Shuttleworth *et al.*
CABI Agriculture and Bioscience (2023) 4:37
https://doi.org/10.1186/s43170-023-00177-z

CABI Agriculture and Bioscience

RESEARCH

Open Access

A comparison of new and existing rootstocks to reduce canker of apple trees caused by *Neonectria ditissima* (Nectriaceae, Hypocreales)

Lucas A. Shuttleworth[1*], Sonia Newman[2] and Ioannis Korkos[3]

Abstract
The grafting of apple rootstocks on to scions confers benefits including reduced tree size/dwarfing for trellis based growing systems, increased tolerance to physiological stress, and pest and disease management. The current study investigated the effect of rootstock selection on canker and tree death using eight common rootstocks M9 337, M9 337 with Golden Delicious (GD) interstock, M9 EMLA, MM106, M116, M26, Geneva® G11 and G41, in addition to six advanced selections from the NIAB East Malling apple breeding programme EMR-001—EMR-006, all grafted with Gala scions. One of the rootstocks, M9 377 was also grafted with a GD interstock. Two locations in England were selected, the first at East Malling, Kent, the second, at Newent, Gloucestershire. Several variables were analysed including cumulative numbers of dead trees per rootstock from 2017 to 2020, number of rootstock 'A type' cankers, number of scion mainstem 'B type' cankers, and number of peripheral 'C + D + E' branch cankers at the Kent and Gloucestershire locations in the fourth and final assessment year of 2020. Kendall's rank correlation was used to test if trunk circumference (a measure of tree vigour) and canker were statistically dependant. Results showed that in Kent, there were significant differences between rootstocks for scion B cankers and peripheral C + D + E cankers. There were no significant differences found between rootstocks for rootstock A cankers at Kent, or any of the three canker types in Gloucestershire. There were up to 31.25% dead trees in Kent (EMR-004), and 30% in Gloucestershire (M9 337 with GD interstock, M26), but there were no significant differences in number of dead trees due to rootstock type in either Kent or Gloucestershire. The Kendall's rank correlation analysis indicated there was almost no dependence of trunk circumference on canker. The three rootstocks with the overall lowest susceptibility to canker were M116, EMR-006, and EMR-004. The industry standard rootstocks M9 EMLA and M9 337 were ranked 12 and 14, and with EMR-001 were the three worst performing rootstocks. The Geneva® rootstocks G11 was ranked 6, and G41 was ranked 11. An interplay of factors are likely involved in the development of canker including location and environmental effects—rainfall, relative humidity, temperature, wind, soil type, topography, aspect, and other seasonal infection processes including pathogen inoculum load, and rainsplash of inoculum between trees and within individual trees, the genetics and physiology of each scion/rootstock combination, water and nutritional status of trees, hormonal and molecular signalling, and orchard management including pruning and removal of cankers.

Keywords European canker, Integrated disease management, Organic production

*Correspondence:
Lucas A. Shuttleworth
lucasashuttleworth@gmail.com
Full list of author information is available at the end of the article

Background

The grafting of plants with a desirable scion cultivar has been used for over two and a half millenia (Hunter 2021). Until the 20th century, scions of apple were commonly grafted on to seedling rootstocks until the discovery of dwarfing rootstocks and canopy management systems/trellising, which enabled more efficient tree management, earlier and higher yield, and increased fruit quality (Marini and Fazio 2018). Currently, all available apple rootstocks have weaknesses such as propagation difficulties, graft incompatibility, and susceptibility to abiotic and biotic stresses including pests and pathogens (Rasool et al. 2020).

European canker, caused by the fungal pathogen *Neonectria ditissima* (Nectriaceae, Hypocreales), is the most important canker disease affecting apple production across Europe, North and South America, Asia and Oceania (CABI 2020). Saville and Olivieri (2019) and Weber and Børve (2021) provide detailed reviews of the disease. Symptoms include lesions on the trunk and branches which often girdle and kill the tree. The proportion of trees dying in the orchard establishment phase can be up to 10% annually (Gómez-Cortecero et al. 2016). Infection of the rootstock during the nursery stage has been reported with wounds in the grafting process providing entry points for the pathogen (Børve et al. 2018). Once trees are planted out in the orchard, the main source of infection under conducive environmental conditions is from neighbouring orchards (McCracken et al. 2003). Rain is also an important factor in the infection process within an orchard, both between trees and within individual trees (Swinburne 1975). Visible cankers can develop after planting out in the field up to 3 years after the initial nursery infection (Weber 2014). The most important canker resulting from nursery infection is a lateral canker on the main trunk, with up to 25% of trees having to be removed after the first growing season (Weber and Børve 2021).

Certain scion cultivars are known to exhibit reduced susceptibility to canker, with Golden Delicious, Elstar, and Jonathan being key examples (Van de Weg 1989, Weigl 2021). Cultivars with higher susceptibility to canker include Gala, Braeburn, Cameo, Kanzi, Scifresh, Rubens, and Zari (Weber 2014). In a recent field study in the UK, Xu et al. (2022), assessed canker incidence on cultivars including Royal Gala, Braeburn, Scifresh, Nicoter, Civni, Grenadier, and Golden Delicious, with Grenadier and Golden Delicious having the lowest incidence across three sites. In New Zealand, whole plants were inoculated with *N. ditissima*, and eight weeks after inoculation the rootstock cultivar Robusta 5 was lesion-free, while Sciearly, Golden Delicious, Braeburn and M9 had moderate symptoms, and Royal Gala was severely cankered (Scheper et al. 2018). Lower susceptibility of cultivars to canker has been reported, however, no cultivar is fully resistant. There appear to be low to moderate effects of genetics on apple rootstock and scion selection in relation to canker susceptibility, and it has been suggested that multiple quantitative trait loci (QTL) need to be considered to improve canker resistance in apple breeding programs (Karlström et al. 2022).

Currently, there are limited options available for growers to manage canker, particularly in the UK and Europe, with conventional chemical options being increasingly restricted each year. Alternative disease management methods including rootstock selection were discussed in a recent review article by Shuttleworth (2021). Rootstocks have been reported to reduce susceptibility to pathogens and disease complexes including *Phytophthora* spp., *Erwinia amylovora* (fireblight), *Venturia inaequalis* (apple scab), and apple replant disease (ARD) (Utkhede and Smith 1994; PSU 2018; Deakin et al. 2019). Korba et al. (2000) investigated the susceptibility to fireblight of 24 apple rootstocks and 4 scion cultivars (96 rootstock/scion combinations in total) and found that the degree of susceptibility depended on the rootstock and season of study, with the highest susceptibility on JTE-E and M9 rootstocks, and lowest on the rootstock TE 52. For the remaining 21 rootstocks there was no difference in fireblight susceptibility, indicating there is some variation in the effect of rootstock on fireblight susceptibility.

The current study investigated the effect of rootstock selection on canker of apple grafted with a panel of eight commonly used rootstocks in commercial production, in addition to six advanced selections from the National Institute of Agricultural Botany (NIAB) East Malling apple breeding programme.

