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ORIGINAL ARTICLE

Progression of European canker and wound types favoring *Neonectria ditissima* infection in apple trees of different ages in Brazil

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Abstract

Epidemiological knowledge in the apple-*Neonectria ditissima* pathosystem is fundamental to optimize disease management practices. Knowledge about disease progress and infection process can be useful for predicting European canker and improving disease management decisions. Thus, the objectives of the present study were (1) to monitor the European canker progression in apple trees established under different time intervals and (2) to study wound types that can be invaded by *N. ditissima*. European canker incidence (% of apple trees with symptoms) was monitored and recorded over time on apple trees established in the years 1990, 2009, and 2011. During the autumn of 2018 to 2020, each age group of the apple trees in the orchard were monitored and the canker numbers and wound types (leaf scars, pruning cuts, fruit picking wounds, and others) that caused *N. ditissima* infection were recorded. Results showed that European canker incidence significantly increased over time, regardless of the age of the apple trees. Fruit picking wounds were the most susceptible natural and/or artificial openings leading to *N. ditissima* infection followed by pruning cuts, leaf scars, and others among the apple trees of different ages over time. The pruning wounds were more important in younger apple trees than older ones because the apple trees were more affected by European canker over time. To the best of our knowledge, this is the first study in Brazil to monitor the European canker progression and determine the main wound types naturally infected by *N. ditissima* under field conditions in the apple orchard established in different years over time.

Keywords *Cylindrocarpon heteronema* · *Malus domestica* · *Nectria galligena* · A2 quarantine pest · Epidemiology · Integrated control

Introduction

European canker caused by *Neonectria ditissima* (Tul. & C. Tul.) Samuels & Rossman (syn. *Nectria galligena* Bres), anamorph *Cylindrocarpon heteronema* Berk. & Broome) Wollenw is an important apple disease that leads to the death of buds, shoots, spurs, and branches and, ultimately, the whole apple tree, when the canker girdles the main trunk (Beresford and Kim 2011; Weber and Børve 2021). Another important symptom caused by *N. ditissima* is eye rot of fruits, which is also a serious problem in apple-producing regions with favorable climatic conditions for the pathogen development (Xu and Robinson 2010; Beresford and Kim 2011; Weber 2014).

In Brazil, the fungus *N. ditissima* is an A2 quarantine pest, which means that the pathogen is already present in the country but exhibits restricted distribution and is targeted by an official control program (Carbonari and Rissi 2019; Gelain et al. 2020). However, despite this official control program, the disease incidence continues to increase in the main apple-producing regions in Brazil (Araujo et al. 2019a, b). Such elevation of disease incidence increases production cost because of the specific management measures needed to control the European canker (Araujo et al. 2019a, b). Furthermore, productivity is reduced due to the need to eliminate symptomatic

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branches and/or all apple trees in order to contain epidemic progress in the orchards (Araujo et al. 2019a, b).

In infected apple plants, *N. ditissima* produces conidia from sporodochia and ascospores from perithecia (Weber 2014; Campbell et al. 2016; Amponsah et al. 2017a). The *N. ditissima* needs either natural or artificial injuries in apple trees to penetrate and to colonize the tissues, and there are several wound types available during the productive cycle in orchards (Amponsah et al. 2015; Alves and Nunes 2017). Wounds caused by leaf fall in autumn, pruning cuts, petal fall, and fruit harvest are considered the most important natural and artificial openings for European canker development (Weber 2014; Alves and Nunes 2017). Successful infection of *N. ditissima* in plant injuries varies with local climates and wound types in apple-producing regions worldwide because temperatures, wetness, and lesion sizes affect differently the germination rates of spores (Latorre et al. 2002; Beresford and Kim 2011; Weber and Børve 2021). Plant ages and seasonal wound susceptibility to *N. ditissima* infection also have relative significance to European canker incidence (McCracken et al. 2003; Gómez-Cortecero et al. 2016; Alves and Nunes 2017; Amponsah et al. 2017b).

Epidemiological studies in pathosystem apple-*N. ditissima* are fundamental to optimize management practices. Understanding epidemics in the field can be useful to predict European canker infection risk and improve disease management decisions (Amponsah et al. 2015, 2017a). The favorable climatic conditions, the high amount of inoculum in orchards, in addition to a lack of experience by Brazilian fruit growers in the disease management may explain the increase of European canker in the southern region of Brazil (Araujo et al. 2019b; Czermainski and Alves 2019; Nunes and Alves 2019). Furthermore, in Brazil, no study has explored the patterns of European canker progression and wound type preference to natural infection by *N. ditissima* in apple orchards of different tree ages to date.

Thus, the objectives of the present study were (1) to monitor the European canker progression in apple trees established in different interval times and (2) to study wound types that facilitate *N. ditissima* penetration and further infection of the plant.

