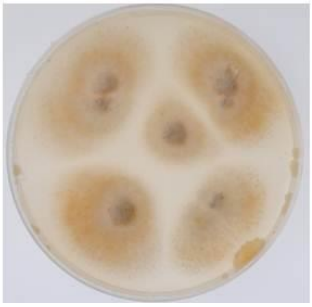
A review of our current knowledge of *Neonectria ditissima* and identification of future areas of research

A study funded by The Horticultural Development Company



Robert Saville, East Malling Research, East Malling, Kent, ME19 6BJ

Contents

1. The Problem

1.1 General introduction

Nectria canker, caused by the fungus *Neonectria ditissima* (formerly *Nectria galligena* and also commonly known as European canker or apple canker), is one of the most important diseases of apple and pear. The losses resulting from this pathogen are very difficult to quantify as they occur at all stages of production, from the tree nursery to the fruit store. Most of the established apple cultivars are very susceptible to the disease and the more recently introduced cultivars such as Jazz, Braeburn, Reubens, Cameo, Kanzi and Zari are also particularly susceptible. The propagation phase in the nursery presents a high risk period for infection due to the large number of wounds created as part of the production process: these act as entry points for the pathogen which can then persist asymptomatically until planted in the growing site, where plant stress often promotes the expression of the disease. The fungus attacks trees in the orchard, causing cankers and die back of young shoots, resulting in loss of fruiting wood and an increase in pruning costs. Apple canker can be particularly damaging in young orchards in the first few years of orchard establishment, as a result of trunk cankers, particularly following exceptionally wet or cold winters. In some years, up to 10% of trees can be lost annually. *N. ditissima* also causes a fruit rot that can occur both pre and post-harvest. Infection at flowering or early fruit development may develop into eye rot in orchards, though most infection stays latent and only develops during long-term storage. Post-harvest rot can result in significant losses as high as 10% or more in stored fruit. Nectria rot, which is often found at the fruit stalk end, is also difficult to spot on the grading line, but becomes obvious during marketing and gives rise to rejection of fruit consignments.

The last major review of the literature was in (Swinburne, 1975). Since then, some significant bodies of work have been undertaken and advances in research tools have been made. With the incidence of Nectria canker on the increase due, in part, to increased plantings of susceptible cultivars, it is pertinent that the latest literature is reviewed 40 years on and new research areas identified to mitigate the losses caused by this increasingly significant disease.

1.2 Geographical distribution

The disease occurs worldwide in the apple growing regions of Europe, Australia, New Zealand, Canada and the United States and South America (CAB international, 1985), where it is favoured by wet temperate climates within these areas. In the UK, climatic conditions at crucial development stages of the host, such as blossom period, pre-harvest and leaf fall, are often favourable for the spread and infection of the disease enabling inoculum to multiply, making this disease a perennial problem. This is in contrast to areas such as Pakistan where, although canker susceptible varieties such as Red Delicious among other delicious types are widely grown, rainfall crucial for inoculum spread and infection is largely restricted to the monsoon season. This coincides with periods of relatively reduced

susceptibility of the host, so the pathogen does not multiply and the disease is negligible in these growing areas (pers comm., Angela Berrie, East Malling Research). Canker expression may also be influenced by local factors such as water availability, microclimate and soil type (e.g. prone to inducing stress conditions) resulting in localised hotspots of canker expression even at the orchard level within farms.

2. The Fungus

2.1 Nomenclature

Neonectria ditissima (Tul. & C. Tul.) Samuels & Rossman, formerly *Nectria galligena* (Bres.), Anamorph; *Cylindrocarpon heteronema* (Berk. & Broome) Wollenw. *Neonectria ditissima* is an Ascomycete fungus within the Class Sordariomycetes. The Sordariomycetes contain some of the most important plant pathogens of horticultural crops including Verticillium and Fusarium. The complete classification of the fungus is listed below:

Scientific classification	
Kingdom:	Fungi
Phylum:	Ascomycota
Class:	Sordariomycetes
Subclass:	Hypocreomycetidae
Order:	Hypocreales
Family:	Nectriaceae
Genus:	Neonectria
Species:	*Neonectria ditissima*

2.2 Life cycle

A simplified disease cycle of *N. ditissima* on apple is depicted in Figure 1. The pathogen survives in orchards primarily in the form of perithecia and mycelium on cankered twigs and branches during the winter. Cankers sporulate after the onset of moist conditions. Two distinct spore types are produced by cankers; conidia (asexual) and ascospores (sexual). On newly formed/young cankers, sporodochia produce asexual spores whilst the perithecia (producing sexual spores) are not usually formed until the canker is 1-2 years old and beyond. The white-cream coloured sporodochia release conidia from early in the growing season all the way through summer and early autumn. Conidia are splash dispersed within the tree or to adjacent trees. The bright red perithecia contain ascospores which are disseminated either by being actively ejected from the perithecium and dispersed by wind or

extruded in a sticky mass and splash dispersed. This makes ascospores capable of local and long distance dispersal. There are wide discrepancies in the literature (e.g. Cayley 1921, Munson 1939, Swinburne 1971) about the maximum period of ascospore release reflecting a region to region and/or year to year variability. Under conducive climatic conditions, spores may infect any wound on woody tissues, including growth cracks, pruning wounds and leaf scars. Both spore types are also able to infect fruit.

