HISTOLOGICAL ALTERATIONS DUE TO GRAPEVINE ESCA

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Summary
This work aimed to study the histological characteristics of Esca symptomatic leaves petioles and internodes sampled from grapevine plants of 'Cabernet Sauvignon', 'Sangiovese' and 'Trebbiano'. Materials were collected during three-year period from: symptomatic vines (SV); vines that never showed Esca symptoms (NES); vines that in the previous years resulted symptomatic and that could be expected to show again foliar symptoms (PYS). On these vines, before Esca appearance, were selected three shoots from which petioles were collected from basal and apical portions. After leaf symptoms appearance, petioles were collected from the apical leaves that had not yet exhibited Esca symptoms. The tissues were fixed in FAA, embedded in Histoplast and transversally sectioned (7μm) by a Shandon microtome. The thin sections were stained with Crystal violet and Erythrosin B to identify the lignified cell walls.
Histological observations were carried out by optical microscope and showed different characteristics in relation to vine health. The main feature of tissues infected by Esca disease was the minor lignification of vascular tissues. This deficiency was detected revealing the presence of lignin by the specific stain. Specifically, this feature was observed in petiole tissues before appearance of visual Esca symptoms. The opportunity to utilize in the future the histological examination of petiole tissues as a method for the early detection of Esca infections was hypothesized.

Introduction

Esca is a complex pathology which has been known ever since the first grapevine cultivations. In Europe and in other parts of the world, it is considered one of the most harmful grapevine wood pathologies. Esca consists of a series of symptoms that arise from structural and physiological modifications that cannot be explained simply by cause and effect relationships. Therefore seemingly similar symptoms can derive from different causes (Mugnai *et al.*, 1999). The symptoms can appear from June to September, and are either mild or severe, although there are cases where both types can be observed on the same plant (Surico *et al.*, 2000; Dubos, 2002). The symptoms of mild cases are characterized by a deterioration of the leaf canopy starting in the basal sections of the branches; the leaves initially beginning to yellow in small interveinal areas which eventually undergo necrosis. The grape bunches can also show symptoms on their skins, and in any case do not fully ripen. Severe Esca cases result in rapid grapevine death, called apoplexy: it is caused by a very quick drying out of the grapevine that causes the death of the entire plant or part of it (Larignon and Dubos, 1997, Surico *et al.*, 2000).

In the last 10 years Esca has continue to receive more attention in all wine producing European countries, where the incidence of the pathology has significantly increased. In Italy it has been observed in all the viticultural regions, and even very different incidence percentages have been recorded annually, in the most severe cases there can death of all the vines in the vineyard. The fluctuations in symptom appearance make it difficult to study this pathology (Mugnai *et al.*, 1999).
Despite numerous studies completed on Esca, histological alterations in the plant tissues remain an uncovered argument which could provide additional insight into the complex mechanisms that are at the root of the Esca pathology. The objective of this study was to observe the characteristics of petioles and internodes coming from symptomatic shoots from a histological point of view

MATERIALS AND METHODS

The experimental trials were completed over three years 2005-2007 in a vineyard in its twenty-fourth year of production found in Colignola (Pisa), in the experimental fields of the Department of Woody Species Cultivation and Defense (Altitude 6 m, 43.02 N, 10.36 E). The 3 x 1m vineyard being composed of 4 varieties (Sangiovese, Cabernet Sauvignon, Trebbiano, Chardonnay), trained as free cordons, positioned perpendicular relative to the vineyard length in four randomized blocks of 15 vines each. The research activities were completed on three of the four vineyards *Sangiovese*, *Cabernet Sauvignon*, *Trebbiano. Cabernet Sauvignon* is a variety that is known to be susceptible to Esca, and *Sangiovese* and *Trebbiano* are two varieties that are nationally widespread, one being a red grape and the other white.