Material and methods

Experimental area

The present study was conducted in an experimental orchard of the Agricultural Research and Rural Extension Company of Santa Catarina (Epagri) in São Joaquim (SC), Brazil (28°17′39" S, 49°55′56" W, 1415 m altitude). The climate of the region is classified as humid (Cfb), according to Köppen, with mild summers and harsh winters. Figure 1 shows the precipitation and temperature data during the experimental period. An automatic weather station (ISIS, S1220-M, Squitter do Brasil®) installed at the experimental orchard was used to measure climate data every 10 min (Araujo et al. 2019a). Apple trees of three ages were used in this study. The orchards were established in three time periods (1990, 2009, and 2011) with the cultivars "Fuji" and "Gala" grafted on Marubakaido rootstock, although assessments began only in 2015. The apple trees from the different age groups were established side-by-side (neighbors). The trees (*Malus domestica*) were planted in a central-leader system, without a support system. "Fuji" was the pollinator, with one "Fuji" tree for every five "Gala" trees. The apple trees established in 1990 were planted with 5-m spacing between the rows and 3-m spacing between the trees, totaling 666 plants per

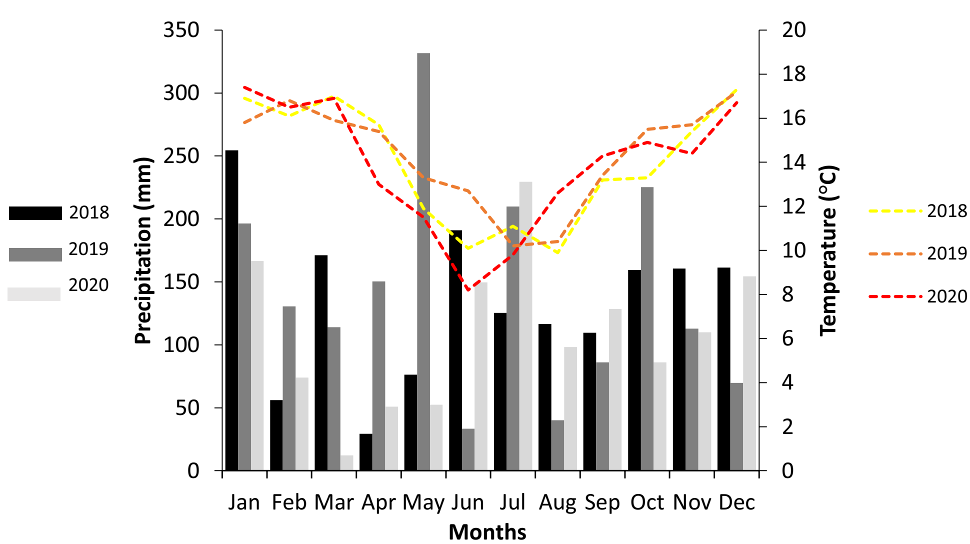

Fig. 1 Weather conditions (monthly temperature and precipitation based on daily means) of the experimental orchard located at the municipality of São Joaquim, in the state of Santa Catarina, Brazil. Data were collected every 10 min from an automatic weather station installed at the experimental station of São Joaquim/Epagri. São Joaquim, SC

hectare. Apple trees established in 2009 and 2011 were planted with 4.5-m spacing between the rows and 1.5-m spacing between the trees, totaling 1482 plants per hectare. Apple trees established in 2009 (Fig. 2a) and 2011 (Fig. 2b) have similar sizes, whereas apple trees planted in 1990 were larger (Fig. 2d). The soil of the experimental area was classified as Humic Cambisol (Araujo et al. 2020a, b). The experimental orchard was managed with reduced applications of pesticides (about half a commercial orchard) for diseases, insects, and weed control (Araujo et al. 2020a, b). Approximately, 15 fungicide and eight insecticide applications per season were performed to control scab and fruit rot, as well as the oriental fruit moth and fruit fly, respectively.

Fig. 2 Experimental orchards where the European canker disease progression was monitored over time. Apple trees cultivars Gala and Fuji established in 2009 (**a**), 2011 (**b**) and 1990 (**c**, **d**) naturally infected by *Neonectria ditissima* in an experimental orchard located at the municipality of São Joaquim, in the state of Santa Catarina, Brazil. Red arrows indicate where symptomatic branches were pruning during the European canker monitoring (twice a year). In apple trees established in 1990, the neighboring branches were very close (**c**, **d**) than those planted in 2009 (**a**) and 2011 (**b**). Front view of apple trees established 2011 (left) and 1990 (right) (**e**)

Experimental design

A 4 × 3 × 3 factorial experiment consisting of wound types (wound leaf scars, pruning cuts, fruit picking, and others) × evaluation years (2018, 2019, and 2020) × orchard establishment year (different plant ages) selected randomly to represent a completely randomized design with four replications of apple trees planted in 2009 and 2011 and five replications of apple trees planted in 1990. Each replication consisted of a row with 40 randomly selected apple trees. Data for European canker incidence (percentage of apple trees with symptoms relative to asymptomatic trees) and canker numbers (were counted the cankers relative only to symptomatic trees) were transformed to square root of x before statistical analysis. The data were analyzed by an analysis of variance (ANOVA) and treatment means was compared using Tukey's test ($P \leq 0.05$) using the R statistical environment.

