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HyTan chestnut tannin: an effective biostimulant for the nursery production of high-quality grapevine planting material

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Introduction: HyTan, a new chestnut tannin extracted from chestnut wood via hydrodynamic cavitation at low temperature in water only, is an effective biostimulant for the nursery production of high-quality grapevine planting material.

Methods: Experiments were carried out in Sicily with HyTan aqueous extracts obtained at different temperatures (45, 60 and 75°C) and dosage (pure extract or diluted with water) on the above-ground and below-ground characteristics of cv. Zibibbo cuttings grafted on rootstock 1103 Paulsen.

Results and discussion: HyTan tannin extracted at low temperature (45°C) favored the growth of both thinner root fraction and total above-ground plant as well as the leaf chlorophyll content. These findings open the route to the widespread use in agriculture of this newly extracted chestnut tannin rich in ellagic acid, nonhydrolyzed ellagitannins (castalagin and vescalagin) and gallic acid.

KEYWORDS

biostimulant, tannin, ellagitannins, HyTan, chestnut tannin, grapevine planting material, hydrodynamic cavitation (HC)

1 Introduction

Generally classified in eight major classes (humic substances, complex organic materials, beneficial chemical elements, inorganic salts, seaweed extracts, chitin and chitosan derivatives, free amino acids and other nitrogen-containing substances), plant biostimulants are substances that applied to plant, seeds or growing substrates modify the physiological processes providing benefits to growth, development and stress response (du Jardin, 2012). In the 27 EU countries, Regulation 2019/1009 defines plant biostimulants as products "stimulating plant nutrition processes independently of the product's nutrient content with the sole aim of improving one or more of the following characteristics of the plant or the plant rhizosphere: nutrient use efficiency, tolerance to abiotic stress, quality traits, and availability of confined nutrients in soil or rhizosphere" (European Union, 2019). In line also with the UN global sustainable development goals for the agricultural sector (Food and Agriculture Organization of the United Nations, 2022), a considerable body of research has been devoted in the last two decades to the use of biostimulants as eco-friendly alternatives to agrochemicals improve the sustainability of agriculture (Calvo et al., 2014; Canellas et al., 2015; Halpern et al., 2015; Andreotti, 2020; Basile et al., 2020; Miele et al., 2020; Shahrajabian et al., 2021).

In this context, pioneered in Italy since the late 1990s, the use of hydrolysable tannins such as tannin obtained from chestnut wood as environmentally friendly pesticide and biostimulant has emerged as one of the promising alternatives to improve root system development, counteract parasites and to enhance plant resilience toward abiotic stresses (Bargiacchi et al., 2012). Successful use has been reported with several annual and perennial crops including carrot, tomato, tobacco, olive, orange and kiwi (Miele et al., 2020; Campobenedetto et al., 2021; Roccuzzo et al., 2021).

The word "tannin" indicates a class of water-soluble oligomeric polyphenolic compounds generally sourced from wood originally used for leather tanning and in winemaking due to ability to bind and precipitate proteins and antioxidant properties, and minor yet important application in the cosmetic and mining industries (Aires, 2020). In the early 2000s, unexpected new applications of chestnut tannin, first in animal (cow, poultry and pig) nutrition and subsequently in agriculture as biopesticide and biostimulant emerged. The market demand for chestnut tannin quickly grew to saturate the production capacity of the extraction plants, today mostly based in Europe (Ciriminna et al., 2024).

The use of chestnut tannin extracts in nursery's management practices has not yet been explored. The production of high-quality nursery material in modern agriculture is essential for providing fruit growers with plants of guaranteed quality from a genetic, phytosanitary and agronomic point of view. On the other hand, the speed and ease with which pathogens spread globally via propagation materials, combined with the ever-increasing availability of new varieties and the need to quickly adapt to market demands, has driven substantial applied research efforts in the nursery sector.

In viticulture, awareness of the importance of plant quality is currently widespread throughout the world and both established and emerging producing countries have developed application tools for the definition of suitable standards and protocols on vine propagation materials. However, standards have been developed focusing mainly on phytosanitary aspects and on the certification procedures for safe exchange of grapevine propagation material (Halleen and Fourie, 2016; New Zealand Winegrowers, 2021; International Organisation of Vine and Wine, 2022).

Other relevant physical aspects of grapevine planting material including completely healed graft union, good plant architecture, uniformity of size, absence of scars or damaged buds, healthy untwined downward pointing roots with fibrous cream-colored branch roots, etc. can be crucial in terms of the success of vineyard plantations or the long-term survival of plants. Yet, to date, they have received little attention, except for the minimum regulatory criteria defined by law in country of origin such as soundness of the graft union, minimum number of roots and length and diameter of the scion lignified stem (Carrere et al., 2022). Furthermore, little is known about the effect of nursery management practices on most of these aspects that in their turn influence the production of good quality grafted vines.

Although technical progress has allowed nurseries to increase production, the quality of the plant material, mostly grafted, is not yet of consistently high level (Waite et al., 2015). On the other hand, it is well known that the quality of planting material affects vine vegetative and reproductive parameters and that quality nursery vines establish quickly and contribute to the uniformity of the vineyard (Pisciotta et al., 2016).