- Before and at the first signs of Esca symptoms (from flowering to fruit set) the material samplings were taken from *Cabernet Sauvignon,* a genotype known to be very susceptible to Esca. Before the appearance of symptoms, three shoots were selected, having similar numbers of nodes (between 24 and 27), from vines that were never symptomatic, considered to be healthy and used as controls (NES) and from vines (5) that were symptomatic the previous year (PYS); from these the petioles of basal and apical leaves were taken. After sampling materials of the same age, for each shoot portion the petioles were collected from the same shoot section consisting of 3 nodes. Upon the appearance of the first foliar symptoms in the basal part of the shoot, samples were taken from asymptomatic apical leaves.

- **After Esca symptom appearance,** (from fruit set to veraison) samples of petioles and internodes (n=10) on symptomatic vines (SV) and asymptomatic vines (NES) of *Cabernet Sauvignon, Sangiovese and Trebbiano* were taken from the median and apical portions of shoots composed of a similar number of nodes, between 30 and 33. After the vegetal material sampling, which was as homogenous as possible in terms of age and of vigor characteristics of fruit bearing shoots, the samples were taken following the same criteria as previously described. The sampled materials were fixed in FAA (Ethanol, Acetic Acid and Formaldehyde in a ratio of 8:1:1 v/v) and following dehydration in a series of increasing alcohol degree solutions, were embedded in Histoplast and sectioned with a microtome (Shandon) into cross sections (7µM). In order to identify the lignification of vascular elements, the cross sections were stained using Crystal violet and Erythrosine B (Clark, 1981). Crystal violet is a cationic colorant which has a high affinity for lignin (Drnovsek and Perdih, 2005), and stains the lignin in a blue-violet colour. The histological slides were observed with an optical microscope (Nikon, Fluophot) under polarized light equipped with a digital camera to record images.

RESULTS AND DISCUSSION

From the microscopy studies on the cross sections taken from the different internode and petiole parts, different characteristics were observed according to the initial material health.

Histological characteristics of plant tissues before the appearance of Esca symptoms.

At the beginning of flowering of the *Cabernet Sauvignon* vines (Figure 1A, D), when the foliar symptoms were not yet visible, the tissues of the basal and apical leaves taken from the vines which had shown Esca symptoms in the previous year (PYS) were all similar to the control tissues (NES). For the basal petioles the cellular walls of the vessels and fibers of the meta-xylem were lignified (Figure 1A), showing a normal violet stain. For the apical petioles the vascular elements showed a weak staining corresponding to the juvenile state of the leaf (Figure 1D)