Methods

Orchard locations

Two orchard locations in England were selected. The first was at NIAB, East Malling, Kent, (Grid reference: 51.287861, 0.43831340). The second was at Herridges Orchard, Poolhill nr. Newent, Gloucestershire (Grid reference: 51.966956, − 2.3953805). Long term climate averages (1991 to 2020) for East Malling and Ross-on-Wye (nearest to Newent) were obtained from the Meteorological Office (Met Office 2023a; 2023b, respectively). East Malling and Ross-on-Wye had an annual rainfall average of 670.20 and 764.30 mm, respectively. Thus, Ross-on-Wye had 94.1 mm more rainfall than East Malling. The average maximum temperature at East Malling was 15 °C, and at Ross-on-Wye was 14.77 °C. The soil type at the East Malling, Kent site is classified as a Luvisol. A Luvisol has a clay-enriched subsoil, high base status, and high-activity clay (LandIS 2023a). The soil type at Newent, Gloucestershire is a Regosol. A Regosol is a relatively young soil, or a soil with little or no profile development (LandIS 2023b). Regosols have low moisture retention, susceptibility to erosion and compaction, and are not very productive for crops, unless intensively managed.

Rootstock selections, nursery propagation, planting and husbandry

Eight commercial rootstock cultivars and six advanced selections developed by the NIAB East Malling Apple Breeding Consortium coded EMR-001 to EMR-006 were selected (Table 1). Rootstocks were bench grafted to a common scion cultivar Gala in February 2016 and grown on in 4 L pots in Westland John Innes No.3 mature plant compost (Westland, Dungannon, County Tyrone, Northern Ireland). Gala is a commercially important cultivar and is known to be susceptible to canker. To promote feathering of the maidens the apex shoot was pinched out to remove apical dominance. Once the trees were dormant in January 2017 they were prepared as bare rooted trees and stored under commercial conditions, specifically, 2 °C in the dark with roots wrapped in damp hessian and watered regularly until planting. Trees were planted at East Malling on 29th March 2017, and at Newent on 14th March 2017. Regarding husbandry, there were no fungicides applied during the experiments and trees were not irrigated. Orchards were mowed at regular intervals during the spring and summer months to manage the grass cover near the trees.

Experimental design

A randomised complete block design was used at both locations. At the Kent location, 448 trees were included in the experiment which consisted of 4 replicates of 8 tree plots, 32 trees per rootstock selection, arranged in four blocks, while at the Gloucestershire location, 560 trees were included in the experiment which consisting of 4 replicates of 10 tree plots, 40 trees per rootstock selection, arranged in 4 blocks. Several of the trees in the study died as a result of other factors not related to canker, e.g., transplant shock, and water stress as trees were not irrigated. The spacing of trees in the Kent experiment was 3.5 m in the in-row × 1.75 m between rows, while in the Gloucestershire experiment was 1.83 m in the in-row × 3.66 m between rows.

Source of *N. ditissima* inoculum

Inoculum for infection was from natural sources in surrounding orchards including wind and rainsplash

Table 1 The fourteen rootstock selections used to compare canker susceptibility including 8 commercially available cultivars and six elite selections from the NIAB East Malling apple rootstock breeding program

Rootstock	Description
M9 337	Dwarfing rootstock selection from M9 virus-free certified clonal stock developed by the East Malling Research Station, Kent, England. M9 337 is very precocious, the measure of time to flowering within the life span of the tree, and is tolerant to a wide range of soil and climatic conditions. Clones of M9 are the most widely planted dwarfing apple rootstock globally (PSU 2018)
M9 337 with GD interstock	Rootstock with characteristics as described above. Golden Delicious (GD) used in the interstock is known to have lower susceptibility and may provide additional protection from canker
M9 EMLA	Developed by the East Malling Long Ashton breeding program to exclude virus infection through micro-propagation. M9 EMLA is free of known viruses and slightly more vigorous than the original M9
MM106	Semi-vigorous, producing free-standing, early bearing trees. Susceptible to collar rot when planted in wet soils and not recommended for poorly drained sites (PSU 2018)
M116	Medium vigour. Described as having increased resistance to *Phytophthora,* powdery mildew and apple replant disease (ARD). Improved yield compared to MM106 (Alston 2008)
M26	A more vigorous dwarfing rootstock than M9 resulting from a cross between M9 and M16. Precocious and very productive. Susceptible to *Phytophthora* and fire blight. Reported to be susceptible to collar rot and fire blight and should not be planted on wet sites (PSU 2018)
G11	A cross between M26 and Robusta 5. Similar in size to M26. Fireblight resistant, and tolerant of *Phytophthora*, partial tolerance to ARD. Productivity/yield efficiency is better than M9. Low amount of suckering and burr knots. Geneva® rootstocks are reported to increase productivity and tree yield efficiency similar or higher than current commercial standards M9 and M26 (PSU 2018; Fazio et al. 2015)
G41	A cross between M27 and Robusta 5, similar in size to M9. Reported as highly resistant to fire blight and *Phytophthora*, tolerant of ARD (PSU 2018; Fazio et al. 2015)
EMR-001	NIAB East Malling advanced selection
EMR-002	NIAB East Malling advanced selection
EMR-003	NIAB East Malling advanced selection
EMR-004	NIAB East Malling advanced selection
EMR-005	NIAB East Malling advanced selection
EMR-006	NIAB East Malling advanced selection

dispersed ascospores and conidia. The Kent experiment at NIAB East Malling had adjacent orchards of apple with canker. The Gloucestershire experiment was adjacent to orchards of mature cultivar Cox apple orchards with established canker. The inoculum in the neighbouring orchards was not quantified, however the blocking of the experiment should have accounted for any variation in inoculum.

Tree death from canker

The total number of dead trees caused by canker for each rootstock in the Kent and Gloucestershire experiments was recorded. Tree death from other causes such as mowing damage in the Kent experiment was also recorded.

Canker assessments

Canker assessments were completed once annually between 2017 and 2020, existing cankers and new cankers were included each year. In 2017, the assessment of naturally infected trees was completed in the Kent and Gloucestershire experiments in late August and early October, respectively, while assessments of naturally infected trees in 2018 to 2020 were completed in mid-May. Cankers were counted when trees were not in full leaf to ensure cankers were visible. Cankers were identified visually based on the typical reddish brown coloured lesions produced by *N. ditissima* and counted according to their position on each tree. These included rootstock 'A' cankers, scion 'B' cankers (i.e., central leader of the scion excluding the apex), 'peripheral C' branch cankers (branches originating from the central leader including the apex), 'peripheral D' branch cankers (branches originating from C branches), and 'peripheral E' branch cankers (branches originating from D branches. Peripheral branch cankers for each tree were added together and labelled as 'peripheral C+D+E' cankers (Fig. 1). In November 2020, trunk circumference 10 cm above the graft union of each tree at both sites was used as a measure of tree vigour.

Statistical analyses

Genstat version 22.1.0.532 (VSN International Ltd, Hemel Hempstead, England) was used for data analyses. A Shapiro–Wilk test for normality was used to determine if datasets were normally distributed, and a Bartlett's test to determine homogeneity of variances. The Shapiro–Wilk test showed that none of the tree death or canker datasets at either location were normally distributed ($p<0.001$, Additional file 1: Table S1), and the Bartlett's test for homogeneity of variances showed variance was not equal across rootstocks ($p<0.05$), except for those in the experiment in Kent in 2020 for rootstock A canker (1) (Additional file 1: Table S2). Datasets had Poisson distributions and therefore this was selected in subsequent analyses.