The most significant challenge toward modernization of the nursery sector remains the ability of nurseries to maintain a consistent supply of healthy and uniform quality vines. Good nursery practices preserve and enhance the quality of cuttings as they proceed through the propagation process (Stamp, 2001; Hunter et al., 2003). The production of high-quality viticultural nursery material, in brief, is critical to the future viability of the vineyard and for the whole viticulture nursery sector. Only in the EU countries, every year approximately 400 million grafted cuttings are produced, of which approximately 150 million in Italy alone (Blando et al., 2023).

We now report that "HyTan", a new aqueous tannin extract rich in gallic acid, ellagitannins vescalagin/castalagin and ellagic acid extracted in water only from chestnut wood via hydrodynamic cavitation (HC), is an effective biostimulant for the nursery production of high-quality grapevine planting material for the production of grafted vines, cv. Zibibbo/Paulsen 1103.

2 Materials and methods

The study was conducted in Italy between early April and December 2021 at the "Giacomo Mannone" nursery located in western Sicily in the countryside of Petrosino (37°42'44.15"N 12° 32'32.79"E), 20 m above sea level. Different aqueous extracts of HyTan derived from chestnut wood were tested on the nursery production of grafted vines, cv. Zibibbo/Paulsen 1103, to assess the influence of tannin applications for the nursery production of sustainable above and below-ground high-quality grapevine planting material as a viable alternative solution to routine chemical fertilizer-based nursery management.

2.1 Preparation of HyTan extracts

Aqueous extracts of HyTan were obtained from 1.8 kg of dried sawdust of sweet chestnut wood of *Castanea sativa* Mill trees growing in Calabria, Italy, ground by a cutting mill (Figure 1).

The extraction was carried out on March 1, 2021 in tap water only using a batch hydrodynamic cavitation-based extractor comprising a closed hydraulic circuit of total volume of 150 L, equipped with a centrifugal pump and a circular Venturi-shaped reactor with circular section as the key components. The details of the extractor, along with the cavitation number (CN) as a measure of cavitation intensity and regime, were described in a previous study described in detail in a previous study reporting the HC-based optimized extraction of waste orange peel (Meneguzzo et al., 2019). Electricity was the only energy employed. Cavitation started at room temperature (20°C) and proceeded smoothly.

Only one extraction was carried out undertaking the HC of 1800 g of sweet chestnut sawdust (from chestnut trees grown in Calabria, Italy) in 150 L water. The temperature of the circulating mixture progressively increased. Samples were withdrawn at different process times corresponding to t = 33, 74 and 116 min, when temperature went from 45, to 60 and 75°C. No active heat dissipation method was used. Power and energy consumption were measured by means of the model D4-Pd three-phase digital power meter (IME, Milan, Italy). Samples of HyTan extracted at different temperatures were thus stored at room temperature and subsequently used in the nursery experiments as such or diluted with water (see below).

FIGURE 1
Chestnut wood next to the HC-based reactor prior to undergoing hydrodynamic cavitation.

Table 1 details the main features of the HyTan aqueous extracts used throughout the study. A subsequent study has recently resulted in a similar HC-based extraction process in water only employing higher amounts of wood (Meneguzzo et al., 2023).

2.2 Preparation of planting material and growing assay

For each HyTan extract tested, we used the extract as such (100% dosage), or diluted using distilled water at 75% and 50% v/v. The first step in the propagation process was the harvesting of cuttings of vines, cv. Zibibbo/Paulsen 1103, and their transport from the mother vine block to the nursery. In order to control trunk diseases in asymptomatic material cuttings, all were subjected to treatment with ambient temperature water (15-16°C for 12 h) in conjunction with fungicide drenches (thiophanate methyl at 800 mL/1000 L of water concentration). Before grafting, to prevent dehydration and minimize risk of cross-contamination, cutting scion buds and rootstocks were covered with a clean damp cloth. After grafting (omega grafting), the cuttings were moved to the incubation room where temperature and relative humidity were kept at fixed values (T = 34°C, RH = 98%) for 15 days to complete the callusing process. Forcing practice was in water. After callusing, grafted cuttings were acclimated at external temperature for approximately 5 days.

Prior to plantation, a portion of the total grafted cuttings obtained were hydrated at their bottom with HyTan tannin solutions using HyTan obtained at three different extraction temperatures and at different dilution values (Table 1). All the grafted cuttings, cv. Zibibbo/Paulsen 1103 rootstock, including the untreated portion used as control, were planted in rows, northeast-southwest oriented, spaced 1.0 m apart (Figure 2).

Distance in the row was 0.07 m. Planting was done on April 4, 2021 in a sandy loam soil in the hot climate conditions of the Mediterranean vineyard previously used to investigate the response of grapevine to above ground and subsurface drip irrigation under arid conditions (Pisciotta et al., 2018). Extirpation was performed

TABLE 1 Basic features of the HyTan extraction[a].

Time (min)	Process temperature (°C)	Energy consumed (kWh)	Power (W)
0	20	0	7183
8	25	0.96	7183
15	35	2.91	7276
33	45	5.21	7304
74	60	8.74	7251
116	75	13.35	6982

[a]Cavitation of 1800 g of sweet chestnut sawdust in 150 L water.
Bold indicates extracts subsequently used.

