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A technical Dossier analysing the potential use and applicability of TT (Thermal Treatment) therapy for *Xylella fastidiosa* on plant propagation material at commercial scale

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Author(s): Pierfederico La Notte, Antonella Labbate, Laura C. Rosso (P5-CNR) and Josep M. Pagés (P19 - ENA)

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1. INTRODUCTION

The emergence and spread of *Xylella fastidiosa* in Europe require effective integrated management strategies to protect the agricultural and nursery sectors. Within the framework of the Horizon Europe BeXyl project, an entire Work Package (WP3) is dedicated to the development and large-scale implementation of Thermal Treatments (TTs). These treatments act as a sustainable, pesticide-free method to ensure the production and safe trade of *X. fastidiosa*-free plant propagation materials.

This document, constitutes a technical dossier analyzing the applicability of these thermal therapies at a commercial and industrial scale. While preliminary experiments have established the biological efficacy of Hot Water Treatments (HWT) against *X. fastidiosa*, transitioning to routine nursery application introduces complex logistical, physiological, and financial variables. The primary aim of this dossier is to link the experimental validation and the real-world implementation. It achieves this by integrating thermodynamic modeling, evaluations of industrial machinery layouts, economic and environmental assessments (including CAPEX/OPEX and Life Cycle Assessment), and a socio-economic survey (using a questionnaire for nurseries) capturing the current readiness of the European nursery sector.

To facilitate the adoption of thermal treatments across the European nursery industry, this dossier is structured to serve as a practical decision-support tool on two main fronts:

- **Operational support for nurseries:** The dossier provides a functional framework for operators to appropriately integrate thermotherapy into their facilities. By systematically categorizing hundreds of host species based on their climatic hardiness, propagation methods, and physical properties (such as stem diameter), the document guides operators in selecting optimal, species-specific time-temperature protocols. This approach is designed to balance pathogen devitalization with the preservation of plant viability. Furthermore, it outlines engineering guidelines for adopting more sustainable plant architectures to reduce energy consumption.
- **Baseline data for institutional frameworks:** By documenting the current structural constraints of the sector, including equipment investment costs, energy requirements, and market pricing limits, the dossier supplies the necessary quantitative data for public institutions and regulatory bodies. This technical and economic foundation is intended to assist authorities in defining feasible phytosanitary standards and in structuring appropriate support mechanisms for the industry, topics which are specifically examined in the concluding sections of this report.

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2. CLASSIFICATION AND NURSERY INTEREST OF *X. FASTIDIOSA* HOST PLANTS

2.1. Methodological framework: macro-categories for host plant classification

A fundamental requirement for the commercial applicability of TTs is a comprehensive characterization of the vast range of *X. fastidiosa* host plants. To this purpose, the EFSA *Xylella* spp. host plant database (XHPD), reaching the 14th update (EFSA, 2026), was enriched incorporating botanical, agronomic, economic (production and trade), and climatic data. This evolution supports the transition toward predictive monitoring and proactive phytosanitary strategies (Mikulová et al, 2026).

The following analysis integrates the work carried out within Task 3.4 to categorize more than 700 host species based on their functional traits, including habitus, propagation methods, and climatic characteristics. By crossing these parameters with a dedicated ranking of nursery interest, developed in collaboration with ENA, this study identifies priority species for the large-scale industrial adoption of thermal therapies, providing the technical framework necessary to ensure the continuity of safe trade and the production of *X. fastidiosa*-free materials in the European market.

Building upon the physiological and economic framework of the BeXyl project, the methodology developed focuses on the systematic categorization of the host plant species, currently identified in the EFSA XHPD. This classification is designed to transform a comprehensive inventory into a functional tool for assessing the commercial feasibility of Thermal Treatments (TT). To achieve this, four macro-categories were defined to group host plants based on botanical, agronomic, and propagation characteristics. The first macro-category, Plant Type (Habitus), classifies species according to their biological form and foliage persistence. Host plants are divided into evergreen or deciduous and further categorized as woody trees, woody shrubs, woody climbers, herbaceous annuals, herbaceous perennials, or suffrutex. Characterizing the habitus is critical for TT therapy, as the physical structure of the plant, specifically the size and diameter of the woody material, directly affects the rate of temperature transmission to the internal xylem tissues where *X. fastidiosa* live. Furthermore, the distinction between evergreen and deciduous species helps determine the optimal seasonal windows for treatment, particularly for protocols designed for the dormant material.

The second category, Botanical Category, evaluates the ecological and agricultural context of the species. This involves distinguishing between cultivated crops (agricultural species), wild flora (spontaneous vegetation), and weeds. This distinction is vital for phytosanitary logistics; while cultivated species represent the primary target for commercial-scale TT implementation, wild flora and weeds often act as environmental reservoirs for the bacterium and its vectors, such as *Philaenus spumarius*.

The third classification tier, the Nursery Category, aligns host plants with professional market segments. Species are grouped into categories such as fruit trees (crop fruit), ornamentals, forest plants, flowers, aromatic herbs, and vegetables. This professional grouping allows for the evaluation of nursery interest, which is ranked on a 6-level scale (from 0 "no interest" to 5 "very high interest"). By identifying high-value commercial segments, such as the Olea, Prunus, and

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Vitis sectors, the project can prioritize the development of industrial TT protocols for the most economically significant species.

Finally, the Propagation System category is perhaps the most important for the practical application of thermal therapies. Host plants are classified by their primary means of multiplication: seed, agamic grafting, or agamic rooting (cuttings). The applicability of TT is highly dependent on the type of propagation material involved, such as bare root plants, cuttings for rooting, or buds for grafting. This functional classification ensures that the technical solutions proposed are correctly positioned along the production process, tailored to the specific physiological requirements and logistical flows of modern nursery production.

2.2. The EFSA *Xylella* spp. host plant database as a predictive tool

The technical framework for this deliverable is grounded in the evolution of the XHPD, which has transitioned from a static inventory (2015–2020) into a dynamic, open-access resource under the 2021–2026 mandate (Mikulová et al, 2026). Updated semi-annually to reflect ongoing global surveillance and peer-reviewed research, the current Version catalogs 728 host plant species. This extensive dataset, encompassing 319 genera and 91 botanical families, serves as the primary technical foundation for assessing the commercial scope of TTs.

Within the BeXyl project, this scientific repository is transformed into a robust predictive tool for decision-making by integrating big-data components that link literature and nursery production/trade. A core architectural enhancement is the inclusion of quantitative commercial statistics, specifically production volumes and economic values, provided through the collaborative efforts of ENA. This integration allows the prioritization of species for large-scale TT protocols based on actual market flows rather than biological susceptibility alone.

Furthermore, the database's integration of precise climatic indicators and spatial occurrence data facilitates risk-based surveillance. By crossing the host list with geographical parameters and functional traits, the tool could allow phytosanitary authorities to perform spatial risk analyses and implement proactive monitoring in high-risk territories. Ultimately, this enhanced database architecture provides the technical infrastructure necessary to estimate species-specific tolerance to thermal protocols at a commercial scale, safeguarding European agricultural biodiversity and ensuring the continuity of international safe trade.

2.3. Climatic characterisation: hardiness and heat-zone indices

The transition from a taxonomic and commercial classification toward a functional evaluation of host plants requires the systematic integration of environmental tolerance data, which serves as a cornerstone for determining the applicability and safety of thermal treatments on an industrial scale. Within the framework of Task 3.4, this climatic characterization was primarily achieved through the intersection of cold resilience indicators and heat-tolerance indices, providing a multidimensional view of a species' physiological limits. The baseline for determining the geographical envelope and baseline robustness of host plants is established using Hardiness Indices, specifically the USDA Hardiness Zones and the Royal Horticultural Society (RHS) system. The USDA standard, which divides territories into zones based on average annual minimum winter temperatures, remains the global benchmark for defining the survival range of plant species. For a higher precision the RHS system was incorporated to provide a more targeted European perspective; unlike the USDA's reliance on averages, the RHS index

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(ranging from H1 to H7) focuses on absolute minimum temperatures and the duration of cold exposure a plant can withstand before suffering irreversible tissue damage. This distinction is critical for the BeXyl project's predictive modeling, for instance, while an evergreen species like *Nerium oleander* might be assigned to USDA Zone 8, the more stringent RHS H3 rating highlights its vulnerability to short-term, severe frost events. This level of detail allows the project to assess whether propagation materials are sourced from regions where high cold-tolerance traits might influence their baseline metabolic state during winter dormancy.

Complementing these cold-tolerance metrics, the introduction of the AHS Heat Zones (American Horticultural Society) provides the innovative physiological mirror necessary for the commercial deployment of thermal therapies. While traditional botany focuses on cold limits, the AHS system measures the duration of heat stress, specifically the number of days per year a location exceeds the critical threshold of 30°C. This threshold is scientifically significant because, beyond 30°C, the secondary metabolism of many temperate species begins to slow, and cellular proteins risk degradation unless the plant can maintain efficient transpiration. In the context of WP3, these heat zones are used as a proxy for innate thermal resilience. Species adapted to high AHS zones, such as sclerophyllous Mediterranean plants like Olea europaea or Cistus spp., possess structural and physiological adaptations, such as specialized xylem architecture and heat-shock protein pathways, that potentially render them more resilient to the high-temperature protocols (e.g., 45°C for 60 minutes or 48°C for 45 minutes) required to eliminate *Xylella fastidiosa*.

Ultimately, the power of this methodology lies in the convergence of these predictive parameters. By crossing a plant's habitus/characteristics, its propagation requirements/process and the specific AHS/USDA ratings, the researchers can estimate the "viability window" and conditions for thermal treatment. This cross-referencing allows for the prediction of species-specific sensitivity to hot water or hot air or steam treatments before costly industrial trials are initiated. For example, technical reports from WP3 indicate that the viability of dormant material is heavily influenced by the phenology of the cultivar (early vs. late varieties) and its intrinsic cold hardiness. By integrating these climatic indicators into the EFSA database architecture, the project transforms a simple host list into a decision-making tool. This enabling framework allows for the calculation of a Resilience Score, which identifies which priority commercial species can be safely subjected to large-scale TT protocols without compromising their sprouting capacity or subsequent nursery performance, thereby theoretically driving the production of *X. fastidiosa*-free materials across the diverse climatic zones of the European market.

2.4. The Resilience Score

Building upon these climatic indices, the BeXyl project proposes a multidimensional Resilience Score designed to quantify the physiological safety margin for industrial-scale thermal sanitation. By integrating these parameters, the database provides a predictive metric to select commercial hosts that are ideal candidates for standardized thermotherapy, ensuring high viability and sprouting capacity even under rigorous industrial protocols.

2.5. Nursery interest ranking and priority species

Drawing on the physiological and climatic characterization of host plants, the commercial feasibility of Thermal Treatments (TT) is ultimately determined by the market dynamics and economic weight of the species involved. A shift from purely biological parameters to a

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professional assessment of nursery interest and commercial relevance seems to be to rank the applicability and the experimental priorities among the huge *X. fastidiosa* host plant list. An example of the resulting multidimensional classification, for a set of priority commercial species, is given in Table 1.

Table 1. Example of the multidimensional Resilience Score: cold hardiness (USDA zone), heat tolerance (AHS heat zone) and nursery interest for a selection of priority Xylella fastidiosa host species.

Host Plant Species	USDA Hardiness (Cold)	AHS Heat Zone (Heat Proxy)	Nursery Interest	Potential Resilience
Olea europaea	8 - 10	9 - 6 (Mediterranean)	5 (Very High)	**High**
Quercus ilex	7 - 10	10 - 7 (Mediterranean)	5 (Very High)	**High**
Viburnum tinus	8 - 10	9 - 6 (Mediterranean)	5 (Very High)	**High**
Nerium oleander	8 - 10	12 - 1 (Subtropical)	5 (Very High)	**Very High**
Prunus avium	3 - 7	7 - 1 (Temperate)	5 (Very High)	**Medium**

To overcome the lack of official quantitative data regarding nursery trade, fundamental was the role of ENA in conducting a comprehensive survey among the 23 national nursery associations across 22 European countries to gather insights into production volumes, market values, and the willingness of professional operators to adopt thermal sanitation protocols. Despite the limited number of answers and the prevalence of ornamental nurseries, this stakeholder engagement was essential for identifying the "economic biomass" most at risk and for aligning technical research with the logistical needs of the European market.