Several null hypotheses were tested, the first was that the cumulative number of dead trees from 2017 to 2020 per rootstock in the experiments in Kent, and Gloucestershire were equal.

The second null hypothesis was that in the 2020 assessment, the number of cankers for rootstock A canker,

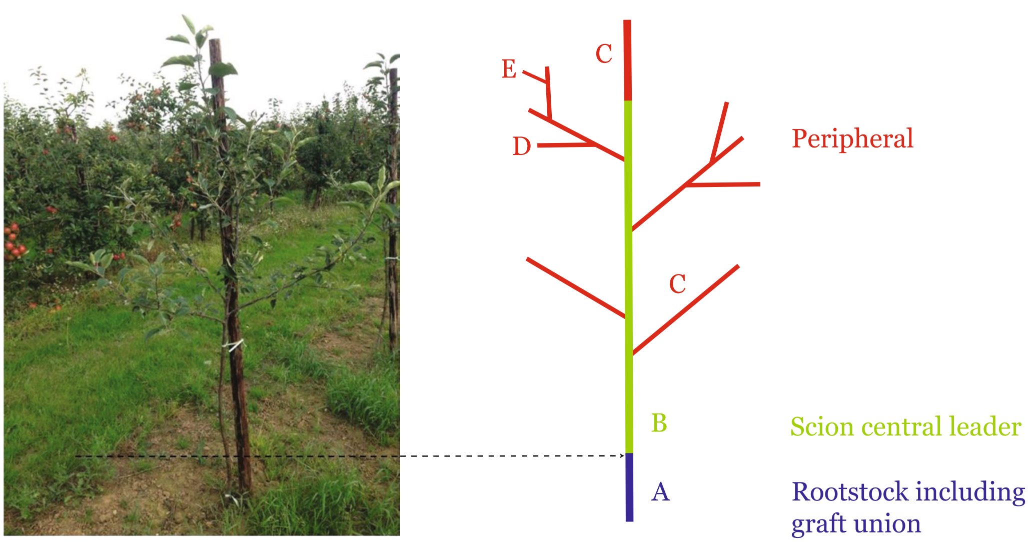

Fig. 1 Cankers were identified visually based on their position on the tree. These were rootstock 'A' cankers, scion 'B' cankers (i.e., central leader of the scion excluding the apex), 'peripheral C' branch cankers (branches originating from the central leader including the apex), 'peripheral D' branch cankers (branches originating from C branches), and 'peripheral E' branch cankers (branches originating from D branches)

scion B canker, and peripheral C+D+E canker of each rootstock in the experiment in Kent were equal, and in the experiment in Gloucestershire were equal. To test the first and second sets of null hypotheses, a Generalised Linear Mixed Model (GLMM) (Schall et al. 1991) was selected, with Rootstock as a fixed effect, and Block and Block×Rootstock as random effects. A Poisson distribution, with a logarithm link function were selected. Rootstock susceptibility was further ranked using the backtransformed means of dead trees from the canker assessments in the Kent and Gloucestershire experiments, and backtransformed means of rootstock A canker number, scion B canker, and peripheral C+D+E canker in the Kent and Gloucestershire experiments in 2020. The ranking value for each rootstock for each variable were added together to form an overall ranking value (Additional file 1: Tables S3, S4, S5, S6, S7, S8, S9, S10). The overall ranking value was used to infer susceptibility to canker.

The third null hypothesis tested if tree vigour, measured as trunk circumference 10 cm above the graft union, was correlated with rootstock A canker, scion B canker, and peripheral C+D+E canker at Kent, and Gloucestershire. Kendall's rank correlation coefficient (non-parametric correlation, Kendall 1938) was selected. Kendall's rank correlation is used to establish whether two variables may be regarded as statistically dependent. It is non-parametric, as it does not rely on any assumptions regarding x and y, or the distributions of x and y. It is a measure of rank correlation, the ordinal association between the two variables represents the binary relation and then normalises the distance such that it will take values between − 1 and + 1. Values close to 0 indicate little to no dependence of the variables. The number of trees used in the analysis was 349 and 280 for the Kent, and Gloucestershire experiments, respectively.

Results

Dead trees

In the 2020 assessment, considering all rootstocks, the Kent site had 15.18% of trees dead due to canker, while the Gloucestershire site had 16.78% dead (Table 2). At the

Table 2 The cumulative number of dead trees from canker per rootstock at the Kent and Gloucestershire sites assessed from 2017 to 2020

Rootstock, Kent	Number, and percentage (in parentheses) of dead trees per rootstock due to canker	Number, and percentage (in parentheses) of dead trees with no canker, and those dead from mower damage	Rootstock, Gloucestershire	Number, and percentage (in parentheses) of dead trees per rootstock due to canker	Number, and percentage (in parentheses) of dead trees with no canker
EMR-006	2 (6.25)	1 (3.13)	EMR-006	2 (5)	0 (0)
G41	2 (6.25)	5 (15.63), 2 (6.25) from mower	EMR-005	2 (5)	0 (0)
M116	2 (6.25)	1 (3.13) from mower	G11	4 (10)	0 (0)
EMR-005	3 (9.38)	1 (3.13) from mower	M116	4 (10)	0 (0)
EMR-002	3 (9.38)	1 (3.13) from mower	EMR-004	5 (12.50)	0 (0)
G11	3 (9.38)	0 (0)	G41	5 (12.50)	1 (2.50)
MM106	4 (12.50)	1 (3.13) from mower	MM106	5 (12.50)	1 (2.50)
EMR-001	5 (15.63)	3 (9.38), 2 (6.25) from mower	EMR-003	7 (17.50)	0 (0)
M26	5 (15.63)	0 (0)	EMR-002	8 (20)	1 (2.50)
M9 337	5 (15.63)	2 (6.25), 1 (3.13) from mower	M9 EMLA	8 (20)	0 (0)
EMR-003	8 (25)	2 (6.25)	EMR-001	10 (25)	0 (0)
M9 EMLA	8 (25)	1 (3.13)	M9 337	10 (25)	0 (0)
M9 337 with GD interstock	8 (25)	2 (6.25), 1 (3.13) from mower	M26	12 (30)	0 (0)
EMR-004	10 (31.25)	1 (3.13)	M9 337 with GD interstock	12 (30)	0 (0)
Total	68/448 (15.18)	19/448 (4.24), with 10/448 (2.23) from mower		94/560 (16.78)	3/560 (0.05)

Rootstocks are ordered lowest to highest based on the number of trees with canker. At planting the number of trees per rootstock at the Kent site was 32, and at the Gloucestershire site 40. The percentage of dead trees per rootstock, calculated by the number of dead trees over the number of trees at planting are included in parentheses. The number and percentage of dead trees with no canker symptoms is also included. The non-canker deaths were mainly caused at the Kent site by physical damage to trees from grass mowing

Kent site, EMR-006, G41 and M116 had equally the lowest percentage of dead trees, while at the Gloucestershire site, EMR-006 and EMR-005 had the equal lowest percentage of dead trees, with G11 and M116 ranking equal second lowest.