FIGURE 2
Planted grafted vines, cv. Zibibbo/Paulsen 1103 untreated (*left*) and treated with 50% diluted HyTan extracted at 60°C (*right*) photographed on the same day (May 16, 2021).

on December 12, 2021. Four replicates of each treatment were arranged in a randomized block design and distributed in four rows. Each replicate included a plot of 20 plants. All grafted cuttings were regularly drip irrigated since planting to uprooting (0-228 days after plantation, DAP) with holes placed every 10 cm on the pipeline, and a flow rate of 1.14 L h^{-1}. By mid-May all the cuttings had sprouted.

The vines of the tannin treatments group received a total of 9576 m^3 ha^{-1} of irrigation water and 4 successive foliar applications (38, 52, 68 and 88 DAP) of the different tannin solutions sprayed manually up to dripping. Water was applied 3 times (3 h each time) on April, 6 times on May, 5 times in June and July, 4 times in August, 3 times in September and 2 times in October.

2.3 Analytical assays and statistical analysis

According to routine protocol applied by the nursery, control vines received, during the vegetative season, a total of 9571 m^3 ha^{-1} of irrigation water and 60 kg ha^{-1} N, 38 kg ha^{-1} P_2O_5, 15 kg ha^{-1} K_2O, 24 kg ha^{-1} Ca, 13 kg ha^{-1} Mg, 7.2 kg ha^{-1} Fe fertilizers. At 60 DAP all vines were topped at about 45 cm from the grafting point. At 41, 55, 71 and 91 DAP, data on leaf chlorophyll, flavonols, anthocyanins content and the Nitrogen Balance Index (NBI) were measured spectrophotometrically with portable FORCE-A instrument (Dualex Scientific+, Paris, France) on four plants/replica and on a fully expanded leaf per vine (Goulas et al., 2004). Data are given as µg per cm^2 of leaf surface. At the end of observation period (228 DAP), data regarding shoot and laterals length and fresh and dry weight were collected on four uprooted plants/replica, together with the evaluation of the root system architecture regarding fresh weight, dry weight, and total length of roots. To this end, the following 4-class root diameter scale was applied:

Class 1 (Cl.1): ø ≤ 1 mm
Class 2 (Cl.2): 1<ø≤ 2 mm
Class 3 (Cl.3): 2<ø≤ 3 mm
Class 4 (Cl.4): ø>3 mm

Root measurements per class were performed by using a digital caliper. The total shoot and root length were calculated as the sum, respectively, of the lengths of shoots and laterals, and of all root classes of each plant. After length measurements, shoots, laterals and root samples for each class were weighed and then dried in a forced-draft oven at 60°C until constant weight.

Statistical data analysis was performed by one-way analysis of variance (one-way ANOVA), where 'Temperature' of tannin extraction and 'Dilution' were considered as the main parameters. To assess the significant main effects and their interaction, mean statistical differences were determined using Tukey's honestly significant difference (HSD) test, (p = 0.05). The Minitab 19 statistical software (Minitab, State College, PA, USA) was used to perform the statistical analyses, including comparison with control samples.

3 Results and discussion

The statistical significance of the two main factors, 'Tannin extraction Temperature' and 'Tannin Dilution', and their interaction is reported in Table 2.

Treatments with HyTan did not significantly affect the successful percentage of cuttings. Significant statistical effects could be observed for the tannin extraction temperature onto the selected shoot parameters. In contrast, tannin dilution did not show any significant effect for all the observed above-ground parameters. Limited effects, once again related only to the factor "temperature extraction", can be observed regarding some below-ground (root) parameters for different root classes (Table 3).

Finally, results in Table 4 concerning the significance of temperature and dilution on the leaf variation of spectrophotometrically measured parameters. Significant effects of tannin extraction temperature can be observed on all parameters measured for nearly all observation dates. In contrast, dilution showed a significant effect only on flavonols on a single survey date (41 DAP).

In line with the ANOVA test results, the effects of tannin extraction temperature on selected above-ground and below-ground parameters, including the leaf biochemical profile spectrophotometrically detected, are significant (Tables 4-7). Generally, the successful percentage for all treatments (data not

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TABLE 2 Results of two-way ANOVA tests for the factors "Tannin extraction Temperature" (T), "Tannin Dilution" (D) and their interaction (T x D) on the variation of rooting success and above-ground (shoots and laterals) vine parameters.

Parameter	Source	Significance
Rooting (%)	T	n.s.
	D	n.s.
	T x D	n.s.
Total vine shoot length (cm)	T	**
	D	n.s.
	T x D	n.s.
Total vine shoot fresh weight (g)	T	*
	D	n.s.
	T x D	n.s.
Total vine shoot dry weight (g)	T	*
	D	n.s.
	T x D	n.s.

Asterisks indicate statistical significance levels: *p ≤ 0.05, **p ≤ 0.01, n.s., not significant.

shown) was 90%. With regards to the effects of temperature on the individual selected epigeal parameters reported in Table 5 only the lowest extraction temperature (45°C) had a positive effect on total above-ground growth (both in terms of shoot length and weight).

TABLE 3 Results of two-way ANOVA tests for the factors "Temperature" (T), "Dilution" (D) and their interaction (T x D) on the variation of below-ground (root) vine parameters, by root diameter class (Cl.1-4).

