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The effects of inoculum dose, duration of wet period, temperature and wound age on infection by *Nectria galligena* of pruning wounds on apple

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Abstract

Four experiments were conducted with potted trees of several apple cultivars to study the effects of several factors on the incidence of canker and the length of the incubation period following the inoculation of pruning cuts with conidia of *Nectria galligena*. These factors included wound age (the interval between pruning and inoculation), inoculum dose and environment (wet-period temperature and duration). The most important factors affecting the incidence of canker and the incubation period were inoculum dose, cultivar and wound age. Low inoculum dose resulted in low incidence of canker. The incidence of canker decreased as the age of the pruning wound increased. The incubation period lengthened with low inoculum dose and increasing age of the wound. The degree of resistance related to the age of the wound varied with cultivar; likewise, it also varied with the time of year, but this was not due to temperature alone. On fresh wounds, the incidence of canker and the incubation period were not affected by temperature during the wet period. The effect of duration of wetness on canker incidence was significant in only one out of three experiments: the longer wet periods resulted in a slightly lower incidence on fresh wounds. In another experiment, wet periods longer than 2 h resulted in shorter incubation periods. The results are discussed in relation to wound healing.

Introduction

Nectria galligena causes cankers on the shoots, main branches and trunks of apple trees and also rots apple fruits (Swinburne, 1975). Apple cultivars differ in resistance to the fungus (Alston, 1970; Krähmer and Schmidle, 1979; Krüger, 1983; Borecki and Czynczyk, 1984; Van de Weg, 1989; Van de Weg et al., 1992). Leaf scars and pruning cuts are important sites for infection (Kennel, 1963; Swinburne, 1971). In the U.K., conidia, ascopores and entry sites can be present for much of the year and so trees are at risk of attack for long periods (Swinburne, 1975). Infection can occur over a wide range of temperatures (Kennel, 1963) and is associated with wet weather (Wiltshire, 1921; Wilson and Nichols, 1964; Wilson, 1966; Dubin and English, 1975b; Graf, 1981). The incidence of canker following the inoculation of freshly-wounded leaf scars increases with the duration of a wet period (Wilson, 1966; Dubin and English, 1974). Natural and artificial wounds become increasingly resistant to infection by *N. galligena* as they age (Marsh, 1939; Wilson, 1966; Burmeister and Kennel, 1967; Dubin and English, 1974; Krähmer, 1980). This resistance associated with wound age was believed to be due to the suberization process following wounding, which can be well predicted by accumulated day-degrees during the healing periods (Krähmer, 1980; Biggs, 1986).

However, the combined effects of wound age and of the duration of and the temperature during the wet periods on infection of pruning cuts by *N. galligena* have not been reported. Understanding such effects enables prediction of the risk of infection by *N. galligena* thus leading to improved management of canker. This paper describes four experiments that investigate these effects using potted apple trees.

Materials and methods

General

Experiment 1 was conducted at Horticulture Research International (HRI) – Efford and Experiments 2–4 at HRI-East Malling.

Two-year-old trees, on MM106 rootstocks and in 30 cm diameter pots (15 litre), were grown on sand-beds at HRI-East Malling. Fungicides were not applied to trees at any stage. For each experiment, plants were moved either to a polythene tunnel or to a glasshouse compartment a few days before pruning and several 1-yr-old shoots were labelled on each plant. Labelled shoots were pruned to about 10 cm from their base at the appropriate time. Trees were inoculated and wetted in glasshouse compartments. At the end of the wetting period, the inoculated trees were moved out of the wetting compartment. The inoculated shoots were examined twice weekly for canker lesions and the date on which each lesion was first seen was recorded. Recording was discontinued when no new lesions were seen for three consecutive weeks. To minimise damage to plants, new lesions and underlying stained wood were excised immediately.