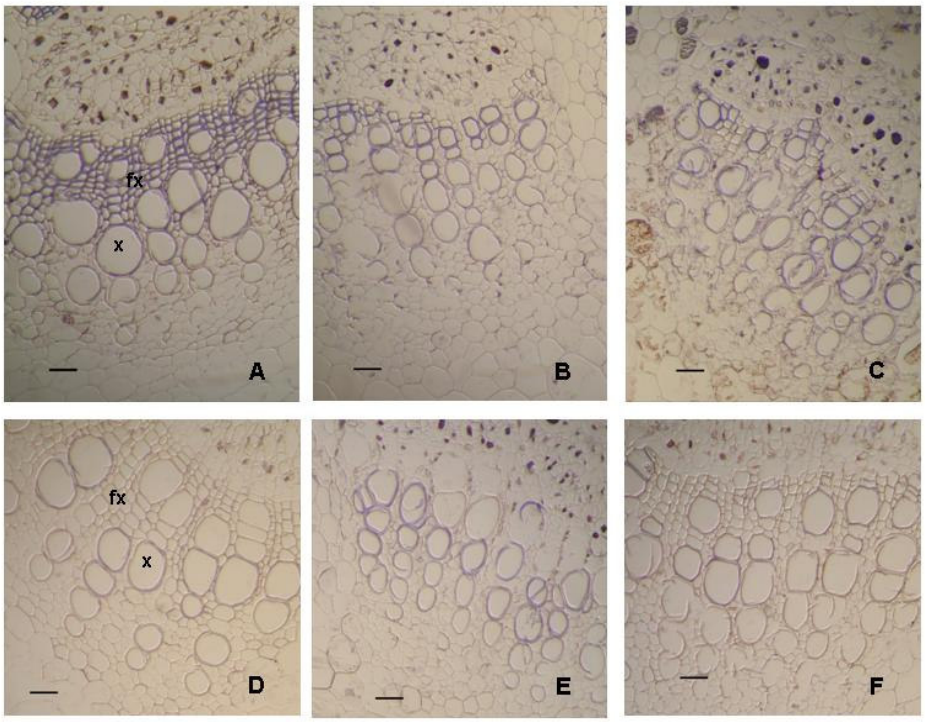

Figure 1. Cross sections representing parts of the vascular elements taken from Cabernet Sauvignon vines which in the previous year were symptomatic. Petioles sampled from from the basal (A, B, C) and apical parts (D, E, F) of the shoot at flowering (A, D), fruit set (B, E) and veraison (D, F).
x: xylem vessels of the meta-xylem, fx: xylem fibers.
Scale: 50µm

During the initial phases of fruit set (Figure 1B, E) the PYS vines still did not shown Esca symptoms, however the basal and apical petioles had xylem tissues that were not as lignified as the previous sampling. This defect is identified by a scarce staining intensity of the vascular elements which means a lesser deposition of lignin in the cell wall. This same characteristic was observed in the petioles of symptomatic leaves which had the cellular walls of the vessels (x), xylematic fibers (fx), and sclerenchyma fibers (fs) that were poorly lignified (Fig. 5 and 6). At the beginning of veraison (Figure 1 C, F), when the foliar symptoms were clearly visible only in the basal parts of the branch, the petioles of the apical leaves which were still asymptomatic showed poorly lignified and partially damaged vascular tissues.

Histological Characteristics of Tissues After the Appearance of Esca Symptoms.
The main characteristic of the tissues affected by Esca that was found in all the varieties studies was less tissue lignification, in particular of the vascular tissues.

Less lignification of the internode tissues was clearly visible in the fine cross sections of *Sangiovese* taken at veraison (Figure 2 and 3), when typical foliar markings were already recognized. In Figure 2 (A) the characteristics of a median internode cross section taken from a healthy plant are shown. It is possible to see the notable thickness of the vascular and parenchyma tissues in comparison to those tissues damaged by Esca (Figure 2C), characterized by a poor staining. The same details were observed for the apical parts of the shoot (Figure 3 A, C). In particular for the samples affected by Esca the lesser lignification noted for the sclerenchyma fibers was especially visible in the apical part of the shoot in comparison to the median (Figure 2D).

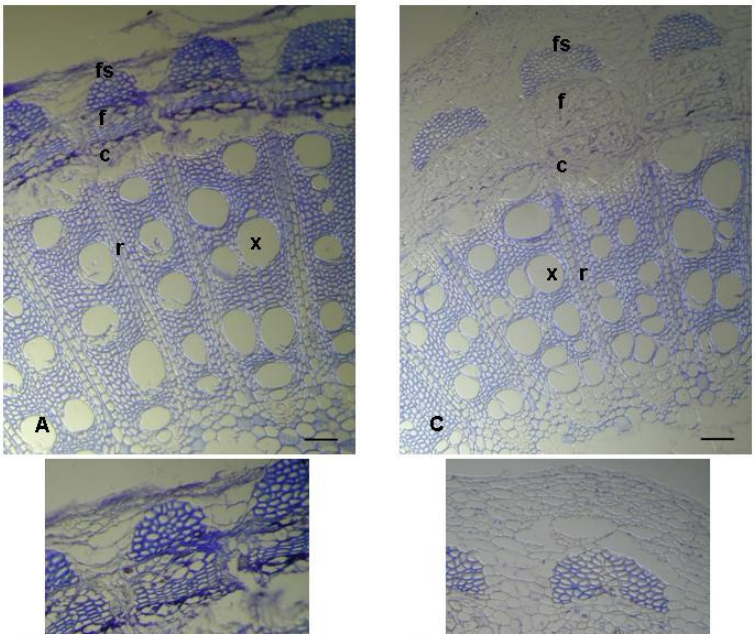

Figure 2. Cross section parts of median internodes taken at veraison from Sangiovese vines: healthy (A), with Esca symptoms (C) and respective particular elements (B and D).
fs: sclerenchyma fibers; f: phloem; c: cambium; x:xylem; r: parenchyma rays. Scale: 100 µm