Parameter	Source		Significance			
		Cl.1	Cl.2	Cl.3	Cl.4	TOTAL
Root length	T	*	n.s.	n.s.	***	n.s.
	D	n.s.	n.s.	n.s.	n.s.	n.s.
	T x D	n.s.	n.s.	n.s.	n.s.	n.s.
Root fresh weight	T			n.s.	***	n.s.
	D			n.s.	n.s.	n.s.
	T x D	n.s.	n.s.	n.s.	n.s.	n.s
Root dry weight	T	n.s.	n.s.	n.s.	n.s.	**
	D	n.s.	n.s.	n.s.	n.s.	n.s.
	T x D		**	n.s.	n.s.	n.s.
Root fresh weight per cm	T		n.s.	n.s.	***	**
	D	n.s.	n.s.	n.s.	n.s.	n.s.
	T x D	n.s.	n.s.	n.s.	n.s.	n.s.
Root dry weight per cm	T	n.s.	n.s.	**	***	**
	D	n.s.	n.s.	n.s.	n.s.	n.s.
	T x D	n.s.	***	n.s.	n.s.	n.s.

Asterisks indicate statistical significance levels: *p ≤ 0.05, **p ≤ 0.01, n.s., not significant.

TABLE 4 Results of two-way ANOVA tests for the factors "Temperature" (T), "Dilution" (D) and their interaction (T x D) on the variation of leaf chlorophyll (CHL), flavonols (FLAV), anthocyanins (ANTH) and Nitrogen Balance Index (NBI) 41, 55, 71 and 91 days after planting (DAP).

Parameter	Source	Significance			
		DAP 41	DAP 55	DAP 71	DAP 91
CHL ($\mu g/cm^2$)	T	*	**	*	*
	D	n.s.	n.s.	n.s.	n.s.
	T x D	n.s.	n.s.	n.s.	n.s.
FLAV ($\mu g/cm^2$)	T	*	***	n.s.	n.s.
	D	***	n.s.	n.s.	n.s.
	T x D	n.s.	n.s.	n.s.	n.s.
ANTH ($\mu g/cm^2$)	T	**	*	**	*
	D	n.s.	n.s.	n.s.	n.s.
	T x D	n.s.	n.s.	n.s.	n.s.
NBI	T	*	**	n.s.	*
	D	n.s.	n.s.	n.s.	n.s.
	T x D	n.s.	n.s.	n.s.	n.s.

Asterisks indicate statistical significance levels: *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$; n.s., not significant.

However, the tannin treatment did not show statistically significant differences compared to the untreated control.

Treatments with chestnut HyTan extracts obtained at different temperatures variously affected root parameters according to their diametric class (Table 6). Treatment with HyTan extracted at the lowest temperature (45°C) favored a significantly higher length of the thinner root fraction (Cl.1), that reached the maximum value measured (375.5 cm, 40.8% of the total length). On the other hand, the lowest root development (67.4 cm) was observed after treatment with HyTan tannin extracted at 75°C, for the thickest fraction (Cl.4).

The extraction at the intermediate temperature (60°C) also favored a significantly higher length of the thinner root fraction (Cl.1), that reached 277.2 cm, 16.1% higher than length of the control roots, as well for the Cl.2 diameter class. Overall, also the treatment with HyTan extracted at 60°C resulted in higher total root development (Figure 3). Yet, whereas the latter improvement amounted to 5% with respect to the untreated cuttings, treatment with HyTan extracted at 45°C resulted in a nearly 31% improvement in the root development. To the contrary, the lowest root development (67.4 cm) was observed for the thickest fraction (Cl.4) after treatment with HyTan tannin extracted at 75°C.

In line with the larger development in length of the Cl.1 roots observed after treatment with HyTan extracted at 45°C, a similarly higher value of the fresh weight could be observed for both root thinner fractions (Cl.1 and Cl.2), compared to the control. In contrast, an opposite effect of tannin extraction temperature was observed both on the total dry weight and on that of the first two diameter fractions for which a higher dry weight was recorded upon treatment with HyTan extracted at 75°C, compared to the control.

TABLE 5 Variation of above-ground (shoots and laterals) vine growth parameters: total shoot length (cm), fresh (g) and dry weight (g), after treatment with HyTan extracted at different temperature.

Treatment	Total vine shoot length (cm)	Total vine shoot fresh weight (g)	Total vine shoot dry weight (g)
T75	106.2 ± 8.3 (b)	11.2 ± 1.2 (c)	5.4 ± 0.8 (b)
T60	137.8 ± 5.7 (ab)	16.5 ± 0.7 (b)	6.9 ± 0.7 (ab)
T45	133.7 ± 10.3 (a)	21.7 ± 0.8 (a)	8.2 ± 0.9 (a)
CTRL	119.6 ± 6.8 (ab)	17.4 ± 0.9 (ab)	6.2 ± 0.7 (ab)

Different letters within a column indicate significant differences (Tukey's test, *p*-value 0.05). n.s., not significant. Values are reported as means ± SE (standard error); T75, T60 and T45 indicate tannin extraction temperature (75°C, 60°C and 45° respectively).