Inoculum

Three isolates of *N. galligena* were sub-cultured onto plates of Sugar Nutrient Agar and Yeast (SNAY) (Experiment 1) or Potato Dextrose Agar (PDA) (Experiments 2–4). Plates were incubated in the dark at 20 °C for 1 week and then placed (c. 40 cm) under fluorescent (65 W) light tubes supplemented with UV (366 nm, 36 W) tubes in the ratio 2:1, and given an 18 h:6 h light:dark regime at 18–20 °C until required, about 1 week later. On the day of inoculation, sporulating cultures were washed with distilled water and a mixed spore suspension was prepared from the three isolates. Macro- and microconidia in the suspension were counted using a haemocytometer and the final concentration for each experiment was adjusted on the basis of macroconidia. The spore concentration was about 5×10^5 macroconidia ml^{-1} except in Experiment 1 where two concentrations (about 5×10^3 and about 5×10^5 macroconidia ml^{-1}) were used. For all experiments, germination was > 30% in samples of spore suspension in 0.1% sucrose maintained at room temperature for 24 h.

Experiment 1

Potted trees of cvs Bramley's Seedling (Bramley), Cox's Orange Pippin (Cox), Golden Delicious (GD) and McIntosh, at autumn leaf-fall stage, were inoculated at the surface of fresh pruning wounds. Thirty-two trees of each cultivar were moved from East Malling to an unheated glasshouse at Efford one week before inoculation. For each cultivar, there were 32 treatments: four temperatures T1–T4 (nominally 10, 15, 20, 25 °C), four wetness periods (6, 12, 24, 48 h) and two inoculum doses (about 100 and 10000 macroconidia per cut surface). For each cultivar, there were two trees for each temperature × wetness treatment. On each tree, four shoots were inoculated; two were assigned randomly to the high dose and two to the low dose of inoculum. This gave 4 shoots per temperature × wetness × inoculum cultivar combination.

Four adjacent glasshouse compartments, each about 40 m^2 and with water absorbent floor matting, were allocated randomly to T1–T4. Heating was controlled, but with no refrigeration facilities; the mean temperatures achieved in treatments T1–T4 were 15.5, 16.2, 20.2 and 23.5 °C, respectively). Wetting was achieved by six overhead nozzles activated when the relative humidity, recorded at 1 m height in the centre of each compartment, dropped below 99% for 2.5 min. Eight trees of each cultivar were moved to each compartment one day before inoculation. Trees were inoculated on consecutive days: T1 and T3 on 24 October, and T2 and T4 on 25 October 1990. Tree by tree, the four labelled shoots were pruned and each cut surface was inoculated immediately with two 10 μl drops of the appropriate spore suspension.

At the end of each wet period, measured from the median inoculation time in each compartment, trees were moved to the glasshouse corridor and held there for 1 week before being moved to a polytunnel at East Malling.

Experiment 2

Pruning cuts of two ages were inoculated on cvs Cox and Fiesta (recently renamed Red Pippin) at bud-break stage, with 48 trees for each cultivar. For each cultivar, there were eight treatment combinations: four wetness durations (2, 4, 6, 12 h) and two wound ages (fresh and 1-d-old). On each plant, eight shoots were inoculated, four being assigned randomly to each of the two wound ages. This gave 48 shoots per wetness × wound age combination.

A single glasshouse compartment (about 25 m^2) was used for the inoculation and wetting. The temperature was set at 20 °C and a single misting nozzle maintained high humidity ($\geq$ 99%). Because of the limited glasshouse space, 16 trees of each cultivar were inoculated on 9, 10 and 11 March 1993. The trees were moved to the compartment and randomly positioned 1 d before inoculation; the four shoots on each tree for 1-d-old cuts were then pruned. Before inoculating, the trees were wetted with distilled water. Tree by tree, the four shoots to be inoculated as freshly cut were pruned and each of the eight cut surfaces was inoculated with two 10 μl drops of spore suspension. At the end of each wet period, the trees were moved to a polytunnel.

