Investigations on yellowing of chestnut crowns in Trentino (Alps, Northern Italy)

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Yellowing of part or the whole canopy of chestnut trees was observed during the summer of 2014 in the areas of Tenno, Pranzo and Drena in Trentino, where chestnut cultivation is traditional on the lateral moraine deposit in a context of a prevalent limestone lithological substrate. Symptoms were observed on chestnut trees of different ages, either grafted or not, scattered or in stripes along the maximum slope. Investigations were carried out in the form of field surveys, chemical and molecular analyses of soil and leaves and greenhouse trials, to assess the damage evolution and its causes. No known pathogens emerged during the field surveys, and laboratory tests excluded the presence of phytoplasma infections. A comparison of yellow and asymptomatic leaves evidenced significant deficiencies of manganese (Mn) and iron (Fe) in the yellow leaves, though the level of the two nutrients was the same in the soil below green and yellowed trees. Experimental run-off trials in the greenhouse reproduced the foliage damage with a continuous basic water regime; open-field fertilizations with Mn and Fe led to a slightly recovery on the part of some treated leaves, thus confirming the hypothesis of a probable, sudden deficiency of the mentioned microelements. A possible explanation of this phenomenon could be, on one side, the high precipitation level of 2014 and, on the other side, the previous outbreaks of the Asian chestnut gall wasp, that had reduced foliage volume and thus the organic substance in the soil. The natural recovery from symptoms was clearly visible in trees after four years in a context of normal rainfall regime and increasing organic acid in the soil, after effective biological control of wasp. Even if related to a particular and rare geological condition, this is the first description of Mn deficiency in Castanea sativa.

Keywords: Chestnut Yellowing, Castanea sativa, Phytoplasmas, Foliar Deficiencies, Manganese

Introduction
Chestnut orchards are an important legacy in the Italian and Mediterranean mountains, where they have constituted one of the main assets for the survival of human populations for more than one thousand years (Pezzi et al. 2019, Conedera et al. 2004). Nowadays, chestnut orchards are still a key component of the Italian Alps and Apennines landscape, covering large surfaces, though most are now abandoned or were coppiced in the fifties of the last century. A total surface of 788,408 ha (INFC 2007a, 2007b) of chestnut woods (9% of the forest surface in Italy) is concrete evidence of such a long history. Indeed, in several areas, chestnut orchards still represent an interesting, and sometimes fundamental or unique, economic resource, especially where “marron” production was maintained and carried on. Moreover, the presence of giant old trees, sometimes quite monumental, confers to chestnut orchards an interesting role for the maintenance of the biodiversity of plants, lichens, insects, and birds. This persistent economic and ecological importance enhances the concern about problems or perturbations able to affect chestnut. Of course, this concern is due to recent history, as these trees have dealt with three biological invasions in less than two hundred years (ink disease – Phytophthora cambivora (Petri) Buism.; chestnut blight – Cryphonectria parasitica (Murr.) Yasum.; and the Asian gall wasp – Dryocosmus kuriphilus Yasumatsu), affecting both the plants and the confidence of growers. The general survival of chestnut stands to ink disease, the natural spread of hypovirulence (Turchetti et al. 2008), and the introduction of a specific parasitoid (Torymus sinensis Kamijo) for the biological control of wasps (Quacchia et al. 2008) have saved the orchards and their cultivation, but phytopathological problems have nonetheless influenced the management of stands, increasing their abandonment in many areas (Pezzi et al. 2011, 2017). Therefore, the appearance of any new symptoms must not be ignored, especially in the still cultivated areas. That is what happened in Trentino during the summer of 2014 when a sudden

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leaves were randomly collected from the crown of each plant in July, every year from 2014 to 2016, and then in 2018. All samples were put into separate plastic bags, which were stored in a refrigerator bag and transported to the laboratory. Analyses were performed immediately. For each sample, all leaves were first examined for disease symptoms and yellowing degree, then five leaves were tested for phytoplasma presence and 20 leaves were analysed for macro and micro-nutrients determination. In total, 60 samples of symptomatic leaves and 40 of healthy leaves were collected. During this period, some trees were cut, and therefore not all 28 points were sampled each year.

Soil samples were collected in 2015 and in 2018 at the aforementioned 28 sites; each sample consisted of a mixture of four sub-samples collected all around the crown of the chosen tree. In Pranzo, a larger amount of soil was picked up for the greenhouse trials. In this case, the soil was collected below symptomatic and green trees.