European canker progression in the orchard

Disease incidence was recorded by visual assessment of typical European canker symptoms. The tree number affected by European canker was observed and recorded over time (2015 to 2020 year). Two observations for disease incidence were performed in June and September in each year. In the experimental orchard established in 2009 and 2011, four rows with 50 apple trees for each age group were examined. In the experimental orchard established in 1990, five rows with 40 apple trees were observed. In all the three experimental orchards, a total of 600 trees were inspected (200 apple trees in each age group). Symptomatic apple trees naturally infected with *N. ditissima* under field conditions were recorded for disease incidence. Disease incidence was recorded as the percentage of apple trees with European canker symptoms relative to asymptomatic trees using the methodology of Adnan et al. (2017). The fungus *N. ditissima* found in the orchard established in 1990 and 2009 was known to have been introduced from new apple trees planted in 2011 (Personal communication Iran Souza Oliveira), since the first symptomatic apple trees were observed only in 2015. Infections of trees from the nurseries may enter a state of latency from which they can break out up to 3 years after the trees have been planted into the commercial orchard (Weber 2014).

Wound types and *Neonectria ditissima* infection in apple trees established in different years

During the autumn of 2018 to 2020 in each age groups of apple trees (orchard established in 1990, 2009, and 2011), the canker numbers and wound types that *N. ditissima* uses to penetrate and cause infection in symptomatic apple tissues were recorded. The wound types visually observed were wound leaf scars, pruning cuts, fruit picking wounds, and others (rasp wound, broken branch, hail, and insect). European canker was observed by visual assessment of typical disease symptoms or when in doubt, a hand lens was used to check the presence of fruiting bodies of the fungus (sporodochial and/or perithecia). Symptoms of European canker and signs of *N. ditissima* were examined on the whole apple tree in each age group (in total 600 apple trees). Cankers without fruiting visible bodies (visual assessment) had the symptoms confirmed through identification of *N. ditissima* in the laboratory. Here, internal necrotic tissues from field samples were disinfected by immersing in 70% alcohol solution for 1 min, followed by 1% sodium hypochlorite solution for 3 min, and three rinses with sterilized distilled water. Pieces of about 5 mm from internal necrotic and healthy tissues (area of transition) was plated on agar medium (dehydrated agar; 15 g/L; Difco; USA) supplemented with streptomycin (streptomycin sulfate; 40 mg/L; Sigma Chemicals; USA). When colonies started to form (about 4 days), mycelial plugs were transferred to Petri dishes with potato dextrose agar (PDA; potato extract 10%, glucose 51%, agar 39%; 39 g/L; Scharlau; Spain) medium, and incubated for 15 days at 20 °C in the dark using the methods of Ghasemkhani et al. (2016) and Gelain et al. (2020). Upon isolation on PDA culture medium, all isolates were confirmed positive for *N. ditissima* by characteristics of colony color and conidia type, according to Campos et al. (2017). The isolates obtained were preserved using Castellani's method (Dhingra and Sinclair 1995). During the autumn of 2015 when we found the first symptomatic trees, the trees were removed (61 apple trees). However, during the autumn of 2016 to 2020, apple trees affected by European canker were no more removed, but symptomatic branches were quantified as described previously before removing from apple trees inspected (twice a year).

Results

Climatic conditions

The trends of temperature were similar in 2018, 2019, and 2020 (Fig. 1). The total rainfall recorded in 2018 (1611 mm) and 2019 (1700 mm) were similar but apparently different from that observed in 2020 (1312 mm) (Fig. 1).

European canker progression in the orchards

The factor evaluation years and orchard establishment year were significant for European canker incidence (Fig. 3). The factors (evaluation years) × (orchard establishment year) interactions were not significant for European canker incidence (data not shown). European canker incidence