Experiment 3

Pruning cuts of four ages were inoculated on 48 trees of each of the three cultivars (cvs Cox, Spartan and Fiesta) at the winter dormant stage. For each cultivar, there were 14 treatment combinations: four wetness durations (2, 6, 12, 24 h) and four wound ages (fresh, 3-d, 6-d, 9-d); the combinations 6 h × fresh and 12 h × fresh were omitted. Twelve shoots were inoculated on each tree. On trees allocated to 6 or 12 h wet periods, four shoots were assigned randomly to each of the wound ages 3, 6 or 9 days, giving 48 shoots per wetness × wound age × cultivar combination; on trees allocated to 2 or 24 h wet periods, three shoots were assigned randomly to each of the four wound ages, giving 36 shoots per wetness × wound age × cultivar combination.

Two weeks before the first pruning cut was made (to obtain 9-d-old wounds), all the trees were moved into a polytunnel and the shoots were labelled for each wound age. To obtain 3-d, 6-d and 9-d-old wounds, the appropriate shoots were pruned in the polytunnel 3, 6 and 9 days before inoculation. The glasshouse compartment used for the inoculation and wetting was similar to the one used in Experiment 2 but wetting was maintained by two mobile humidifiers. The temperature was set at 20 °C. Due to the lack of glasshouse space, the trees were inoculated over a 3-day period (27–29 January, 1994). On each day, the 48 trees of just one of the three cultivars were positioned randomly in the compartment 1 h before inoculation and wetted with distilled water. Tree by tree, the shoots labelled for fresh wounds were pruned and each of the 12 cut surfaces was inoculated with two 10 μl drops of spore suspension. At the end of each wet period, the trees were moved to a polytunnel.

Experiment 4

Forty-eight trees of cv Spartan, at the stage of summer extension growth, were used. Pruning cuts of twelve ages, ranging from fresh to 24-d-old (fresh, 1-d, 2-d, 3-d, 6-d, 13-d, 14-d, 15-d, 17-d, 20-d, 22-d, 24-d), were inoculated, with four trees allocated randomly to each of twelve pruning ages. Ten to fifteen shoots were inoculated on each tree.

On each of eleven pruning days (excluding trees to be freshly pruned), four trees were moved from the sandbed to a polytunnel and the shoots were labelled and pruned. The temperature in the polytunnel was recorded at 24 min intervals with a Tinytalk logger (Orion Components (Chichester) Limited, West Sussex, England). The glasshouse compartment for the inoculation and wetting was similar to the one used in Experiment 2 but wetting was achieved by three misting nozzles. On the day of inoculation (15 August, 1996), the temperature was set at 20 °C and the nozzles were activated. One hour before inoculation, the 48 trees were positioned randomly in the compartment and wetted with distilled water. Tree by tree, each cut surface received two 10 μl drops of spore suspension; the fresh wounds were made by pruning immediately before inoculation. After 24 h, the trees were moved to a polytunnel.

Data analysis

Logistic regression analysis (Cox and Snell, 1989), which is based on the logit transformation of the proportion (P) of shoots cankered ($1n(^{p}/_{(1-p)})$), was used to assess the effects of treatments on the final incidence of inoculated shoots with canker lesions. The number of cankered shoots per treatment within each tree was assumed to be binomially distributed.

In Experiment 1, the logistic regression analysis was unable to reflect the nesting in the experiment design because some data were missing. Instead, the logistic regression analysis ignored the nesting and also only assessed main effects and two-factor interactions. Although significance levels may not be strictly accurate, the analysis highlights the relative importance of the different factors. Experiment 2 was analysed as a split-plot design, where the three inoculation dates were regarded as blocks. In this type of analysis, the effects of cultivar, duration of wet period, temperature, and their interaction were tested

Table 1. Final incidence (%) of *Nectria* canker lesion on inoculated fresh pruning cuts, and median and interquartile range (IQR) of incubation period (days) in Experiment 1