The central tool for this commercial prioritization is a Nursery Interest Ranking system, which evaluates species on a standardized 6-level scale:

- 0: No interest
- 1: Very low interest
- 2: Low interest
- 3: Medium interest
- 4: High interest
- 5: Very high interest

By applying this ranking to the initial dataset of 665 species categorized from the EFSA database (in 2024), the study identified 301 species as having significant nursery interest. The distribution of these results provides a roadmap for the industrial deployment of TT; while 59% of the interested species were ranked as "very low," a select group of high-value plants emerged as the primary targets for large-scale implementation. Notably, *Quercus ilex* (Holm Oak) was identified as the only species possessing "very high interest" (Level 5) across the sampled nursery sectors, highlighting its status as a critical node in both ornamental and forestal

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production. Following this, 10 species were categorized as having "high interest" (Level 4), representing pillars of the European nursery trade such as *Laurus nobilis*, *Arbutus unedo*, and *Viburnum tinus*, along with major ornamental and forest trees like *Liquidambar styraciflua*, *Liriodendron tulipifera*, and several *Magnolia* and *Quercus* species. Parallel to these ornamental and forestal priorities, the analysis confirms the paramount importance of the major Mediterranean perennial fruit crops. The sectors for olive (*Olea europaea*), almond (*Prunus dulcis*), grapevine (*Vitis vinifera*), walnut (*Junglans regia*), and *Citrus* spp. are identified as strategic targets for the BeXyl thermal therapies. The high economic value and massive trade volumes associated with these plants, particularly the nursery materials for *Vitis* and *Prunus*, justify the intensive development of species-specific TT schedules to ensure the production of *X. fastidiosa*-free propagation material. This integrated ranking ensures that technical solutions and logistics are tailored to the species that define the economic backbone of the European nursery and agricultural industries.

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3. SYNTHESIS OF PLANT BEHAVIOUR AND VIABILITY UNDER THERMAL TREATMENTS

This paragraph summarizes the findings from the extensive experimental trials, mainly well-established technique of Hot Water Treatment (HWT), conducted on the propagation materials of various *Xylella fastidiosa* host species, assessing their physiological behavior and viability following thermal treatments, with the final goal to identify optimal temperature and exposure time combinations that effectively devitalize the pathogen without irreversibly inhibiting the plants' capacity to regenerate, root, or sprout. The analysis of the results highlighted that heat tolerance is not an absolute parameter, instead, it depends on a complex interaction involving the botanical species, the specific cultivar's characteristics, and, crucially, the architecture and phenological stage of the treated plant material (e.g., softwood or hardwood cuttings, bare-root plants, dormant buds, or scions for grafting).

3.1. Sensitivity and variability in olive (*Olea europaea*)

The olive tree served as one of the primary study models due to its evergreen habit, its economic importance and high susceptibility to the epidemic. Several large-scale HWT trials conducted by three different partners in different years and conditions revealed a strong interaction between genotype, cutting type, and thermal stress intensity. Tests covering a wide range of temperatures (from 42 °C to 50 °C) and exposure times (from 20 to 100 minutes) established clear survival thresholds. A temperature of 50 °C, even for just 20 minutes, proved almost universally lethal for olives, with negligible survival rates (1-3%) observed only in exceptionally hardy cultivars like 'Coriana' and 'Koroneiki'. Temperatures of 48 °C resulted in 100% mortality across all trials, regardless of the exposure time. At more moderate regimens (42 °C - 44 °C), genotypic variability became prominent. The cultivar 'Arbequina' consistently demonstrated high tolerance, maintaining rooting rates around 40-50% after treatments at 42-44 °C for 40 minutes. Conversely, cultivars like 'Arbosana' and 'Picual', although capable of moderate rooting at 42 °C, experienced a drastic drop in survival (mortality exceeding 80%) when the temperature increased to 44 °C for extended periods.

Beyond genotype, the nature of the propagation material plays a crucial role: hardwood cuttings generally ensured significantly higher survival rates than softwood cuttings, resisting prolonged heat damage more effectively under identical immersion conditions (e.g., 48.0% average survival for hardwood versus 43.8% for softwood at 42-46 °C).

3.2. Response of almond and other stone fruits (*Prunus* spp.)

For stone fruits, HWT showed interesting potential commercial applications alongside notable limitations depending on the plant portion treated. For almond (*Prunus dulcis*), using dormant buds intended for grafting proved to be a successful approach if finely calibrated. Thermal treatments at 50 °C for 40 minutes caused total tissue browning and necrosis, but regimens at 45 °C for 30 or 40 minutes allowed commercially viable graft success rates (exceeding 70%). A well-defined varietal tolerance scale was recorded: the cultivar 'Pentacebas' showed over 90% success at 45 °C, followed by 'Avijor' and 'Vialfas', while 'Isabelona' was the most sensitive (65-70%). In subsequent trials on *P. dulcis*, temperatures of 48 °C for 45 minutes heavily depressed budbreak in cv. 'Tuono' (dropping to 28%), while cv. 'Genco' maintained excellent viability (76%) despite showing more contained shoot growth.

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Similar outcomes for commercial applications were obtained for nectarines (*Prunus persica*). Treating dormant buds subsequently grafted onto micropropagated rootstocks identified 45 °C for 40 minutes as the optimal regimen, yielding a final viability of 56.3%. Surprisingly, reducing the exposure time to 20 minutes dropped viability to 29%, demonstrating that post-hydrothermal shock cellular reactivation dynamics do not always follow linear patterns. Greater challenges emerged with sweet cherry (*Prunus avium*) where scions and buds proved to be extremely delicate: treatments at 45 °C for 60 minutes lowered grafting success to 50% in cv. 'Ferrovia' and 41.7% in cv. 'Sweet Heart'. When increasing the stress to 48 °C for 45 minutes, 'Sweet Heart' suffered total lethality (0% survival), highlighting the inadequacy of severe HWT. However, using rootstocks or bare-root plants of the *Prunus* genus (e.g., *Prunus mahaleb* and the GF677 hybrid) revealed good resilience, with sprouting percentages often exceeding 70-90% at 48 °C for 45 minutes, although 50 °C completely inhibited vegetative recovery in the GF677 hybrid.

3.3. Resilience of fig (*Ficus carica*), mulberry (*Morus alba*) and walnut (Juglans regia)

Exploring alternative fruit crops, plant architecture at the time of hot water immersion was the main success factor. Walnut (*Juglans regia*) and mulberry (*Morus alba*), when treated as entire dormant bare-root plants, exhibited good resistance: at both 48 °C and 50 °C for 45 minutes, sprouting remained consistently at 100%, with no statistical differences compared to the untreated control. The only drawback was a slight delay in spring shoot elongation. Conversely, mulberry cuttings prepared for rooting showed a marked dependence on wood maturity. Cuttings from early or intermediate-ripening cultivars ('Bianco' and 'Alba') tolerated 48 °C well but suffered a collapse in viability at 50 °C. In contrast, the late-ripening cultivar 'Rosa' handled the 50 °C thermal stress for 45 minutes, achieving near 96% rooting. This demonstrates how an extended autumn lignification cycle provides superior constitutional thermotolerance. For fig (*Ficus carica*), varietal differences emerged clearly at higher regimens. Hardwood cuttings and bare-root plants of the cultivars 'Dottato' and 'Nero di Terlizzi' perfectly tolerate 48 °C for 45 minutes (rooting and sprouting close to 100%). However, an increase of just two degrees (50 °C) breached this tolerance threshold: cv. 'Dottato' saw its viability halved (48%), confirming that the operative TTs conditions must be carefully adapted by genus and variety.

3.4. Ornamental and forest species

Considering nursery trade flows, the study extended to some Mediterranean shrubs and forest species. Oleander (*Nerium oleander*), frequently infected in nature and good evergreen model plants for its high rooting capacity, stood out as an ideal target for hydrothermal baths. Hardwood and semi-hardwood cuttings immersed at 42 °C and 45 °C for 30 minutes maintained excellent rooting percentages (62-77%), statistically indistinguishable from healthy control material. Even with intensified protocols (48 °C and 50 °C for 45 minutes), oleander cuttings exhibited stable viability and callogenesis indices (around 62%), making this species highly suitable for standardized commercial sanitation. In contrast, other ornamental sector species proved incompatible with hydrothermal immersion: *Polygala myrtifolia* and *Lavandula* spp. suffer total necrosis even at 42 °C, while laurel (*Laurus nobilis*) shows extensive failure rates. In the forestry sector, young bare-root trees treated at 50 °C for 45 minutes displayed diametrically opposed behaviors depending on the species' ecology. Vallonea oak (*Quercus*

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vallonea) and elm (*Ulmus minor*) maintain excellent vegetative returns (70%), demonstrating rapid post-immersion recovery. Conversely, sclerophyllous species like Macedonian oak (*Quercus trojana*) and especially the evergreen cork oak (*Quercus suber*) cannot tolerate the HWT, the latter dropping to a negligible and unsustainable 10% survival rate. A synthesis of all the HWT viability trials carried out within WP3, by species, type of propagation material and temperature–time combination, is provided in Table 2.

Table 2. Summary of the hot water treatment (HWT) viability trials carried out in BeXyl WP3: species, number of cultivars tested, type of propagation material, temperature–time combinations applied and period of treatment.

Plant species	N° cvs tested	Material type	HWT Conditions (°C/ Time in minutes)	Evaluation date
O. europaea	**10**	Cuttings (Hardwood and Softwood)	**42, 44, 46, 48, 50 °C / 20' to 100'**	July 2023
O. europaea	**18**	Cuttings	**42, 44 °C / 40' & 60'**	March 2026
P. dulcis	**1**	Bare-root plants	**50 °C / 45'**	April 2023
P. dulcis	**4**	Dormant buds (pre-grafting)	**40, 45, 50 °C / 30' & 40'**	July 2023
P. dulcis	**2**	Dormant buds / Stems	**45, 48 °C / 45' & 60'**	March 2024
P. dulcis	**2**	Buds (inoculated/healthy)	**40, 45 °C / 30'**	August 2024
P. dulcis	**2**	Bare-root plants / Scion wood	**48, 50 °C / 45'**	March 2026
P. persica	**2**	Budsticks for grafting	**50 °C / 30'**	April 2023
P. persica	**1**	Dormant buds (on GF677)	**45 °C / 20' and 40'**	April 2025
P. armeniaca	**2**	Budsticks for grafting	**50 °C / 30'**	April 2023
P. avium	**3**	Bare-root plants / Budsticks	**50 °C / 30' and 45'**	April 2023
P. avium	**2**	Dormant buds / Stems	**45, 48 °C / 45' and 60'**	March 2024
P. avium	**1**	Scion wood	**48, 50 °C / 45'**	March 2026
GF677	**1**	Bare-root plants	**50 °C / 45'**	April 2023
GF677	**1**	Bare-root plants	**48, 50 °C / 45'**	March 2026
P. mahaleb	**1**	Bare-root plants	**48, 50 °C / 45'**	March 2026
F. carica	**1**	Cuttings for rooting	**50 °C / 45'**	April 2023
F. carica	**2**	Dormant buds / Stems	**45, 48 °C / 45' and 60'**	March 2024
F. carica	**2**	Bare-root plants / Cuttings	**48, 50 °C / 45'**	March 2026
M. alba	**1**	Cuttings for rooting	**50 °C / 45'**	April 2023
M. alba	**1**	Dormant buds / Stems	**45, 48 °C / 45' and 60'**	March 2024
M. alba	**3**	Bare-root plants / Cuttings	**48, 50 °C / 45'**	March 2026
J. regia	**1**	Bare-root plants	**50 °C / 45'**	April 2023
J. regia	**1**	Dormant buds / Stems	**45, 48 °C / 45' and 60'**	March 2024
J. regia	**1**	Bare-root plants / Cuttings	**48, 50 °C / 45'**	March 2026
N. oleander	**1**	Cuttings (hardwood/semi-hardw.)	**42, 45 °C / 30'**	March 2025
N. oleander	**1**	Cuttings	**45, 48, 50 °C / 30'-45'**	March 2026
***Lavandula* spp.**	**1**	Cuttings	**42, 45 °C / 30'**	March 2025
L. nobilis	**1**	Cuttings	**42, 45 °C / 30'**	March 2025
P. myrtifolia	**1**	Cuttings	**42, 45 °C / 30'**	March 2025
Q. suber	**1**	Plants (young)	**50 °C / 45'**	Febr. 2026
Q. trojana	**1**	Plants (young)	**50 °C / 45'**	Febr. 2026

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Q. vallonea	**1**	Plants (young)	**50 °C / 45'**	Febr. 2026
Ulmus minor	**1**	Plants (young)	**50 °C / 45'**	Febr. 2026

3.6. Perspectives on steam thermal treatments

Given the physiological challenges observed for more delicate or metabolically active crops during prolonged liquid immersion, recent investigations (winter 2026) have explored the use of hot steam as a thermal transfer media. Although still in initial stage, the results have revealed highly interesting prospects. On grapevine (*Vitis vinifera*), dormant canes subjected to steam at 53 °C (for 45 and 60 minutes) returned 100% budbreak, although displaying a noticeable slowdown in the phenological recovery kinetics compared to the traditional hydrothermal bath. The advantage of steam, however, proved decisive in evergreen species like *Nerium oleander*, where graduated heat (53 °C for 45-60 min) ensured significantly higher rooting and development rates compared to the severe necrotic trauma caused by the rapid thermal transfer of hot water.