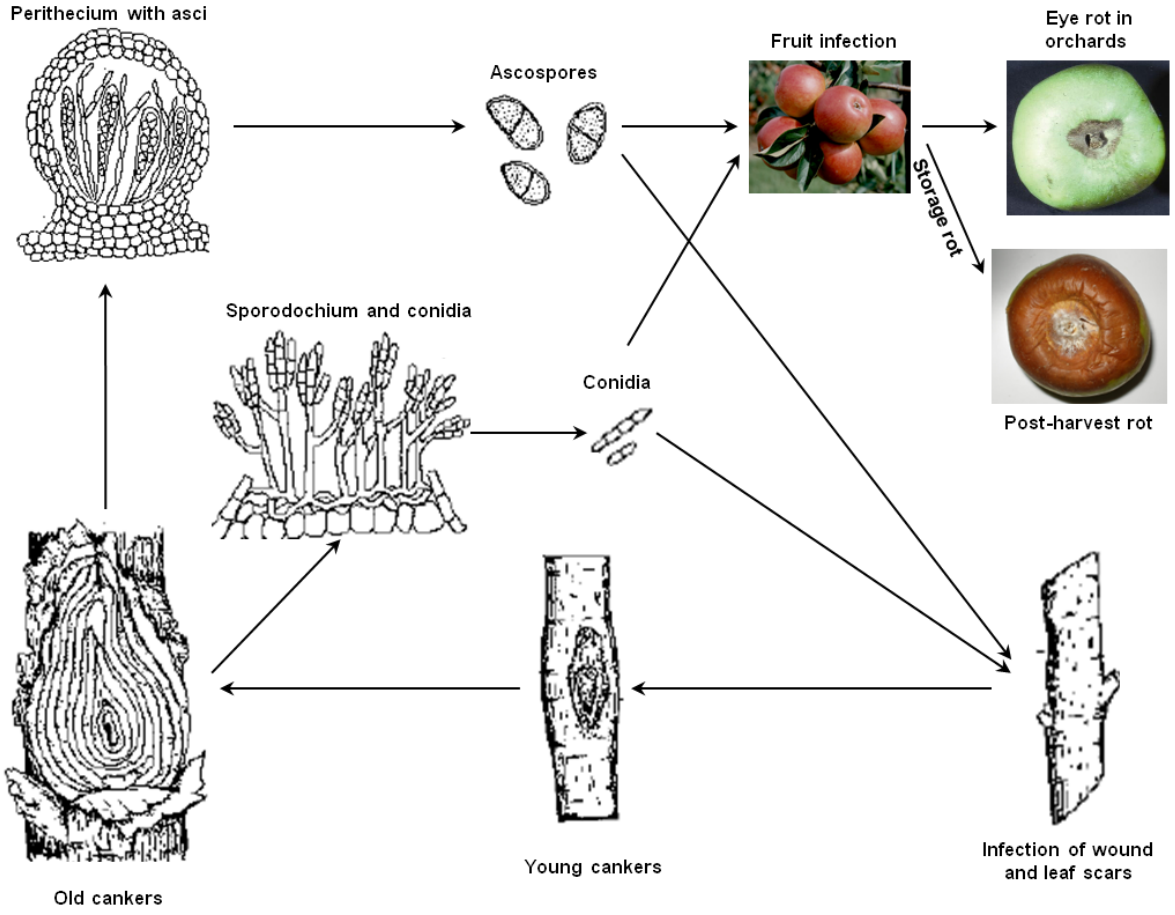


Figure 1 Disease cycle of apple canker and apple fruit rot caused by *Neonectria ditissima* (adapted from Agrios, 1988)

2.3 Spore morphology

Macroconidia are produced in white to cream-coloured sporodochia arising from white mycelium which bear the multi-branched conidiophores (Figure 2). The macroconidia are cylindrical, straight or curved, with rounded ends of average size 52-62 x 4.5-5.5 µm with varying degree of septation (ranging from 1 to 7 septa). Microconidia (4-8 x 2-3 µm) are hyaline, aseptate and cylindrical, with rounded ends, and are produced by abstraction from hyphal branches (Zeller 1926). Perithecia are ovoid-pyriform and bright red. The asci are clavate and stalked, typically containing eight ascsopores (14-22 x 6-9 µm). Ascospores are hyaline, bicellular, oval, ellipsoidal, or spindle-shaped, and frequently slightly constricted at the central septum (Seemüller 1988).

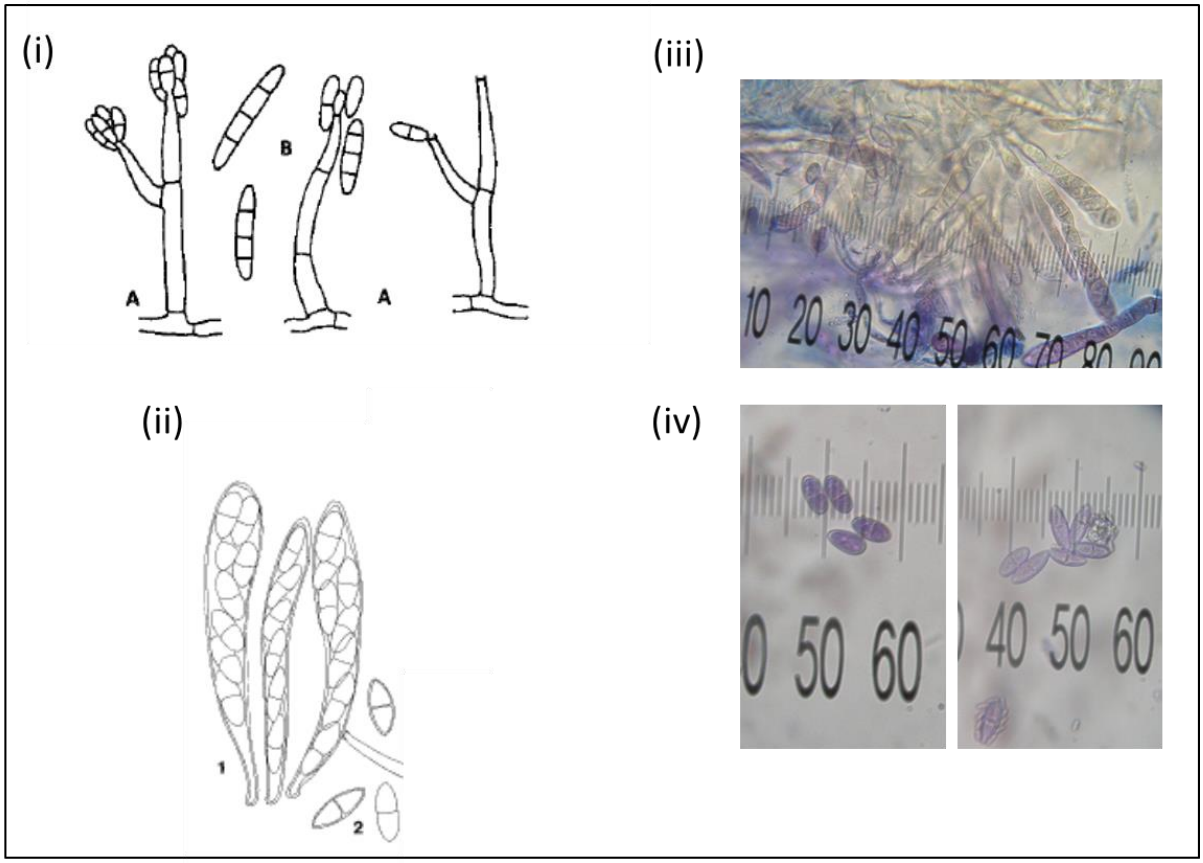


Figure 2 Spore morphology of *Neonectria ditissima* (i) anamorphic (Cylindrocarpon) and (ii) telomorphic states. (i) conidiophores with attached conidia (A), microconidia and macroconidia (B). (ii) asci (1 and iii)) and ascospores (2 and iv). Picture credits; (i) Barnett and Hunter (1998); Snowdon (1990); microscope images (iii & iv) Laure PESTEIL, Station d'Etudes et d'Expérimentations Fruitières de La Morinière.

2.4 Molecular diagnostics

Molecular diagnostics is an increasingly utilised tool in phytopathology research, to increase our understanding of the epidemiology of plant diseases. It is also now being made available for diagnostic applications in the field. There are two major challenges in the adoption of molecular techniques for *N. ditissima* research; (1) DNA extracted from the lignified woody tissue of host substrate contains inhibitors which affect the polymerase chain reaction (PCR) and (2) sampling strategies to ensure a representative result.

The development of a DNA extraction protocol using magnetic capture hybridisation to reduce the effects of inhibitors (Langrell and Barbara 2001) and the subsequent development of *N. ditissima* specific primers, Ch1 and Ch2, enabled the semi quantitative tests for fungal biomass (Langrell 2002). The Ch1 and Ch2 primers are designed to amplify a 412 base pair product from the internal transcribed spacer (ITS) region, a highly polymorphic region of DNA present in all eukaryotes which enables distinction at a species level. Ch1 and Ch2 primers have improved specificity compared to primers 'specific' to *C. heteronema* (*N. ditissima* anamorph) described by Brown *et al.* (1993) which were shown to cross amplify other closely related species within *Nectriaceae* (Langrell 2002). More recently, a qPCR assay has been developed which enables the amount of fungal biomass to

be quantified rather than simply analysing presence/absence (qualitative) (Garkava-Gustavsson *et al.* 2013). A monoclonal antibody, from cell line NG-IE4, was able to detect *N. ditissima* in woody tissue using ELISA (Dewey and Swinburne 1995) and may offer the potential for a field-based diagnostic device as has been developed for *Phytophthora* testing.