Field fertilization trial

Different fertilization treatments were applied to 18 out of the 20 yellowed trees previously chosen for foliage analysis. Pelleted chicken manure (3.5 kg per plant) was scattered under the crown of six trees. The crown of six other trees was sprayed with liquid manganese fertilizer (1.2 gr 1\(^{-1}\)), while six others were treated with liquid iron fertilizer (1.0 gr 1\(^{-1}\)). The treatments were carried out in July 2015. The crown condition was assessed with a visual evaluation in September 2015.

Greenhouse trials

Two-year-old chestnut seedlings were potted with three different types of soil: peat, soil collected in the field under calcareous rocks, and peat, soil collected in the field under calcareous rocks. Greenhouse trials were carried out in the last six years. The goals were to: (i) define the observed symptoms correctly; (ii) understand the possible environmental and pathological factors involved; (iii) suggest proper and efficient management techniques of the affected trees and orchards.

Materials and methods

Site description

Yellowing of part or the whole crown was reported in only three chestnut sites in Trentino: Tenno (Loc. Pranzo), Campi and Drena. All these sites were located in lateral basins of the Lake valley (Sarca valley). Calcareous rocks belonging to the Calcari grigi group constitute the main lithological substrate. Glacial activity defined the valley’s morphology, where small patches of chestnut orchards were planted in the range of the lateral moraine deposit (Castellarin et al., 2005).

These stands are centenary and represent a legacy of this traditional culture.

Meteorological data

Monthly accumulated precipitation data for the period 2002-2019 were obtained from the meteorological stations closest to the affected sites: Tenno (415 m a.s.l.), located at the basis of the affected slopes (Meteotrentino network), and Cavedine (552 m a.s.l. – FEM agro-meteorological network), located less than 7 km away from the study site of Drena. Only raw, non-validated, non-gap-filled meteorological data series were used.

Field investigations and sampling

In July 2015, after observing that crown yellowing was still present on the affected plants, field surveys were carried out in order to: (i) accurately describe the observed symptoms; (ii) examine affected plants for the presence of chestnut blight and ink disease symptoms; (iii) collect leaf samples from affected and healthy trees for laboratory analyses.

Fifty yellowed trees were accurately examined for disease symptoms: 30 in Tenno, 16 in Campi, and 4 in Drena. The presence of different types of cankers (Turchetti et al. 2008) was recorded and accurate inspections of the collar area were carried out to assess the presence of ink disease symptoms.

Twenty-eight sampling points (corresponding to 20 symptomatic and 8 asymptomatic trees, all marked and georeferenced) were selected in the affected area and 25 symptoms.

out to assess the presence of ink disease symptoms. 2008 of different types of cankers (16 in Campi, and 4 in Drena. The presence of phytoplasmas was assessed also in the symptomatic trees surveyed in this study.

Phloem tissue of leaves was obtained by following the midribs with a scalpel. Then, by lyophilization and homogenization, the total DNA was purified from around 15-20 mg of dry material with the commercial kit NucleoSpin® Plant II (Macherey-Nagel GmbH & Co. KG, Düren, Germany). The presence of phytoplasmas was assessed using a nested PCR assay with phytoplasma universal primers F1 (Deng & Hiruki 1991) /P7 (Schneider et al. 1995), followed both by primers FUsU1 /Us (Seemüller et al. 1998) and R16F2n/R16R2 (Lee et al. 1993). The nested-PCR products were electrophoresed on a 1.5% agarose gel stained with Midori Green Advance® (Nippon Genetics Europe, Düren, Germany) in TAE buffer and then visualized with a GelDoc® UV-transilluminator (BioRad, Segrate, Italy).

Chemical analysis of soil

Soil samples were air-dried and manually sieved (<2 mm). Soil reaction was determined in a 1:2.5 soil to water suspension using a pH-meter equipped with a SenTix®41 pH Electrode (INOLAB LEVEL 2,WTW, Weilheim, Germany). Total inorganic carbon was measured by a volumetric method with a Dietrich-Fruehling calcimeter according to ISO-10693 (1995) and expressed as g of CaCO\(_3\) per kg of soil, as well as active lime that was quantified by titration after reaction with ammonium oxalate 0.1 M following the Drouiné method (Drouiné 1942).

The available fractions of Fe and Mn were extracted with a DTPA 0.005M, CaCl\(_2\), 0.01M and triethanolamine 0.1M solution at pH 7.3 (Lindsay & Norwell 1969) and measured with an inductively coupled plasma optical emission spectrometer (ICP-OES, Optima 8300, Perkin Elmer, Waltham, USA), after calibration with certified standard solutions.