The root fresh weight per cm was very similar across the different extraction temperatures tested, but statistically greater than the control. The highest values of root dry weight per cm were observed for tannins extracted at the highest temperature (75°C and 60°C) in all diameter classes, while for tannin extracted at 45°C this parameter was not statistically different from the control (except for Cl.2).

Table 7 shows the effect of the tannin extraction temperature on the distribution of the dry matter accumulated in the shoots and roots of the vine at the date of grubbing, along with the ratios of dry matter between shoots and roots. Opposite trends regarding shoots and roots can be attributed to the increase in tannin extraction temperature. The higher the extraction temperature, the lower the accumulation of dry matter in the shoots, and the larger the accumulation of dry matter in the roots. Overall, tannin treatments resulted in larger total dry biomass accumulation in the vine compared to the control. Increasing values of the shoot/root (S/R) ratio were observed with the application of HyTan extracted at lower extraction temperature, with the lowest S/R value observed for the T75 samples.

The chlorophyll (CHL) content was higher for nearly all observation dates in the leaves of the vines treated with HyTan extracted at 45°C, compared to the other treatments and to the control (Table 8). In contrast, flavonols (FLAV) and anthocyanins

TABLE 6 Variation of selected below-ground vine growth parameters by root diameter class (Cl.1-4)[a], after treatment with HyTan extracted at different temperatures.

Parameter	Treatment	Cl.1		Cl.2		Cl.3		Cl.4		TOTAL	
Root length (cm)	T75	217.3 ± 19.4	b	273.5 ± 17.0	a	113.9 ± 10.5		67.4 ± 13.1	b	672.1	
	T60	277.2 ± 19.1	ab	244.1 ± 17.1	ab	142.4 ± 13.2		75.4 ± 6.4	ab	739.1	
	T40	375.5 ± 35.4	a	236.9 ± 37.6	ab	152.4 ± 14.2		154.9 ± 17.0	a	919.7	
	CTRL	238.7 ± 15.6	b	175.1 ± 26.4	b	144.5 ± 14.5	n.s.	144.5 ± 13.9	ab	702.8	n.s.
Fresh root weight (g)	T75	1.5 ± 0.2	ab	5.8 ± 0.6	a	5.5 ± 0.5		6.2 ± 0.9		19	
	T60	1.8 ± 0.2	ab	5.0 ± 0.3	a	7.7 ± 0.6		7.1 ± 0.8		21.5	
	T40	2.4 ± 0.3	a	4.9 ± 1.0	a	7.5 ± 0.8		7.4 ± 0.8		22.3	
	CTRL	1.0 ± 0.1	b	2.3 ± 0.4	b	6.5 ± 1.0	n.s.	6.5 ± 1.0	n.s.	16.3	n.s.
Dry root weight (g)	T75	1.0 ± 0.1	a	4.1 ± 0.4	a	3.8 ± 0.4		4.1 ± 0.4		13	b
	T60	1.1 ± 0.1	a	3.1 ± 0.2	ab	4.6 ± 0.4		4.8 ± 0.7		13.6	b
	T40	1.0 ± 0.2	ab	2.2 ± 0.4	b	3.5 ± 0.4		3.4 ± 0.4		10.1	bc
	CTRL	0.4 ± 0.0	b	1.0 ± 0.2	c	3.3 ± 0.5	n.s.	3.5 ± 0.5	n.s.	18.3	a
Fresh root weight per cm (mg)	T75	6.8 ± 0.5	a	20.9 ± 1.3	a	52.1 ± 2.8		101.9 ± 14.2	a	181.7	a
	T60	6.4 ± 0.4	a	21.1 ± 1.2	a	55.7 ± 3.6		92.8 ± 4.3	a	176	a
	T40	6.2 ± 0.4	a	19.8 ± 1.2	a	51.2 ± 4.2		49.7 ± 4.0	b	126.9	b
	CTRL	4.4 ± 0.3	b	12.6 ± 0.9	b	43.2 ± 4.5	n.s.	47.1 ± 5.6	b	107.3	c
Dry root weight per cm (mg)	T75	4.7 ± 0.3	a	14.9 ± 0.9	a	36.2 ± 2.1	a	66.9 ± 8.3	a	122.7	a
	T60	3.9 ± 0.3	a	12.8 ± 0.8	a	32.6 ± 2.9	a	54.2 ± 3.0	a	103.5	a
	T40	2.5 ± 0.2	b	8.5 ± 0.7	b	23.6 ± 2.0	b	26.2 ± 3.8	b	60.8	b
	CTRL	1.7 ± 0.2	b	5.1 ± 0.5	c	22.9 ± 2.1	b	26.2 ± 3.8	b	55.9	b

[a]Different letters within a column indicate significant differences (Tukey's test, *p*-value 0.05). n.s., not significant. Values are reported as means ± SE (standard error); T75, T60 and T45 indicate tannin extraction temperature (75°C, 60°C and 45°C respectively).

TABLE 7 Shoot (S) and Root (R) dry matter partitioning (%); total (S+R) dry matter accumulation; Shoot/Root (S/R) ratio for different vine samples after treatment with HyTan extracted at different temperature.