N. ditissima infected tissue is usually easily identifiable by visual symptoms alone (see section 3.1). Molecular diagnostic applications can be deployed to detect asymptomatic infections e.g. in a nursery certification scheme. However, sampling tissue for analysis is destructive and thus cannot be applied to each individual tree. Furthermore, the selection of tissues to sample is another problem because this pathogen can infect many host tissue types. It is therefore an important research target to develop sampling strategies to assess asymptomatic cankers in order to increase our knowledge of the epidemiology of the pathogen and for application in diagnostics.

3. Disease symptoms

3.1 Canker

The term 'canker' refers to the symptom of an open wound surrounded by swollen bark. Cankers can form on a range of woody hosts and are caused by a number of pathogens (fungi, bacteria and virus) and pests (e.g. woolly aphid). On *Malus* and *Prunus* species, cankers on woody tissue can result from infection by *Neofabraea* species, *Monilinia fructigena*, *Erwinia amylovora* and other pathogens, but *N. ditissima* is responsible for the majority of cankers in pome fruit orchards in the UK. Old/main stem cankers (> 2 years) caused by *N. ditissima* are oyster shell like in appearance as lesions expand more rapidly longitudinally than transversely (Swinburne 1975) with radial layers of host produced phellogen radiating from the initial point of infection in the centre of the canker. Younger/peripheral cankers (< 2 years) develop from infected leaf scars, bud-scale scars or other wounds (Swinburne 1975). Such cankers, present on young 1 – 2 year growth, tend to girdle the vascular system, particularly on susceptible cultivars, resulting in branch death. New cankers (< 2 years) produce conidia (asexual spores) and will seldom produce perithecia (fruiting bodies producing sexual ascospores) until cankers have matured (> 2 years following infection). The conidia of *N. ditissima* are evident as whitish pustules forming narrow, roughly parallel lines, whilst the perithecia form in clusters of bright red, pear shaped bodies (Figure 3).

Although not infected directly, leaf symptoms can also be indicative of the presence of *Neonectria* canker. Staining of internal wood tissue and the death of distal leaves, associated but distal of trunk cankers (Wormald 1935; Figure 3) is speculated to result from the production of a yet to be identified diffusible toxin produced by the pathogen at the site of infection. Trees with trunk cankers which have not girdled the main trunk completely can show wilting symptoms and these trees will invariably be associated with brown internal staining (personal experience) providing further evidence that the fungus is able to produce a toxin. Certain plant pathogens produce toxins as virulence factors, weakening the host's resistance response and toxin production may be upregulated in response to a host resistance response. Toxin production and its effects are well characterised for the fungi

associated with grapevine trunk diseases (Andolfi et al. 2011) but not yet so for apple diseases.

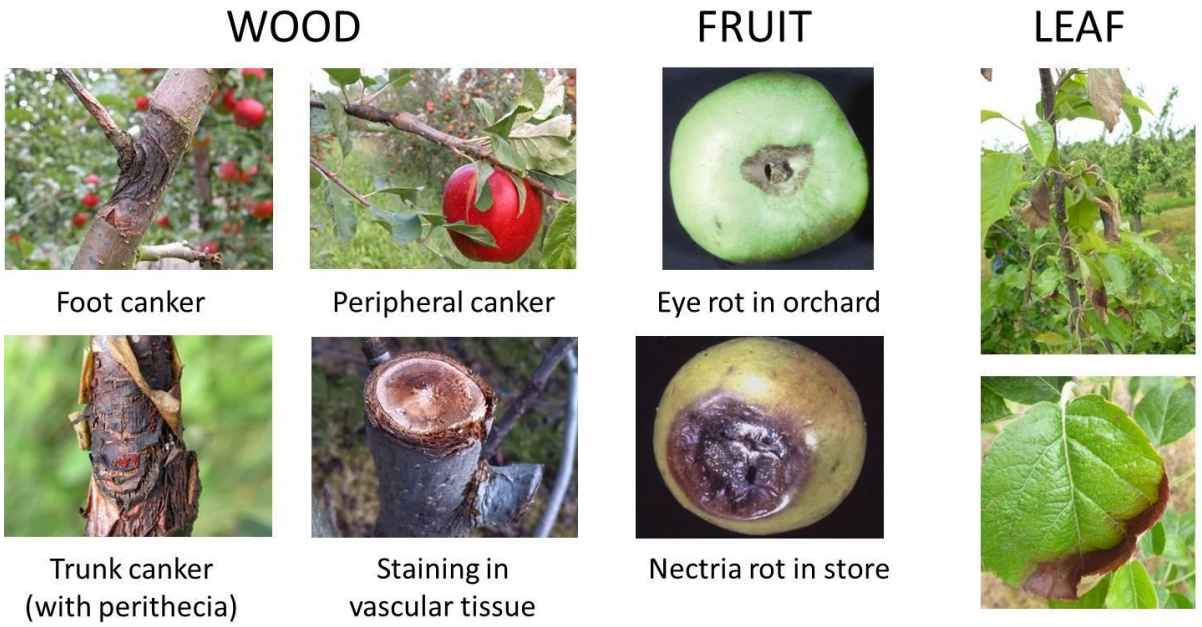


Figure 3 Symptoms of *Neonectria ditissima* infection on apple tissues

3.2 Fruit Rot

N. distissima also infects apple fruits resulting in a fruit rot either in the orchard or, more commonly, in store. The route of infection is either through the calyx end (particularly of open calyx varieties), through the stalk end or through lenticels, scab lesions and wounds. Sometimes infection may develop into an eye rot in orchards (Figure 3) though most infection remains latent and only develops during long-term storage (Figure 3). The fruit rot occurs on the eye, the stalk end or on the cheek depending on the site of infection which is in turn influenced by the timing of infection. The rots are soft, slightly sunken, with the rotted part easily scooped out from the sound flesh. Cheek and stalk-end rots are circular, brown with pale brown centres, with the rot colour depending on cultivar and storage conditions. Rots on fruit stored in low oxygen tend to be green in colour with little sporulation, whereas those in higher oxygen storage tend to be brown with white/creamy sporing pustules.

4. Epidemiology

4.1 Inoculum sources and overwintering

The cankers are the overwintering substrate of the disease. The fungus survives primarily in the form of perithecia and mycelium from which spores are produced during periods when climatic conditions are suitable. The conidia are produced more commonly early in the season but also in the summer and early autumn. Perithecia appear in the canker in late summer and autumn. The ascospores are either forcibly discharged from the perithecium

and dispersed by wind or they ooze from the perithecium during periods of high humidity and are washed by rain or carried by insects (Swinburne 1975).

4.2 Factors affecting infection

Three factors are required for disease initiation for any plant pathogen; inoculum, host and environment: this is referred to as the disease triangle (Figure 4). Conidia and ascospores can be produced from a canker throughout the year as discussed in section 2.2. Therefore disease causing propagules (inoculum) are available all year round. Pathogen entry points (i.e. wounds on the host), are another limiting factor of infection, but are available throughout the year. High risk growth stages include bud burst, bloom, fruitlet drop, summer leaf drop and in particular autumn leaf fall. High risk orchard operations include pruning, mechanical flower and fruitlet thinning and fruit harvest and high risk weather events such as hail, strong winds and frost damage can also induce wounds. Host susceptibility, particularly in the case of fruit infection, does vary with developmental stage and this will be discussed further in section 4.5. The final limiting factor of disease initiation is climatic conditions, in particular rain, due to the role of water in spore dispersal and for providing conditions conducive for spore germination and infection. Due to the omnipresent availability of spores and wounds throughout the growing season, the periods of peak infection generally coincide with rainfall, so can vary geographically and from year to year. In the UK, rainfall often coincides with periods of high wound availability (eg. autumn leaf fall), making this a critical period for disease development.