The rootstocks with the highest percentage of dead trees due to canker, at the Kent site were EMR-004 (31.25%), followed by M9 337 with GD interstock, M9 EMLA and EMR-003 (all 25%). At the Gloucestershire site, the equal highest percentage of dead trees due to canker were M9 337 with GD interstock and M26 (both 30%), and equal second highest were EMR-001 and M9 337 (both 25%).

At the Kent site the percentage of dead trees due to causes other than canker was 4.24% (no visible canker). Of these 10 trees (2.23% of total trees) had died due to mower damage. (Table 2). The trees dead due to causes other than canker were excluded from the analyses.

The analysis of the cumulative number of dead trees from 2017 to 2020 showed no significant differences between rootstocks at Kent (Wald statistic 16.67, df 13, chi pr 0.215; Additional file 1: Table S3) and Gloucestershire (Wald statistic 20.66, df 13 F pr 0.08; Additional file 1: Table S4).

Canker number

In the Kent experiment in 2020, the total number of rootstock A cankers was 12, scion B cankers was 620, and peripheral C + D + E cankers was 4397 (Fig. 2).

In the Kent experiment there was no significant difference among numbers of rootstock A cankers in 2020 (Wald statistic 3.69, n.d.f. 13, *F* statistic 0.28, d.d.f. 130.4, F pr 0.994; Additional file 1: Table S5), however there was a significant difference among rootstocks for numbers of scion B cankers (Wald statistic 40.68, n.d.f. 13, *F* statistic 3.13, d.d.f. 35, F pr 0.004; Additional file 1: Table S6). The numbers of peripheral C + D + E cankers were also significantly different among rootstocks (Wald statistic 47.99, n.d.f. 13, *F* statistic 3.69, d.d.f. 38.4, F pr < 0.001; Additional file 1: Table S7).

In the Gloucestershire experiment in 2020, the total numbers of rootstock A cankers was 87, scion B cankers was 110, and peripheral C + D + E cankers was 123. There was no significant difference in the numbers of rootstock A cankers (Wald statistic 7.77, n.d.f. 13, *F* statistic 0.6, d.d.f. 36.2, F pr 0.84; Additional file 1: Table S8), scion B cankers (Wald statistic 17.2, n.d.f. 13, *F* statistic 1.32, d.d.f. 34.8, F pr 0.249 Additional file 1: Table S9), or peripheral C + D + E cankers (Wald statistic 16.67, n.d.f. 13, *F* statistic 1.28, d.d.f. 42.4, F pr 0.264; Additional file 1: Table S10).

Effect of trunk circumference on canker number

The Kendall's rank correlation coefficient (τ) for trunk circumference and the 2020 Kent experiment rootstock A canker numbers was − 0.01; for the scion B canker numbers was − 0.07, and for the peripheral C + D + E canker numbers was 0.07. For the 2020 Gloucestershire experiment rootstock A canker numbers the τ was 0.04, for the scion B canker numbers τ was − 0.03, and for the peripheral C + D + E canker numbers τ was 0.09 (Fig. 3).

Overall rootstock rankings for canker susceptibility

The overall ranking of the three rootstocks conferring the lowest susceptibility to canker were M116, EMR-006, and EMR-004 (Table 3). The industry standard rootstocks M9 EMLA and M9 337 were ranked 7 and 14 out of the 14 rootstocks tested, while Geneva® G11 was ranked 5, and G41 was ranked 8. The three most susceptible rootstocks were M9 337, EMR-001 and M9 EMLA.

Discussion

This study showed that rootstock selection had a significant effect on scion B and peripheral C + D + E canker numbers in the Kent experiment. The rootstocks with lowest overall ranking value for tree death and canker, and hence considered the best performing, were M116, and the NIAB East Malling advanced selections EMR-006, and EMR-004. M116 is known for its resistance to canker and can be used in low-cost orchard systems with trees supported by a single horizontal wire on small stakes every 10 m (Hutchinsons 2023). EMR-006 and EMR-004 are promising for lower canker susceptibility, however data on the effects of parameters such as suitability for training on high density trellis systems, fruit yield, yield efficiency, fruit quality, suckering, graft compatibility, nutrient uptake efficiency, precocity, tolerance to specific soil types, susceptibility to pests and other diseases, and responses to any other unique stress events are needed (Fazio et al. 2015).

Our results showed little evidence for statistical dependence of trunk circumference (as an indicator of tree vigour) and the three canker types in either the Kent or Gloucestershire experiments. It is not known if an association may develop as the trees age. In an experiment by Johnson et al. (1982), differences in tree size alone did not account for differences in canker numbers of the four scion cultivars Summerland Red McIntosh, Spartan, Harrold's Delicious, and Golden Delicious. In an inoculation experiment of apple with *N. ditissima*, Amponsah et al. (2017) found that scion cultivars, Braeburn, Scilate, Fuji, Golden Delicious, Jonathan, and Royal Gala, grafted with rootstocks M793 and M9 showed