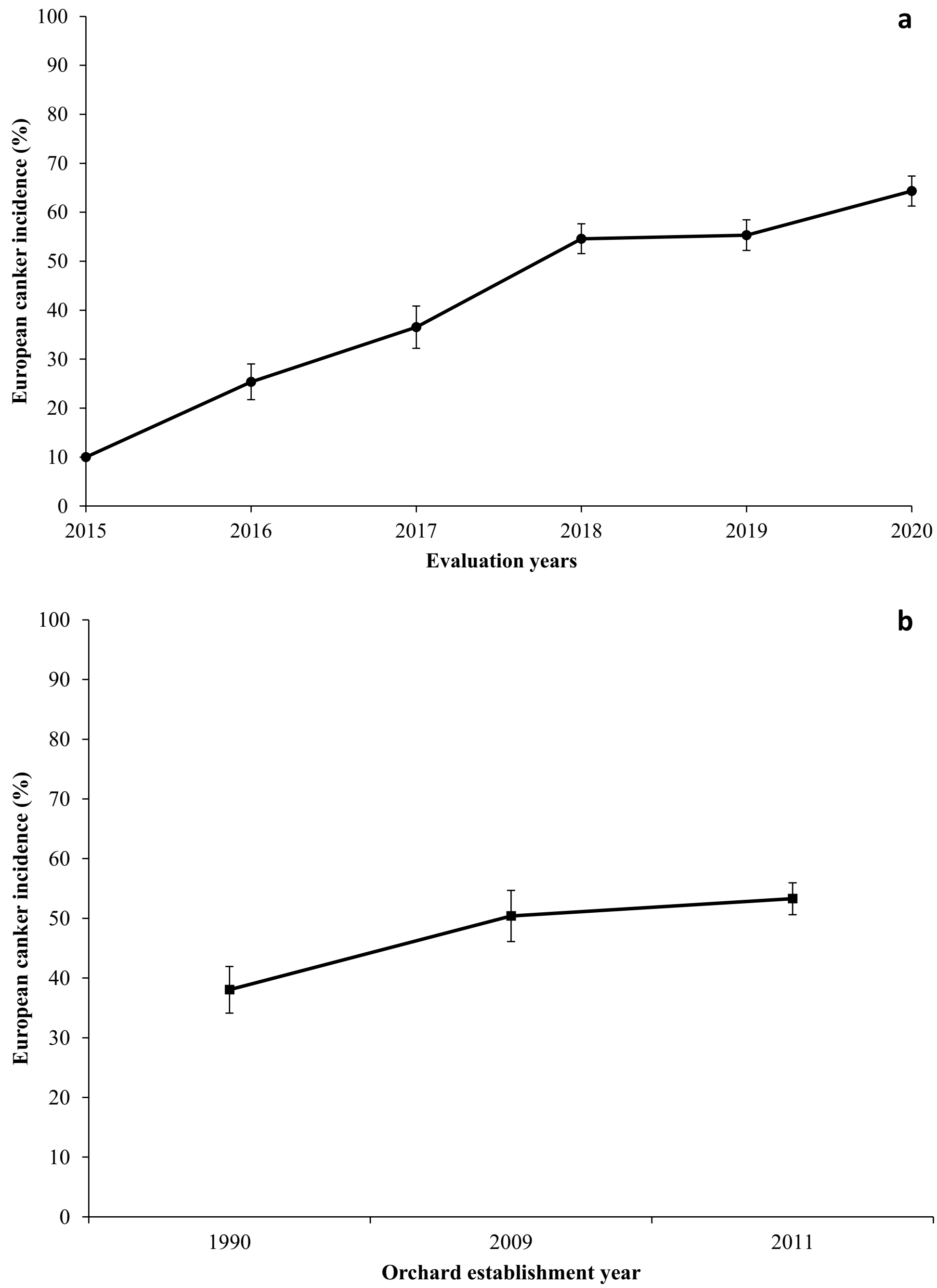

Fig. 3 European canker incidence monitored over time (**a**) in apple trees cultivars Gala and Fuji established in 1990, 2009, and 2011 years (**b**) naturally infected by *Neonectria ditissima* in an experimental orchard located at the municipality of São Joaquim, in the state of Santa Catarina, Brazil. Disease incidence was recorded as the percentage of apple trees with European canker symptoms relative to asymptomatic trees. Means within each column followed by the same letter are not significantly different ($P \leq 0.05$; Tukey's test)

significantly increased over time (Fig. 3a). In Fig. 3a, there was no statistical difference between evaluation years 2016 (25%) and 2017 (36%), which were statistically lower that evaluation years 2018 (54%), 2019 (55%), and 2020 (64%) which were statistically the same. European canker incidence in apple trees established in 2011 (53%) and 2009 (50%) was higher than that observed in apple trees planted in 1990 (38%) year (Fig. 3b).

Lesions of European canker in apple trees established in different years over time

The factors for wound types, evaluation years, and orchard establishment year (Fig. 4), as well as the factor interaction (data not show) were significant for canker numbers. The breakdown of the interactions among the factors studied was shown separately (Figs. 5 and 6).