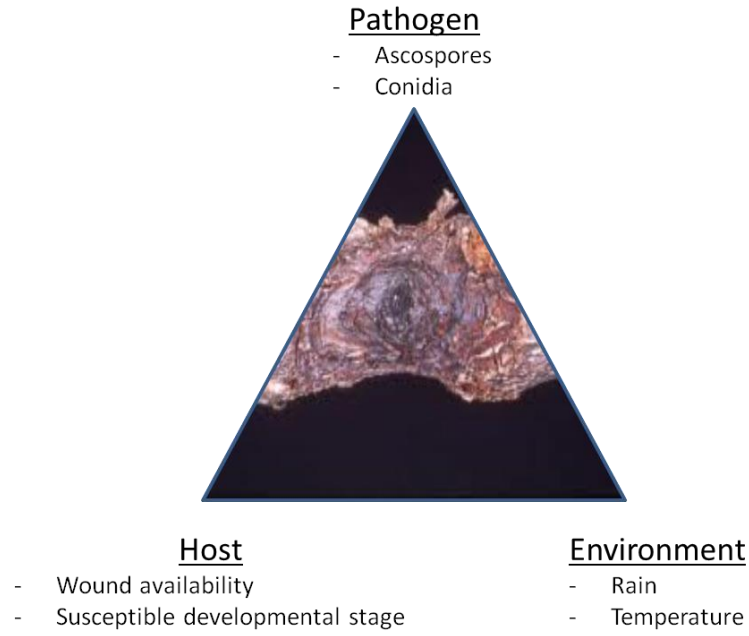


Figure 4 Disease triangle; factors necessary for *Neonectria ditissima* disease initiation

4.3 Nursery and orchard infection

McCracken *et al*. (2003) conducted an epidemiological study in the UK with the principal objective of determining the relative significance of nursery versus orchard infection of *N.*

ditissima. A total of 27 rootstock x scion combinations from three nurseries were planted out at three locations and canker development was recorded. The positions of infected trees within the orchard and the position of the canker within each tree (main-stem or peripheral) were recorded, and the source of infection (i.e. nursery or orchard) was inferred from (1) canker position, (2) distance from neighbouring orchards, and (3) fungal population comparisons. *N. ditissima* was isolated from the cankers and analysed using molecular techniques to determine the source of infection by determining the population structure of the isolates. The isolates collected from peripheral cankers in the different geographical areas were from different populations, suggesting that these infections occurred locally (i.e. in the orchard); in contrast the isolates collected from the main stem cankers at the three sites were genetically similar suggesting that these infections originated from a common source, i.e the nursery, but remained symptomless until planting in the orchard.

This study also demonstrated that nursery borne infection can remain symptomless for at least three years following propagation. The study concluded that infection in newly planted orchards can result from inoculum brought in on asymptomatic nursery stock but primarily from external inoculum from neighbouring orchards. Although less significant in incidence, main stem cankers resulting from infection occurring during propagation represent a serious loss to growers as these trees act as a source of inoculum to surrounding trees and have to be grubbed out. In contrast, when cankers form on side shoots or minor branches (i.e. orchard borne infections) the damage can be relatively less severe (dependent on cultivar tolerance), and infections can be pruned out and control achieved through autumn and spring applications of fungicides (McCracken *et al*. 2003). In this particular trial infection occurring during the propagation phase was low. However the authors suggest that the significance of nursery borne infection can change from year to year and apple growers have reported years when they have experienced devastating losses of young trees caused by canker development at or near the base of the main stem (i.e. most likely to originate from nursery infection). Cultural practice of propagation and climatic conditions to which trees are exposed to during the propagation phase, may be determining factors influencing incidence.

4.4 Disease spread

Once disease is present in the orchard, wind, rain splash and to a lesser extent insects, are considered the principal carriers of spores (Swinburne 1975). The active ejection of the ascospores from the perithecia enables wind and air currents to further disseminate the spores. Airborne ascospores are thought to be responsible for the long distance spread of the disease (Swinburne 1971) and distances of up to 125 m have been recorded in windy conditions. The distance of dissemination of viable spores is limited by humidity. Localised dissemination of spores is aided by rain splash. Dissemination distances of spores can be very short with this method (generally < 15cm per splash event) but continual re-splashing of spore-carrying droplets can distribute spores effectively throughout the canopy (Madden 1997). Fruit infection is particularly prone to this method of spore dispersal and increased susceptibility coincides with periods of favourable conditions for rain splashed spores. Insects, such as woolly apple aphids (*Eriosoma lanigera*, Reding *et al.* 1997) and ants (Glime 2007), have been implicated in disease dispersal due to their tendency to pick up and carry spores on their bodies and the creation of wounds providing entry sites for the pathogen.

4.5 Factors Influencing Infection

A wide range of temperatures for the germination of *N. ditisima* spores *in vitro* has been reported (e.g. 5 to 32°C; Latorre *et al.* 2002). In the field the optimum temperature for infection of leaf scars following inoculation was reported as 15°C (Latorre et al. 2002). Compared to *in vitro* studies, field infection appears to occur over a narrower range (Dubin and English 1975) suggesting other factors are influencing infection. Dubin and English (1975) found that the number of hours per day between 11 and 16°C was significantly associated with leaf scar infection and also with the number of ascospores in the spore traps (indicating increased spore release). Spore release was also significantly associated with the number of hours per day at 5 to 10°C.

Sporulation, spore dispersal and infection of *N. ditisima* are favoured by rainfall. Dubin and English (1974 and 1975) consider frequency and duration of rainfall rather than amount of rainfall more important for all of the above processes. The periods of wetness required for spore release have been quantified in a study by Butt *et al.* (1994); The number of ascospores discharged from individual cankers within six hours following 30 minutes wetting varied greatly, ranging from *c.* 4,000 to *c.* 45,000 and the number of conidia, released from individual cankers within six hours after 12 hours wetting ranged from *c.* 17,000 to *c.* 35,000. The importance of the duration of leaf scar (Wilson 1966; Dubin and English 1974), pruning wound (Xu *et al*. 1998) and fruit (Xu and Robinson 2010) surface wetness on infection rate has also been demonstrated.

Wound age has been extensively investigated as a factor affecting *N. ditissima* susceptibility. It is well known that wounds on woody hosts become increasingly resistant to infections by pathogens as they age (e.g. El-Hamalawi and Menge 1994) which is important to consider for effective disease management in the field (i.e. the period of time fungicide cover is required to protect the leaf scars following leaf fall or the optimum time to prune trees to minimise wound infection by *N. ditissima*). Wilson (1966) showed that 6% of the leaf scar wounds were infected when inoculated 28 days following leaf drop, as compared with a 20% of infection for fresh leaf scar wounds. Similarly the incidence of canker lesions caused by *N. ditissima* was greater following the inoculation of fresh pruning wounds than older cuts as demonstrated in a study by Xu *et al*. (1998). Interestingly, this study also found a significant interaction between cultivar and age of pruning wound on the incidence of canker lesions, suggesting that cultivars differ in their rates of wound healing. Xu *et al*. (1998) suggest that natural selection has led to rapid healing of leaf scars and the evolution of leaf scar associated defence responses whilst pruning wounds are non-natural thus a healing/defence response is slower and thus pruning wounds are more susceptible to *N. ditsissima* than leaf scars. It would be interesting to investigate if cultivar differences in leaf scar healing/defence response exist.

