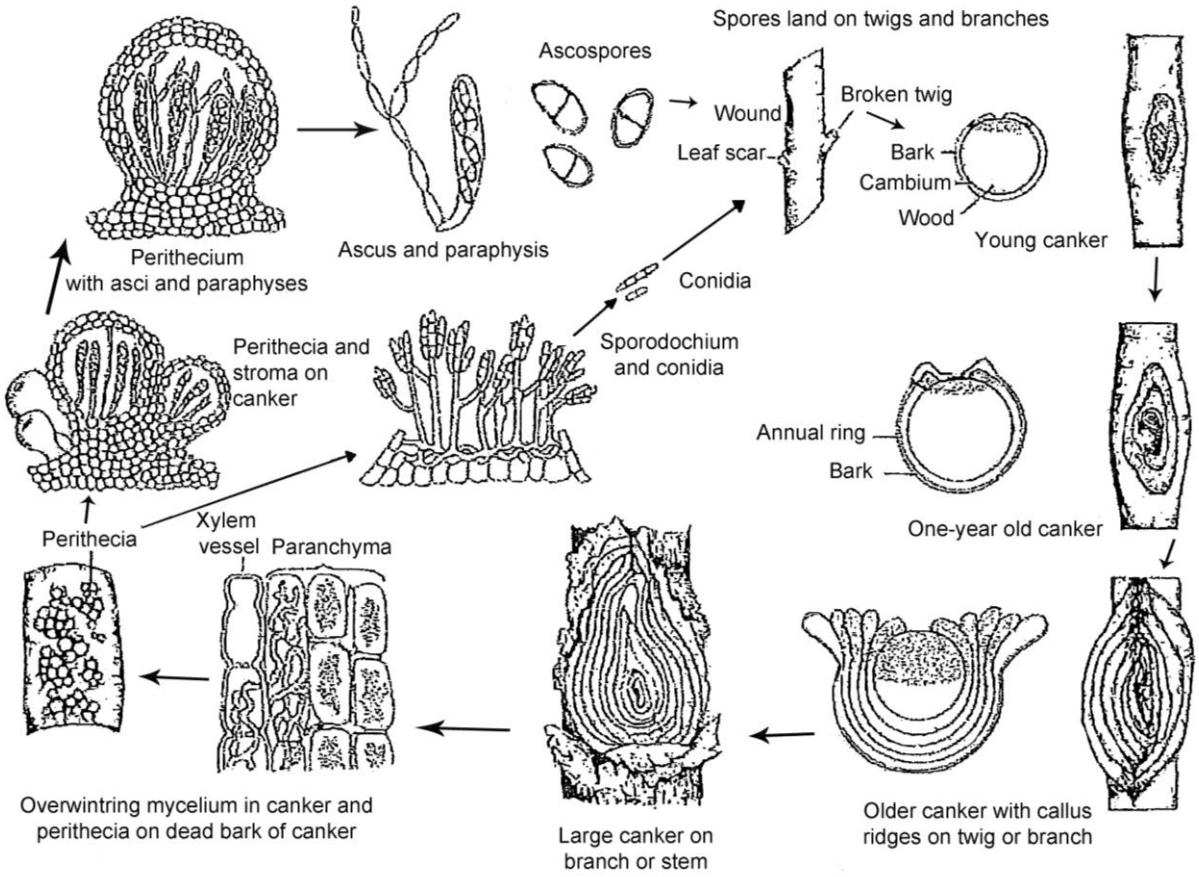
9.3. Rain splash

The spores are probably disseminated by rain splash and enter through bark lenticels or small insect wounds. This disease can thus spread in the field with naturally occurring rain (Madden 1997).

10. Disease cycle

The fungus overwinters in cankered limbs, twigs and branches, as perithecia and mycelium during the winter and under adverse environmental conditions. Sporodochia are usually produced when the young cankers develop in the first year. Therefore, the first spores to be produced in a new canker wound are conidia. The year after canker formation, perithecia develop and appear in the cankers in late summer and autumn. If there are favorable conditions throughout the year, cankers may continue to produce both conidia and ascospores (Xu and Butt 1994), which are capable of causing infection (Fig. 9).