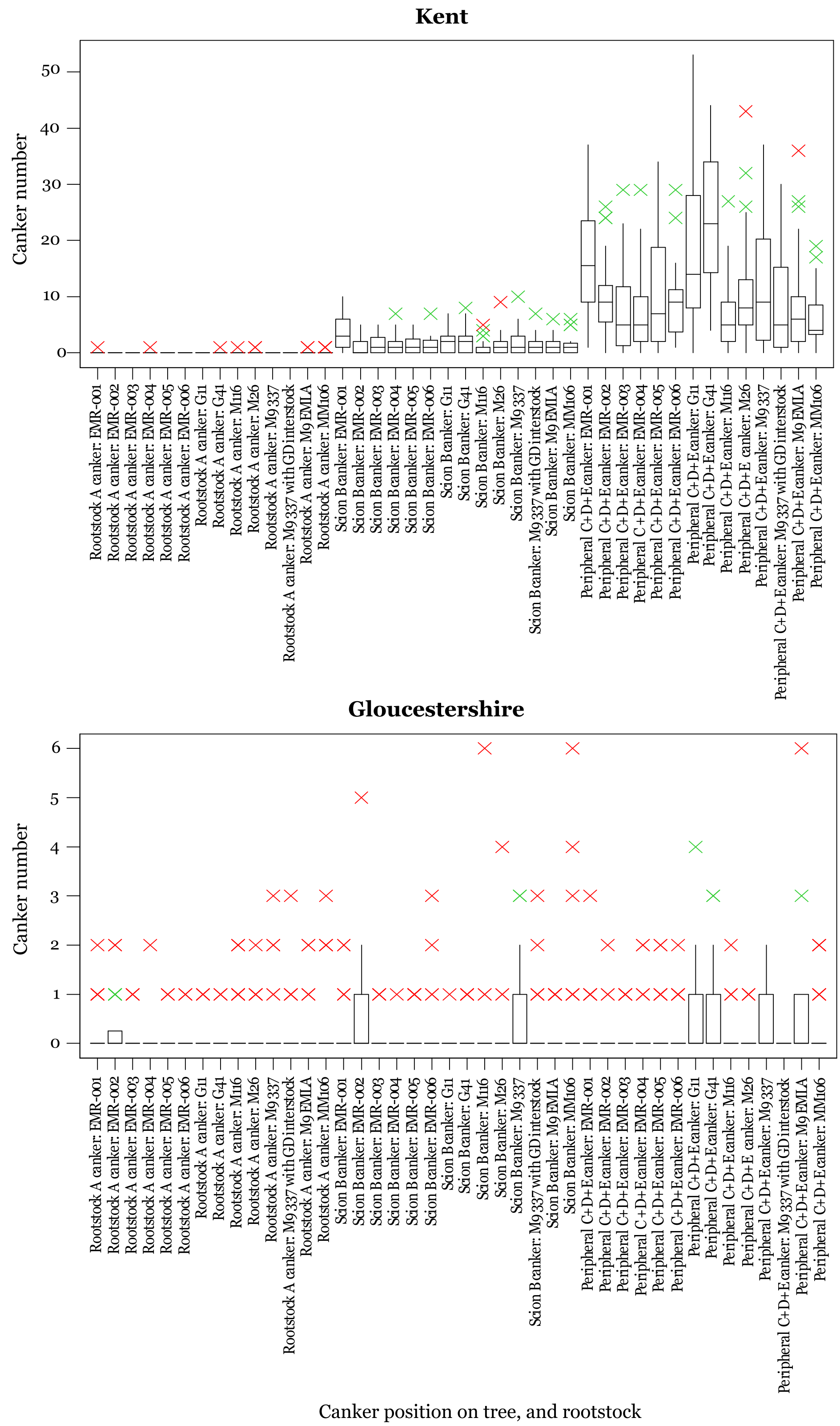

Fig. 2 Canker counts on apple trees grafted with fourteen different rootstocks in 2020 in the Kent and Gloucestershire experiments. Cankers were counted according to their position on the tree and include rootstock A cankers, section B cankers and peripheral C + D + E cankers

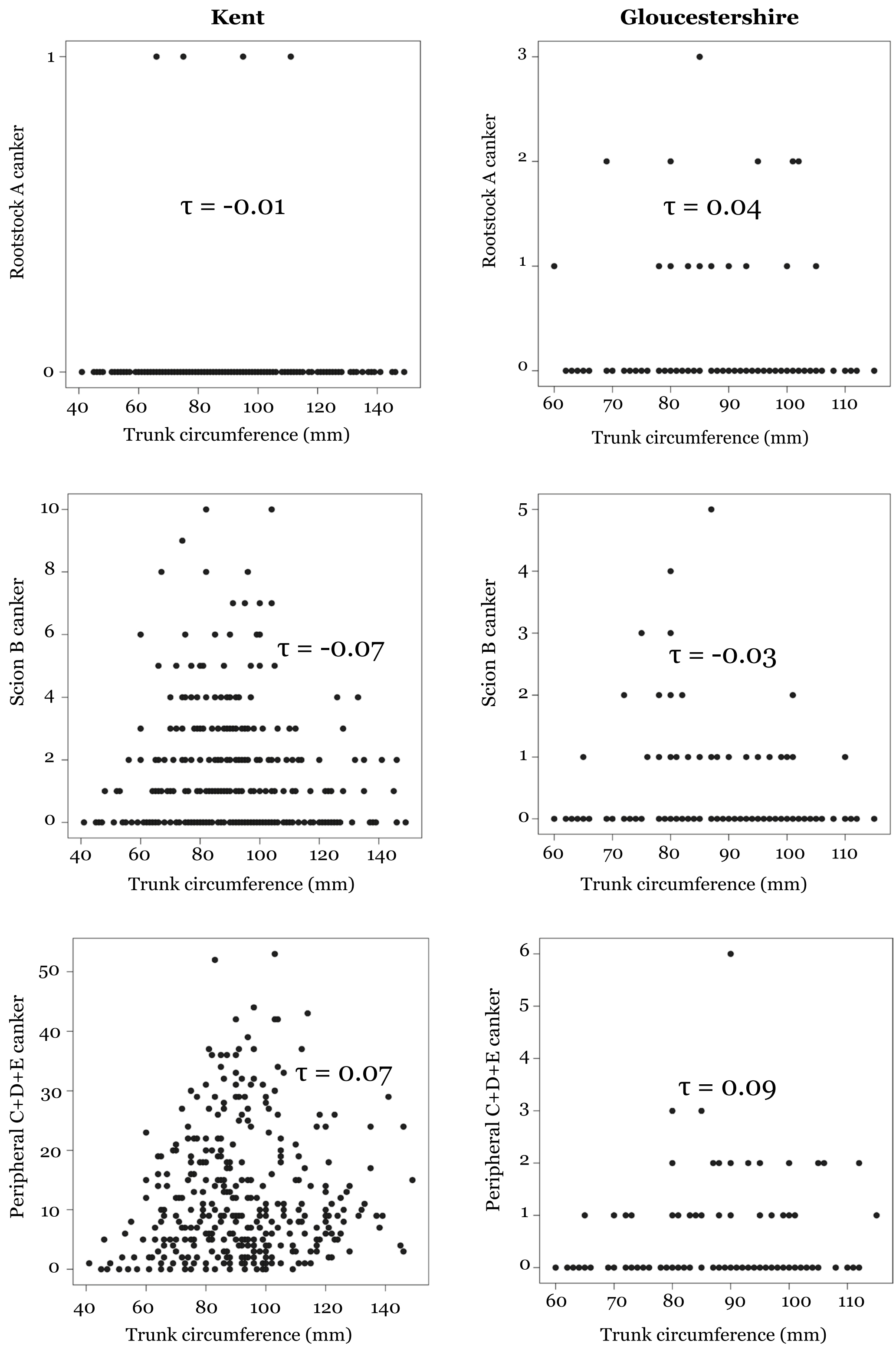

Fig. 3 The association measured using Kendall's rank correlation coefficient (τ) between apple trunk circumference and rootstock A canker, scion B canker and peripheral C + D + E canker numbers in 2020 in the experiments in Kent and Gloucestershire, respectively. Kendall's rank correlation coefficient (τ) for each variable are displayed on graphs. It is a measure of rank correlation, the ordinal association between the two variables represents the binary relation and then normalises the distance such that it will take values between − 1 and + 1

Table 3 The overall ranking of apple rootstocks for different variables including cumulative dead trees from canker, rootstock A canker numbers, scion B canker numbers, and peripheral C + D + E canker numbers in the Kent and Gloucestershire experiments assessed in 2020