Xu and Robinson (2010) investigated interacting factors (wetness duration and fruit maturity) influencing Nectria fruit rot. The study showed that fruit maturity was the main factor influencing infection (Figure 5); young fruit were most susceptible, with 50% of fruit infected when inoculated up to four weeks after full bloom, the majority of infections leading to Nectria eye rot, expressed in the orchard. Susceptibility decreased as the fruit matured until

approximately two months after full bloom (< 10% fruit infection) and then increased gradually until harvest (up to 30% fruit infection). Most infections during this period led to storage rot expressed in store. However, it should be noted that early infection can remain symptomless and hence lead to post-harvest fruit rot. The effect of wetness duration on infection was only significant when young fruit were inoculated (five wetness periods between 6 – 48 hours post inoculation were tested) but not for those inoculated near harvest. These results have assisted decision based management for the pre-harvest treatment, storage and marketing time of fruit to mitigate the losses due the Nectria fruit rot.

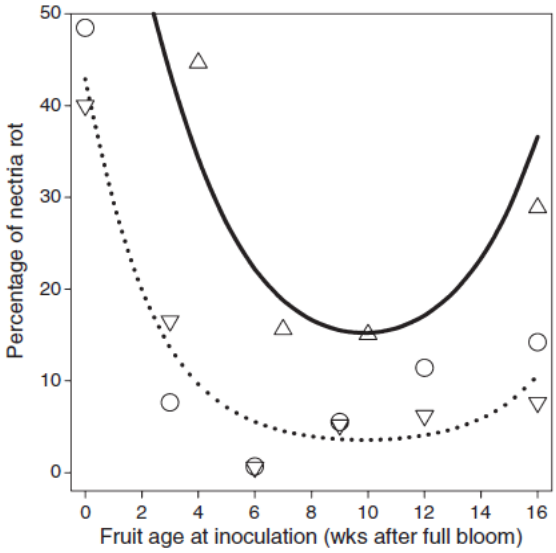


Figure 5 Incidence of Nectria rot (preharvest and postharvest) following inoculation of apple fruit with conidia of *Neonectria ditissima* at different times following full bloom. 2006 =△, 2007 = ▽, 2008 = ○, solid line = fitted model for 2006 and dotted line = fitted model for 2007 and 2008. Figure adapted from Xu and Robinson 2010

Modelling the epidemiology of diseases using data collected in studies such as those described above can help growers adopt a rational decision-based strategy for control options. Such epidemiological models have been built for apple scab (Xu *et al*, 1994), apple powdery mildew (Xu and Butt 1993) and Nectria canker and fruit rot (Xu and Butt 1994) into a programme called ADEM (Apple Disease East Malling (Xu and Butt 1996). The Nectria model is no longer available commercially within ADEM because of the lack of independent field data to validate the model, particularly the leaf-scar model. Another model, Patfrut©, which is used commercially, was developed by a Chilean group (Latorre et al. 2002). Field validation of Patfrut© demonstrated that the severity, but not the incidence of cankers, can be reduced when fungicide treatments are applied in accordance with the warnings determined by the model. For example, on a Red King Oregon crop tested in 1999 the model managed plot received three applications of Cuprodul 50 WP (Copper oxide) during leaf fall in response to the model warnings whilst the standard programme received three applications of Cuprodul 50 WP using a calendar based spray program (<5%, 50% and >80% leaf fall). The disease incidence was not statistically different (model managed = 4.6%, standard 10.8%) whilst the severity was; 0.8% in model managed plot versus 2.6% in the standard plot. Statistical differences in either incidence or severity were not observed in

a repeated experiment on Richard Red Delicious the following year, highlighting the season to season variability of this disease.

4.6 Other hosts

Other hosts can provide reservoirs of inoculum which need to be considered for integrated disease control. In addition to cankers on the commercial crop within orchards, common hedgerow species on the periphery of the orchard are likely to act as inoculum sources. These include wild *Malus* and *Pyrus* species and a large number of woody hosts including *Acer, Betula, Carpinus, Carya, Crataegus, Cydonia, Fagus, Fraxinus, Jugulans, Magnolia, Populus, Prunus, Quercus, Salix* and *Sorbus*. It is not known whether *N. ditissima* isolates are specialised to particular host species (i.e. specialised pathotypes), which may be less virulent on non-host species although, using genetic fingerprinting, isolates present in pear orchards have been identified in neighbouring apple orchards (Defra project report, OC9518).

5. Control

Canker is currently controlled by a combination of cultural methods to remove canker lesions and the use of protectant fungicides. Identification of genetic resistance to *N. ditissima* is a research target of several groups around the world but this is a long term goal yet to come to fruition. With susceptible varieties being a main stay for growers the need for an integrated control programme will be crucial for future control of this disease.

5.1 Cultural control

The cultural control of canker, through pruning out of new cankered lesions, is currently the most effective and widely adopted practice due to the lack of alternative control measures against this disease. Covering of pruning wounds or pared back cankers can provide temporary protection while the tree develops its own protective callus layer. To be effective, paints must be applied to the wound or pared back canker, immediately, and at the very least within one hour of pruning. Although recommended, especially in young orchards where it is vital to keep trunk and scaffold branches canker-free, painting wounds is seldom practiced due to the increased time and cost of implementing such a practice. Wound paints offer a physical barrier to infection, provide fungicidal activity or both. Clay and silicon based products, which form physical barriers against spores, are available for other crop groups (e.g. Scaniavital® Silica, for wound dressing in protected tomato), but no commercial products are available for use in apple orchards. Products with fungicidal action include Bordeaux paste (a mixture of copper sulphate and slaked lime), disinfectants (e.g. sodium hydroxide) and a formulated fungicide product, Bezel (tebuconazole), the only approved formulated fungicide product available for fruiting trees. Formulated biological products are also used as wound treatments in other crop groups (e.g. Vine Vax Wound Dressing approved for use on grape contains *Trichoderma*).

Technologies which simultaneously prune and apply treatment have been available for many years. Seaby and Swinburne (1975) described a device which when fitted to conventional

secateurs applied a small quantity of fungicide to the wound surface during the cutting action. Although commercially available, the devices (Figure 6a) are yet to be widely adopted in the industry, probably due to the lack of registered products available for this application technique.

Researchers in the Netherlands are currently trialling novel pruning protectant treatments and application technologies (including the modified secateurs) to target *N. ditissima* (pers comm., M. Wenkner, PPO, Wageningen). There is a potential for knowledge exchange with the vine industry in this regard which faces a number of canker causing grapevine trunk diseases including *Eutypa* and *Botryosphaeria* spp. (Úrbez-Torres *et al*. 2006) which are epidemiologically similar to *N. ditissima*. A product used for sealing wounds in grape crops following pruning is VitiSeal (Figure 6b) and this product will be assessed, along with others, in the Dutch trial.

Growth cracks are a site of entry for *N. ditissima* and successful infection leads to cankers localised in the crotches of branches and junctions of different seasons' growth (Swinburne 1975). Growth cracks can be promoted by increased growth rate. Adapting nitrogen and irrigation programmes in young orchards and the use of growth regulators to manipulate growth rate may reduce the incidence of cankers borne from growth cracks. However, further work needs to be carried out in this area to determine the benefits.

Figure 6 Pruning wound solutions, technologies and treatments being trialled at PPO, Wageningen to protect pruning wounds from *N. ditissima* infection

The propagation phase is a high risk period for *N. ditissima* infection and can lead to high losses in the orchard following the planting of asymptomatic infected trees. The risk of *N. ditissima* infection during propagation can be reduced by limiting the numbers of wounds created during propagation. The current industry standard is the Knipboom method of propagation, whereby the first year's growth is cut down to 80 cm producing a 1 year old feathered leader the following year. Some nurseries are reverting to producing maiden trees, the original technique, for the propagation of particularly susceptible new cultivars (Pers.

comm., M. Wenkner, PPO, Wageningen) to limit the wounds created during the propagation phase.