The spores can be transported over considerable distances, up to 125 m in windy conditions. Conidia and ascospores produced by the fungus are dispersed not only within the same tree, but also to neighboring trees and thus cause infection during prolonged periods of rainy weather (Latorre et al. 2002; Beresford and Kim 2011). Maximum production of both spore types occurs at 10–16 °C in autumn.



(Agrios, 1997)

Figure 9. Disease cycle of fruit tree canker caused by *N. ditissima*

When spores reach an injured tree with a favorable site, usually natural or artificial holes in the bark, they grow and germinate instantly. The fungus then penetrates into the bark tissue, and may become established in the bark tissue within 3 to 4 hours (Brandt 1964), and can then engage in both sexual and asexual reproduction. Conidia produced during summer spread to other trees and start another cycle of infection. During the growing season, this cycle, i.e. the asexual stage, is repeated many times. In winter time, the sexual stage is started by production of ascospores. In the next spring, ascospores again infect trees, in a primary infection attack.

11. Infection

Neonectria ditissima plays a role as a secondary pathogen following infection by other pathogens such as scab fungus (*Venturia inaequalis*) and another canker fungus, *Neofabrea malicorticis* (Crowdy 1949).

Infection of *N. ditissima* usually occurs through wound sites e.g., leaf scars, pruning wounds, fruit scars due to chemical thinning or natural abscission, twig stubs, in the crotches of limbs, and even apple scab lesions (Xu et al. 1998; Naqvi 2004). The pathogen has also been observed in the lenticels of the cork tissue (Dewey and Swinburne 1995).

Lesions can start to grow whenever the infection has been established. New wood grows around the infected area and forms a protective boundary, i.e., a wound callus, preventing the spread of infection into the new tissue. The boundary tissue consists of two zones; wound wood which is a hard tissue consisting of tightly packed polyhedral cells close to the canker area, and restricting the spreading of the fungus by gum barriers, and a soft parenchymatous tissue located outside the woody zone, and easily recognizable from normal peripheral tissue by the absence of fiber bundles. This tissue behaves like normal phloem and cortex in response to infection (Zeller 1926; Crowdy 1949).

Sometimes, infection is not successful because wounds are not sufficiently deep and do not reach the wood, thus confining the fungus to the cortex.

11.1. Spread in the xylem

The fungal hyphae invade the xylem vessels, tracheids (but not the lignified walls of these tissues), fibers, and medullary rays and it then stays dormant (Crowdy 1949). Langrell (2000) reported that the hyphae of *N. ditissima* were detected in the xylem. The hyphae are frequent and strong in the lesion but become weaker and less frequent further away from the center of infection (Sakamoto et al. 2004). Pathogen penetration has also been seen in the soft tissues outside of the xylem. At this stage, peripheral tissue is stimulated by the fungus and forms a phellogen barrier, which blocks the

vascular tissue thereby temporarily preventing further damage to the plant (Clatterbuck 2006) and restricting the spread of the pathogen toxins.

However, the pathogen soon produces a large aggregate of mycelium close to the barrier, resulting in barrier breakage. It has been claimed that either the mechanical pressure or an abnormal concentration of toxins produced by the aggregated mycelium may break the barrier. The new phellogen is then formed and this progress is continuously repeated (Zeller 1926).

11.2. Spread in the phloem

The mycelium cannot penetrate directly into live tissue of phloem. Instead, pathogen secretion first kills living cells, and then, the fungal hyphae can penetrate into the lumen of the phloem fiber cells. Wound phellogen is formed by the host near the active margin of the lesion in response to the infection thereby separating the infected tissue from the healthy. Spread of the pathogen in the phloem fiber is similar to spread in the xylem fiber. At an early stage, the mycelium grows within cells and moves from one cell to another through the pits. In later stages, the mycelium develops intercellularly and hyphae grow spirally around small groups of fibers within the bundles (Zeller 1926; Crowdy 1949).