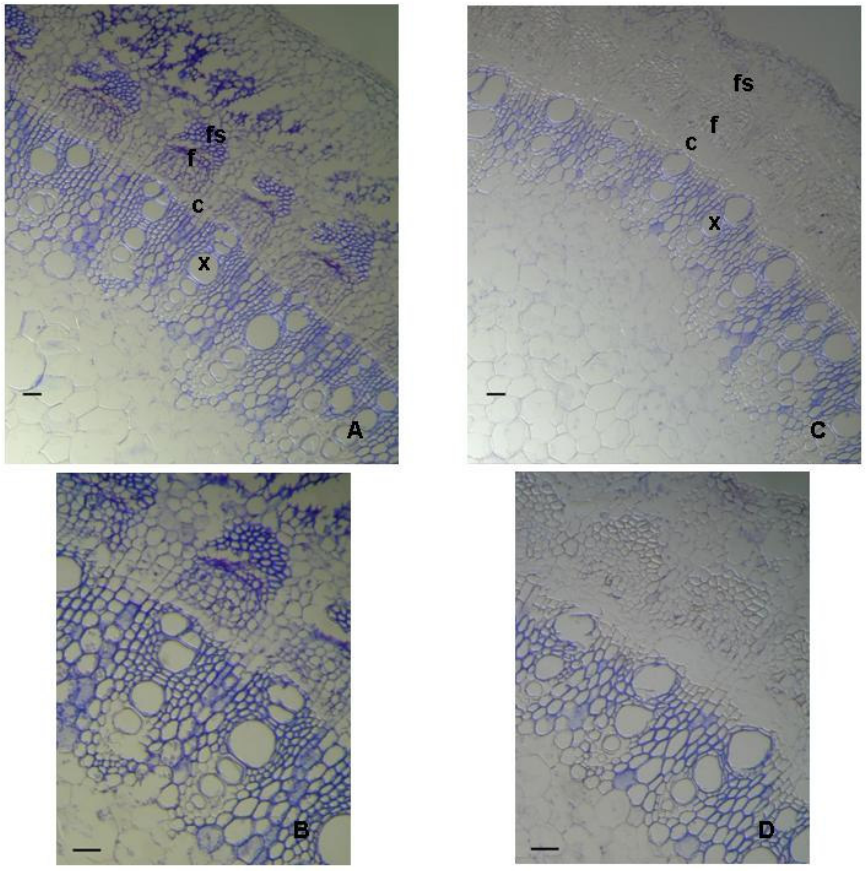

Figure 3. Cross section parts of apical internodes taken at veraison from Sangiovese vines: healthy (A), with Esca symptoms (C) and respective particular elements (B and D).
fs: sclerenchyma fibers; f: phloem; c: cambium; x: xylem vessels
Scale: 50µm

Similar characteristics were also observed in the median internode samples taken at fruit set from symptomatic *Trebbiano* vines (Figure 4): the xylem elements and the sclerenchyma fibers showed less thickened cell walls, given their scarce or absent coloration.