Evaluation of the effects of rootstocks on susceptibility will also be an important consideration. M9 is considered to be relatively susceptible to *N. ditissima* whilst M1 and M12 have been reported to be less susceptible (Moore 1960). Whether the variation in disease susceptibility is inherent or a result of differences in tree vigour conferred by the rootstock, is an area yet to be explored.

The apple rot risk assessment concept, published in the Apple Best Practice Guide (Webster *et al.* 2001), describes risk factors and advice on control options to mitigate losses caused by the main apple rots in store. For Nectria fruit rot, the main orchard factor affecting rotting in store is the incidence of cankers in the orchard. The guide recommends that 20 trees should be assessed per orchard and each orchard scored then ranked for risk based on the following criteria;

Percentage of trees with canker	Risk
>25	**High**
5-25	**Moderate**
<5	**Low**
0	**No risk**

For orchards with a low incidence of cankers (<5%), fungicide application specifically for control of Nectria rot may not be necessary and these fruit can be allocated for long term storage. For orchards with a medium level of sporulating cankers (5-25%), control during early fruit development is essential during periods in which weather conditions are conducive to *N. ditissima* infection. A pre-harvest treatment targeting *N. ditissima* may be considered if rainfall is above average and the fruit from these orchards should be stored for the medium term. For orchards with a high incidence of sporulating cankers (>25%), in addition to early control measures, pre-harvest treatment may also be necessary particularly if weather conditions are conducive to *N. ditissima* infection. Early marketing of the fruit would also be recommended to mitigate losses in store.

5.2 Fungicides and biocontrol

The primary objective of the chemical control of *N. ditissima* is to reduce infection of new woody tissues and reduce sporulation on old cankers. Fungicides are usually applied at several key growth stages such as bud-break, petal-fall and leaf-fall (10% and 50%) in autumn (Webster *et al*. 2001). In orchards with moderate to high canker incidence, an extra application of fungicide at 90% leaf fall may be necessary. Copper fungicides give good prolonged protection of leaf scars and bud-scale scars against *N. ditissma*, but can be phytotoxic, can only be used post-harvest and pre-bud burst and there use is restricted in some countries (e.g. the Netherlands). Fungicides that are mainly active against apple scab such as dodine, dithianon and captan also have some protectant action against *N. ditissima.* Carbendazim was a key fungicide used for the management of *N. ditissima.* However this

fungicide was withdrawn from commercial use in apple production in the UK. Tebuconazole (Folicur) has been identified as a possible alternative product to carbendazim and has recently been granted an EMUA (2159/2008) for use post-harvest during leaf fall. Despite this, products effective against *N. ditissima* that can be used in the growing season are limited. An HDC project is currently underway to evaluate the efficacy of a new group of chemicals (SDHI group) which may be active against *N. ditissima,* together with 'alternative' treatments. One such 'alternative' treatment to be tested is a foliar nutrient containing copper (e.g. 42PHI Cu - ammonium phosphate and copper phosphite). The low dose of copper in such products allows for the treatments to be applied throughout the season without the phytotoxic effects experienced with higher dose copper products (Paul Bennett, Agrovista, Pers comm.).

Up until the late 1990's, rotting in stored apple was controlled primarily by post-harvest fungicide drenches in the UK. Such treatments were only partially effective on Nectria rot. Although not banned in the UK, post-harvest drenching is no longer practiced commercially, partly because of the resulting fungicide residues, which, although usually below the Maximum Residue Level, are not acceptable to consumers. In unpublished work at East Malling Research, the importance of control of Nectria infection during the flowering period was demonstrated in the orchard, which reflects the findings of Xu and Robinson (2010) which demonstrated on potted trees that apple fruit is most susceptible to infection by *N. ditissima* up to four weeks following pollination. This study also showed that apple fruits become susceptible again as they reach maturity (section 4.5, Figure 5), Pre-harvest treatments such as Captan, Switch, Bellis and Cercobin are also recommended if a high risk for Nectria rot development in store is predicted using the rot risk assessment concept.

Biocontrol agents have long been recognised as potential antagonists to *N. ditissima*. The feasibility of using BCAs to protect the leaf scar from infection by *N. ditissima* was first realised in the 1970's (Swinburne *et al.* 1973, 75 and 76). In these early studies, isolations of the microbial communities residing in the tissues of leaf scars were made and an isolate of *Bacillus subtilis* was found to be highly antagonistic to *N. ditissima in vitro*. However, the commercial potential has not yet been exploited in the orchard due to the inherent issues of BCA reliability in a field situation. BCA reliability may be especially hard to achieve when applying treatments targeted at leaf fall when climatic conditions are less conducive to BCA establishment.

Most of the commercial BCA's currently available are considered epiphytic, that is they live on the surface of the plant tissues. *Bacillus subtilis* is an example of this*.* Recently there has been increasing interest within the research community of endophytic microbes, which are microorganisms living within the plant tissues. These organisms have real potential for future biocontrol agents as they may be applied to seed or during the propagation phase and persist for the life of the plant. There is also growing evidence that some pathogens may be functioning as endophytes, harmlessly inhabiting the plant until the plant comes under stress, upon which disease is expressed. This is potentially quite a significant stage in the epidemiology of *N. ditissima* which to date has not been considered and, if proved correct, would affect how this disease is controlled, such as the inoculation of an antagonistic endophytic BCA, or mixture, during propagation to outcompete latent pathogenic organisms such as *N. ditissima*.

Previous work in the area of endophytes in apple trees has been carried out by Li (1995), identifying endophytic profiles in apple trees using traditional culturing techniques. The use of traditional culturing techniques has limitations when working with species which live in such close association with plants. A proportion of endophytes will be obligate biotrophs (i.e. require a living host to survive) and thus may not be culturable on artificial media meaning that this technique will not be representative of the endophyte profile as a whole. New molecular methods called metagenomics, utilising next generation sequencing technologies to determine the species present in environmental samples, are being developed for various applications. At East Malling Research this technique is currently being optimised and it is hoped it can be applied to analysing the endophytic profiles of the major horticultural crops. Comparisons with the culturable species profiles reported by Li (1995) will be interesting.

The use of hypovirus infection of *N. ditissima* as a novel biocontrol agent for apple canker has been investigated (Green, 2004). The biocontrol of pathogens using viruses has been demonstrated for the control of chestnut blight. The presence of the virus in the pathogen (*Cryphonectria parasitica*) reduced the virulence on the host (*Castanea sativa*) (Nuss 1992). This method of biocontrol is attractive for natural ecosystems because the virus like genetic element is able to spread horizontally through the fungal population during hyphal anastomosis. Equally this would be an economically attractive control option for orchard diseases (i.e. no need for multiple applications of chemical or BCA products). A survey found that of 102 isolates of *N. ditissima* collected from across the UK, no endogenous virus-like dsRNA elements were found (Green 2004). Transfection with the hypovirus of *C. parasitica* was successful and stable on the non-host, *N. ditissima*. Infected isolates had reduced growth *in vitro*. However, no consistent or appreciable difference in virulence was observed on detached fruit infection assays. Further work is required in this area to yield an effective biocontrol agent.

5.3 Genetic resistance

As reviewed above there is a large amount of information known about the epidemiology of *N. ditissima* and, based on this knowledge, many control strategies have been developed. However, despite the worldwide importance of this disease to the apple growing industry very little is known about the host-pathogen interaction that precedes either successful pathogen infection or successful host defence.