Chemical analysis of foliage

After sampling, the leaf blades (without petiole) were washed with a 0.2% citric acid solution in order to remove dust and soil residues, and doubly rinsed with distilled water. The leaves were then ground and homogenized with a knife mill and oven-dried at 70 °C. P, K, Ca, Mg, S, Mn, Fe, B, Cu, and Zn contents were determined using an ICP-OES spectrometer after acid digestion with concentrated HNO\(_3\) (65%). Total nitrogen was quantified with an elemental analyzer (Vario Macro CN®, Elementar Analysetechnik GmbH, Hanau, Germany).
Statistical analysis
As data were not normally distributed, significant differences in soil characteristics and in the macro and micro-nutrient contents of leaves were highlighted between test and yellowing thesis using the non-parametric Mann Whitney U test.

For SPAD data, factorial ANOVA (with data, type of substrate and pH as factors) and the post-hoc Tukey’s HSD test were performed, complying with the parametric test assumptions.

The statistical elaboration was carried out with Dell Statistica 64® version 13 (Dell Inc., Tulsa, OK, USA).

Results
Symptom description
The appearance of yellowing on the chestnut crown was first reported in July 2014 in all three mentioned sites. The same plants showed symptoms again in 2015 and, in most cases, also in the following years. Yellowing seemed to affect the whole crown in most of the observed trees but in some plants only part of the crown or single branches were interested. In Pranzo and Campi, most of the yellowed trees appeared clustered in clear stripes on the slopes following the maximum slope (Fig. 1a). On the other hand, in Drena the few affected trees were isolated. Both grafted and “wild” trees were affected by the yellowing, as well as young and old trees. The disorder seemed not to spread further during the observation period, remaining localized on the first affected trees: the monitored green ones maintained a normal appearance with no yellowing, while no new patches of yellowed trees appeared on a landscape scale. Only a few dominated and small trees appeared to get worse during the growing seasons, with twig diebacks and reduced foliation. No yellowed trees died during the observation period (2014-2018), but some of them were cut down by the owners.

The observed symptoms were the same for all the monitored trees: new leaves grew pale yellow with clear green veins; in several cases the leaf margins appeared first necrotized and then curled, finally assuming a distorted and scorched appearance that was more evident at the end of the season (Fig. 2). The foliage had a regular distribution on the crown, but yellowed leaves sometimes appeared to be smaller than green ones.

Field investigations
A clear predominance of hypovirulent infections of chestnut blight was observed on the examined trees. Healing and healed cankers (Turchetti et al. 2008) were present on almost all trees, while virulent infections were found sporadically and limited to small branches or sprouts.

Ink disease was observed on a single plant that died in 2016. All other yellowed trees had no evidence of collar coloniza-

Field fertilization experiments
Fertilization trials were carried out only once; they were subsequently abandoned due to the practical difficulty of distributing the fertilizers evenly on the crowns.

In any case, treated trees showed some response: greener leaves were observed in September in two out of six trees treated

Meteorological data
A clear increase of rainfall was reported for 2014, with more than 600 ml above the average for both the examined meteorological stations (Fig. 3). This increase was more evident in the winter season, but continued throughout the vegetative season months. Interestingly, most of the winter precipitation was represented by rain rather than snow. The same trend was observed for the whole Trentino region.

Fig. 1 - (a) Symptoms observed in the summer of 2014 in the Pranzo area: notice the stripes of yellowed trees on the slopes. (b) The area of Pranzo in July 2018: note the complete disappearance of symptoms and the recovery of most of the trees.

Fig. 2 - Symptomatic leaves: yellowed with green bands along the veins and curled and necrotic leaf margin.

Fig. 3 - Meteorological stations in the three monitored areas (a) Pranzo, (b) Drena and (c) Campi showing the increase of precipitation in 2014 with respect to 2013.
For the period 2002-2019 recorded in the meteorological stations of Tenno and Cave-dine.

Fig. 3 - Annual rainfall for the period 2002-2019 recorded in the meteorological stations of Tenno and Cave-dine.

Fig. 4 - A chestnut seedling grown on Pranzo soil with continuous basic irrigation: leaves showed the same yellowing symptoms observed in the field.