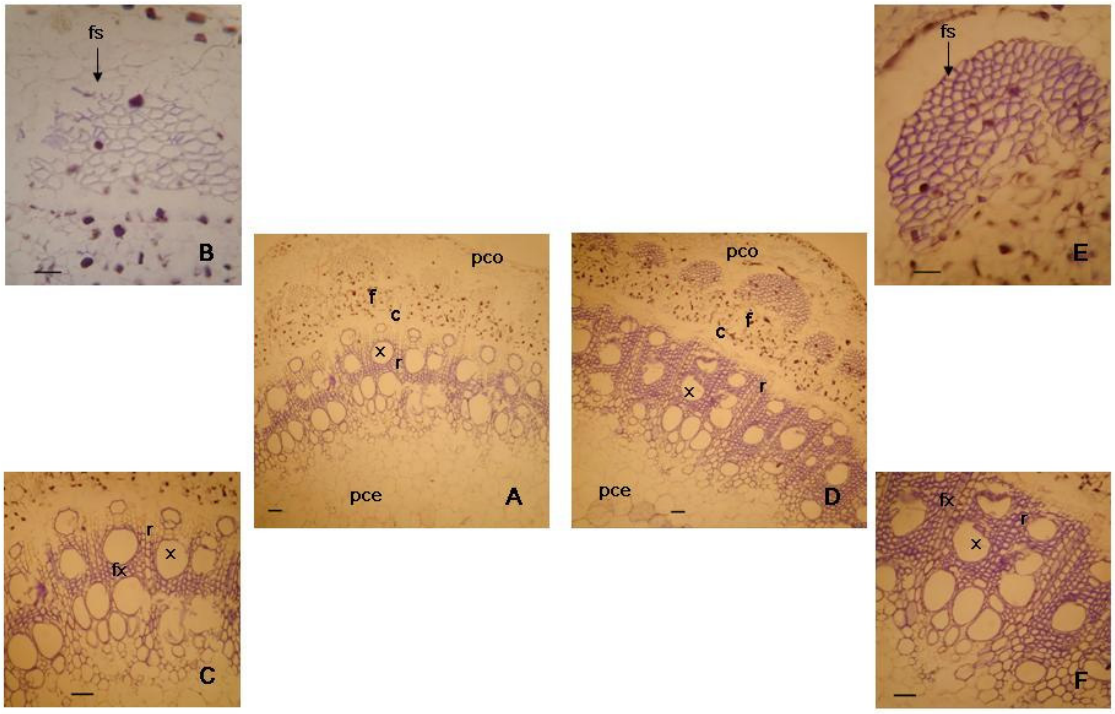

Figure 4. Particular elements of median internode cross sections taken at veraison of Trebbiano vines: symptomatic (A, B, C) and healthy (D, E, F). pco: cortex parenchyma, fs: sclerenchyma fibers; f: phloem; c: cambium; x: xylem vessels; r: parenchyma rays; fx: xylem fibers; pce: central parenchyma. B, E: magnification/scale 30μm; A, C, D, F: scale 50 μm).

The histological examination of the petioles confirmed that which was observed for the internodes (Figure 5 and 6). In the cortex zone of petioles of symptomatic leaves a greater degradation of parenchyma cells is observed (Figure 7C); while, in the central parenchyma, the cells were not turgid and in part collapsed (Figure 7D). On the other hand the petiole tissues from healthy leaves were intact and normally lignified (Figure 5, 6, 7).