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4. TECHNICAL SOLUTIONS AND LOGISTICS FOR COMMERCIAL IMPLEMENTATION

The transition of thermal treatments (TT) from controlled laboratory validations to continuous, large-scale commercial application in nurseries of different working capacity represents an important operational and engineering challenge. While establishing the biological efficacy of a treatment, such as the reference EFSA protocol for *Vitis vinifera* requiring 50°C for 45 minutes, provides the fundamental phytosanitary target, executing this reliably on an industrial scale requires highly sophisticated machinery, rigorous thermodynamic management, and advanced material handling. It is anyway crucial to recognize that the vast biodiversity of *Xylella fastidiosa* host plants and the varied nature of propagation protocols (rooting, grafting, micropropagation) and planting materials (e.g., bare-root dormant or evergreen plants, dormant cuttings, summer budsticks, potted evergreens) preclude a "one-size-fits-all" logistical approach. In our scope this paragraph, through an extensive literature review, several nursery consultations, and especially 4 years of BeXyl trials at different scale, is aimed to: a) describe the characteristics and the TTs technology actually available on the market; b) specify the main applicative requirements in term of volume/ working capacity, layout, period of treatment, logistic needs, and energy source related to the different plant species and propagation materials considered; c) evaluation of investment costs in equipment and operating costs; d) evaluation of alternative energy sources and LCA. A full-scale industrial thermotherapy installation of the type discussed in this section is shown in **Figure 1**.



Figure 1. Industrial hot water treatment facility in a commercial grapevine nursery, showing the insulated treatment tank, the loading gantry and the forklift handling system.

4.1. Review of industrial thermotherapy machinery and layouts

The thermotherapy machines currently available on the market were designed and developed several years ago to meet the specific needs of grapevine nurseries. These nurseries were primarily dealing with phytosanitary issues related to phytoplasmas (such as Bois noir and Flavescence dorée), fungal diseases (like the agents of Esca disease and Petri disease), and certain insects, including the grapevine phylloxera (*Daktulosphaira vitifoliae*). In addition to easily adjustable temperature conditions and treatment times (the Standard EPPO PM 10/18 of 2012 require the submersion in hot water at 50°C for 45 minutes), the requirement to treat

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large quantities and volumes of dormant grapevine wood within a short timeframe has dictated the structural design choices. For instance, this led to the use of large, cubic-shaped metal baskets that can be easily filled with bundles of plants or bags of cuttings and then handled and loaded into the treatment tanks in a discontinuous batch process using forklifts. Below are the main types of machinery available:

4.1.1. Single-tank industrial immersion systems

This configuration represents the backbone of modern thermotherapy. Equipment such as the spanish RG Projects THERMO series (e.g., THERMO T-110, T-220, and T-400) features massive insulated stainless-steel tanks capable of handling large batch volumes. A standard heavy-duty unit might utilize a 9.6 m^3 tank powered by high-capacity primary circuits, such as a 220-kW diesel boiler. Similar configurations are also used by the Italian company Appiano Romano (1 tank model, 2900lt) and the french Chauvin-agro. To satisfy strict phytosanitary regulations, these machines employ multiple high-flow recirculation pumps to prevent thermal stratification and rely on advanced Proportional-Integral-Derivative (PID) algorithms to maintain the water temperature within a strict ±0.5°C tolerance. The main single-tank units currently available on the European market are shown in Figure 2.



Figure 2. Commercial thermotherapy units currently available on the European market: Appiano Romano (IT), RG-Projects (ES) and Chauvin-Agro (FR).

4.1.2. Batch capacity and throughput for grapevine propagation material

To fully understand the logistical efficiency of current industrial Hot Water Treatment (HWT) systems, it is essential to quantify the volume of material that can be processed in a single operational cycle. Grapevine nurseries represent the benchmark for this high-throughput logistics. Current industrial tanks, equipped with large cubic metal baskets, are optimized to maximize the load density of dormant grapevine material. **Table 3** provides an estimation of the average quantities of different grapevine propagation materials, rooted cuttings (barbatelle), rootstock/scion cuttings, and specific grafting buds, that can be safely treated

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during a single standard thermotherapy cycle in a large-scale commercial tank (e.g., 8,000 - 9,600 L capacity).

Table 3. Estimated average load capacity of a single thermotherapy cycle in a large-scale commercial tank (8,000–9,600 L) for the main types of grapevine propagation material.

Type of material	Units
Bare rooted plants (in bunches of 25), variable with the Cv for the diameter and root's development	5.000-7.000 plants
Dormant rootstock cuttings for propagation	10.000-13.000 units
Buds for omega grafting (in bags)	60.000 (12 bags of 5.000 units)
Buds for hand grafting in pots (in bags)	96.000 (12 bags of 8.000 units)

This massive throughput highlights why current machinery is highly cost-effective for the viticulture sector, but also emphasizes the logistical challenges when transferring this technology to other fruit or ornamental species, which often require much smaller daily grafting batches or feature incompatible plant dimensions.

4.1.3. Multi-tank and phased logistics layouts

To increase industrial continuity and minimize thermal shock, multi-tank layouts are highly recommended. Systems such as the Appiano Romano double-tank thermotherapy unit (approximately 2.900 L capacity, utilizing 8 to 13.5 kW electric heating elements) allow for a phased logistical approach. A continuous nursery workflow can be established where material is pre-heated or acclimatized in the first tank, treated at the lethal target temperature in the second, and subjected to rapid anti-inertia cooling in a subsequent stage. An example of such a multi-tank layout is shown in Figure 3.



Figure 3. Multi-tank thermotherapy installation allowing a phased workflow of pre-heating, treatment at the lethal target temperature and rapid anti-inertia cooling.

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4.1.4. Spray and recirculation cabins

While designed for standard pallet-bin logistics and post-harvest fruit operations, other technologies (e.g., the XEDA Hydroheater) distribute hot water over stacked bins by continuously collecting and reheating the run-off. These systems do not appear suitable for densely packed woody nursery propagation materials, which require intensive hydraulic validation, because their heat transfer dynamics differ significantly from full submersion methods.

4.2. Limitations and adaptations of TT equipment for other species and propagation materials

From the experiments conducted within the BeXyl project on various species, such as stone fruits or other species, important technical details have emerged which suggest that current machines are not versatile enough for efficient use, thereby requiring profound structural changes or technical adaptations. Below are some considerations regarding application methods that differ from those used for grapevines:

- **Treatment of dormant bare-root plants.** Unlike grapevines, in the case of mulberry, walnut, fig, stone fruits, and their respective rootstocks, for example, bare-root plants can reach considerable lengths, from 1.5 to over 2 meters. This makes the complete filling of cubic-shaped baskets difficult, whereas rectangular-shaped tanks would be much more suitable. The difficulty of loading long bare-root plants into baskets designed for grapevine material is illustrated in Figure 4.



Figure 4. Loading of long bare-root plants into a cubic basket. Dormant bare-root plants of different species and sizes prepared in bundles for treatment, next to the cubic metal baskets originally designed for grapevine material.

- **Treatment of budsticks for grafting.** Unlike grapevines, where graft buds are single-node, possess a nearly constant diameter, and above all can be cold-stored after treatment, for many other species such as stone fruits, the buds must be used, meaning grafted, immediately after the treatment to avoid reductions in viability and grafting success. Consequently, the quantity of buds to be treated must be reduced according to the daily grafting capacity, which translates to bundles of thin graft cuttings with very small volumes needing to be treated on a daily basis.

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- **Treatment period.** Another important difference concerns the period of treatment for materials of some species propagated by bud grafting or rooting, which typically occurs in two windows, respectively in spring or late summer. During these periods, it is possible to utilize energy from photovoltaic or even solar thermal systems, especially for small-capacity thermotherapy tanks.
- **Level of nursery specialization.** Unlike grapevine nurseries, which are almost entirely specialized in producing tens or hundreds of varieties in different grafting combinations, most fruit and especially ornamental nurseries multiply numerous species, often in medium or low volumes, and across different periods and with different propagation methods. Consequently, there is a need for much smaller, more versatile machines, potentially powered in a more sustainable manner, compared to those currently available on the market.

Given all the above, it is reasonable to foresee for the future that, if the development and use of thermotherapy prophylaxis for propagation materials begin to spread in nurseries across a greater number of species and larger volumes of materials, it will drive technical innovation and the development of new machines suitable for these diverse operational needs.

4.3. Logistical needs, material preparation and handling arrangements

A fundamental logistical bottleneck in commercial nurseries, particularly for the treatment of dormant materials such as grapevines, is the massive seasonal volume of rooted plants and propagation materials: millions of plants must be processed within a very narrow winter window. Since the precise treatment time is dictated by multiple variables, including stem radius, initial material temperature, bath temperature, convective heat-transfer coefficient, and specific wood properties such as density and moisture-dependent thermal conductivity, optimizing the logistical workflow is mandatory to speed up and improve the efficiency of the whole process.

4.3.1. Material sorting and pre-treatment logistics

To prevent catastrophic operational failures, nurseries must rigorously sort and batch their propagation materials before any thermal application. Materials must be grouped by species, type of plant material, and, most importantly, by stem diameter. Mixing thin cuttings with thick rootstocks or large bare-root plants in a single commercial batch will inevitably result in either the severe thermal damage (over-treatment) of the thin materials, or the survival of the pathogen (under-treatment) in the thick materials. Implementing a multi-tank equipment layout or introducing a prolonged pre-conditioning phase at room temperature can significantly reduce the actual immersion working time and improve the overall thermal transfer efficiency. The main categories of propagation material that must be sorted and batched separately are illustrated in Figure 5.

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Figure 5. Types of propagation material requiring separate batching before treatment: bundled cuttings in cages (left), jute bags of budsticks (centre) and single-node grafting buds (right).