Treatment	Shoot (%)	Root (%)	S+R (g)	S/R (g/g)
T75	29.3	70.7	18.4 (b)	0.41 (c)
T60	33.7	66.3	20.5 (a)	0.51 (b)
T45	44.8	55.2	18.3 (b)	0.81 (a)
CTRL	43.1	56.9	14.4 (c)	0.76 (a)

Different letters within a column indicate significant differences (Tukey's test, *p*-value 0.05). n.s., not significant. T75, T60 and T45 indicate the temperature of tannin extraction at 75°C, 60°C and 45° respectively.

(ANTH) were generally the lowest at T45. Finally, NBI (that the Dualex instrument employed automatically determines as the ratio between CHL and FLAV) ranged from a minimum of 14.6 (CTRL at 41 DAP) to a maximum of 31.0 (T 75 at 71 DAP). This parameter, expressed as the mean value of samples undergoing treatment with different HyTan formulations, ranged from a minimum of 20.1 (41 DAP) to a maximum of 29.7 (71 DAP). As an average of all observation dates, NBI was higher (+21%) for tannin treated vine leaves (22.9) compared to the control (18.9). Finally, even if it was beyond the scope of this paper, we observed a certain positive effect of the vine leaves resistance to attack of the cotton leafhopper (*Jacobiasca lybica*) polyphagous species.

In brief, results show evidence that only the tannin extraction temperature had a significant effect on the measured characteristics of the shoot and root, while the applied dilution did not show any significant influence. In the case of polyphenols extracted from chestnut wood with water or with aqueous ethanol the extraction rate is controlled by the internal diffusion of polyphenols in wood matrix (Gironi and Piemonte, 2011). Hence, the amount of extractable polyphenols increases as temperature of water increases from 50°C to 80°C, and doubles again when replacing water with aqueous ethanol 40% in EtOH (Gironi and Piemonte, 2011).

Using the HyTan aqueous tannin extract conveniently obtained from chestnut wood via hydrodynamic cavitation, we found that HyTan extracted at the lowest temperature (45°C) had a significant positive effect on the development of important epigeal and hypogeal qualitative characteristics of the cuttings. Parameters such as the total above-ground weight, both fresh and dry, the length of shoots and the overall development of the roots, were all higher than those obtained by treating the grafted cuttings with HyTan extracted at higher temperature. Oxidation of phenolic compounds caused by prolonged extraction at relatively high temperature has been frequently reported. For example, the extraction of phenolic compounds from grape marc with aqueous ethanol 40% in EtOH is better carried out for 20 h at optimal 45°C rather than more rapidly at 60°C to avoid thermal degradation of the extracted phenols yield and lower antioxidant power of the extract (Spigno et al., 2007).

TABLE 8 Foliar biochemical profile after treatment with HyTan extracted at different temperature: variation of chlorophyll (CHL); flavonols (FLAV); anthocyanins (ANTH); and Nitrogen Balance Index (NBI) 41, 55, 71 and 91 days after planting (DAP).

Parameter	Treatment	DAP 41	DAP 55	DAP 71	DAP 91
CHL (μg/cm^2)	T75	18.3 ± 0.99 (b)	18.7 ± 0.40 (b)	21.6 ± 0.73 (b)	25.8 ± 0.83 (b)
	T60	18.5 ± 1.25 (b)	19.1 ± 0.45 (b)	22.6 ± 0.99 (ab)	25.5 ± 0.61 (b)
	T45	24.5 ± 0.30 (a)	24.3 ± 0.30 (a)	23.4 ± 0.29 (a)	28.8 ± 0.41 (a)
	CTRL	19.5 ± 0.85 (b)	19.3 ± 0.36 (b)	23.4 ± 0.81 (a)	24.3 ± 0.89 (b)
FLAV (μg/cm^2)	T75	1.20 ± 0.07 (a)	1.23 ± 0.04 (a)	0.88 ± 0.08 (b)	1.29 ± 0.05 (a)
	T60	1.26 ± 0.05 (a)	1.32 ± 0.03 (a)	0.89 ± 0.09 (b)	1.27 ± 0.04 (a)
	T45	0.92 ± 0.03 (b)	0.93 ± 0.03 (b)	0.94 ± 0.03 (b)	1.25 ± 0.02 (a)
	CTRL	1.31 ± 0.05 (a)	1.33 ± 0.02 (a)	1.31 ± 0.04 (a)	1.23 ± 0.08 (b)
ANTH (μg/cm^2)	T75	0.16 ± 0.01 (a)	0.14 ± 0.004 (a)	0.11 ± 0.006 (a)	0.12 ± 0.006 (a)
	T60	0.17 ± 0.01 (a)	0.14 ± 0.004 (a)	0.10 ± 0.005 (a)	0.12 ± 0.006 (a)
	T45	0.02± 0.002 (b)	0.02 ± 0.002 (b)	0.02 ± 0.002 (b)	0.01 ± 0.003 (c)
	CTRL	0.16 ± 0.008 (a)	0.15 ± 0.003 (a)	0.12 ± 0.02 (a)	0.10 ± 0.004 (b)
NBI	T75	15.2 ± 1.10 (b)	15.7 ± 0.59 (b)	31.0 ± 4.25 (a)	20.8 ± 1.05 (b)
	T60	14.8 ± 0.98 (b)	14.7 ± 0.38 (b)	30.1 ± 3.99 (a)	20.5 ± 0.69 (b)
	T45	30.2 ± 1.64 (a)	30.1 ± 1.64 (a)	28.0 ± 1.39 (ab)	23.5 ± 0.65 (a)
	CTRL	14.6 ± 0.85 (b)	14.7 ± 0.41 (b)	23.2 ± 1.00 (b)	23.2 ± 1.14 (a)

Different letters within a column indicate significant differences (Tukey's test, p-value 0.05). n.s., not significant. Values are reported as means ± SD. T75, T60 and T45 indicate the temperature of tannin extraction at 75°C, 60°C and 45°, respectively.