	Inoculum dose					
	Low			High		
	Incidence	Incubation period		Incidence	Incubation period	
Cultivar		Median	IQR		Median	IQR
Cox	38	138	76	88	68	35
GD	40	68	91	84	54	41
Bramley	13	138	90	33	135	69
McIntosh	24	166	54	65	135	56

Table 2. Final incidence (%) of *Nectria* canker lesion on inoculated pruning cuts, and median and interquartile range (IQR) of incubation period (days) in Experiment 2

	Pruning cut age					
	Fresh			1 day		
	Incidence	Incubation period		Incidence	Incubation period	
Cultivar		Median	IQR		Median	IQR
Cox	94	49	11	67	55	18
Fiesta	81	58	22	56	67	21

for their significance against the variation among trees within each date, whereas the effects of pruning age and its interactions with the other factors were tested against the residual variation. In Experiment 3, a similar split-plot analysis was applied, except that the effects of cultivar were confounded with inoculation date effects. This does not present any major problem because the main emphasis of this study is on the effects of environmental conditions and pruning age on canker incidence. In Experiment 4, the age of the pruning cut was expressed as the number of accumulated day-degrees above 8 °C (DD_8) from pruning to inoculation. The daily increase in DD_8 was calculated as $\sum^{60}_{i=1} (T_i–8)/_{60}$ where T_i is the mean temperature recorded at 24 min intervals. If $T_i < 8$, then (T_i-8) was replaced by zero.

Analysis of variance (ANOVA) was used to assess the effects of the treatments on the length of the incubation period (logarithmically transformed). The incubation period was the time from inoculation to the day the lesion was first seen. A formal ANOVA was not possible for Experiment 1 because there were no degrees of freedom for error in some of the strata due to missing data and because no cankers developed in some treatments. Instead, a non-hierarchical unbalanced factorial ANOVA was applied, ignoring the nesting in the experimental design. Again, only main effects and two-factor interactions were assessed. An unbalanced factorial ANOVA was applied to Experiment 2 and 3, using the split-plot design described above. In Experiment 4, the effect of pruning age was tested against the variation among trees to assess its significance.

Results

The results of the experiments are described under two headings: 'Incidence of *Nectria* canker lesions' and 'Length of incubation period'. Tables 1–3 show the results for Experiments 1, 2 and 3 respectively. The results for Experiment 4 are plotted in Figure 1.

Incidence of Nectria *canker lesions*

Experiment 1. Cox and GD had a higher incidence of canker lesions than Bramley and McIntosh throughout the recording period. The rate of increase in the numbers of lesions on Cox and GD was greatest about 40–70 d after inoculation, whereas on Bramley and McIntosh, the increase was more gradual. The final

Table 3. Final incidence (%) of *Nectria* canker lesion on inoculated pruning cuts, and median and interquartile range (IQR) of incubation period (days) in Experiment 3

	Pruning cut age											
	Fresh			3 days			6 days			9 days		
	Incidence	Incubation period		Incidence	Incubation period		Incidence	Incubation period		Incidence	Incubation period	
Cultivar		Median	IQR		Median	IQR		Median	IQR		Median	IQR
Cox	99	37	8	95	37	8	91	37	8	85	41	14
Spartan	96	40	0	99	40	3	98	46	5	96	48	11
Fiesta	90	39	4	92	42	8	79	47	8	66	53	16