Variable, year, location	Rootstock													
	M9 337	**M9 337 with GD interstock**	**M9 EMLA**	**MM106**	**M116**	**M26**	**G11**	**G41**	**EMR-001**	**EMR-002**	**EMR-003**	**EMR-004**	**EMR-005**	**EMR-006**
Dead trees from canker assessed in 2020. Kent	7	9	9	5	1	6	3	2	8	4	9	10	3	1
Dead trees from canker assessed in 2020. Gloucestershire	9	11	7	4	2	10	2	5	9	8	6	3	1	1
Rootstock A canker number. 2020. Kent	1	1	7	8	2	6	1	5	3	1	1	4	1	1
Scion B canker number. 2020. Kent	10	6	4	3	1	5	11	12	13	2	7	7	9	8
Peripheral C + D + E canker number. 2020. Kent	11	5	6	1	2	8	13	14	12	9	4	3	10	7
Rootstock A canker number. 2020. Gloucestershire	12	11	9	7	14	10	5	4	8	13	6	1	3	2
Scion B canker number. 2020. Gloucestershire	12	11	9	14	4	5	1	6	8	13	3	2	10	7
Peripheral C + D + E canker number. 2020. Gloucestershire	11	1	13	10	3	4	12	14	9	2	5	7	8	6
Overall ranking value	73	55	64	52	**29**	54	48	62	70	52	41	**37**	45	**33**

Lower ranking values indicate lower susceptibility to canker. The three most resistant were M116 (29 overall ranking value), EMR-006 (33 overall ranking value) and EMR-004 (37 overall ranking value) highlighted in bold in the last row of the table. Data is based on the backtransformed means (Additional file 1: Tables S3, S4, S5, S6, S7, S8, S9, S10) significant wood age effects regarding canker incidence and lesion length. Three year-old wood had a higher number and longer canker lesions, than either 2 year-old or 1 year-old wood, suggesting the older wood of these cultivars when grafted on these two rootstocks, was more susceptible to canker.

From our data it is unclear whether the use of a GD interstock reduced canker numbers. There did appear to be a reduced canker number compared to M9 337 alone, with overall ranking values of 55 versus 73, however based on tree death from canker, M9 337 grafted with GD interstock had a higher percentage of dead trees than M9 337 in both the Kent and Gloucestershire experiments. Research by Johnson et al. (1982) found that apple interstock cultivar was not a significant factor affecting apple tree mortality from canker, whereas scion cultivar was a highly significant factor. Differences in susceptibility to canker between apple scion cultivars are well documented (Van de Weg 1989; Scheper et al. 2018; Weigl 2021; Xu et al. 2022). Our work agrees with these studies that there is a cultivar effect on canker, and specifically, an association between rootstock cultivar and canker of Gala scions, but also depending on the orchard location. In the Kent experiment, by 2020 tree death ranged from 6.25% to 31.25% depending on rootstock, and in the Gloucestershire experiment from 5 to 30% depending on rootstock.

The other potential effects of the observed differences in canker susceptibility include genetic effects of the specific rootstock/scion combination, physiological effects (hormones, water and nutrient transport, gas exchange), and environmental and location effects. Karlström et al. (2022) studied a multi-parental population, comprising 317 individuals from four full-sib and one half-sib family and their parents that were selected based on a subset of progeny from each family showing segregation for resistance to canker. Individual quantitative trait loci (QTL) were found with low to medium effects on resistance to *N. ditissima* and the authors recommended that multiple QTLs be considered to improve canker resistance. Regarding other molecular mechanisms in apple, a phloem RNA transport system has been suggested to be involved between the rootstock and scion of apple trees. Kanehira et al. (2010) analysed cDNAs derived from apple shoot phloem cells and detected several mRNAs previously reported in other plants as phloem transported. The most abundant cDNAs in phloem cells encoded antioxidants that are related to responses to oxidative stress. In a study by Jensen et al. (2012), the effect of apple rootstock on the gene-expression of Gala scions inoculated with *Erwinia amylovora* (fireblight) showed gene expression patterns correlated with differences in rootstock-regulated susceptibility through metabolic pathways involving sorbitol dehydrogenase, phenylpropanoid, protein processing in the endoplasmic reticulum, and endocytosis.

Hormonal responses and sugar transport are additional factors affected by rootstock/scion interaction that may affect canker susceptibility. Kviklys and Samuolienė (2020) investigated the relationship between rootstock cultivar, hormone signalling, and sugar content of apple trees. Scion cultivars Ligol and Auksis were grafted on to five rootstocks M26 (semi-dwarfing), M9 (dwarfing), B396, P67, and P22 (super-dwarfing). The phytohormones zeatin, jasmonic acid, indole-3 acetic acid (IAA), abscisic acid (ABA), gibberellins 1, 3, and 7, and soluble sugars glucose, fructose, and sorbitol were evaluated. Dwarfing rootstocks M9, B396, and P67, as well as super-dwarf P22 produced an altered accumulation of promoter phytohormones, while the more vigorous semi-dwarfing M26 had higher glucose and inhibitory phytohormones, with increased IAA, ABA, and GAs. Rootstock and scion combinations affect phytohormone signalling and sugar transport, and potentially have flow on effects to apple tree health including infection by *N. ditissima*.

Other functional effects of apple rootstocks on the scion include water potential, leaf gas exchange and carbon isotope signatures. Casagrande Biasuz and Kalcsits (2022) studied 9 apple rootstocks grafted with a cultivar Honeycrisp scion. Rootstocks with a high dwarfing capacity had lower stem water potential, lower net CO_2 assimilation, and lower stomatal conductance than the more vigorous rootstocks. Work by Kviklys et al. (2017) found significant differences in leaf and fruit mineral content of trees grafted on various rootstocks and that more vigorous rootstocks accumulated more minerals in leaves and fruits (P, K) than a super-dwarf rootstock. They suggested that rootstock can potentially be used for tolerance to unfavourable soil nutrient conditions.

Nutrients in apple may also be affected by rootstock/scion combinations (Abdalla et al. 1982). Leaf nutrient composition (N, P, K, Ca, Mg, Mn, Fe, Zn, Cu) was measured in cultivar Delicious scion grafted on 21 rootstock with interstock combinations classified according to vigour (standard, semi-standard, semi-dwarf, dwarf). Significant differences in leaf P, K, and Mn were observed between the four vigour classifications. P and K for example were higher in leaves of standard sized trees than in leaves of dwarf trees, however leaves of dwarf trees had higher Mn. Fruit yield was correlated negatively to tree size and leaf K, and positively correlated to leaf Mg and Mn. This indicated that different rootstock/scion combinations and tree vigour impacted nutrient transport in the plants and affected fruit yield. Slowinski and Sadowski (2001) tested 22 apple rootstocks and found that variation in leaf nutrient concentrations due to seasonal effects was often larger than rootstock effects.

Ebel et al. (2000) studied the influence of 8 rootstocks with and without 4 interstocks on foliar concentrations of N, P, K, Ca, Mg, Mn, Fe, Zn, and B on cultivar Golden Delicious 'Smoothee' scion. K decreased to deficiency concentrations by the end of the experiment in all but the more vigorous seedling rootstock. Over the course of the experiment, foliar Ca increased in trees with M9 EMLA and EM26 EMLA rootstocks across years. They also reported that individual rootstocks varied in the rate at which they approach toxicity and deficiency concentrations, with foliar Mn increasing to nearly toxic concentrations in EM27 EMLA and Mark rootstocks, but not in other rootstocks. Amiri et al. (2014) also reported that apple rootstocks had an effect on mineral uptake and translocation to scions of cultivars Royal Gala and Golden Delicious, and impacted vigour and fruit yield. These results suggest that rootstock genotype has a strong influence on the transport of mineral nutrients to the scion. Nutrient testing may be beneficial for monitoring and supplementing the nutritional status of individual rootstock/scion combinations in orchard settings.