FIGURE 3
Roots of grafted vines, cv. Zibibbo/Paulsen 1103 treated with HyTan extracted at 45°C (*right*) compared to control (*left*). Photographs taken on February 1, 2022.

Using HyTan extracted at 45°C we found the lowest dry weight/cm values for both fine and thick roots. This can be explained in terms of a greater capacity to accumulate dry matter in the roots subjected to less overall elongation (as in grafted cuttings treated with T60 and T75 HyTan) because, for such reduced growth in length, smaller quantities of assimilates were diverted for this purpose. Indeed, in addition to indicating a greater total accumulation of dry matter generally determined by tannin, data on the distribution of dry matter in Table 7, also show that treatment with HyTan T45 affords a higher S/R ratio, due to higher accumulation of dry matter in shoots and lower in roots. This higher S/R biomass ratio of T45 cuttings can be considered the effect of better growing conditions of the vines, which allows investments in roots to be minimized (Harris, 1992). Woody plants that grow on "good" sites indeed generally tend to allocate a lower proportion of photosynthate to root production than those that grow on "poor" sites (Schenk et al., 2002).

The result obtained using HyTan extracted at 45°C is in line with the emerging findings suggesting the application of biostimulants even from the very early stages of producing good quality vine plant material, especially because the beneficial effects of biostimulants on plant growth are related to positive on changes in root architecture (Canellas et al., 2015). Together, the favorable S/R ratio of the T45 cuttings and the high root length development can both be considered advantageous for the subsequent establishment of vines in the field (Archer et al., 2018). Furthermore, we also observed in terms of length, a higher Fine-Thick root ratio (Cl.1+Cl.2/Cl.3+Cl.4) of all tannin treatments (2.4, on average) compared to the control (1.4), presumably a more favorable condition for ensuring early better vine performance. Fine roots indeed are the main water and nutrient absorption structures for the vine (Keller, 2020). Hence, their increased formation due to the biostimulation action of HyTan extracted at 45°C leads to a greater volume of explored soil can, in fact, not only promote growth in the nursery but can also help to overcome stress phenomena following planting, optimizing the absorption of water and nutrients from the soil (Archer et al., 2018).

Concerning the potential effects of tannin treatments on the foliar biochemical profile, the observed higher chlorophyll content, the lower amount of flavonols and anthocyanins, and the higher NBI in the leaves of cuttings treated with tannin extracted at 45°C, compared to the other treatments and to the control, all confirm recent observations (Blando et al., 2023) for which the larger presence of nitrogenous substances in the leaf tissues points to the invigorating action exerted by the biostimulants on the physiological activity of the vines. Such likely improvement in the assimilation of soil macro and micronutrients essentials to the chlorophyll synthesis, can explain the role of biostimulants in the improvement of some physiological parameters of the vine (increased net leaf CO_2 exchange rate, leaf chlorophyll concentration etc.) (Popescu and Popescu, 2018).

The composition of HyTan – in which gallic acid is 1.35 times more abundant than castalagin/vascalagin (the nonhydrolyzed forms of ellagitannins) and 3.37 times more abundant than ellagic acid, rich also in grandinin/roburin E and roburin A/roburin D – is more diversified (Table 9) when compared to the composition of commercial chestnut tannin conventionally extracted by the tannin industry. The latter, reported for example by Fulcrand and co-workers in 2019 (Karaseva et al., 2019), reveals that commercial chestnut tannin is mainly composed of gallic acid, castalagin and vescalagin. Ellagic acid (EA) is virtually absent, in industrial wood chestnut tannin, even though EA from chestnut wood readily forms

TABLE 9 Main components of HyTan extract[a].

Compound	Concentration (mg/kg)
Gallic acid	2420.4
Castalagin/Vescalagin	1797.0
Ellagic acid	717.2
Grandinin/Roburin E	488.3
Roburin A/Roburin D	311.3

[a]Valued measured on spray-dried solid extract.
Quantative analysis of the HyTan composition carried out by an independent laboratory (Food and Agriculture Organization of the United Nations, 2024) three years after its extraction.

via hydrolysis of ellagitannin (through the production of hexahydroxydiphenic acid which spontaneously converts via lactonization to EA) (Vekiari et al., 2008).