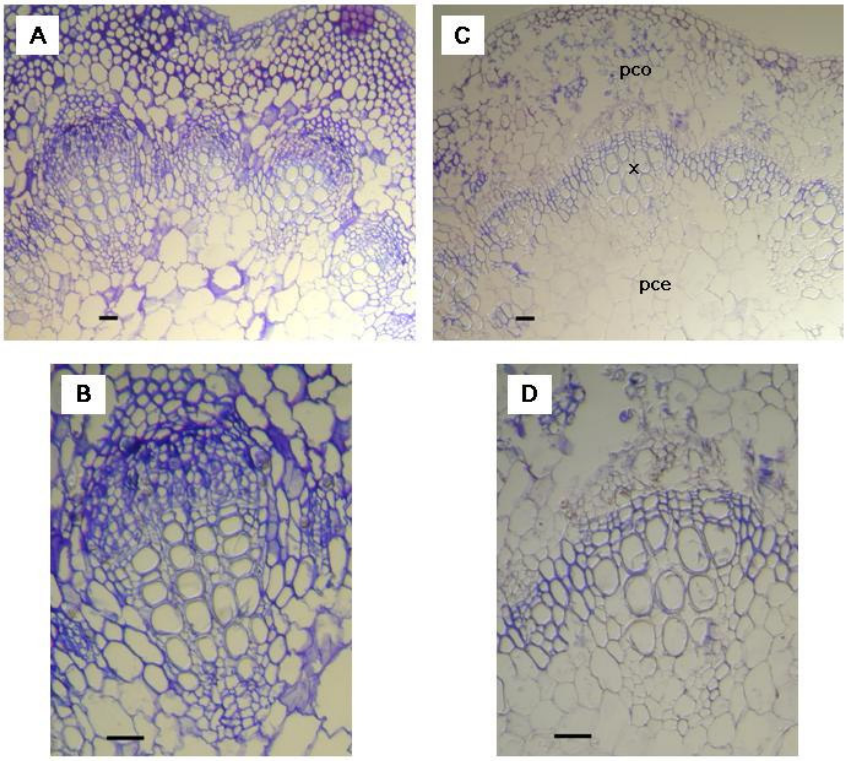

Figure 5. Cross section parts of cribro-vascular elements taken at veraison from Sangiovese vines: healthy (A), symptomatic (C) and respective particular elements (B and D). pco: cortex parenchyma; x: xylem; pce: central parenchyma.
Scale: 50 μm.

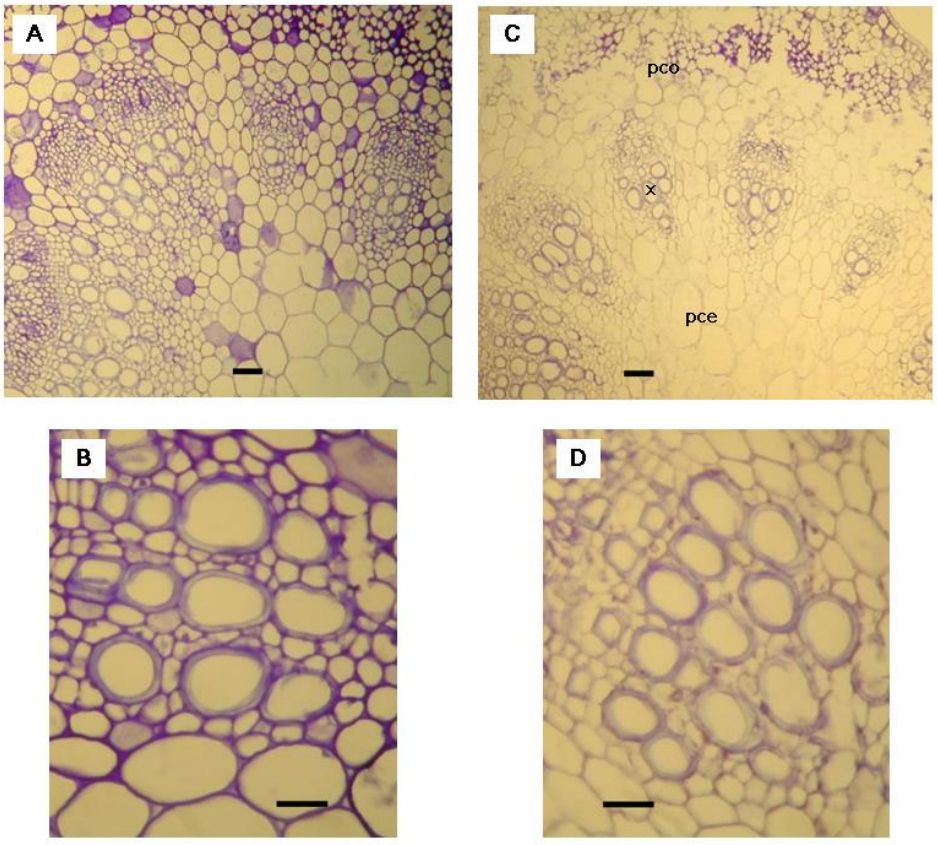

Figure 6. Cross section parts of cribrovascular elements taken at veraison from Cabernet Sauvignon vines: healthy (A), symptomatic (C, D) pco: cortex parenchyma; x: xylem; pce: central parenchyma.
A, C: scale 70 μm; B, D: scale 30 μm