11.3. Leaf scar infection

The most important sites for infection are leaf scars formed during leaf fall in autumn (Fig. 10), and pruning cuts (Dubin and English 1974; Naqvi 2004). Infection has been reported to start from the leaf scar, not from the buds (Wiltshire 1921). The fungus enters through cracks in the leaf scar which appear at the margins. The pathogen uses these small cracks for entrance into the host tissue (Crowdy 1952). Then the host is stimulated to form a protective phellogen barrier, but the pathogen breaks down this layer after a while (Crowdy 1949). Small amounts of water are held in small depressions on the surface of the leaf scars, which help spores to germinate. A small circular dark reddish spot observed at the margin of the leaf scar is the first clear sign of infection. When the sap of the host is exposed to air, it rapidly oxidizes and the

color turns to bright reddish brown. Then the host forms the first phellogen barrier some distance away from the fungus to surround it. Thus the primary scar is formed.

When the fungus enters into the stem, it grows quickly and can girdle the whole stem in a short time. The bark frequently separates from the cortex and a ragged membrane occasionally remains over the infected area. The pathogen grows in the intercellular spaces of the internal tissues close to the leaf base because these sections are looser than the normal cortex. After a while, the hyphae grow inward between the cortical tissues and finally a compound mycelial strand may result. The first immature phellogen barrier is attacked by the fungus, and then the host forms a second phellogen barrier usually with some cracks close to the infected area since the cortical cells under the phellogen start to divide thereby producing new tissue.

The canker can develop rapidly after bud infection, and then all shoots above the infected area are killed immediately. In some cases, the canker develops slowly and it takes several years before it encircles the stem (Wiltshire 1921).

Photo: Natural Resources Canada, www.nrcan.gc.ca

Figure 10. Leaf scar of apple tree

11.4. Infection through other sites

Cracks and frost injuries can act as entrance sites for infection before they are covered by callus. Spores can stay in these sites and germinate, and then the cortical tissues of the bark are damaged by the fungus and infections are more likely to occur (Pijut 2006).

The injuries caused by woolly aphids are also a way of penetration for the pathogen. The aphids produce swollen and soft tissues that crack during winter, allowing the spores to penetrate into the bark (Mols and Boers 2001).

In addition, pruning cuts are a common source of infection unless they are treated by wound dressings or thoroughly soaked with fungicidal spray or covered by dust. The infection caused by such wounds is not as destructive as those caused by leaf scars and winter injuries (Xu et al. 1998).

12. Symptoms

Canker can infect wood of all ages, and the symptoms are very variable, depending on stage of disease development, climate, and type of host plant. The initial symptoms of *N. ditissima* appear as sunken areas of the bark around the buds (Fig. 11), wounds, shoot bases, and leaf scars. The fungus then grows gradually in concentric circles from the central infection point during autumn and winter. When the pathogen penetrates into the host tissue, a marked swelling of the shoot is seen around the canker region, forming a strong callus at the margin of the canker due to activity of the phellogen (Beltra et al. 1969). Concentric ridges are observed in exposed wood of old cankers, caused by differences in the seasonal growth rate of the fungus and the host (Crowdy 1949).

Black areas of the bark are caused by dry, spongy, and dead cortex and phloem tissue and loss of small fragments of superficial bark, leading to exposure of the xylem (Fig. 12) and cracked and roughened bark. Bark canker may cause dieback of younger branches or twigs (Agrios 2005).

White fruiting bodies consist of conidial spore masses that can be recognized on young cankers, especially on the young shoots in summer and early autumn whereas red fruiting bodies or perithecia are observed in autumn, winter, and spring. Apple canker usually expands and girdles a large trunk or limb, and kills all branches above the infected point (Fig. 13). Wilting and browning of leaves and blossoms may occur even before the branch is girdled. Brown staining on the wood and leaf of infected trees are caused by a fungus toxin (Naqvi 2004).

Photo: M. Ghasemkhani

Figure 11. Young branches infected by *N. ditissima*

Photo: Robert L. Anderson, USDA Forest Service

Figure 12. Structure of typical canker; concentric rings of the old canker

13. Histopathology of fruit tree canker

Some research has been carried out on the epidemiology of fruit tree canker (Swinburn 1971a; Cooke 1999) whereas detailed studies of the anatomy of *N. ditissima* attacks are lacking. One anatomical study of Nectria canker on *Fraxinus mandshurica* var. 'Japonia' has, however, been reported (Sakamoto et al. 2004). Based on this research, the concentric rings of the infected xylem are mostly composed of wood fibers, axial parenchyma cells, and a small number of vessels which contain fungal hyphae.