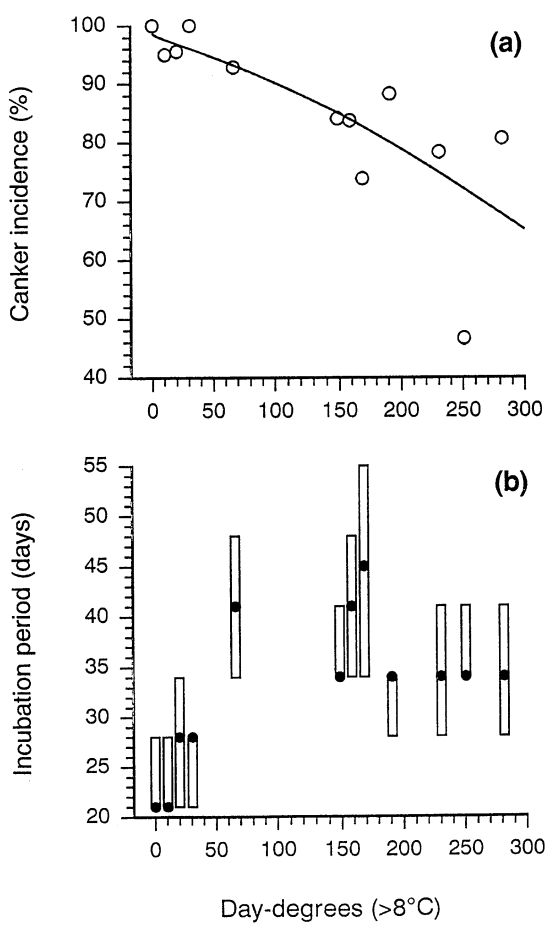

Figure 1. The relationship of the final incidence of *Nectria* canker and the incubation period with the age of wounds (day-degrees above 8 °C from pruning to inoculation) in Experiment 4: (a) incidence of canker, open circle – observed mean incidence of canker lesions for twelve ages of pruning cut, curve – fitted values (see text); (b) incubation period, solid circle – median duration of incubation period, bar – interquartile (25–75%) range of incubation periods. In this study, day-degrees above 8 °C (Krähmer, 1980) can be converted easily to day-degrees above 0 °C (Biggs, 1986) as all temperatures recorded were above 8 °C.

incidence was affected significantly by cultivar and inoculum dose ($P < 0.001$), but not by temperature. The incidence of canker decreased significantly with increasing duration of the wet period ($P < 0.05$); the overall incidence for 6, 12, 24 and 48 h wet periods was 60%, 51%, 48% and 45% respectively. The overall incidence of canker on Bramley, McIntosh, GD and Cox was 23%, 45%, 62% and 63% respectively; only Cox and GD were not significantly different from each other. The overall incidences of canker lesions resulting from low and high doses of conidia were 29% and 68% respectively. There was no significant interaction between inoculum dose and cultivar (Table 1).

Experiment 2. Canker lesions developed on 571 of the 768 shoots inoculated (74%), most appearing within 3 months of inoculation. The final incidence of lesions was affected significantly by cultivar ($P < 0.05$) and wound age ($P < 0.001$). Overall, Cox had a higher incidence (80%) than Fiesta (69%) and more lesions developed on fresh wounds (87%) than on 1-d-old wounds (62%). There was no interaction between cultivar and wound age (Table 2). The duration of wetness did not affect disease incidence significantly.

Experiment 3. Canker lesions developed on 1499 of the 1673 inoculated shoots (90%), most appearing within 2 months of inoculation. The final incidence of lesions was affected significantly by cultivar and wound age ($P < 0.001$) as well as by their interaction ($P < 0.01$), and by the three-way interaction between cultivar, wound age and wetness duration ($P < 0.01$). However, the deviances associated with the interaction terms were much smaller that those associated with the main effects. The main effect of wetness duration on the canker incidence was not significant. Overall, the incidence of canker on Spartan, Cox and Fiesta was 97%, 91% and 81% respectively. Overall, fewer

lesions developed on 9-d-old wounds (82%) than on 6-d-old wounds (89%), which in turn had fewer lesions than on 3-d-old (95%) and fresh wounds (95%) wounds. However, the degree of this wound-age effect on the incidence of canker differed between cultivars. Thus, on Spartan, there were no significant effects of wound age; on Cox, the incidence was significantly higher on wounds less than 9 days old; on Fiesta, the incidence was significantly higher on wounds less than 6 days old (Table 3).