4.3.2. Standard operating workflow for material handling

Implementing these advanced technical solutions require a strict, sequential operating philosophy to ensure both phytosanitary compliance and the biological survival of the plant material. Based on large-scale logistical trials conducted at facilities such as Vivai Murciano (Otranto, IT), the standard workflow should be structured as follows:

1. **Preparation and cold storage:** Following harvest, propagation materials (bare-root plants, cuttings, or budsticks) are sorted by diameter and immediately transferred to cold storage rooms maintained at 4°C. This crucial infrastructural step enforces plant dormancy and preserves internal carbohydrate reserves.
2. **Pre-conditioning and hydration:** Prior to thermotherapy, materials must be removed from cold storage and acclimatized at room temperature for up to 24 hours. Proper hydration is physically critical, as increased moisture content directly enhances the internal thermal conductivity of the wood. For bare-root plants, an essential logistical requirement is thorough washing with high-pressure water to remove all residual field substrate, which would otherwise contaminate the treatment bath and clog the recirculation pumps. This washing step is illustrated in Figure 6.



Figure 6. High-pressure washing of bare-root plants to remove residual field substrate before immersion, preventing contamination of the treatment bath and clogging of the recirculation pumps.

3. **Loading and thermal treatment:** The material is secured in specialized stainless-steel cages or platforms and submerged. Nurseries must account for the fact that the massive introduction of cold wood and trapped ambient air instantly depresses the water

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temperature, requiring heating systems capable of rapid thermal recovery before the official treatment timer can begin.

4. **Unloading and immediate acclimatization (cooling):** Upon completion of the cycle, the platform automatically extracts the material. To prevent latent tissue damage caused by residual internal heat, it is strongly recommended a logistical step to immediately spray the material with water or transfer the material into an adjacent ambient-temperature water bath or a dedicated cooling zone to rapidly halt the heat transfer process. The loading and washing operations of a full basket are shown in **Figure 7**.



Figure 7. Washing and hydration of a loaded basket prior to thermotherapy (still frame from the demonstration video).

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5. ECONOMIC AND ENVIRONMENTAL ASSESSMENT

The transition of thermal treatments (TT) for the elimination of *X. fastidiosa* from a strictly biological and experimental validation phase to an industrial-scale nursery application requires a rigorous economic and environmental sustainability assessment. The feasibility of adopting large-scale HWT (Hot Water Treatment) thermotherapy depends on the capacity of nursery infrastructures to support the significant required energy load without compromising their profit margins and without increasing the carbon footprint of the production cycle. The analyses conducted within BeXyl Task 3.4, and specifically the engineering models developed by the subcontractor Politecnico di Bari, demonstrate unequivocally that merely transferring current fossil fuel-based technology to a large scale is incompatible with the objectives of the European Green Deal and the financial stability of the companies. This section analyzes the economic and environmental impact of thermal treatments, starting from the thermodynamic modeling of energy needs, evaluating renewable alternatives, and quantifying the capital expenditures (CAPEX) and operating expenses (OPEX) necessary for a widespread commercial use of thermotherapy prophylaxis. To size the industrial plant, the energy model study must add up not only the sensible heat required to warm the wood mass (Q_wood), but also the massive energy for the initial heating of the water volume (Q_init), the replenishment of water lost due to drag-out (Q_repl), the thermal transmission losses through the tank walls, and the latent consumption related to surface evaporation during opening cycles. Each of these components translates directly into kilowatt-hours (kWh) consumed per cycle, defining the OPEX profile of the machine.

5.1. Baseline scenario: cost and carbon footprint of fossil fuel dependence

As described in the previous section, the current industrial technological landscape available on the market for nursery thermotherapy heavily relies on the use of diesel boilers, which translates to highly fossil-intensive configurations. The economic and environmental impact of this conventional approach was first analyzed across its phases at the Murciano grapevine nursery (Otranto, ITA) and then quantified using multi-year real thermotherapy data provided by the largest European grapevine nursery, Vivai Cooperativi Rauscedo (Case Study 2). The cooperative nursery operates two large treatment plants with capacities of approximately 8,000 liters and 7,260 liters. To meet an operational demand of six daily treatment cycles, the documented consumption stands at about 50 liters of diesel per day. Modeling this scenario, the energy requirement from diesel amounts to a substantial 535.83 thermal kWh per day, which translates into a very high and concerning operational carbon footprint of nearly 134 kg of CO_2 emitted daily into the atmosphere (corresponding to a Life Cycle Assessment LCA indicator of over 160 kgCO2e/day).

From an economic standpoint, assuming a conservative cost of agricultural diesel at 1.20 EUR/liter, the operational fuel cost alone for the treatments reaches 96.20 EUR per day. On an annual basis and considering the need to sanitize increasingly larger volumes of material to comply with quarantine regulations, this total dependence on fossil fuels exposes company balance sheets to the dramatic volatility of risky international energy markets. Furthermore, the use of natural gas boilers, although it reduces operational emissions to about 101 kgCO2/day and halves daily costs to about 54.49 EUR, does not resolve the issue of fossil fuel

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dependence and non-compliance with European directives on the ecological transition of the agricultural sector. Gas cogeneration (Gas CHP) was also evaluated but discarded due to a combined thermal and electrical efficiency that is not favorable for the intermittent load profiles typical of these treatments.

5.2. Strategic assessment of renewable energy alternatives

To overcome the structural limitations of fossil technologies, an in-depth screening of renewable sources was conducted. Wind energy (difficult to integrate in urbanized or greenhouse-dense contexts), medium-enthalpy geothermal energy (due to a lack of widespread resources in target areas such as Apulia), and green hydrogen (considered not yet economically scalable for nurseries) were preliminarily discarded. Attention focused on local solid biomass, solar thermal, and, predominantly, the integration of photovoltaics (PV) and high-temperature heat pumps (HP).

Direct solar thermal theoretically offers the lowest absolute operational costs (estimated at about 11.60 EUR/day for the large nursery) and an almost zero carbon footprint. However, especially for grapevines and other deciduous species, the analysis highlights a severe seasonal mismatch: peak solar thermal energy production occurs in the summer months, whereas nursery operations of harvesting, processing, and treating dormant propagation material are almost exclusively concentrated in the winter months. This misalignment makes solar thermal a complex option, unless massive inter-seasonal storage basins or extensive greenhouse heating needs are available to absorb the excess summer heat.

Solid biomass boilers, instead, represent a robust and implementable alternative. The use of pellets or, preferably, olive-pit biomass offers excellent operational resilience. In the case of the large nursery, an olive-pit boiler would require about 636 thermal kWh per day, zeroing out operational CO_2 emissions (being a carbon-neutral biomass) and lowering daily costs to about 42.26 EUR. Moreover, using local agricultural waste perfectly aligns with the circular economy principles promoted by European policies.

The solution that unquestionably emerges as the most efficient and ecologically sustainable is the combination of electrical energy generated by photovoltaic systems coupled with high-temperature air-to-water Heat Pumps (HP). In the case of the large nursery, which already boasts a pre-existing 425-450 kW photovoltaic installation capable of producing about 500,000 kWh annually, the energy input drops drastically to only 169.68 electrical kWh per day (thanks to the heat pump's Coefficient of Performance, COP). The LCA indicator plummets to just 8.48 kgCO2e/day and the operational cost falls to 22.14 EUR per day, guaranteeing the highest level of environmental sustainability and economic competitiveness among fully dispatchable options.

5.3. Integrated plant architecture and PCM thermal storage

From a strict technical standpoint, directly powering an energy-intensive and discontinuous industrial process through photovoltaic panels presents the critical issue of solar radiation intermittency. To ensure that environmental sustainability does not compromise nursery productivity, the developed and proposed plant architecture is based on an innovative integrated system. The conceptual architecture does not merely replace the burner but redesigns the entire energy flow. The electrical energy produced by the PV array powers the

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high-temperature heat pump during peak sunlight hours. The latter does not transfer heat directly to the treatment tank but charges an advanced thermal storage system based on Phase-Change Materials (PCM). The PCM storage acts as a vital energy buffer, temporally decoupling electrical generation from batch operations; the storage is designed to maintain forced temperature stratification, preserving the useful thermal energy that will be rapidly released to the secondary circuit of the HWT tank exactly when the treatment cycle begins, limiting the start and stop cycles of the heat pump.

To guarantee the absolute continuity of quarantine operations even during prolonged winter weather disturbances or electrical malfunctions, the architecture integrates a backup boiler fueled by olive pits in parallel. Furthermore, a significant engineering advantage of this layout is the ability to recover the "cold side" of the heat pump (i.e., the chilled air or water expelled during heat generation); this cooling energy can be usefully channeled towards the cold storage rooms that generally abound in nurseries, where plant material must be kept at low temperatures before or after treatment, thereby increasing the overall efficiency of the system to levels unattainable by traditional combustion boilers. The corresponding heat generation and distribution unit is shown in Figure 8.



Figure 8. Heat generation and distribution unit of the integrated plant architecture, combining a high-temperature heat pump with the hydraulic circuit serving the treatment tank.

5.4. CAPEX analysis and impact on unit production costs

Implementing renewable energy architecture entails a radical repositioning of the nursery company's cost structure: there is a dramatic reduction in operating expenses (OPEX) against a massive increase in capital expenditures (CAPEX). By processing commercial price lists, a parametric formula was defined to estimate the initial cost of industrial thermotherapy plants based on the required nominal power: P = 285 * [kW] + 30,000 €.

Applying this economic modeling to Case Study 2, the cost to install an advanced integrated plant of approximately 425 equivalent thermal kW amounts to 151,125 EUR. Focusing specifically on adding a 100 kW high-temperature heat pump to retrofit an existing plant, the estimated cost is around 25,000 EUR. Assuming a savings of 50 liters of diesel per day at a cost of 1.20 EUR/liter (yielding a gross operational savings of about 60 EUR daily), the Payback Time settles at a highly competitive period of approximately 4.5 years, excluding any financial charges or additional costs for electrical infrastructure upgrades.

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What is fundamental for the commercial competitiveness of the European nursery sector is the impact of these investments on the final cost of the single kilogram or single treated woody plant. The processed data shows that for an annual treated volume exceeding 1,850,000 kg of plant material, amortizing the CAPEX over a 10-year horizon, the plant depreciation quota accounts for only 0.008 EUR per kilogram of wood. Added to this is an optimized variable operational cost (energy/fuel), estimated at 0.011 EUR per kilogram, bringing the total cost of thermotherapy to less than 0.02 EUR per kilogram.

These economic parameters demonstrate that, although the financial barrier to entry for purchasing the machine and the related renewable integrations (heat pump, PCM storage) is very high for small and medium-sized enterprises, the unit cost of the process absorbed by the single finished product remains absolutely negligible and easily absorbable within commercial margins. This makes the transition towards sustainable thermal sanitation an important step from a phytosanitary perspective against the threat of *Xylella fastidiosa* and other harmful organisms, but also economically advantageous in the medium-long term, capable of qualifying European germplasm on international markets without structurally burdening the final selling price to the agricultural sector.

5.5. Non-energy operating costs

While energy consumption represents the primary driver of operating expenses, a truly comprehensive and realistic techno-economic modeling must account for all non-energy operational costs (OPEX). A thorough *a priori* evaluation of these variables is essential to properly orient and optimize capital investments in machinery and facility infrastructures.

Specifically, the execution of thermal treatments entails significant water management costs. The continuous processing of plant material, especially bare-root plants, introduces soil and organic debris into the system, requiring periodic water replacement, advanced filtration, and compliant wastewater disposal. Additionally, the process involves extra labor costs to manage the complex material handling and logistical operations required before and after the thermal cycles.

Operating under strict phytosanitary quarantine regimes also introduces essential maintenance and compliance expenses. Commercial units require routine servicing (such as heat exchanger decalcification), periodic calibration of temperature sensors, and dedicated resources for the administrative traceability and reporting demanded by phytosanitary protocols.

Integrating these logistical variables and hidden operational costs into an *a priori* business plan is fundamental. It ensures that the final investment in sustainable thermotherapy architecture is financially viable, seamlessly integrated into the nursery's specific workflow, and perfectly optimized for long-term commercial success.

5.6. Decision-support tool for techno-economic and environmental modelling

In the framework of task 3.4, in collaboration with the subcontractor Politecnico di Bari, a prototype calculation tool has been developed (File: BEXYL_Energy_Heating_Cycles_v17_Case_Study_2_EN.xlsx). This tool uses a dedicated checklist (File: BEXYL_Energy_Consumption_Checklist_EN.xlsx) to gather data and energy

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modeling inputs through interviews and on-site nursery inspections, with the aim of designing new thermotherapy plants or optimizing existing ones.