The area of host pathogen interactions is most advanced in arable crop and disease patho-systems such as wheat rust and potato blight and in model plants such as *Arabidopsis*. Studies in these systems have determined how pathogens evolve (by natural selection of new effectors) to overcome plant defences and how plants counteract by evolving new defence mechanisms (natural selection of new resistance genes). Such studies enable the race structure of pathogens to be determined (pathotypes) and enable knowledge driven selection of resistant breeding progeny using maker assisted selection (MAS) to generate resistant cultivars.

For the apple – *N. ditissima* pathosystem, very little is known about the race structure of the pathogen or the R-gene complement of the host. It is also unknown how resistance may be

expressed in different tissues of the host e.g. wood vs. fruit. It may be that resistance mechanisms are localised at the leaf scar, an area that is vulnerable to pathogen attack.

Malus species and apple cultivars show variation in susceptibility to *N. ditissima* (Alston, 1970, Krahmer and Schmidle 1979, Kruger 1983, Borecki and Czynczyk 1984, Van de Weg 1989 and 1992). Variations in disease susceptibility may partly be a result of disease escape (e.g. the speed of wound healing in relation to *N. ditissima* susceptibility has been shown to differ between cultivars - Xu et al., 1998). Whilst other reports have presented evidence of a genetic based resistance with resistance controlled 'predominantly by additive gene action' (Gelvonauskiene et al., 2007). This offers the potential to mine for quantitative trait loci in progeny segregating for disease resistance (i.e. derived from parents which have high 'resistance' and susceptibility). Once discovered QTL's may be cloned and the underlying resistance mechanism determined. Ultimately, markers designed to the QTL's can be utilised in breeding programmes. Researchers at The New Zealand Institute for Plant & Food Research have been carrying out a QTL mining study (population details unknown as the work is yet to be published) and have discovered QTLs associated with reduced susceptibility to *N. ditissima* (Pers. comm. R Scheper, Plant and Food Research, New Zealand).

Due to the current consumer preference towards Gala/Braeburn types, the majority of the new mainstream cultivars such as Kanzi, Jazz and Envy all have Gala and Braeburn in their parentage (two highly susceptible varieties). With the problems associated with these varieties, in relation to *N. ditissima* susceptibility, now becoming apparent, future breeding programmes would be wise to put *N. ditissima* resistance high on the selection trait agenda to assist in the control of this disease in the future. Accurate phenotyping of *N. ditissima* in the field is challenging, particularly when dealing with non-major gene controlled resistance (e.g. quantitative resistance), as phenotypic variance in the trait can be influenced by a number of factors including developmental age and environmental conditions. The research community have for many years been developing assays for the accurate phenotyping of *N. ditissima* resistance (Alston 1970, Van de Weg 1989 and Garkava-Gustavsson *et al.* 2013) with some success (e.g. selection of highly resistant parent material such as Santana - Garkava-Gustavsson *et al.* 2013). Complementing empirical selection with advances in genomic technology, the aim of a new HDC project (TF 211 - Resources for future breeding of apple utilising genome-wide selection) at East Malling Research, will only speed up the process of bringing a new generation of *N. ditissima* resistant cultivars to market.

6. Future research areas

Future applied research into control options for *N. ditissima* is essential and a top priority for many in the industry (pers comm. various growers and industry representatives). A summary of future research needs in the short, medium and long term as set out below is hoped to form a research strategy moving forward.

Short-term

- Identifying effective fungicide treatments for use during the growing season and pre-harvest.
- Evaluation and use of commercially available BCAs and novel chemicals.
- Development of a diagnostic tool to test nursery material (mother trees and rootstocks).

Medium-term

- Further development, validation and dissemination to growers of models for knowledge based application of treatments rather than relying on calendar based control programmes.
- Better understanding of the plant x pathogen x endophyte/epiphyte interaction. Using metagenomic and traditional culturing techniques to discover potentially antagonistic (to *N. ditissima*) microbes in the host tissue and to increase our understanding of the epidemiology of *N. ditissima* (e.g. testing the hypothesis that *N. ditissima* survives in the plant endophytically).
- Discovery and development of new biocontrol agents.
- Exploring the use of novel application techniques such as tree injection, which has successfully been used for the control of other diseases such as Dutch elm disease (Clifford et al. 1977) and could be deployed to keep nursery mother trees disease-free.
- Assess water/nitrogen regime effects of canker susceptibility/expression. The findings could provide new guidelines for cultural practice in orchards (e.g. knowledge based selection of the best planting sites and orchard establishment for particularly susceptible cultivars to mitigate canker susceptibility/expression).
- Rootstock susceptibility of existing rootstocks and rootstock /scion interaction.

Long- term

- Breeding new scion and rootstock varieties for resistance to *N. ditissima* by utilising the current knowledge from the assessment of resistance in current varieties/species (QTL mining) for selection of parents into the breeding pieline. It would also be interesting to investigate if cultivar differences in leaf scar healing/defence response exist.
- Increasing our understanding of factors affecting the expression of latent infection in stored apple will be of applied and fundamental interest - *N. ditissima* pathosystem is a good model for experimentation.

7. Groups around the world working on problem

Country	Name	Institute
Belgium	Wendy Van Hemelrijck	PC Fruit, Namur Area
	Kjell Hauke	Research Station for Fruitgrowing, Liège Area
	Marc Lateur	Centre Wallon de Recherches Agronomiques, Namur Area
Netherlands	Peter Frans de Jong	Applied Plant Research/WUR
	Peter Roelofs	Applied Plant Research/WUR
	Bart Heijne	Applied Plant Research/WUR
	Jürgen Köhl	Applied Plant Research/WUR
	Marcel Wenneker	Applied Plant Research/WUR
	Henk S.	Applied Plant Research/WUR
	Adrie Boshuizen,	Horti Bureau, Wageningen
Norway	Jorunn Børve	Bioforsk, Hordaland County
Sweden	Anna Zborowska	Swedish University of Agricultural Sciences, Alnarp
	Larisa Garkava Gustavsson	Swedish University of Agricultural Sciences, Balsgård
	Bengt Boysen	Swedish University of Agricultural Sciences, Balsgård
	Marjan Ghasemkhani	Swedish University of Agricultural Sciences, Balsgård
Switzerland	Andreas Naef	Scientist bei Agroscope, Zürich Area,
United Kingdom	Louise Cooke	Agri-food and biosciences institute, Belfast
	Angela Berrie	East Malling Research, East Malling
	Robert Saville	East Malling Research, East Malling
	Xiangming Xu	East Malling Research, East Malling
New Zealand	Nicholas Tabi	Plant and Food Research, Canterbury & West Coast
	Reiny Scheper	Plant & Food Research
	Monika Walter	Nelson, Marlborough & Tasman
	Robert Beresford	Plant & Food Research Ltd, Auckland
Chile	Mauricio Lolas	Universidad de Talca
Brazil	Silvio Alves	Researcher at Embrapa, Vacaria Area
	Rosa Maria Valdebenito- Sanhueza	Pesquisadora na Proterra Engenharia Agronômica, Vacaria Area

This list is compiled from the LinkedIn group for the European Fruit Tree Canker Working Group and is by no means exhaustive of all the researches worldwide who work on the disease.