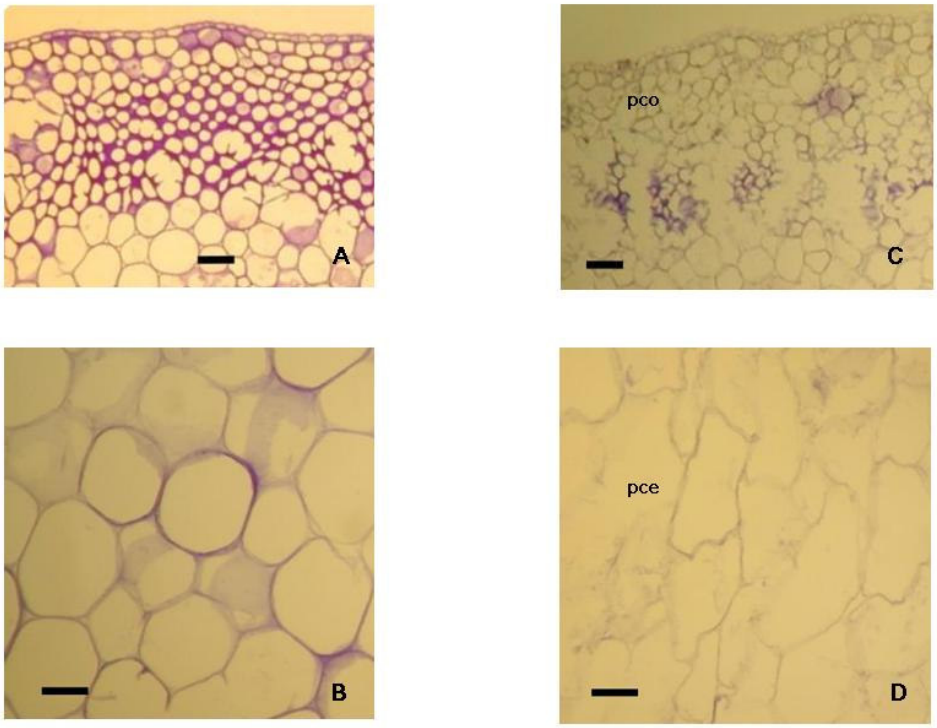

Figure 7. Particular cellular elements of cortex and central parenchyma of petiole cross sections taken at veraison from Cabernet Sauvignon vines: healthy (A, B) and symptomatic (C, D) pco: cortex parenchyma; x: xylem; pce: central parenchyma. Scale: 50 µm.

Conclusions

The main characteristic of the plant tissues affect by Esca, is less lignification, in particular of the vascular tissues. Furthermore the parenchyma tissues showed notable areas affected by cellular degradation. These tissue alterations due to Esca were found in all three varieties under study and were verified over the three years of study. The histological study of petiole and internode tissues of vines affected by Esca demonstrated the histological characteristics for each of these vine structures.
The weaker staining of cellular walls and vascular elements was in part verified by coloration with Safranine and Fast Green (results not presented) however it would be necessary to further validate these results using other specific stains to identify different cell wall lignification. Furthermore if the results were validated it would be necessary to verify that the less lignified cell walls of vascular elements, found before the appearance of foliar Esca symptoms in *Cabernet Sauvignon* vines, can be found in other vines. If it were so, histological examination could be a useful tool for the early detection of the Esca pathology.

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