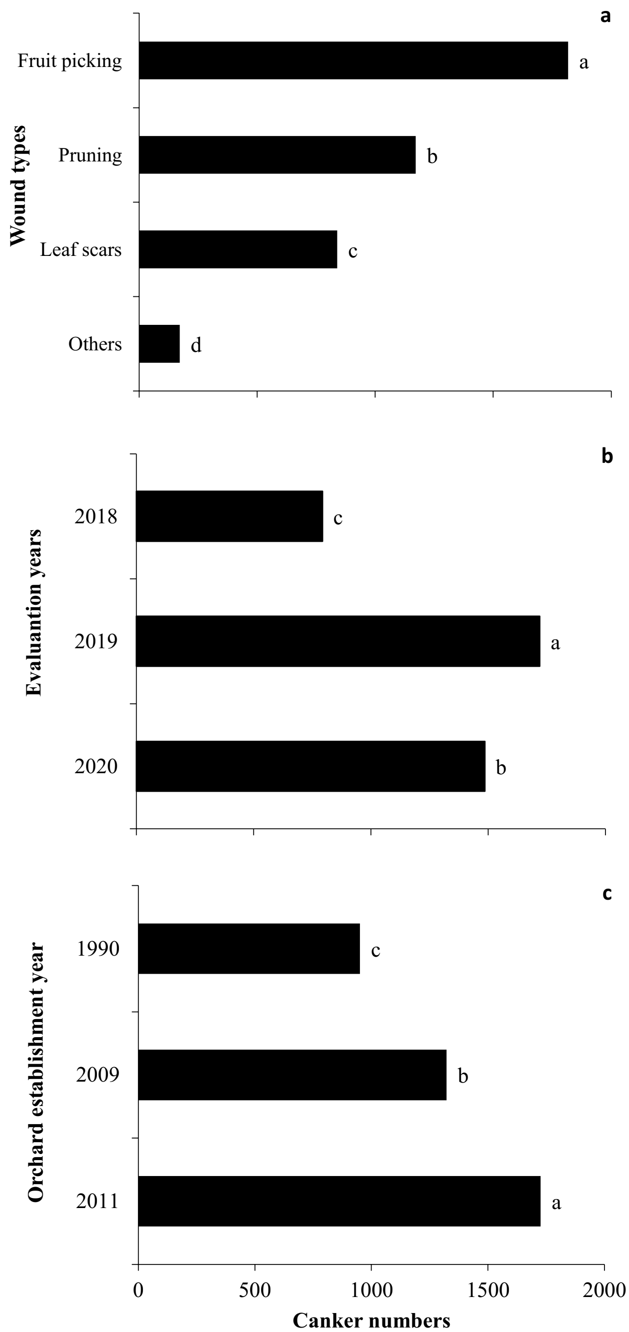

Fig. 4 Number of European cankers in different wound types (**a**) and evaluation years (**b**) in apple trees cultivars Gala and Fuji established in 2011, 2009, and 1990 years (**c**) naturally infected by *Neonectria ditissima* in an experimental orchard located at the municipality of São Joaquim, in the state of Santa Catarina, Brazil. In the experimental orchard was inspected 200 apple trees in each age group of which 168 were Gala and 32 Fuji. Means within each column followed by the same letter are not significantly different ($P \leq 0.05$; Tukey's test)