This system allows users to size new prototypes or rely on the technical specifications of existing commercial products, applying either new or standard operating cycles. It features dynamic lists for supply sources, CAPEX/OPEX estimates based on production capacity, detailed thermal balances, operational emission calculations, Life-Cycle indicators, and economic comparisons. Ultimately, this workbook serves as a valuable decision-support tool to compare commercially available machines and various thermal energy sources from both an economic and environmental perspective.

6. NURSERY SECTOR PERSPECTIVES: QUESTIONNAIRE RESULTS

6.1. Objectives of the survey

Within BeXyl project, specifically Task 3.4, a structured multi-lingual questionnaire was developed and disseminated to evaluate the awareness, interest, and readiness of the European nursery sector to adopt thermal treatments (TTs) for plant health management. This activity was essential to transition from experimental validation to commercial implementation by identifying the primary technical and economic barriers - such as high initial investment costs and specialized knowledge gaps - that currently hinder the large-scale adoption of these sustainable sanitation and preventive protocols. Furthermore, the survey served as a critical tool to collect quantitative data on nursery production and to align future research priorities and regulatory developments with the actual needs and risk perceptions of industry stakeholders.

The survey instrument, in form of a questionnaire, was developed through a collaborative effort between CNR and the European Nurserystock Association (ENA) as a key activity within Task 3.4. The primary goal was to collect quantitative and qualitative data to evaluate the readiness of the European nursery sector to implement thermal treatments (TTs) for plants. Specifically, the activity aimed to assess the current level of knowledge regarding Xylella fastidiosa and the perception of related risks to nursery activities and plant trade. Beyond awareness, the survey was designed to evaluate the interest of operators in adopting thermal treatments for Vitis and other *X. fastidiosa* host species and to identify the primary technical and economic barriers, such as high investment costs, lack of specialized knowledge, or concerns regarding plant damage that currently hinder the large-scale adoption of these techniques. Furthermore, the questionnaire served as a tool to guide future research by identifying priority plant species for expanded TT validation and to establish a robust baseline of quantitative data regarding the volume and value of nursery production per species, filling a significant gap in official EU and national statistics. Finally, the survey aimed to determine the technical, financial, and regulatory support needs required to encourage the implementation of sustainable thermal treatment protocols across the sector.

6.2. Questionnaire design and dissemination

The questionnaire was designed as a structured multi-lingual instrument to ensure broad geographical representation across the European Union, specifically targeting countries significantly impacted by *X. fastidiosa* outbreaks, such as Italy, Spain, France, and Portugal.

The survey was structured into three main sections to ensure a comprehensive assessment:

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1. Nursery Profile: This section collected descriptive data, including geographic location, nursery size, and types of species produced (e.g., deciduous fruit crops, evergreens, or ornamentals), essential to contextualize responses and identify shared needs across specific nursery categories.
2. Respondents' Perception – Awareness and Knowledge of TTs: This core section aimed to quantify familiarity with thermal sanitation protocols and identify the specific challenges perceived by stakeholders.
3. Additional Comments and Future Participation: An open-ended section allowed stakeholders to express specific concerns and register their willingness to participate in future experimental trials or receive project updates.

The survey was hosted on the Microsoft Forms online platform to ensure cross-device accessibility. To reach a wide and diverse panel of stakeholders, dissemination followed a multi-channel approach:

- Direct mailing lists targeting nursery organizations and individual operators.
- Promotion through social media platforms and professional networking sites.
- Promotion through the organization of technical seminars for nurseries and NPPOs.
- Technical articles and project newsletters are designed to stimulate interest within the scientific and professional communities.

To further reduce response bias and encourage transparency, participation was strictly voluntary, anonymous, and confidential, in full compliance with the EU General Data Protection Regulation (2018). The questionnaire was optimized for user experience, requiring approximately 10 minutes to complete, thereby maximizing completion rates among industry professionals. This structured methodology provided an accurate baseline for evaluating the economic feasibility and environmental impact (e.g., carbon footprint) of adopting TTs at a commercial scale. The distribution of the responses obtained across the seven language modules is shown in Figure 9.

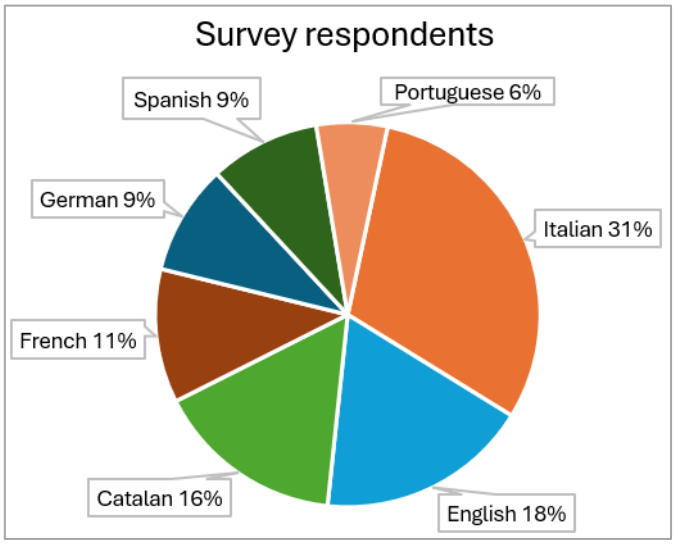


Figure 9. Distribution of the 151 questionnaire responses across the seven language modules.

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6.3. Response rate and data analysis

The survey successfully reached a diverse group of stakeholders across Europe, gathering a total of 151 responses through its seven language-specific modules. The multi-lingual strategy was instrumental in capturing perspectives from regions with varying levels of *Xylella fastidiosa* impact and nursery production traditions. The breakdown of responses by language is as indicated in Table 4.

Table 4. Number of questionnaire responses received per language module (n = 151).

N. of responses for languages							
Italian	**English**	**Spanish**	**French**	**Catalan**	**German**	**Portuguese**	**TOT.**
46	27	14	17	24	14	9	**151**

This distribution highlights strong engagement from the Italian nursery sector, which has been significantly affected by *X. fastidiosa* outbreaks, as well as notable participation from the Spanish/Catalan and French sectors. The duration of the survey ensured a robust period for data collection.

The primary data was processed using descriptive statistics to characterize the nursery profiles and quantify the level of knowledge and perceived risk regarding *Xylella fastidiosa*. This quantitative baseline was then enhanced by a thematic grouping of qualitative feedback from the open-ended sections. By using visual synthesis tools such as Word Clouds, recurring priorities regarding plant species and technical barriers (e.g., "costs," "efficacy," "technical knowledge") were identified directly from the stakeholders' own words. Rather than treating these as separate studies, the analysis employed cross-tabulation to compare responses across different nursery sectors (e.g., grapevine vs. ornamental) and geographic regions. This combined approach ensured that the statistical trends were contextualized by the technical and economic realities reported by operators. Ultimately, the survey findings served as a foundational dataset for the broader SWOT Analysis within Task 3.4, guiding the evaluation of the large-scale feasibility of thermal treatments and helping to define the economic and environmental baseline (e.g., carbon footprint) for future sustainable thermal treatment protocols.

6.4. Profile of the respondent nurseries

Building upon the integrated analytical framework and dissemination methods previously established, the survey data provides a detailed characterization of the European nursery sector's current profile. The professional roles declared by the respondents are summarised in Table 5.

Regarding the professional profile of the sample, the respondents demonstrate a high level of industry expertise, with a significant majority reporting over 20 years of activity in the nursery sector. In particular, the professional landscape of the respondents is characterized by a strong presence of decision-making roles, with a combined total of 53 sole proprietorships and 30 members of family-owned corporations across all surveyed regions. The participation of specialized technical and management figures was also significant, involving 2 partners, 17 technical directors, and 7 pest management (IPM) professionals, in addition to 10 commercial

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managers (*Note: Total responses exceed the number of participants (151) as respondents could select multiple professional roles*).

Table 5. Professional roles declared by the survey respondents. Totals exceed 151 because respondents could select more than one role.

Professional Role	Frequency
Sole proprietorship / Individual enterprise	53
Member of family-owned corporation	30
Other (Altro)	23
Partner in nursery	22
Technical Director / Production Manager	17
Commercial Manager	10
IPM / Pest Manager	7
Member of cooperative	3

This composition underscores that the insights gathered reflect the strategic perspectives of those directly managing nursery operations and technical protocols, providing a robust baseline for evaluating the feasibility of adopting TTs methods. Structurally, the surveyed businesses are predominantly integrated enterprises engaged in both propagation and production activities. Their production focus is primarily concentrated on key woody species, specifically fruit crops, grapevines, and ornamental plants, which represent the core interest groups for the validation and implementation of TTs protocols. The structural classification of the responding businesses and the main plant categories produced are presented in Figure 10 and Table 6.

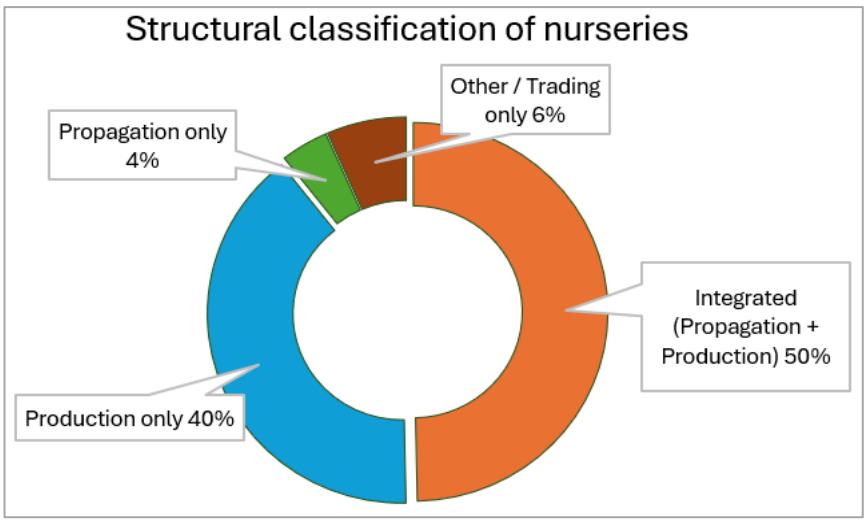


Figure 10. Structural classification of the responding nurseries by type of activity. Main plant categories produced by the responding nurseries (multiple answers allowed).

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Table 6. Main plant categories produced by the responding nurseries (multiple answers allowed, n = 151).

Plant Category	Frequency (n)
Woody ornamentals	83
Fruit crops	53
Grapevines	47
Olive trees	41
Forest plants	16
Herbaceous ornamentals	59
Other	14

This specialization is further evidenced by a strong commitment to modernization, with a large portion of nurseries having adopted innovations in new products and automated processes over the last five years. These established structures and high levels of expertise provide a robust context for assessing the sector's awareness and readiness to adopt the sustainable TTs protocols developed within the BeXyl project. The types of innovation adopted are shown in Figure 11.

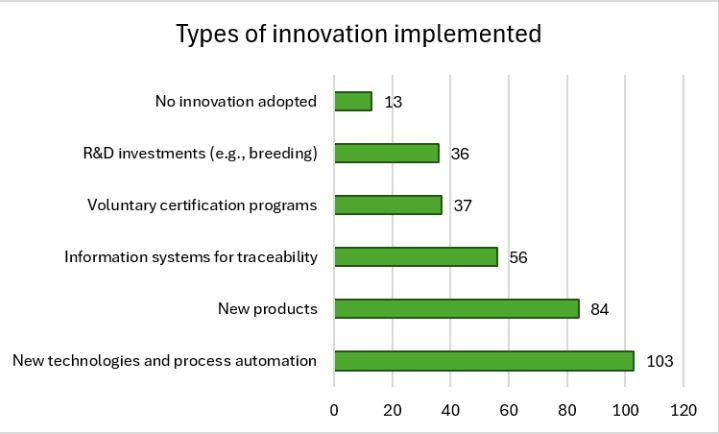


Figure 11. Types of innovations implemented among the responding nurseries.