Photo: Bruce A. Watt Photo: H. Nybom

Figure 13. An apple tree completely girdled by fruit tree canker

Narrow and few vessels and a large number of axial parenchyma cells have been observed in the infected xylem compared to the healthy. An irregular orientation has been seen in all xylem elements, e.g., wood fibers and vessels, axial and ray parenchyma cells. Arrangement of cambial cells is disordered around the infected areas and cambial zones. A large number of parenchyma cells and sclereids are detected in the abnormal phloem.

Differentiation of narrow vessels decreases water conductivity and it is one of the reasons for dieback or debilitation of the infected trees in early spring. Differentiation of these vessels is due to mechanical wounding (Levyadun and Aloni 1993) and is not caused by fruit tree canker in itself.

Application of plant hormones can affect the anatomy; changes of stem anatomy in *Ulmus americana* L. seedlings are thus brought about by application of ethrel (Yamamoto et al. 1987). Possibly, anatomical characteristics can also be altered through hormonal changes caused by canker.

14. Climatic conditions

The geographical distribution of fruit tree canker depends on the climate (Van de Weg et al. 1992; Beresford and Kim 2011); fungus development is favored by mild and wet weather conditions (McCracken et al. 2003; Kim and Beresford 2012). Other factors like rainfall and temperature decisively influence the disease incidence (Dubin and English 1975; Swinburne 1975; Latorre et al. 2002). Production and distribution of spores depend on rainfall (McCracken et al. 2003) with duration of rainfall being more important than amount of rainfall (Dubin and English 1974). An average annual rainfall above 1,000 mm is apparently necessary for development of fruit tree canker in California (Dubin and English 1975), but this threshold is not applicable for all regions, e.g., fruit tree canker is a serious problem in Kent, England where average annual rainfall is 600 to 700 mm (McCracken et al. 2003; Beresford and Kim 2011). Temperature is an important factor for in vitro spore germination (Latorre et al. 2002), and also affects the infection of pruning wounds and leaf scars in the field (Dubin and English 1975; McCracken et al. 2003). Infection of *N. ditissima* occurs in vitro at a wide range of temperatures from 6 °C to 32 °C while the optimum temperature for germination is estimated between 20 °C and 25 °C (Latorre et al. 2002). Nevertheless, natural infection happens over the temperature range from 20 °C to 25 °C in the field (Dubin and English 1975). The most favorable temperature for leaf scar infection is 15 °C (Latorre et al. 2002). Number of hours per day at a temperature from 11 °C to 16 °C is significantly associated to leaf scar infection while number of hours per day from 5 °C to 10 °C is associated with the number of trapped ascospores (Dubin and English 1975).

15. Type of canker

Fruit tree canker lives for several years in the host tissues. The mycelium spends winter or any other inactive period in a dormant state in the infected canker tissue, and then starts to grow and penetrate into healthy tissue. This growth can vary depending on the type of canker; open, closed, or superficial cankers.

15.1. Open canker

The fungus grows under suitable temperature conditions when the tree spends a period of dormancy, and mostly happens during autumn and spring. At this time, the tree cannot recognize the pathogen. After a while, the healthy bark forms new tissue, cork cambium and callus, around the infected areas thereby making the margin of the canker look swollen. At this stage, the tree starts to grow actively and a fissure is formed between the infected bark of tree and healthy bark. Fungal hyphae are observed in the wood formed in the previous year, giving the edges of callus a darker color. Canker grows and spreads more quickly along the length of the stem compared to the stem diameter. The central part of the canker becomes deep and opens after several years, then the old bark adhering to the wood of the first or second year, falls away (Fig. 14). This kind of canker is occasionally observed on apple trees.

Figure 14. Open canker on apple tree

15.2. Closed canker

The infection spreads quickly in the layers adjacent to the cambium and discoloration of these layers is observed when the bark separates from the edges. Canker grows in lateral and longitudinal directions, and concentric rings of the callus are not obvious like in open canker. This type of canker can be found on apple and pear trees with a rough fissured bark (Fig. 15).