8. Acknowledgements

The author would like to thank HDC for the opportunity to review the literature related to *N. ditissima* and to visit Wageningen UP PPO through funding. In addition to this literature review, this project has delivered a presentation of the current knowledge and future prospects of *N. ditissima* research to agronomists at the HDC agronomist's day, strengthened links with horticultural pathologists in the Netherlands and contributed to proposals for future work in this area (HDC tree fruit panel, Agritech proposal and a EU-China IPM collaboration on fruit and vegetables).

9. References

Alston FH (1970) Response of apple cultivars to canker, *Nectria galligena* Report of East Malling Research Station for (pp 147-148), East Malling, UK.

Andolfi, A. Mugnai, L. Luque, J.; Surico, G. Cimmino, A. Evidente, A. (2011) Phytotoxins Produced by Fungi Associated with Grapevine Trunk Diseases. Toxins, 3, 1569-1605.

Barnett H. L. and Hunter B. B. (1998) Illustrated Genera of Imperfect Fungi, Fourth Edition. The American Phytopathological Society, St. Paul, Minnesota. ISBN 978-0-89054-192-0.

Beresford, R M; Kim, K S (2011) Identification of regional climatic conditions favorable for development of European canker of apple. Phytopathology vol. 101 (1) p. 135-46

Brown AE, Muthumeenaski S, Sreenivasaprasad S, Mills PR, Swinburne TR (1993) A PCR primer specific for Cylindrocarpon heteronema for detection of the pathogen in apple wood. FEMS Microbiology Letters 108, 117–120.

Butt DJ, Gunn N, Xu X-M, 1994. Release of Nectria galligena spores from apple cankers. Norwegian Journal of Agricultural
Sciences 17 (Suppl.), 309–16.

CAB International, 1985. Nectria galligena. Distribution Maps of Plant Diseases Map 38, 4th Edn. Wallingford, UK: CAB International.

Cayley DM (1921) Some observations on the life-history of Nectria galligena Bres. Annals of Botany 35:79-92

Clifford, D. R., Cooke, L. R., Gendle, P., and Holgate, M. E. 1977. Performance of carbendazim formulations injected for the control of Dutch elm disease. Annals of Applied Biology 85: 153-156.

Defra project report OC9518 - The significance of nursery inoculum sources in apple canker epidemiology. http://randd.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&Completed=0&ProjectID=5989

Dewey, F. M., Li, R. and Swinburne, T. (1995), A monoclonal antibody immunoassay for the detection of *Nectria galligena* in apple fruit and woody tissues . EPPO Bulletin, 25: 65–73.

Dubin HJ, English H (1975) Epidemiology of European apple canker in California. Phytopathology 65:542-550

Garkava-Gustavsson, L., Zborowska, A., Sehic, J., Rur, M., Nybom, H., Englund, J.-.E., Lateur, M., Van de Weg, E. and Holefors, A. 2013. Sceening for apple cultivars for resistance to European canker, *Neonectria ditissima*. Acta Hort. (ISHS) 976:529-536

Gelvonauskiene D, Sasnauskas A and Gelvonauskis B (2007) The Breeding of Apple Tree Resistant to European Canker (*Nectria galligena Bres.*) Sodininkyste Ir Darzininkyste 26(3).

Glime JM (2007) Bryophyte Ecology. Vol 1. Physiological Ecology. Ebook sponsored by Michigan Technological University and the International Association of Bryologists

Langrell S.R.H. and Barbara D.J. (2001). Magnetic Capture Hybridisation for Improved PCR Detection of Nectria galligena from Lignified Apple Extracts. Plant Molecular Biology Reporter 19: 5-11

Langrell S.R.H, (2002) Molecular detection of *Neonectria galligena* (syn. *Nectria galligena*). Mycological Research 106, 280–292.

Latorre BA, Rioja ME, Lillo C, Munoz M (2002) The effect of temperature and wetness duration on infection and a warning system for European canker (*Nectria galligena*) of apple in Chile. Crop Protection 21:285-291

Li, R. (1995) Studies on the infection of apple trees by *Nectria galligena* Bres. MPhil Thesis, University of London

Madden LV (1997) Effects of rain on splash dispersal of fungal pathogens. Canadian Journal of Plant Pathology 19:225-230

Moore, M. H. (1960) Apple rootstocks susceptible to scab, mildew and canker for use in glasshouse and field experiments. Plant Pathology. 9, 3: 84–87.

Munson, R. G. (1939) Observations on apple canker. Annals of Applied Biology, 26: 440–457.

Nuss, D.L. (1992) Biological control of chestnut blight: an example of virus-mediated attenuation of fungal pathogenesis. Microbiol Rev. 56(4): 561–576.

Reding ME, Alston DG, Zimmerman RJ (1997) Apple Aphids. Orchard IPM Series, Utah State University

Seaby, D. A. and Swinburne, T. R. (1976), Protection of Pruning Wounds on Apple Trees from *Nectria galligena* Bres. using Modified Priming Shears. Plant Pathology, 25: 50–54.

Seemüller, 1988. *Nectria galligena* Bresad. In: Smith, I. M., et al., Eds.), "European handbook
of plant diseases". Ikwell Scientific Publications, pp. 280-282.

Snowdon A. L. (1990) A Colour Atlas of Postharvest Diseases of Fruits and Vegetables: General Introduction and Fruits volume 1. Wolfe Scientific. ISBN 0-7234-0931-5.

Swinburne, T.R. (1973). Microflora of apple leaf scars in relation to infection by *Nectria galligena*. Transactions of the British Mycological Society 66) 389-403.

Swinburne, T.R. (1975) European Canker of Apple. Review of Plant Pathology. 54:10 787-799.

Swinburne, T.R., Barr, J.G., Brown, A.E. (1975) Production of antibiotics by *Bacillus subtilis* and their effect on fungal colonists of apple leaf scars. Transactions of the British Mycological Society. 65,2; 211-217.

Swinburne, T.R. and Brown, A.E. (1976) A comparison of the use of *Bacillus subtilis* with conventional fungicides for the control of apple canker (*Nectria galligena*). Annals of Applied Biology. 82; 365-368.

Úrbez-Torres J. R., Leavitt G. M., Voegel T. M., and Gubler W. D. (2006) Identification and Distribution of *Botryosphaeria* spp. associated with Grapevine Cankers in California. Plant Disease 90:12, 1490-1503

Van de Weg (1989) Screening for resistance to *Nectria galligena* Bres. In cut shoots of apple. Euphytica. 42: 233-240.

Vitiseal marketing brochure – accessed 17/12/2014 http://www.vitiseal.com/Home_files/VSBrochurePages11.5.pdf

Webster A, Cross J, Berrie A et al., 2001. The Best Practice Guide for UK Apple Production. London, UK: Department for Environment, Food & Rural Affairs.

Wilson EE (1966) Development of European Canker in a California apple district. Plant Disease Reporter 50: 182-186.

Xu XM., Butt DJ (1994) Biology and epidemiology of *Nectria galligena* and an infection warning system Norwegian Journal of Agricultural Sciences. No 17. P. 317–324

Xu, X.-.M. and Butt, D.J. 1996. ADEM™ A PC-based multiple disease warning system for use in the cultivation of apples. Acta Hort. (ISHS) 416:293-296.

Xu, X.-M., Butt, D. J., Ridout, M. S., 1998. The effects of inoculum dose, duration of wet period, temperature and wound age on infection by *Nectria galligena* of pruning wounds on apple. European Journal of Plant Pathology 104, 511-519.

Xu, X.-M., Robinson, J. D., 2010. Effects of fruit maturity and wetness on the infection of apple fruit by *Neonectria galligena*. Plant Pathology, 59, 542-547.

Zeller SM (1926) European canker of pomaceous fruit trees. Bulletin Oregon Agricultural Experiment Station, p 52