6.5. Awareness of *Xylella fastidiosa* and perceived economic impact

One of the questions asked of nursery gardeners called for a self-assessment of the level of knowledge on the topic *Xylella fastidiosa*: it turned out that the European nursery sector generally considers itself well informed. Across the various geographic regions, a significant majority of respondents described their level of knowledge as "fairly well-informed" or "moderately informed". Specifically, aggregated data shows that 54,3% of the total sample possesses a solid understanding of the pathogen, combining those who feel "fairly well" and "extremely well" informed. For nurseries operating in Italy, which represents the largest respondent group (n=46), awareness is particularly high: over 50% of participants claimed to be fairly well-informed, with a notable portion stating they were extremely well-informed. This high level of awareness is a critical baseline for Task 3.4, as operators who understand the gravity of the threat are statistically more inclined to evaluate and adopt advanced preventing technologies, such as thermal treatments. The self-assessed level of knowledge of the pathogen is shown in Figure 12.

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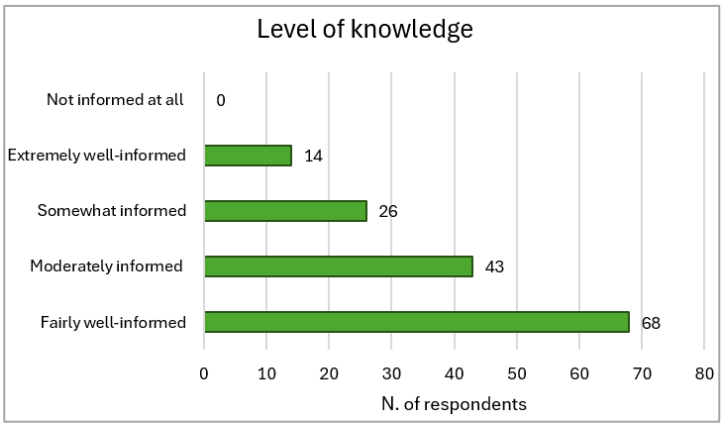


Figure 12. Self-assessed level of knowledge of *Xylella fastidiosa* among the responding nurseries.

Despite this high level of awareness, the economic impact of the pathogen is already a tangible reality for a substantial portion of the industry. Nearly one-third of the total European sample (31,8%) has already experienced the economic consequences of the pathogen. This impact is significantly more pronounced in high-risk zones or countries with major export flows, reaching 39,1% among Italian operators and 28,9% in Spain. In contrast, northern European countries like Germany and central regions like France report a lower direct impact, with damages often limited to administrative costs or precautionary changes in the species propagated. This geographic disparity underscores that the demand for thermal treatments is currently driven by a core group of affected nurseries in the Mediterranean basin, although the perceived risk remains a cross-border concern for the entire EU market. The distribution of the nurseries reporting damage, by country, and the main types of damage reported are given in Table 7 and Figure 13.

Table 7. Nurseries reporting economic damage attributable to *Xylella fastidiosa*, by country, with the resulting impact rate.

Country	Total Respondents (n)	Nurseries reporting damages (Yes)	Nurseries reporting no damage (No)	Impact Rate (%)
Italy	64	28	36	43.8%
Spain	38	11	27	28.9%
France	16	2	14	12.5%
Germany	12	0	12	0.0%
Portugal	9	7	2	77.8%
Others (Mixed EU)	12	0	12	0.0%
TOTAL	**151**	**48**	**103**	**31.8%**

The nature of these damages is primarily indirect and commercial. Respondents frequently identified export restrictions and limitations on trade with third countries as the most significant impacts. Among the operators operating in Italy, a total of 20 nurseries explicitly cited commercial damages due to export limitations, while others highlighted increased production costs linked to new certification requirements and preventative measures. The cross-border distribution of these impacts highlights that trade-related restrictions and

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administrative burdens represent the primary economic drivers for the adoption of BeXyl protocols. This perception of risk is not limited to biological infection but extends to the administrative and bureaucratic burden that follows outbreaks, which is seen as a major threat to the competitiveness of the European nursery supply chain.

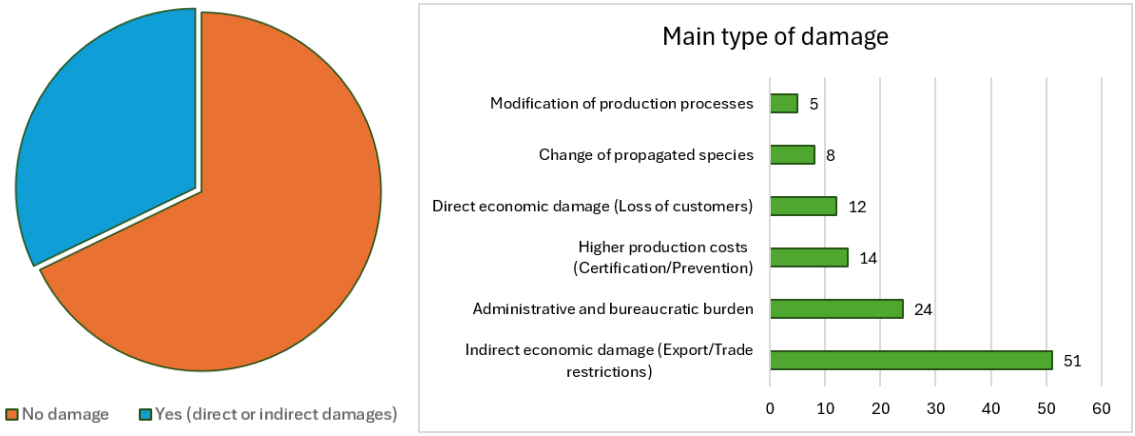


Figure 13. Nurseries reporting economic damage attributable to *Xylella fastidiosa* and main types of damage reported by the nurseries affected.

6.6. Familiarity with, and current use of, thermal treatments

Coming to the central core of the research, namely heat treatments, the investigation focused on the level of familiarity with this process and the extent/type of investments required. The analysis of technical familiarity reveals a significant gap between the theoretical awareness of the pathogen and the practical knowledge of the technologies. Despite the high awareness of *X. fastidiosa* risk, the level of expertise regarding thermal treatment methods (e.g., hot water treatment, cryotherapy) remains relatively low across the European nursery sector. Aggregated data shows that 39,7% of respondents are "not familiar at all" with these techniques, while only a small elite (8,6%) considers itself "extremely well-familiar." This underscores a primary need for technical dissemination and standardized protocols to fill the knowledge gap. The self-assessed level of familiarity with thermal treatments is shown in Figure 14.

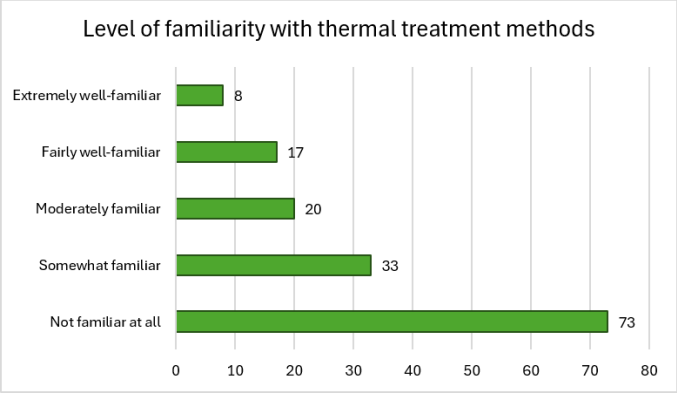


Figure 14. Self-assessed level of familiarity with thermal treatment methods among the responding nurseries.

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In terms of practical application, the current adoption of thermal treatments is still limited to a minority of specialized operators. Only 18,5% of the nurseries surveyed (28 out of 151) have ever implemented thermal treatments in their facilities. However, there is a clear strategic interest in technology: among those who do not currently use TT, a significant majority (52,3% of the total sample) stated they would consider adopting them in the future, provided they are proven to be cost-effective and efficient. Among the current users, the preferred implementation strategy is direct investment in equipment (24 nurseries), particularly in Italy, while outsourcing the treatment to external service providers is a common alternative in other regions, such as those represented in the English-language module. This data suggests that while technology is perceived as a valid phytosanitary tool, the high initial investment remains a pivotal factor in the decision-making process. The current use of thermal treatments and the openness to future adoption are shown in Figure 15.

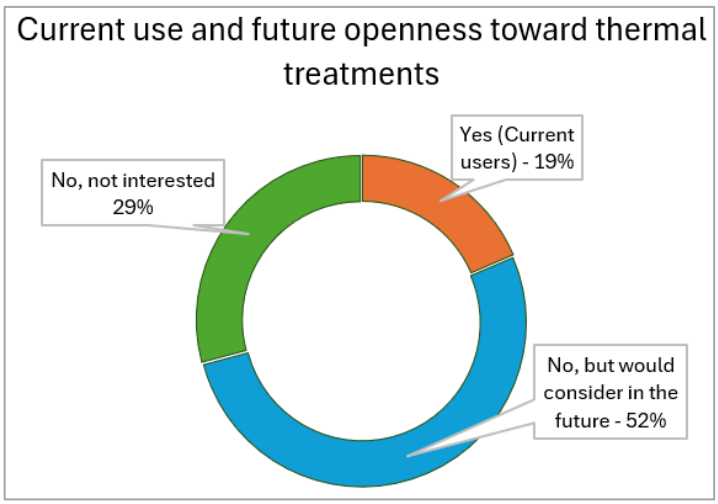


Figure 15. Current use of thermal treatments and openness to future adoption among the responding nurseries.

6.7. Drivers of adoption

The decision to adopt thermal sanitation protocols is primarily driven by regulatory compliance and market access. Aggregated data across all geographic regions indicates that the most compelling reason for considering TT is the ability to provide a guaranteed "Xylella-free" status for outgoing plants. Due to the survey's skip logic, this specific assessment was addressed only to the subgroup of 107 respondents who already use thermal treatments or are open to future adoption. Within this target cluster, nearly 82,2% of operators (88 out of 107) identified the delivery of Xylella-free plants as a top priority, confirming that BeXyl protocols align with essential market demands rather than just regulatory obligations. This is closely followed by the need to meet strict EU regulatory requirements, particularly for nurseries located in or trading with demarcated areas. For many operators, technology is viewed not just as a phytosanitary tool, but as a strategic commercial asset to maintain competitiveness in an increasingly restricted market. Environmental sustainability also emerges as a significant driver, with many respondents valuing TT as a method to reduce chemical use and improve overall plant health. The relative weight attributed to each driver is reported in Table 8.

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Table 8. Drivers of the adoption of thermal treatments, ranked by the number of respondents rating each as "very important" or "important" (subgroup of 107 current or prospective users).

Driver	Very important	Important	Tot. Positive
Best option to deliver Xylella-free plants	58	30	88
Meeting regulatory requirements	49	35	84
Improving plant health	38	42	80
Environmentally friendly method	27	38	65
Reducing chemical use	24	37	61

6.8. Barriers to adoption

Conversely, several critical barriers hinder the widespread implementation of these treatments. The primary concern across the sample is the uncertainty regarding treatment efficacy and the risk of potential plant damage. The complete ranking of the barriers identified is given in Table 9.

Table 9. Barriers to the adoption of thermal treatments, ranked by the number of respondents rating each as "very important" or "important" (n = 143).

Barrier	Very important	Important	Tot. positive	% su 143
High initial investment	51	46	97	64,2%
Concerns over potential plant damage	53	51	104	68,9%
Uncertainty about treatment effectiveness	41	51	92	60,9%
Lack of technical knowledge/expertise	29	42	71	47%
Impact on the final sale price	32	55	87	57,6%
Time-consuming nature of the process	21	40	61	40,4%

This is particularly evident in the Italian and Spanish samples, where operators expressed significant worry that high temperatures might reduce the viability or rooting capacity of sensitive species, such as stone fruits or specific ornamentals. Financial constraints also play a major role: the high initial investment in specialized equipment and concerns regarding the recovery of these costs through plant sales are cited as major deterrents, especially for small and medium-sized enterprises (SMEs). Furthermore, a widespread lack of technical knowledge and standardized protocols remains a substantial hurdle, highlighting the gap between research-scale validation and commercial-scale application.