Analyzing chestnut ellagitannins by matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry, Pash and Pizzi in 2002 suggested that the varying composition of chestnut tannins obtained across countries using different extraction methods is actually due to the hydrolysis of the real structure of the tannin as present in nature: a high molecular weight random series of pentagalloylglucose oligomers of the repeating unit shown in Figure 4, affording castalagin, vescalagin, vescalin, castalin, gallic acid and ellagic acid (Pasch and Pizzi, 2002)

Numerous recent studies have identified the highly beneficial role of EA in promoting plant growth and protecting from stressful conditions. For instance, seed pretreatment with EA and ellagic acid foliar spraying afford increased flavonoid, anthocyanin, and protein output in soybean plants (Arab et al., 2022). Under salt stress, canola soaked in ellagic acid developed more quickly and was less damaged by salinity (Khan et al., 2017). Ellagic acid in plants aids the antioxidant response of the cell due to its pronounced free radical scavenging ability and *also* by stimulating the pentose phosphate pathway that in the process produces reducing equivalents ($NADPH_2$) that support cellular antioxidant enzyme response (Vattem and Shetty, 2005). Similarly, numerous recent studies found that gallic acid (GA) has a substantial effect on root development due to its antioxidant and auxin-like promoter activity (Negi et al., 2005), reflected in an increase in growth and antioxidant defenses in GA-treated roots of plants subjected to salt stress (Campobenedetto et al., 2021). Auxin, we briefly remind, is the name of a class of molecules capable to drive growth responses in plants (Teale et al., 2006).

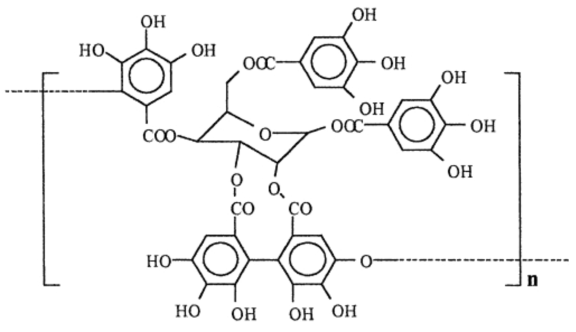

FIGURE 4
Structure of the pentagalloylglucose repeating unit underoging oligomerization in *n* monomers. Source: Reproduced from Pasch and Pizzi (2002) with kind permission.

4 Conclusions

In conclusion, we have discovered that the application of chestnut tannin "HyTan" extracted at low temperature (45°C) via hydrodynamic cavitation from sweet chestnut wood in the nursery process of vine planting material affords a vigorous vine plant material with well-balanced shoot:root ratio and excellent above- and below-ground characteristics allowing to sustain a consistent nursery growth and thus, prospectively, better performance of the vineyard, without the use of chemical fertilizers. In brief, the HC-based extraction process, carried out in water only at low temperature and ambient pressure rather than in superheated water at 110°C under pressure (2 bar) as it happens in the industrial extraction process, allows to quickly obtain a new chestnut tannin aqueous extract particularly rich in ellagic acid and gallic acid, as well as in the nonhydrolyzed ellagitannins (castalagin and vescalagin), whose composition markedly differs from that of commercial chestnut wood tannin commercialized by the tannin industry (Karaseva et al., 2019). As first shown in this study, HyTan can be diluted with an equivalent volume of water retaining its powerful bioactivity. This, *inter alia*, eliminates the need for concentration through expensive membranes as done for instance with 50% chestnut tannin extracted via the conventional extraction process subsequently commercialized for use in agriculture (Proras, 2000), and opens the door to the long-awaited bioeconomy of forest regions based on tannin, a versatile substance whose large-scale employment has been hindered by limited supply (Pagliaro et al., 2021).

The extraction mechanism does not rely on the diffusion of extractable tannins and polyphenols from wood to the aqueous phase as it happens in the conventional extraction (Khatib et al., 2023). Indeed, the conventional extraction of tannin from wood and barks with superheated water in connected steel extraction vessels uses a substantial amount of energy and expensive vessels, valves and pipes (Pizzi, 2003). In contrast, the low-cost extraction of HyTan from chestnut wood at room temperature and atmospheric pressure quickly affords in one pot a ready-to-use aqueous tannin extract. Finally, besides use as plant biostimulant, numerous new applications of HyTan in agriculture can be anticipated, including application as biopesticide and fungicide (Tegli, 2016). As new researches are unveiling a number of new applications and properties of chestnut tannin (Ciriminna et al., 2024), applications of HyTan might include animal feeding, nutraceutics and hopefully medicine (Pizzi, 2021). Further researches in course in our Laboratories will be reported in due time. The process, in conclusion, enables the circular bioeconomy of chestnut tannin because it enables quick and convenient recovery of tannins from chestnut forest residues (Aires et al., 2016).

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding authors.

Author contributions

AP: Conceptualization, Formal analysis, Methodology, Resources, Writing – review & editing. RC: Conceptualization, Formal analysis, Methodology, Writing – review & editing. DP: Conceptualization, Formal analysis, Methodology, Writing – review & editing. DM: Formal analysis, Investigation, Methodology, Writing – review & editing. EB: Formal analysis, Methodology, Writing – review & editing. RD: Formal analysis, Methodology, Writing – review & editing. SP: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – review & editing. LT: Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – review & editing. AS: Investigation, Resources, Writing – review & editing. LA: Formal analysis, Resources, Writing – review & editing. FM: Formal analysis, Visualization, Writing – review & editing. MP: Conceptualization, Formal analysis, Resources, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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