Factors including the location of an orchard and the environmental conditions affect the incidence of canker (Xu et al. 2022; Saville and Olivieri 2019; Liu et al. 2018). In our datasets, trees in the Kent experiment had 15.7 times higher total canker number (5029) compared to those in the Gloucestershire experiment (320). This suggests location has a strong influence on canker, particularly peripheral cankers that are more likely to occur in the orchard after planting. Interestingly, there was a similar overall percentage of tree death due to canker in the Kent experiment (15.18%) and the Gloucestershire experiment (16.79%), indicating this variable may be less influenced by location, and may be more likely due to infections occurring at the nursery stage (Børve et al. 2018).

While canker susceptibility is an important consideration in apple rootstock breeding programs, there are other factors for growers to consider when selecting rootstocks. These include effects of tree vigour and suitability for training on high density trellis systems, fruit yield, yield efficiency, fruit quality, suckering, graft compatibility, nutrient uptake efficiency, precocity, tolerance to specific soil types, susceptibility to pests and other diseases, and responses to any other stress events (Fazio et al. 2015).

Conclusion

Rootstock selection may be an alternative management method to conventional chemicals for reducing canker in the scion of cultivar Gala but may depend on the orchard location. We identified M116, and the NIAB East Malling advanced selections EMR-006 and EMR-004 as having the lowest numbers of cankers, and dead trees from canker. There is likely an interplay of factors affecting canker such as orchard location effects including rainfall, relative humidity, temperature, wind, soil type, topography, aspect, and other seasonal pathogen-related processes such as inoculum load, and rainsplash of inoculum between trees and within individual trees. The genetics and physiology of each scion/rootstock combination, water and nutritional status of trees, hormones and molecular signalling may also be involved, and further work is needed to elucidate the specific effects of these factors on canker.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43170-023-00177-z.

Additional file 1: Table S1. Results of Shapiro-Wilk test for normality of dead tree data, rootstock A canker, Scion B canker and C+D+E canker data from Kent, and Gloucestershire. The Shapiro-Wilk tests the hypothesis that the distribution of the data deviates from a normal distribution. The test rejects the hypothesis of normality when $p \leq 0.05$. **Table S2.** Results of Bartlett's test for homogeneity of variances to test if rootstocks within a variable have equal variances. Some statistical tests, for example the analysis of variance, assume that variances are equal across samples/groups. If the p-value that corresponds to the test statistic is less than some significance level $p \leq 0.05$, then we reject the null hypothesis and conclude that not all groups have the same variance. **Table S3.** Results of the generalised linear mixed model analysis of the cumulative numbers of dead apple trees due to canker in the experiment at East Malling, Kent, in 2020. Apple trees of cultivar Gala were grafted onto different rootstocks. **Table S4.** Results of the generalised linear mixed model analysis of the cumulative numbers of dead apple trees due to canker in the experiment at Poolhill, Gloustershire, in 2020. Apple trees of cultivar Gala were grafted onto different rootstocks. **Table S5.** Results of the generalised linear mixed model analysis of the numbers of rootstock A cankers on apple trees of cultivar Gala grafted onto different rootstocks in the experiment at East Malling, Kent, in 2020. **Table S6.** Results of the generalised linear mixed model analysis of the numbers of scion B cankers on apple trees of cultivar Gala grafted onto different rootstocks in the experiment at East Malling, Kent, in 2020. **Table S7.** Results of the generalised linear mixed model analysis of the numbers of peripheral C + D + E cankers on apple trees of cultivar Gala grafted onto different rootstocks in the experiment at East Malling, Kent, in 2020. **Table S8.** Results of the generalised linear mixed model analysis of the numbers of rootstock A cankers on apple trees of cultivar Gala grafted onto different rootstocks in the experiment at Poolhill, Gloucestershire, in 2020. **Table S9.** Results of the generalised linear mixed model analysis of the numbers of scion B cankers on apple trees of cultivar Gala grafted onto different rootstocks in the experiment at Poolhill, Gloucestershire, in 2020. **Table S10.** Results of the generalised linear mixed model analysis of the numbers of peripheral C + D + E cankers on apple trees of cultivar Gala grafted onto different rootstocks in the experiment at Poolhill, Gloucestershire, in 2020.

Acknowledgements

We acknowledge the four reviewers for advice and suggested changes to the manuscript, Robert Saville for assistance with project funding and experimental design, Xiangming Xu for helpful comments, Nigel Kitney and Chris Nicholson for management and assessments at the Gloucestershire site, Feli Felicidad Fernández Fernández for supplying rootstocks, and Tom Passey, Jennifer Kingsnorth, Sarah Cohen for assistance with the field experiment in Kent.

Author contributions

LAS wrote the article, completed the Kent assessments, data analysis and was the primary contact during the review process, SN completed the Gloucestershire assessments and assisted with writing the article, IK completed data analysis and wrote the article. All authors approved the manuscript prior to submission.

Funding
This work was funded by the Agriculture and Horticulture Development Board (AHDB), UK project TF223 'Improving integrated pest and disease management in tree fruit'.

Availability of data and materials
Datasets are available on reasonable request from the corresponding author.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The author declares that there are no commercial or financial competing interests.

Author details
[1]Department of Pest and Pathogen Ecology, National Institute of Agricultural Botany (NIAB), East Malling, Kent ME19 6BJ, UK. [2]RSK-ADAS Ltd., Helsby, Cheshire WA6 0AR, UK. [3]London South Bank University, London South Bank University Business School, London SE1 0AA, UK.

Received: 22 October 2022 Accepted: 8 September 2023
Published online: 28 September 2023