Experiment 4. Canker lesions developed on 529 of the 624 inoculated shoots (85%), most appearing within 50 d of inoculation. Logistic regression analysis showed that the final incidence of canker was affected significantly by wound age (measured as day-degrees from pruning to inoculation). The effect of wound age on the incidence of canker lesions on Spartan was well described by the equation

$$ln(\frac{p}{1-p}) = 4.397 - 0.2177\sqrt{DD_8}$$

where p is the incidence of canker lesions and DD_8 is defined in the Materials and Methods section. The observed and fitted values are plotted in Figure 1a. The observed incidence differed markedly from the fitted value only when DD_8 was approximately 250. The rate of increase of resistance to *Nectria* with increasing wound age was slow: the incidence of canker was > 70%, even on 20-d-old wounds.

Length of incubation period

Tables 1–3 show, for Experiments 1, 2 and 3, respectively, the median incubation period (the number of days from inoculation until 50% of the final number of canker lesions had been recorded) and the numbers of days from the 25% to the 75% quartiles (interquartile range) of canker lesion appearances. The median value for combined treatments cannot be derived from those of individual treatments in the tables. Incubation periods for Experiment 4 are plotted in Figure 1b. Overall, the median incubation period and the interquartile range were greatest in Experiment 1.

Experiment 1. The incubation period was affected significantly by cultivar and inoculum dose, and by their interaction ($P < 0.001$), but not by temperature and wetness duration. Apart from a non-significant difference between McIntosh and Bramley, all pairwise comparisons between cultivars were significant ($P < 0.001$). The median incubation time was 55, 75, 138 and 138 d on GD, Cox, McIntosh and Bramley respectively. Overall, the incubation periods on shoots inoculated with the low and the high doses of conidia were 138 and 82 d respectively. The interaction between cultivar and inoculum dose was mainly due to the incubation periods being similar for both doses of conidia on Bramley. The interquartile range was generally narrower for the high dose than for the low dose.

Experiment 2. The incubation period was affected significantly by cultivar and wound age ($P < 0.001$), but not by duration of wet period. The median incubation time was 52 d on Cox but 61 d on Fiesta; the median incubation time was 54 d on fresh wounds but 57 d on 1-d-old wounds.

Experiment 3. The incubation period was significantly affected by the cultivar and wound age ($P < 0.001$), by the duration of wet period ($P < 0.05$), and by the interaction between cultivar and age ($P < 0.001$) and between wound age and duration of wet period ($P < 0.05$). However, the proportions of variance accounted for the interaction terms were much smaller than those by associated with the main effects. Overall, the median incubation time on Cox, Spartan and Fiesta was 37, 43 and 45 d respectively. The median incubation periods were 40, 40, 45 and 47 d following the inoculation of fresh, 3-, 6- and 9-d-old pruning cuts respectively. On Cox, the median incubation time associated with 9-d-old wounds was significantly longer than for younger wounds; on Spartan and Fiesta, the median incubation times associated with 6- and 9-d-old wounds were similar and significantly longer than for fresh and 3-d-old wounds. For all ages of pruning cuts, the interquartile range on Spartan was shorter than on Cox and Fiesta; the interquartile range increased with increasing wound age. The significant effect of wet period was mainly due to the longer incubation period (c. 3 days) at 2 h in comparison with the other wet periods.

Experiment 4. The incubation period was affected significantly by wound age ($P < 0.001$). The incubation time increased with increasing wound age up to about 170 day-degrees; above this, incubation time decreased and varied very little (Figure 1b).

Discussion

In this study, the effects of wound age and of the duration of and temperature during the wet period on infection of pruning cuts by *N. galligena* were investigated. To assess the effects of these factors on canker development, the incidence of canker and the length of the incubation period were recorded. Generally, these two measures were negatively correlated, i.e. lower canker incidence was associated with a longer incubation period and vice versa. However, tree-to-tree variation was substantial, especially for the length of the incubation period. Thus, canker incidence is likely to be a better indicator of treatment effects than is the length of the incubation period.