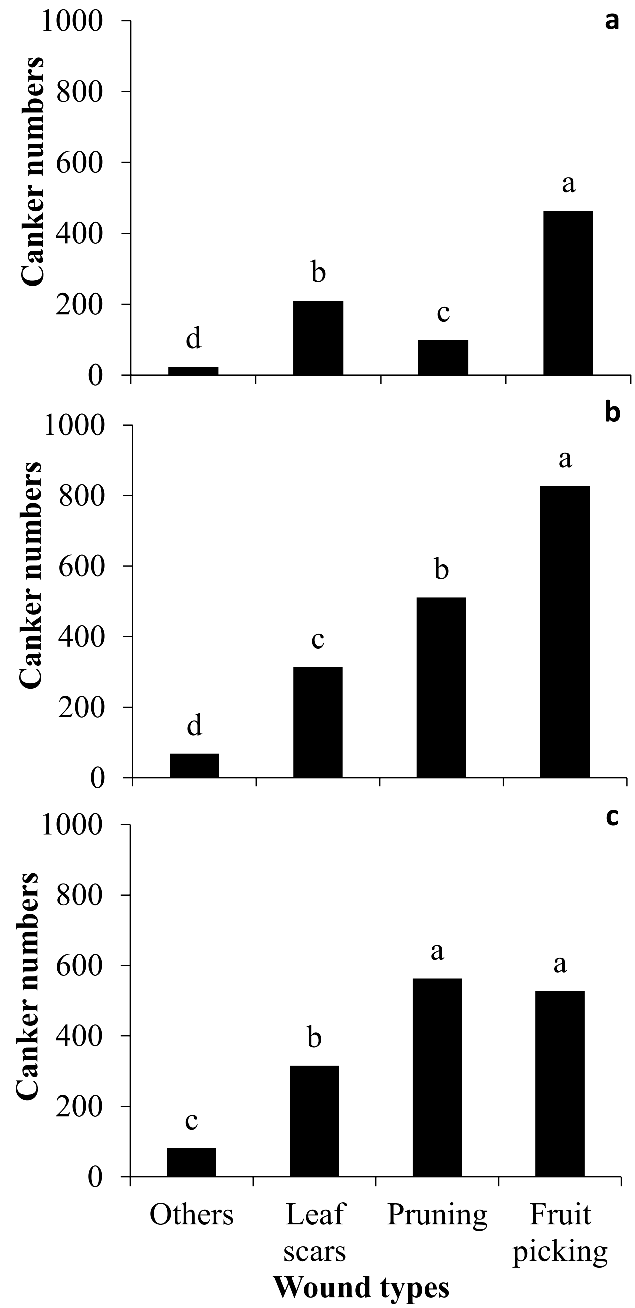

Fig. 5 Number of European cankers in different wound types in apple trees cultivars Gala and Fuji naturally infected by *Neonectria ditissima* in an experimental orchard located at the municipality of São Joaquim, in the state of Santa Catarina, Brazil at 2018 (**a**), 2019 (**b**), and 2020 (**c**) years. The total canker number from apple plants in each evaluation year was summed, independent of each age group. In the experimental orchard was inspected 200 apple trees in each age group of which 168 were Gala and 32 Fuji. Means within each column followed by the same letter are not significantly different ($P \leq 0.05$; Tukey's test)

For the wound type factor, the main European canker wounds identified in order of importance were in fruit picking (1.817), pruning cuts (1.172), leaf scars (839), and others (172) (Fig. 4a). For the observation year factor, the canker number was significantly higher for 2019 (1.720) followed by 2020 (1.486) years in comparison to cankers observed in

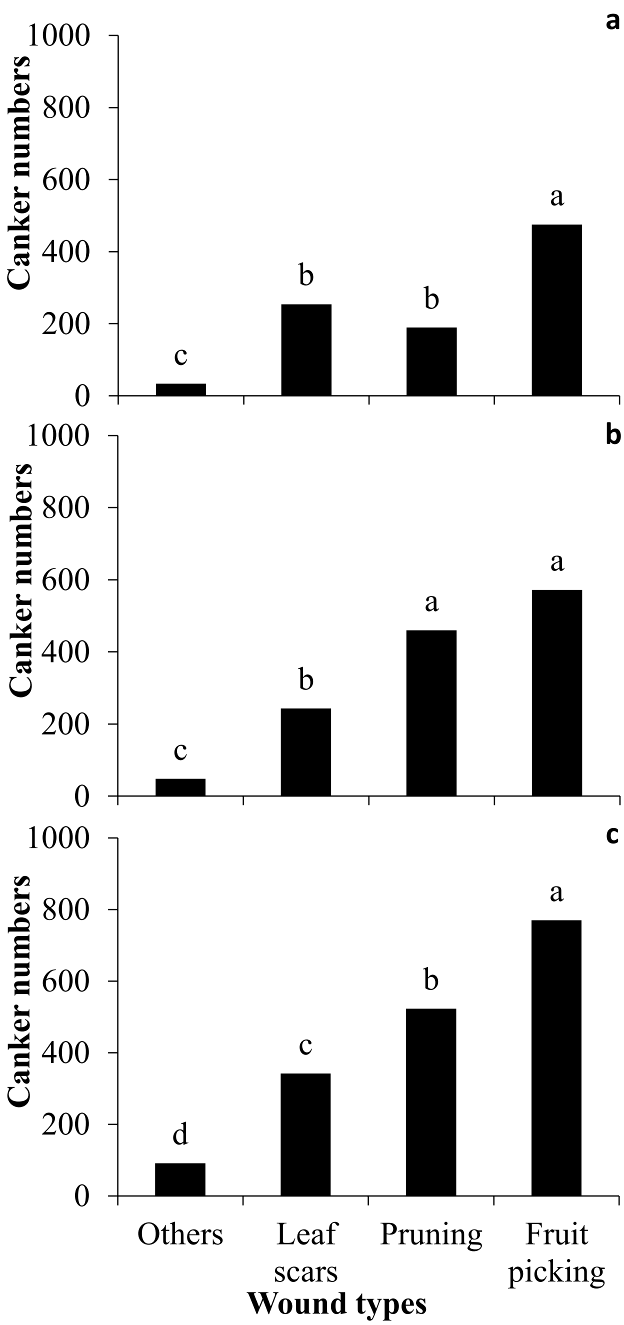

Fig. 6 Number of European cankers in different wound types in apple trees cultivars Gala and Fuji established in 1990 (**a**), 2009 (**b**) and 2011 (**c**) year, naturally infected by *Neonectria ditissima* in an experimental orchard located at the municipality of São Joaquim, in the state of Santa Catarina, Brazil. The total canker number from apple plants in each age group was summed, independent of each evaluation year. In the experimental orchard was inspected 200 apple trees in each age group of which 168 were Gala and 32 Fuji. Means within each column followed by the same letter are not significantly different ($P \leq 0.05$; Tukey's test)

2018 (794) year (Fig. 4b) which was the least. For orchard establishment year factor, the canker number was higher in apple trees established in 2011 (1.726), followed by 2009 (1.323) than that observed in the apple trees planted in 1990 (951) (Fig. 4c) which was the least.