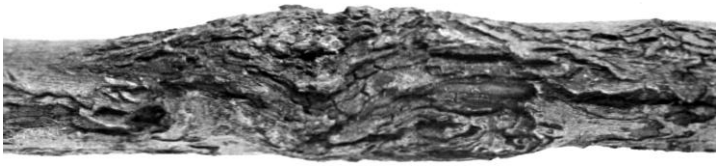


Figure 15. Closed canker on apple tree

15.3. Superficial canker

During the first year, infection spreads on the trunk, and there is no evidence of cambium infection. The canker develops during the late autumn, winter, and early spring. In the second year, infection may spread into the cambium. This kind of canker has not been ascribed to *N. ditissima* (Zeller 1926).

16. Control of fruit tree canker

Eradication by cutting out all infected tissue and covering the wound with e.g., Bordeaux paste or other disinfectants has been recommended. At the early stages of infection, where cankers occur only on side shoots or minor branches, application of fungicides during autumn and spring can also provide some damage control (Swinburne et al. 1975; Cooke 1999). For this purpose, fungicides based on copper such as Bordeaux, Copper-Count-N, and Cuprofix Ultra 40 are used (English et al. 1979). In some countries, these copper-containing products are, however, prohibited, especially in organic production. Recently, the Swedish product Scaniavital Kambium has been commercialized for treatment of fruit tree canker (www.nordiskalkali.se). This is a natural sea bottom-derived paste containing silica, calcium and various organic components.

Unfortunately, none of the described control measures can prevent the occurrence of epidemics completely.

17. Breeding and biotechnology associated with fruit tree canker

Breeding cultivars with a high level of resistance to canker would be a great help towards an improved control of this disease. To date, very little research has been focused on this disease but it is known that *Malus* species and apple cultivars show variable levels of resistance to *N. ditissima* (Garkava-Gustavsson et al. in press; Lateur and Populer 1994; Kozlovskaya et al. 1999; Sasnauskas et al. 2006) although complete resistance to this disease has not yet been reported (Van de Weg 1989). It has been claimed that some cultivars among cider apples can be very resistant and ‘M.1’ and ‘M.12’ have been recognized as resistant among rootstocks (Moore 1960).

Conventional plant breeding is time-consuming, but the recent development of molecular techniques has enabled great advances towards our understanding of the structure of plant genomes.

17.1. Molecular markers

Molecular markers are a useful tool for the genetic improvement and genetic analysis of complex agronomic traits, and can speed up the breeding programs (Stankiewicz et al. 2002). They simplify identification of specific genotypes, as well as desirable traits linked to the gene(s) among related species. By contrast, traditional breeding methods usually use the whole genome along with desirable and undesirable gene(s), and the elimination of undesirable gene(s) is then necessary through backcrossing. Thus, molecular markers allow eliminating of 'undesired' genome regions in a few generations.

Efficiency of molecular markers is usually evaluated as the ability to identify variation in a population, known as marker polymorphism. They can be classified into two groups; a) biochemical markers which can identify variation at the functional gene level such as changes in amino acids and proteins, and b) DNA markers that detect and analyze diversity at the DNA level like nucleotide changes.

17.1.1. Biochemical markers – Isozymes and allozymes

Biochemical markers such as isozymes have been ascertained as reliable genetic markers in plant breeding and genetic studies because of stability in expression, regardless of environmental factors (Kumar et al. 2009). Isozymes markers, which are the oldest among the molecular markers, were defined as multiple molecular forms of an enzyme with the same catalytic function and they have been widely used for different research purposes, e.g., to determine phylogenetic relationships, to evaluate genetic variation, and to study taxonomy and population genetics (Weeden 1989; Gelvonauskiene et al. 2005; Petrokas and Stanys 2008; Kumar et al. 2009).

High levels of polymorphism and high heterozygosity have been detected in apple isozymes, making them useful for cultivar identification (Manganaris and Alston 1989;

Samimy and Cummins 1992; Biruk and Kazlovskaya 2008). Additionally, linkage maps have been developed with some important traits being linked to isozyme loci (Weeden and Lamb 1987).