This study showed that the incidence of canker lesions caused by *N. galligena* was greater following the inoculation of fresh pruning cuts than older cuts, as observed by others (Marsh, 1939; Saure, 1962; Seaby and Swinburne, 1976). It is well-known that wounds on woody trees become increasingly resistant to infection by pathogens as they age (Krähmer, 1980; Biggs and Miles, 1985, 1988; Doster and Bostock, 1988a, b; El-Hamalawi and Menge, 1994). This type of resistance is related to the wound healing process which leads to the formation of boundary zone tissue and wounds periderm (Mullick, 1975, 1977; Krähmer, 1980).

The effects of wound age on the incidence of canker differed between Experiments 2 and 3: reduction in canker incidence with wound age was greater in Experiment 2 than in Experiment 3. This difference is not explained by differences in temperatures during the healing periods. Doster and Bostock (1988b) also found that resistance to infection by *P. syringae* of aged wounds of almond in winter did not consistently correspond to temperatures in the healing period. The difference observed in the present study is more likely due to an interaction between healing rate and tree growth stage. Trees in Experiment 2 were at bud-break when pruned whereas trees in Experiment 3 were dormant. The response of the host to wounding is normally faster for trees which are active metabolically (Crowdy, 1949; Mullick and Jensen, 1976; Tamura and Saito, 1982; Baudoin and Exkert, 1985; Biggs and Miles, 1988). Thus, wounds remained susceptible to infection for longer when the wounds were made in winter (Marsh, 1939; Grant and Spaulding, 1939). Immature developing apple fruits have an extensive and rapid wound reaction that results in periderm formation, whereas on mature fruits this reaction is reduced resulting in the absence of periderm (Skene, 1981). A better understanding of age-related mechanisms of resistance of wounds to pathogens is needed.

There were significant interactions between cultivars and ages of pruning wounds on the incidence of canker lesions, which implies that cultivars differ in their rates of wound healing, as shown for other woody species (Biggs and Miles, 1988; Doster and Bostock, 1988a, b). The interactions between age-related wound resistance, cultivar and host physiology may have implications for resistance breeding and canker management. Selection for resistance to *N. galligena* is usually based on incidence and size of cankers following the inoculation of fresh wounds (Borecki and Czynczyk, 1984; Van de Weg, 1989; Van de Weg et al., 1992). It may be necessary to improve screening by inoculating wounds of various ages on trees at different physiological stages. In the U.K., *N. galligena* spores are present in winter (Swinburne, 1975) and readily germinate at low temperatures (Dubin and English, 1975a). It may be advisable, therefore, to restrict winter pruning to canker-free orchards or cultivars with fast-acting defence mechanisms.

It is well known that cultivars differ in their susceptibility to *Nectria* (Zagaja et al., 1971; Van de Weg, 1989; Van de Weg et al., 1992; Pedersen et al., 1994). The observed moderate to high susceptibility of McIntosh, Cox and Spartan agrees with previous studies (Zagaja et al., 1971, Borecki et al., 1978, Borecki and Czynczyk, 1984), as does the low susceptibility of Bramley (Pedersen et al., 1994). Previous studies showed that Golden Delicious has low susceptibility to *Nectria* (Borecki et al., 1978; Borecki and Czynczyk, 1984; Van de Weg, 1989; Van de Weg et al., 1992). However, in this study Golden Delicious was found to be highly susceptible. This difference may have several causes. Firstly, most previous studies used canker size as a resistance criterion, whereas in this study canker incidence and the length of incubation period were recorded. The relationship among canker incidence, the length of incubation period and canker size may depend on cultivar and experimental conditions. Van de Weg (1989) found the significant difference in the incidence of canker between cultivars, whereas in another study cultivars did not differ in canker incidence but in size, and the incidence was also not affected by initial incubation temperature whilst in contrast canker size decreased with increasing temperature (Van de Weg et al., 1992). Secondly, experimental methods differed among the various experiments. Some studies were based on nat-