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with Mn and in one out of six of those treated with Fe (Tab. 1). Only the lower branches showed this slight improvement, while most of the crown maintained the yellowish appearance. Chicken manuring had no effect on crown recovery. Controls, yellow and green ones, showed no change in their crown condition.

In 2018 most of the monitored yellow trees showed a complete or partial recovering (Tab. 1, see also Fig. S1 in Supplementary material), while no spread of the symptoms was observed in the monitored green ones. This recovery appeared evident in the whole area of Pranzo and Campi (Fig. 1b), while the affected trees of Drena showed little or no recovery: two trees maintained the yellow appearance of the whole crown, while in the other only single branches continued to show yellow foliage.

Greenhouse trials
Both grafted and non-grafted seedlings exhibited a similar behaviour during their growth: yellowing and a reduced leaf dimension were observed in the treatment growing on soil collected in the field; these symptoms were recorded only in the plants with a basic water regime, while trees irrigated with acidic water showed a quite regular growth and no anomalies in the foliage. The symptoms developed in the greenhouse resembled those observed in the field (Fig. 4).

SPAD data enhanced the different behaviour of the treatments. In general, seedlings planted in soil coming from the affected area and with basic irrigation water (“Pranzo pH=8”) showed the worst performance, in terms of chlorophyll content, during the whole period of observation. The average SPAD values for this treatment (30) were significantly (p<0.05) lower than “Peat pH=8” (mean value = 34) and “Control pH=6.6” (mean = 33), whereas the difference with “Peat pH=6.6” (mean = 39) and “Pranzo pH=6.6” (mean = 38) was highly (p<0.001) significant. On the contrary, the difference with “Control pH=8” (mean value = 32) was not statistically significant. “Peat pH=6.6” generally showed the best results, but “Pranzo pH=6.6” also performed well, the SPad values of these 2 theses being significantly (p<0.001) higher than all others and performing distinctly better than their homologous in basic conditions. Interestingly, an intermediate behavior was recorded for the seedlings under “Peat pH=8” treatment. The differences among treatments were more evident and significant after the middle of July (Fig. 5). The great difference in the SPAD values recorded at pH=6.6 and at pH=8 for “Pranzo” suggests a higher influence of the acidity of the soil solution collected under symptomatic trees compared to the “Control” and “Peat” (Fig. S2 in Supplementary material).
Laboratory tests

Phytoplasmas detection

A total number of 83 leaf samples (59 collected from yellowed trees and 24 from asymptomatic ones) were analysed for the presence of phytoplasmas using the universal primers P1/P7, followed by the fU5/rU3 and R16F2n/R16R2 primers. No sample tested positive for the presence of phytoplasmas, either in direct or nested PCR.

Chemical analysis of soil

The soil reaction for healthy trees ranged between 5.4 to 7.8 (median 7.3) whereas for yellowing trees range between 6.5 and 8.1 (median 7.6). Total inorganic carbon ranged between non-detectable (<10 g CaCO$_3$ kg$^{-1}$) and about 400 g CaCO$_3$ kg$^{-1}$ for both groups, with median values of 84 and 104 g CaCO$_3$ kg$^{-1}$, respectively, in the growing soil of test and yellowing trees. Active lime varied between <10 and 63 g CaCO$_3$ kg$^{-1}$ (median <10 g CaCO$_3$ kg$^{-1}$) for test soils and <10 and 90 g CaCO$_3$ kg$^{-1}$ (median 11 g CaCO$_3$ kg$^{-1}$) for yellowing soils. Available Fe and Mn ranged respectively from 10 to 134 mg kg$^{-1}$ (median 30 mg kg$^{-1}$) and from 4.4 to 32.9 mg kg$^{-1}$ (median 13.3 mg kg$^{-1}$) in test soils and from 19.4 to 41.3 mg kg$^{-1}$ (median 26.4 mg kg$^{-1}$) and from 9.3 to 10.3 mg kg$^{-1}$ (median 9.5 mg kg$^{-1}$) in yellowing soils. All these differences were not statistically significant (p > 0.05 – Tab. S2 in Supplementary material).

Chemical analysis of foliage

Fig. 6 shows macro and micronutrient content distributions in test and yellowing leaves. Fe and Mn contents were significantly (p<0.001) higher in the test than in yellowing leaves while P, K, and S (p<0.05), as well as Ca, Mg and B (p<0.01) were significantly higher in the latter. N, Zn, and Cu were not significantly different in the two groups (Tab. S2 in Supplementary material).