17.1.2. DNA markers

DNA markers are used extensively for different purposes, e.g., cultivar identification, determination of genetic variation among and within population(s), marker-assisted breeding (tagging important trait(s) in a breeding program) and molecular mapping.

Different DNA markers such as RAPD (Random Amplified Polymorphic DNA), SSR (Simple Sequence Repeats), ISSR (Inter Simple Sequence Repeat), AFLP (Amplified Fragment Length Polymorphism), and RFLP (Restriction Fragment Length Polymorphism) have now almost completely replaced isozymes as molecular markers in apple research. These markers have been used for cultivar identification and for assessment of genetic relationships among *Malus* species and cultivars (Goulao and Oliveira 2001; Tignon et al. 2001; Laurens et al. 2004; Galli et al. 2005; Garkava-Gustavsson et al. 2008; Adebayo et al. 2009; Gharghani et al. 2009; Guo et al. 2009) and for evaluation of genetic diversity also in fungal pathogens.

17.1.2.1. DNA markers in assessment of *Neonectria* diversity

RAPD markers and ribosomal DNA polymorphism have been used for comparison of genetic diversity of *Neonectria* species (Plante et al. 2002). Mantiri et al. (2001) used mitochondrial ribosomal DNA sequences for phylogenetic relationships in *Neonectria* species (anamorph; *Cylindrocarpon*) and suggested that the mitochondrial small subunit (mtSSU) rDNA region is suitable for phylogenetic analysis of *Cylindrocarpon* and *Neonectria*. Langrell et al. (2000) developed specific primers for detection of *N. ditissima* in apple wood. Those are especially valuable if the apple trees are infected with a mix of closely related species.

17.1.2.2. DNA markers associated with desired genes

Detection of markers linked to desirable traits is another useful application of DNA markers, and thus many different monogenic traits have been identified; 76 genes linked to morphological traits, and 69 genes encoding enzymes related to disease and pest resistance (Maric et al. 2010). Markers for major resistance genes to economically important diseases of apple, have been identified and can be used as a powerful selection tool to develop new cultivars with durable resistance, e.g., by pyramiding of resistance genes. Resistance genes to powdery mildew, apple scab, and woolly apple aphid have been identified by genetic markers, with 15 and 7 resistance genes to apple scab and powdery mildew, respectively (Maric et al. 2010) but genes controlling resistance to fruit tree canker in apple have not yet been identified (Alston et al., 2000). Gelvonauskiene et al. (2007) reported that resistance to fruit tree canker is mainly controlled by additive gene action and they introduced the apple cultivars 'Kaunis' (Lithuania) and 'Tellissa' (Estonia) as sources of resistance to *N. ditissima* in breeding programs.

17.1.2.3. DNA markers and QTL mapping

DNA markers can also be applied to construct genetic maps. The first genetic map of apple was combined of RFLP, RAPD, and isozyme markers (Hemmat et al. 1994) while several saturated genetic maps of the apple genome have been developed recently using molecular markers such as RFLP, SCAR, SSR, and AFLP (Maric et al. 2010).

In addition, markers linked to quantitative (continuous) traits are used as a tool to assay quantitative trait loci (QTL). They can provide information about the genetic basis of quantitative traits, e.g., mode of gene action such as dominance and additive, the effects of individual genes, and the number and chromosomal locations of quantitative traits (Angaji 2009; Xu 2010). Quantitative traits are generally polygenic, i.e. controlled by more than one gene. Environmental changes can also play a significant role in the phenotypic variance and make study of quantitative traits more difficult than monogenic traits. Traditional methods use pedigree and phenotypic

information to evaluate the collective effect of all QTL but they cannot separate the effects of individual loci.

Several QTLs have been detected that are associated with disease resistance in apple, e.g., fire blight (Liebhard et al. 2003; Khan et al. 2006; Le Roux et al. 2010), apple scab (Hemmat et al. 2000; Hemmat et al. 2003), and powdery mildew (Stankiewicz-Kosyl et al. 2005) but quantitative trait loci controlling related to fruit tree canker resistance have not yet been studied.

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