ural infection (Zagaja et al., 1971; Pedersen et al., 1994) and others on artificial inoculation (Borecki et al., 1978; Krüger, 1983; Borecki and Czynczyk, 1984; Van de Weg, 1989). In artificial inoculation studies, some used mycelium as inoculum (Borecki et al., 1978; Borecki and Czynczyk, 1984) and others used a spore suspension (Krüger, 1983; Van de Weg, 1989; Van de Weg et al., 1992); the duration of the wet period (high humidity), the means of achieving the wet period, and the initial incubation temperature also differed. Indeed, Van de Weg (1989) demonstrated the effects of inoculation techniques on canker incidence. Thirdly, cultivar resistance to *Nectria* may also vary with the type of entry site. Most previous studies inoculated fresh wounds around leaf scars or tree trunks, whereas pruning wounds were inoculated in this study. A better protocol for screening resistance to *Nectria*, which also incorporates healing rate and tree physiological state, should be developed.

Higher inoculum doses resulted in higher canker incidence as observed by Van de Weg (1989). This has also been observed by Carter and Moller (1971) for *Eutypa armeniacae* on pruning wounds of *Prunus* species. In the present studies, an inoculum dose of c. 100 macroconidia (c. 200 spores including microconidia) resulted in about 32% incidence of canker on fresh pruning cuts. To achieve a similar level of disease on freshly wounded leaf-scars, more than 500 conidia were needed (Dubin and English, 1974). The incidence of canker on pruning cuts up to 9-d-old did not depend on the duration of wetness except in Experiment 1 in which incidence was significantly reduced by prolonged wet periods. In contrast, the incidence of canker on freshly wounded leaf-scars increased with prolonged wetness (Wilson, 1966; Dubin and English, 1974). These differences in the effects of inoculum dose and duration of wetness on the incidence of cankers between pruning cuts and leaf scars could be due to differences in inoculation methods, incubation conditions and cultivars used. Another likely explanation is the difference in anatomy and physiology between these two types of entry site. A small wound at a leaf scar may hasten the formation of the abscission layer or other host defensive responses; such a fast response may result from natural selection because large numbers of leaf scars are present naturally every year. In contrast, trees may respond to pruning wounds more slowly because pruning is not natural. Heinrich (1982) showed that pruning wounds are more susceptible to *Nectria* than leaf-scars.

Spore suspension may be drawn immediately into the host when applied to fresh pruning cuts (X.-M. Xu unpub.). Furthermore, observations at HRI – East Malling have shown that fresh apple pruning cuts inoculated with a conidial suspension become cankered without imposing any period of wetness. If germinated conidia have to penetrate the developing defensive barriers in order to infect ageing wounds, longer wet periods would be expected to result in higher incidences of canker and possibly shorter incubation periods, because more spores would have successfully penetrated. In the present study, the incidence of canker did not, however, increase with increasing duration of wetness on ageing wounds. Also the length of incubation period did not decrease with increasing temperature or duration of wetness except in Experiment 4 where wet periods longer than 2 h resulted in a slightly but significantly shorter incubation time. Thus, it is possible that *Nectria* may not be able to penetrate the developing defensive barriers but may enter only through 'unhealed' areas of the site. Alternatively, a period of wetness as short as 2 h at these ageing sites may be sufficient to support the penetration of the developing defensive barriers.

This study shows that the incidence of canker and the length of the incubation period on pruning wounds are mainly affected by inoculum dose, cultivar and wound age. High inoculum dose and young pruning wounds resulted in short incubation periods as well as a high incidence of canker. This correlation between the incidence and the length of the incubation period is consistent with the nature of canker pathogens. The greater the numbers of spores that land on a single wound site and the younger the site, the greater the invading fungal biomass and the greater the probability of infection, thus leading to rapid colonisation and expression of symptoms.

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