Discussion and conclusion

Chemical analyses clearly evidenced how the observed symptoms were related to a mineral deficiency involving Fe and, mainly, Mn. The hypothesis was confirmed by the result of the greenhouse trials and by the slight recovery of the leaves treated with liquid Mn or Fe fertilizer. Moreover, the foliage appearance of symptomatic trees resembles that described for other species suffering from Mn deficiency: the yellow-whitish colour with the marked green of...
the veins is reported as characteristic for this deficiency, and so are the necrosis and the curling of the leaf margin (Costello et al., 2003). This provides support to the hypothesis that these new symptoms observed in C. sativa were, in the examined localities, strictly related to a mineral deficiency involving the adsorption of Mn. Even if the current knowledge about the nutritional status of C. sativa is still insufficient, B and Mg deficiencies have been reported in Portugal by Portela et al. (2010). These authors suggested a strong Mn component in the symptoms observed in Calçots, while Larroche et al. (1997) reported that the Mn content in foliage was strongly and negatively affected by the pH increase also in chestnut seedlings. In our study, the yellowing leaves showed lower Fe and Mn and higher Ca and Mg contents than the healthy ones. In detail, Mn content in both theses is decidedly lower than the values reported in the literature as adequate (Toprak & Seferoglu 2013, Arrobas et al., 2018, Toprak 2019). Chestnut grows preferentially on acidic soil and, for this reason, a Mn deficit is rarely reported, while antagonism or toxicity phenomena are highlighted (Portela et al. 2010). However, on calcareous soils like those of the investigated stands, a pH increase could lead to a decrease in Mn availability. In all cases, the link between Mn availability and its related high pH value with Mn deficiency is well known (Costello et al. 2003) and here, for the first time, it is reported also in C. sativa in relation to symptomatic manifestations.

The presence of yellows diseases was observed in the Japanese chestnut, Castanea crenuta Sieb. and Zucc., in the 1950s in Japan (Shimada & Kouda 1954) and then associated with phytoplasmas by Okuda et al. (1974). Phytoplasmas causing diseases on chestnut were observed also in Korea (Han et al. 1997, Jung et al. 2002) and more recently in China (Lin et al. 2011).

What we observed in Trentino is similar, if not equal to the chestnut yellowing described by Mittempferger & Sfalanga (1998) in the northern Apennines and in two Trentino sites, far from the ones investigated in this paper. Antonaroli & Perna (2000) reported similar phenomena in both the Emilia Romagna and Marche regions: foliage symptoms were reported, including yellowish, browning and curling of the leaf margin exactly like those observed in Trentino. In both cases, there was no evidence of soil disorders or weather anomalies, even if the mentioned Trentino sites were located in a limestone area. Moreover, no phytoplasmas or other agents were identified in the affected trees. Again, in the Marche region, similar symptoms proved to be transmissible in grafting trials, but neither viruses nor phytoplasmas were detected in the affected plants (Vetraino et al. 2005). Interestingly, also in all these cases the symptoms were very localized (mainly on single trees or stands) and were not spreading further during the years. At present, yellowing on C. sativa in Italy seems not to be related to phytoplasmas, as confirmed by our laboratory tests, and its symptoms seem dissimilar and different from those reported in Japan, Korea or China. Probably due to its reduced spread, its unclear or never confirmed association with pathogens, such as phytoplasmas, and its sudden disappearance, “Chestnut yellows” was therefore removed from the EPPO Alert list in 2001 (EPPO 2001).

Even the natural recovery of most trees seems to confirm the lack of microelement uptake deficiency. The pattern of the yellowing distribution along the slope suggests the possible role of the main drainage routes in creating a preferential area for this disorder. Anyway, a basic soil solution on a continental scale. Vegetation History and Archaeobotany 13: 161-179. - doi: 10.1007/s00334-004-0058-7


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References


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Drouineau J (1942). Dosage rapide du calcaire ac-tif de sols [Rapid dosage of active limestone in soils]. Annales Agronomiques 12: 441-450. [in French]


Mittempergher L, Sfalanga V, Turchetti C, Ferretti F, Maresi G (2018). Assessing the impact of

iForest 13: 466-472

Fig. S1 - Tree no. 19 in 2015 (a) and after a complete recovery in summer 2018 (b).

Fig. S2 - Overall effect of different irrigation regimes on the three tested soil thesis.

Tab. S1 - Chemical characteristics of soils under healthy and yellowing trees.

Tab. S2 - Macro and micro-nutrient con-tents of healthy and yellowing leaves.

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