6.9. Willingness to pay and support needs

The final assessment of the survey focuses on the industry's readiness to integrate thermal treatments (TT) and the enabling conditions required for a successful transition. Among respondents who do not currently use technology, there is a significant strategic openness to future adoption, provided that the protocols are proven to be both efficient and economically sustainable. When evaluating the willingness to pay (WTP) for outsourced thermal sanitation services, most of the European samples (N=59) expressed a clear preference for a limited cost impact. Specifically, the majority of potential users (69,5%) stated they would only accept a

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price increment of 5% while 98.3% of respondents set a definitive limit at 10% on the final sale price of the plants. This high price sensitivity underscores that the large-scale implementation of BeXyl protocols must be accompanied by optimization of energy costs and logistics to avoid compromising the market competitiveness of nursery products. The maximum acceptable price increment declared by the respondents is shown in Figure 16.

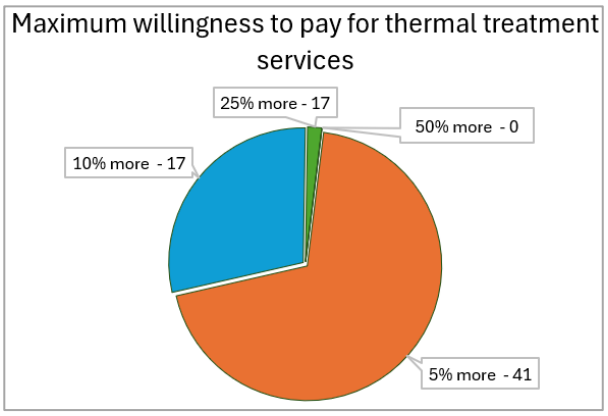


Figure 16. Maximum acceptable price increment for outsourced thermal treatment services (n = 59).

To bridge the current adoption gap, nursery operators identified financial incentives and technical capacity building as the most urgent support needs. Aggregated data reveals that subsidies, grants, and specialized technical workshops are considered the primary facilitators for adopting thermal treatments. This finding indicates that the industry perceives the transition not only as a financial challenge but also as a technical one, requiring a transfer of expertise from research institutions (like CNR and IVIA) to the practitioners. Interestingly, while there is caution regarding direct costs, there is overwhelming support for the promotion of certified "Xylella-free" plants within the sector. Nearly 77% of the total sample (116 out of 151) would welcome such a certification scheme, with a predominant preference for voluntary programs (77 respondents) over compulsory mandates (38 respondents). This suggests that the European nursery sector views thermal treatments as a proactive tool to enhance brand reputation and guarantee plant health in the single market. The types of support requested, and the support for a certification scheme and its preferred form, are shown in Figures 17 and 18.

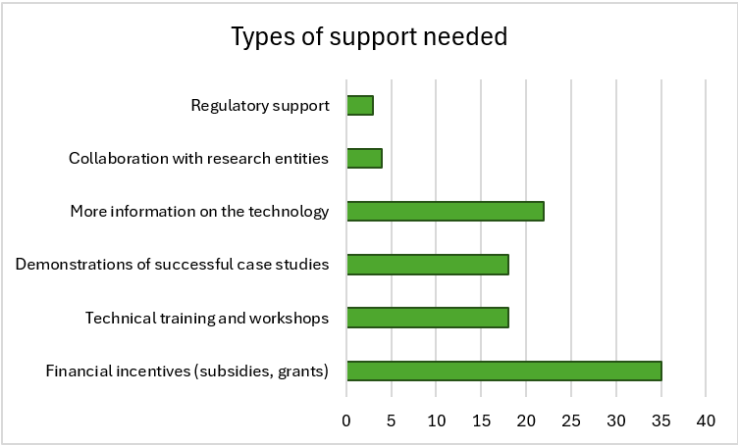


Figure 17. Types of support identified by nurseries as necessary to enable the adoption of thermal treatments.

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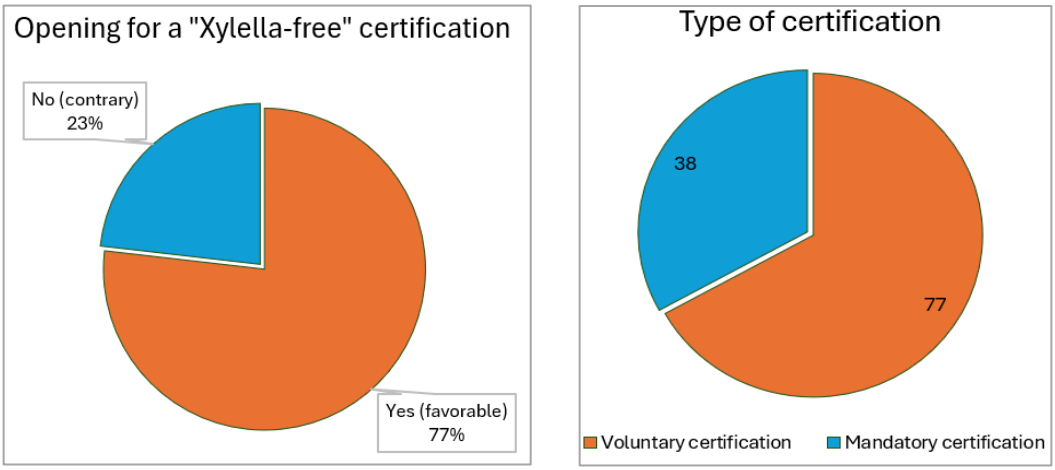


Figure 18. Support for the introduction of a "*Xylella-free*" certification scheme and Preferred type of certification scheme: voluntary versus mandatory.

6.10. Cross-analysis: production sector versus perceived barriers

To refine the implementation strategy for BeXyl protocols, a cross-tabulation was performed between the main plant categories produced (Q7) and the perceived barriers to adoption (Q22). The analysis reveals a clear divergence in risk perception across sub-sectors. The results of this cross-tabulation are shown in Figure 19.

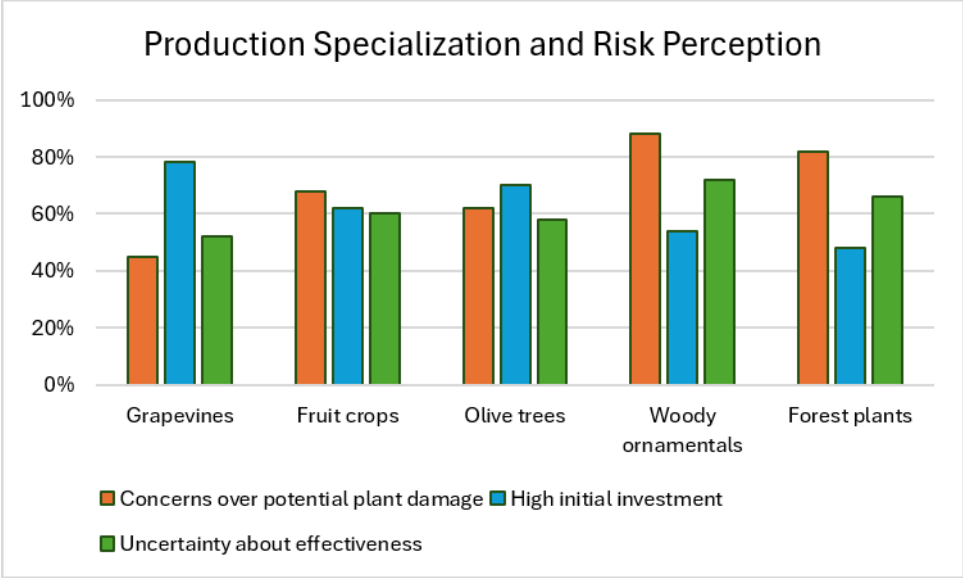


Figure 19. Cross-analysis of production specialisation (Q7) and perceived barriers to adoption (Q22).

For grapevine nurseries, the primary obstacle is economic, centered on the high initial investment for equipment. This is consistent with the sector's existing technical familiarity with heat treatments used for other pathogens like Flavescence dorée of grapevine. Conversely, for the ornamental and forest plant sectors, the primary barrier is biological uncertainty. As shown in the updated data, 68.9% of the total sample identifies potential plant damage as a critical threat. This concern is particularly acute for high-value woody ornamentals and evergreen

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shrubs, where any reduction in plant viability or aesthetic quality is commercially unacceptable. These findings provide a strong scientific justification for the focus of Tasks 3.1 and 3.2. The demand for standardized, safe "time-temperature" protocols is not just a research goal but a non-negotiable market requirement to overcome the "biological fear" that currently hinders the adoption of thermal treatments beyond the viticulture sector.

6.11. Cross-analysis: willingness to pay versus support needs

Furthermore, a strategic cross-analysis was conducted between the maximum willingness to pay for outsourced thermal treatments (Q24) and the types of support identified as essential for technology adoption (Q25). The results highlight a critical dependency between price sensitivity and public policy intervention. The results of this cross-analysis are shown in Figure 20.

The data reveals that most nurseries with the lowest price tolerance, those only accepting a 5% price increment (69.5% of the subgroup), identify financial incentives (subsidies and grants) as their primary requirement. For these operators, the adoption of BeXyl protocols is perceived as economically unfeasible without external financial cushioning. Conversely, respondents with a slightly higher price tolerance (10% increment) prioritize technical training and specialized information. This correlation confirms that the large-scale implementation of thermal treatments faces a "sustainability trap": while the industry recognizes the phytosanitary value of technology, the narrow margins for cost recovery (98.3% of the sample rejects increases above 10%) mandate the integration of TTs into National Rural Development Programs (RDPs). Without targeted subsidies to cover initial capital expenditures and energy costs, the transition toward a "Xylella-free" certified nursery chain will remain limited to a small segment of high-value niche productions.

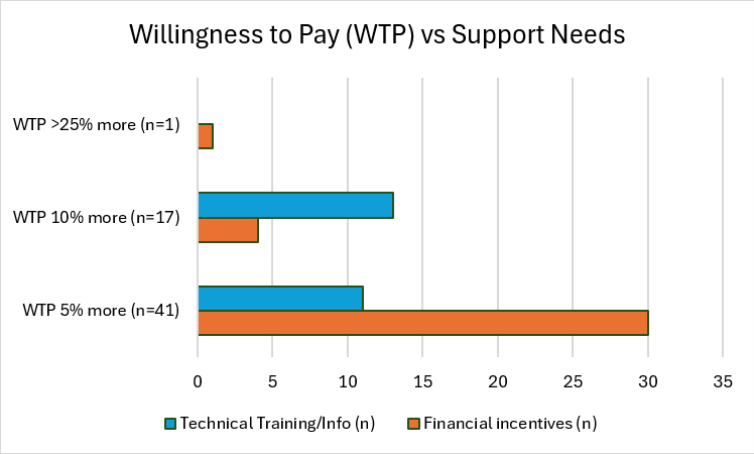


Figure 20. Cross-analysis of maximum willingness to pay (Q24) and the type of support considered essential (Q25).

6.12. Discussion of the survey findings

The transition toward standardized TTs protocols represents a crucial shift for the European nursery industry. While the technical efficacy of TT is supported by scientific literature and ongoing BeXyl experiments, its commercial integration faces a complex landscape of stakeholder perceptions and economic constraints.

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The findings indicate that the industry is at a critical transition point: there is high awareness and strategic interest, but adoption is hindered by technical uncertainties and narrow economic margins.

The nursery sector's perception of thermal treatments is evolving. While initially introduced as a mandatory precautionary measure for the movement of Vitis within demarcated areas, the survey confirms that the industry now views TT as a proactive market asset. The overwhelming support for a certified "Xylella-free" label suggests that thermal sanitation can be transformed from a bureaucratic burden into a competitive advantage that rewards plant health excellence.