References

Abdalla OA, Khatamian H, Miles NW. Effect of rootstocks and interstems on composition of 'delicious' apple leaves. J Am Soc Hortic Sci. 1982;107:730–3.
Alston F. Apple rootstock tree named 'M116.' United States Plant Patent. Patent No. US PP18618P2. 2008. https://patents.google.com/patent/USPP18618P2/en
Amiri ME, Fallahi E, Safi-Songhorabad M. Influence of rootstock on mineral uptake and scion growth of 'golden delicious and "royal gala" apples. J Plant Nutr. 2014;37:16–29.
Amponsah NT, Scheper RWA, Fisher BM, Walter M, Smits JM, Jesson LK. The effect of wood age on infection by *Neonectria ditissima* through artificial wounds on different apple cultivars. N Z Plant Prot. 2017;70:97–105.
Børve J, Kolltveit SA, Talgø V, Stensvand A. Apple rootstocks may become infected by *Neonectria ditissima* during propagation. Acta Agric Scand B Soil Plant Sci. 2018;68:16–25.
CABI. NeoInvasive Species Compendium. *Neonectria ditissima* (Nectria canker (apple, pear)). 2020. https://www.cabi.org/isc/datasheet/35964.
Casagrande Biasuz E, Kalcsits LA. Apple rootstocks affect functional leaf traits with consequential effects on carbon isotope composition and vegetative vigour. AoB PLANTS. 2022;14(4):plac020.
Deakin G, Fernández-Fernández F, Bennett J, Passey T, Harrison N, Tilston EL, et al. The effect of rotating apple rootstock genotypes on apple replant disease and rhizosphere microbiome. Phytobiomes J. 2019;3:273–85.
Ebel RC, Caylor AW, Pitts JA, Wilkins BS. Mineral nutrition during establishment of golden delicious 'smoothee' apples on dwarfing rootstocks and interstems. J Plant Nutr. 2000;23:1179–92.
Fazio G, Robinson TL, Aldwinckle HS. The geneva apple rootstock breeding program in janick j plant breeding reviews. Oxford: Wiley-Blackwell Oxford; 2015.
Gómez-Cortecero A, Saville RJ, Scheper RWA, Bowen JK, Agripino De Medeiros H, Kingsnorth J, et al. Variation in host and pathogen in the *Neonectria/Malus* interaction; toward an understanding of the genetic basis of resistance to European canker. Front Plant Sci. 2016;7:1365.
Hunter P. The molecular biology of grafting. EMBO Rep. 2021;22: e54098.
Hutchinsons. Designs on light—The fruit grower. Hutchinsons—Crop Production Specialists. 2023. https://www.hlhltd.co.uk/designs-on-light/
Jensen PJ, Halbrendt N, Fazio G, et al. Rootstock regulated gene-expression patterns associated with fireblight resistance in apple. BMC Genom. 2012. https://doi.org/10.1186/1471-2164-13-9.
Johnson DL, Doust JL, Eaton GW. The effect of European canker and its spatial pattern on four apple cultivars in British Columbia. J Appl Ecol. 1982;19:603–9.
Kanehira A, Yamada K, Iwaya T, Tsuwamoto R, Kasai A, Nakazono M, et al. Apple phloem cells contain some mRNAs transported over long distances. Tree Genet Genom. 2010;6:635–42.
Karlström A, Gómez-Cortecero A, Nellist CF, Ordidge M, Dunwell JM, Harrison RJ. Identification of novel genetic regions associated with resistance to European canker in apple. BMC Plant Biol. 2022;22:452.
Kendall M. A new measure of rank correlation. Biometrika. 1938;30:81–93.
Korba J, Patáková K, Kůdela V. Effect of rootstock clones on fire blight susceptibility in scion apple cultivars. Plant Prot Sci. 2000;38:552–4.
Kviklys D, Samuolienė G. Relationships among the rootstock, crop load, and sugar hormone signaling of apple tree, and their effects on biennial bearing. Front Plant Sci. 2020. https://doi.org/10.3389/fpls.2020.01213.
Kviklys D, Lanauskas J, Uselis N, Viškelis J, Viškelienė A, Buskienė L, et al. Rootstock vigour and leaf colour affect apple tree nutrition. Zemdirbyste. 2017;104:185–90.
LandIS. World reference base: Luvisol. 2023a. https://www.landis.org.uk/soilsguide/wrb.cfm?wrbc=LV
LandIS. World reference base: Regosol. 2023b. https://www.landis.org.uk/soilsguide/wrb.cfm?wrbc=RG
Liu J, Abdelfattah A, Norelli J, Burchard E, Schena L, Droby S, et al. Apple endophytic microbiota of different rootstock/scion combinations suggests a genotype-specific influence. Microbiome. 2018;6:18.
Marini R, Fazio G. 2018. Apple Rootstocks. In: Warrington. (eds). Horticultural Reviews. John Wiley Sons. Hoboken
McCracken A, Berrie A, Locke B, Cooke L, Phelps K, Swinburne T, et al. Relative significance of nursery infections and orchard inoculum in the development and spread of apple canker (*Nectria galligena*) in young orchards. Plant Pathol. 2003;52:553–66.
Met Office. UK Climate averages. 2023a. UK Climate averages. East malling. 1991–2020. https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/u107dpz9j
Met Office. UK Climate averages. 2023b. UK Climate averages. Ross-on-Wye. 1991–2020. (Herefordshire). https://www.metoffice.gov.uk/research/climate/maps-and-data/uk-climate-averages/gcnpm68t8
PSU. Apple rootstocks: Capabilities and limitations. PennState Extension. 2018. https://extension.psu.edu/apple-rootstocks-capabilities-and-limitations
Rasool A, Mansoor S, Bhat KM, Hassan GI, Baba TR, Alyemeni MN, et al. Mechanisms underlying graft union formation and rootstock scion interaction in horticultural plants. Front Plant Sci. 2020. https://doi.org/10.3389/fpls.2020.590847.
Saville R, Olivieri L. Fungal diseases of fruit: apple cankers in Europe. In: Xu X, Fountain M, editors. Integrated management of diseases and insect pests of tree fruit. Cambridge: Burleigh Dodds; 2019.
Schall R. Estimation in Generalized Linear Models with random effects. Biometrika. 1991;78(4):719–27.
Scheper R, Fisher B, Taylor T, Hedderley D. Detached shoot treatments cannot replace wholetree assays when phenotyping for apple resistance to *Neonectria ditissima*. N Z Plant Prot. 2018;71:151–7.
Shuttleworth LA. Alternative disease management strategies for organic apple production in the United Kingdom. CABI Agric Biosci. 2021. https://doi.org/10.1186/s43170-021-00054-7.
Slowinski A, Sadowski A. Mineral element content in leaves of different apple rootstocks and of 'Elise' scion cultivar on the same rootstocks. Acta Hortic. 2001;564:309–16.
Swinburne T. European canker of apple (*Nectria galligena*). Rev Plant Path. 1975;54:87–799.
Utkhede RS, Smith EM. Field resistance of apple rootstocks to *Phytophthora cactorum* infection. J Hortic Sci. 1994;69:467–72.
Van de Weg WE. Screening for resistance to *Nectria galligena* Bres. in cut shoots of apple. Euphytica. 1989;42:233–40.

Weber R. Biology and control of the apple canker fungus *Neonectria ditissima* (syn. *N. galligena*) from a Northwestern European perspective. Erwerbs-Obstbau. 2014;56:95–107.
Weber R, Børve J. Infection biology as the basis of integrated control of apple canker (*Neonectria ditissima*) in Northern Europe. CABI Agric Biosci. 2021. https://doi.org/10.1186/s43170-021-00024-z.
Weigl T. Susceptibility of different apple cultivars to European canker. Masters Thesis. Norwegian University of Life Sciences. Faculty of Biosciences. 2021. https://nmbu.brage.unit.no/nmbu-xmlui/bitstream/handle/11250/2766964/Theresa_Weigl_Master.pdf?sequence=1
Xu X, Passey T, Robinson-Boyer L, Mclean H, Saville R, Papp-Rupar M. Development of European apple canker on different cultivars in relation to planting time at three sites in the UK. Front Hortic. 2022. https://doi.org/10.3389/fhort.2022.995776.

Publisher's Note