In the year 2018, the most wound types found to be infected with cankers were fruit picking wounds (463), followed by leaf scars (210), pruning cuts (98), and others (23) (Fig. 5a). In the year 2019, the most wound types in cankers were also fruit picking wounds (827), followed by pruning cuts (511), leaf scars (314), and others (64) (Fig. 5b). However, in the year 2020, the most wound types infected with cankers were pruning cuts (563), followed by fruit picking wounds (527), leaf scars (315), and others (81) (Fig. 5c).

In the apple trees planted in 1990 (Fig. 6a), the most wound types infected by *N. ditissima* found were fruit picking wounds, followed by leaf scars, pruning cuts, and others. In the apple trees established in 2009 (Fig. 6b) and 2011 (Fig. 6c), the most wound types found were fruit picking wounds, followed by pruning cuts, leaf scars, and others.

The damage (architecture) of younger apple trees (Fig. 2a, b) were more than older trees (Fig. 2c, d), due to the excessive pruning of main branches with disease symptoms during the European canker monitoring.

Discussion

The results of the present study showed that European canker incidence in Brazil significantly increased over time, regardless of the apple tree ages. Fruit picking wounds were the most favorable natural and/or artificial openings to *N. ditissima* infection followed by pruning cuts, leaf scars, and others in apple trees of different ages over time. To the best of our knowledge, the present epidemiological study is the first in Brazil to monitor European canker disease progression and to determine the main wound types naturally infected by *N. ditissima* under field conditions in apple tree established in different years over time.

In the experimental orchard with apple trees of different ages, we observed that the European canker incidence increased from 10 to 64% overtime (5 years). According to Adnan et al. (2017), apple orchards with disease incidence exceeding 25% are classified as high risk for European canker and can be severely affected by fungus. In orchards with medium to high European canker incidence, the disease can increase the production cost to around 21%, reduce the productivity about 15% and decrease the longevity of apple trees from 20 to 12 years (Lazzarotto and Alves 2015). In this same scenario, the replanting of a new orchard may be the best option for the fruit growers due to the low production potential of apple trees severely affected by European canker (Lazzarotto and Alves 2015). In Brazil, mountainous regions in the state of Santa Catarina are the most suitable for apple production because of favorable climatic conditions (highest temperatures and precipitations); however, these conditions also favor the development of diseases (Araujo et al. 2019a, 2020a, b). In Balochistan, higher canker incidence was observed in apple orchards established in warmer and wetter areas (Adnan et al. 2017). In the present study, elimination of symptomatic branches (twice a year) were not efficient for preventing European canker progression in the experimental orchards, most likely due to favorable climatic conditions to European canker disease development in the country. Thus, rigorous management measures should be adopted to prevent European canker dissemination in Brazilian apple orchards, such as frequent monitoring of symptoms in orchards for early detection and diagnosis of the disease. In Brazil, we developed an app for cell phones in the Android (Cancontrol 2022a) and IOS (Cancontrol 2022b) versions and a web system (Cancontrol 2022c) to help technicians and fruit growers in the early detection and identification of European canker symptoms. European canker identification, integrated management measures such as repeated pruning-out of cankers, removal of tissues with symptoms, and prompt and timely treatment with effective fungicides to protect fresh natural or artificial wounds, are considered the basis for the successful control of European canker (Weber and Børve 2021). This should also be a practice to be implemented in Brazil.

European canker incidence in apple trees established in 2009 and 2011 was higher than that observed in apple trees established in 1990. However, the wood age can be involved with higher levels of European canker incidence (McCracken et al. 2003; Amponsah et al. 2017b), other factors may be discussed in relation to different susceptibility of apple trees established in different periods of time, such as spacing between plants and excessive vegetative growth (Weber and Børve 2021), and/or different date/local of orchard European canker introduction (Weber 2014) in the experimental orchards. The older apple shoots (3 years) from Royal Gala cultivar was more susceptible than the younger shoots (1 and 2 years) artificial infected by *N. ditissima* (Amponsah et al. 2017b). However, European canker can be more damaging in young than in oldest orchards where in some years, up to 10% of trees can be lost annually in the first few years of orchard (McCracken et al. 2003; Gómez-Cortecero et al. 2016). Damage is greater in young plants because often *N. ditissima* infects the stem (central-leader) or main branches, whereas in older plants the fungus affect preferably the branch minors (Lazzarotto and Alves 2015). In the present study, the architecture of younger apple trees was more affected than older apple trees due to the excessive pruning of main branches with disease symptoms during the European canker monitoring. Furthermore, the first European canker symptoms in the experimental orchard (used in this study) were observed in apple trees planted in 2011 (Personal information Iran Souza Oliveira).

In our study, the spacing between the plants adopted was greater for the older apple trees (3 m) than the younger (1.5 m) established in different periods of time. However, older apple trees were larger in comparison to younger and have neighboring branches very close (largest vegetative growth). Iorio et al. (2019) indicated a clustered spatial pattern of European canker in an apple orchard with high disease incidence. Non-random spatial patterns can infer processes influencing the management of the disease (Iorio et al. 2019). Thus, some explanations for the existence and subsequent changes in non-random spatial patterns are as simple as tree removal and new block planting; other explanations involve multiple environmental (e.g., rainfall and humidity), management (e.g., fungicide, pruning, and plant spacing) and apple cultivar factors including the interactions between these (e.g., timing of pruning and inoculum availability) (Iorio et al. 2019). In European canker lesions, after rain conidia and ascospores, respectively, are released which can travel around 1 to 4 m and few kilometers from the inoculum source, respectively (Weber 2014; Gómez-Cortecero et al. 2016; Nunes and Alves 2019; Weber and Børve 2021). These dispersal mechanisms lead to non-random spatial patterns and progression of the disease (Campbel et al. 2016). European canker was first recorded in Brazil in 2002 in nursery trees; however, it was only in 2012, that it was confirmed in apple orchards (Carbonari and Rissi 2019). Canker number and lesion length caused by *N. ditissima* can differ from orchard to orchard by possible factors: inoculum level/disease pressure, differences in cultivar susceptibility, time of infection during the year, plant age when inoculum is introduced, and local climatic conditions (Weber 2014; Amponsah et al. 2017b). Thus, the spacing between plants, as well as the date establishment (1990) of older apple trees may have contributed to the lowest levels for lesion number and European canker incidence in comparison with plants implanted in 2009 or 2011 (younger apple trees).

In younger apple trees (established in 2011 and 2009), the most susceptible wounds to *N. ditissima* infection were fruit picking wounds, pruning cuts, leaf scars, and others, whereas in older plants (established in 1990) it was in fruit picking wounds, leaf scars, pruning cuts, and others. Differences in wound susceptibility to *N. ditissima* infection occurred throughout the year, between trees, orchards and apple cultivars (Amponsah et al. 2015; Alves and Nunes 2017). According to Amponsah et al. (2015) pruning cut wounds were the most susceptible, followed by fruit picking wounds and leaf scars using artificial inoculation of *N. ditissima* conidia. In contrast, in commercial orchards under natural infection with *N. ditissima*, fruit picking wounds were more found in comparision to pruning cuts (Amponsah et al. 2015). Often the presence of few spores of *N. ditissima* on large wound can result in infection with the shorter latent period (Walter et al. 2016). However, because pruning wound can be protected in commercial orchards with paints and pastes which contained fungicides, minor wound such as fruit picking can be considered the most important entry sites for *N. ditissima* in apple trees (Alves and Nunes 2017; Weber and Børve 2021). Although, Alves and Nunes (2017) observed differences in the susceptibility of infection sites of apple trees to *N. ditissima* (pruning and picking wounds are very susceptible to the pathogen), when monthly inoculations of this fungus were performed on various wound types (bud scar, petal scar, thinning scar, leaf scars, picking wound scars, and pruning wounds) on cvs "Gala" and "Fuji" throughout the year in Brazil. In this study of Alves and Nunes (2017), in warmer months the apple wounds were less susceptible to the *N. ditissima* in comparison to colder months because the metabolism is accelerated, and apple plants can be more effective to wound healing. According to Amponsah et al. (2017b), the European canker incidence and progression can be affected with wood age and apple tree cultivar. Thus, in this present study, high inoculum source in the experimental orchard, as well as the different conditions climatic (rainfall) observed over time, explain, at least in part, the final canker number in wounds on "Gala" apple trees established in three periods of time. The data also confirm that the pruning wounds were more important in younger apple trees than in older ones.

In conclusion, the present study demonstrated the need for the control of *N. ditissima* inocula throughout the year and the protection of wounds (fruit picking, pruning and leaf scars) on apple trees with fungicides all year long, including periods (autumn/winter) that Brazilian fruit growers hardly sprayed orchards. Furthermore, our data reinforce the need to ensure greater care to younger apple orchards than older ones because the younger apple trees were more affected by European canker over time.

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Author contribution All the authors contributed to the study conception and design. Epidemiological data were collected by Leonardo Araujo, Felipe Augusto Moretti Ferreira Pinto, Paulo Henrique da Silva Nogueira, and James Matheus Ossacz Laconski. The first draft of the manuscript was written by L. Araujo, and other authors commented on the previous versions of the manuscript. All the authors read and approved the final manuscript.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

Informed consent Informed consent was obtained from all the individual participants included in the study.

Conflict of interest The authors declare no competing interests.

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