Furthermore, experimental data from BeXyl Tasks 3.1 and 3.2 underscore that a "one-size-fits-all" approach to thermal treatments is unfeasible. While the standard 50°C for 45 minutes is robust for dormant grapevine material, other high-value crops show higher and variable sensitivity. These results justify the industry's concern regarding plant viability (cited by 56% of respondents) and highlight the urgent need for the finalized BeXyl protocols to provide safe, validated "time-temperature" combinations for the most sensitive Mediterranean germplasm.

The survey identifies also a strict "psychological price ceiling", with almost all operators unwilling to accept price increments exceeding 10%. To overcome the high capital and operational costs:

- Energy Efficiency: Task 3.4 must continue prioritizing low-cost energy solutions, such as waste heat recovery or biomass from pruning, to keep the sanitation cost within the 5% margin preferred by the majority of users.
- Added Value: The promotion of TT should emphasize its multipurpose efficacy. Beyond *X. fastidiosa*, TT is a proven tool against Vitis phytoplasmas, wood fungi, mites, and phylloxera. This "total sanitation" approach increases the return on investment for nurseries by reducing overall pest management costs.

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7. SWOT ANALYSIS FOR THE LARGE-SCALE APPLICATION OF THERMAL TREATMENTS IN THE EU NURSERY SECTOR

To strategically evaluate the positioning of this technology, the following framework identifies internal factors and external dynamics that will influence its adoption. Based on the comprehensive work conducted within the framework of the BeXyl project, incorporating the re-engineering of the EFSA host plant database, extensive in vivo biological validations, engineering assessments of alternative energy sources, and a wide-scale European nursery survey (n=151), the following detailed SWOT analysis evaluates the large-scale implementation of Thermal Treatments (TTs). The transition of thermal treatments from controlled experimental procedures to routine commercial applications represents a profound evolution for the European nursery sector. This analysis evaluates the feasibility and strategic positioning of technology by examining the interplay between technical demands and market realities. The internal and external factors identified are summarised in **Tables 10** and **11**.

7.1. Strengths

The primary internal strength of thermal treatments, particularly Hot Water Treatment (HWT), lies in their proven phytosanitary efficacy. Scientific literature and BeXyl experiments confirm that these protocols can successfully eliminate Xylella fastidiosa along with other critical pathogens such as Vitis phytoplasmas, wood-boring fungi, insects, nematodes and mites, offering a "total sanitation" approach superior to many chemical alternatives. Furthermore, the project has transformed the EFSA database into a predictive tool. By crossing functional traits with nursery interest, researchers have identified species-specific "viability windows," providing a robust technical foundation that moves beyond static lists to guide commercial planning. From a regulatory standpoint, TTs ensure direct compliance with EU emergency measures, enabling the safe movement of plants from demarcated areas. This technical reliability is recognized by the industry as a high-value market asset, with 82.2% of potential users viewing the "Xylella-free" status as a priority for restoring commercial competitiveness.

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Table 10. SWOT analysis: internal factors (strengths and weaknesses) of the large-scale implementation of thermal treatments in the EU nursery sector.

STRENGTHS (Internal)	WEAKNESSES (Internal)
Phytosanitary Efficacy: Proven ability to eliminate *Xylella fastidiosa* and other key pathogens in dormant material (e.g., *Vitis*, *Ficus*, *Prunus*).	**Genotype Sensitivity:** Significant variability in thermal tolerance even within the same species (e.g., 'Arbequina' vs 'Picual' olives), leading to reduced rooting or grafting capacity.
Predictive Methodology: Integration of functional traits (habitus, cold hardiness, heat tolerance) into the EFSA database allows for the identification of species-specific "viability windows" before testing.	**Physiological Shock:** Prolonged Hot Water Treatment (HWT) causes severe stress in non-dormant evergreen species.
Optimization Matrices: Scientific validation of exposure times based on stem diameter and wood density, preventing both energy waste and incomplete sanitation.	**Financial & Knowledge Barriers:** High initial investment for precision equipment and a widespread lack of technical familiarity among operators (40% "not familiar").

7.2. Weaknesses

Despite their efficacy, several internal weaknesses hinder the widespread adoption of thermal protocols. The most significant barrier is the high capital expenditure (CAPEX) required for specialized, high-precision equipment capable of maintaining temperature stability within 0.1°C, a burden cited by 64.2% of nurseries. This financial hurdle is compounded by an evident technical knowledge gap, as 39.7% of surveyed operators report having no familiarity at all with the technology, leading to fears that improper application could cause irreversible damage. Furthermore, BeXyl in vivo experiments have highlighted physiological sensitivity and varietal variability as critical weaknesses. For instance, certain almond and cherry cultivars (e.g., *Prunus avium* cv. Sweetheart) showed high sensitivity, resulting in significantly reduced budburst or rooting capacity when subjected to standard 50°C protocols. Additionally, while effective for dormant material, prolonged water immersion can cause severe physiological shock in non-dormant evergreen species, limiting the technology's universal applicability.

7.3. Opportunities

External opportunities provide strategic pathways to overcome existing adoption barriers. The ongoing collaboration with the European and Mediterranean Plant Protection Organization (EPPO) to draft official international standards is a major opportunity to facilitate trade. These standards will provide the legal framework needed to unlock export barriers toward Third Countries, a vital necessity starting from the 51 nurseries in the study sample currently affected by trade restrictions. Market-driven certification schemes also represent a significant

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opportunity, given the 76.8% mass consensus for a voluntary quality label that rewards healthy production. On the technical side, energy optimization models developed by the Politecnico di Bari explore alternative sources, such as waste heat recovery and biomass from pruning, to ensure that operational costs remain competitive. Finally, technological innovation, such as the development of Hot Vapor Lethal Treatment (HVLT) prototypes, offers a solution for sensitive evergreen hosts like *Nerium oleander*, potentially reducing physiological stress while maintaining sanitation efficacy.

Table 11. SWOT analysis: external factors (opportunities and threats) of the large-scale implementation of thermal treatments in the EU nursery sector.

OPPORTUNITIES (External - Factors to exploit)	THREATS (External - Risks to mitigate)
Certification Schemes: Mass consensus (76.8%) for a voluntary quality label to enhance brand reputation and guarantee "Xylella-free" status.	**Price Sensitivity:** Extreme market rigidity; **98.3%** of nurseries reject price increases above 10%, while nearly **70%** accept a maximum of only 5%.
Policy Integration: Potential to include TTs in National Rural Development Programs (RDPs) to facilitate access to subsidies and financial incentives for initial equipment.	**Technical Uncertainty & Biological Damage:** The fear of reduced viability remains the primary barrier for **68.9%** of nurseries, as confirmed by high sensitivity in specific cultivars like almond and cherry.
Trade Facilitation: Development of official EPPO standards to provide the legal framework necessary to unlock export barriers toward Third Countries for the 51 affected nurseries.	**Varietal Variability:** Highly genotype-specific survival rates (e.g., differences between olive or cherry varieties) requiring tailored, experimentally verified protocols.
Energy Optimization & Sustainability: Use of alternative energy (waste heat recovery, biomass from pruning) to ensure operational costs remain within the acceptable 5% margin.	**Administrative Burden:** Risk of excessive bureaucracy and monitoring requirements associated with new certification and traceability protocols.
Technological Innovation: Development of Hot Vapor Lethal Treatment (HVLT) prototypes to reduce physiological shock in sensitive evergreen hosts like *Nerium oleander*.	**Operational Limitations:** Technical difficulty in treating large-sized adult plants or materials sourced from wide, unprotected field areas.

7.4. Threats

The commercial success of TTs faces several external threats, most notably extreme price sensitivity. The European nursery market exhibits high rigidity, with 98.3% of nurseries rejecting

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price increases above 10% and nearly 70% accepting a maximum increment of only 5%. If the costs of implementation cannot be kept within these narrow margins, the technology risks widespread market rejection. This economic constraint is intensified by the "biological fear" of damage, which remains the primary concern for 68.9% of nurseries. This threat is validated by experimental data showing that improper time-temperature combinations can be devastating for specific Mediterranean germplasms. Furthermore, there is a perceived administrative and bureaucratic burden associated with new certification and monitoring protocols. Operators fear that additional regulatory layers could compromise the competitiveness of a supply chain already strained by existing phytosanitary mandates. Finally, the operational limitations of treating large-sized adult plants or materials from wide field areas remain a technical challenge for nurseries specializing in late-stage ornamental growth.

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8. STRATEGIC SYNTHESIS OF THE WORK DONE

1. From Static Lists to Predictive Tools: By crossing functional traits with commercial interest, the project transformed the EFSA database into a decision-making tool. This allows for the calculation of a Resilience Score, identifying which of the 750+ host species can safely undergo TT without compromising performance.
2. Experimental Validation of Viability: Trials on dormant material (Task 3.2) confirmed that while *Vitis* is highly resilient, other species like almond and cherry show sensitivity that requires specific "time-temperature" adjustments (e.g., 45°C for 60 instead of 50°C) to avoid budburst failure.
3. Technological Pivot for Evergreens: The recognition of the limitations of HWT for evergreen hosts led to the strategic exploration of steam (HVLT), which preliminary tests show significantly reduces plant stress while maintaining efficacy.
4. Economic Realism: The energy assessment is the necessary response to the survey's "sustainability trap": since nurseries cannot transfer more than 5% of the cost to the final price, the transition depends entirely on energy efficiency and potential integration into National Rural Development Programs (RDPs).

This integrated SWOT demonstrates that BeXyl has provided the technical and economic "bridge" needed to move thermal treatments from specialized grapevine applications to a standardized protocol for the wider Mediterranean nursery chain.

In conclusion, the integrated analysis confirms that thermal treatments have successfully transitioned from niche experimental measures to a technically mature reality for several major Mediterranean crops, including *Vitis*, *Ficus*, *Morus*, *Juglans* and hopefully, in the near future, also some *Prunus* spp. The BeXyl project has provided the essential 'technical and economic bridge' required for large-scale adoption by transforming the EFSA host plant database into a predictive decision-support tool and validating energy-efficient models capable of maintaining operational costs within market-acceptable margins. While physiological sensitivity in certain cultivars remains a technical challenge that necessitates continued experimental refinement, the strategic path forward is clearly defined. The large-scale success of these protocols will depend on two external catalysts: the finalization of internationally recognized EPPO standards to facilitate trade with Third Countries, and the establishment of a voluntary certification framework that rewards early adopters for their commitment to plant health. By aligning validated science with the socio-economic expectations of nursery operators, these protocols are positioned to become a standardized pillar of a resilient and competitive European nursery chain.

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9. EMERGED RESEARCH NEEDS AND FUTURE PERSPECTIVES

The extensive experimental validations and large-scale trials conducted throughout the BeXyl project have established, in specific experimental conditions, the phytosanitary efficacy of thermal treatments against *Xylella fastidiosa*. However, the transition from controlled experimental conditions to routine, continuous commercial application has illuminated a complex matrix of biological, physical, and engineering variables that demand further scientific investigation. The formulation of official standards and the ultimate acceptance of these protocols by the European nursery sector greatly depend on resolving these technical uncertainties. This section details the critical research trajectories and future perspectives required to optimize the TTs procedures, minimizing plant physiological stress while maximizing operational efficiency, drawing heavily upon Task 3.2, 3.3 results and the foundational parameters established in Milestone 13 “A protocol with conditions established to perform TTs at large-scale”.

9.1. Diversification of thermal transfer mechanisms: beyond hot water

Six potential thermal treatment (TT) systems were hypothesized, tailored to the diverse physiological requirements and botanical characteristics of the different *X. fastidiosa* host plants: Hot Water Lethal Treatment (HWLT), Hot Air Sublethal Treatment (HAST), Cold Air Lethal Treatment (CALT), Cold Air Sublethal Treatment (CAST), Quick change of sublethal temperature, and Hot Vapor Lethal Treatment (HVLT). While current standardizations primarily rely on HWLT (submersion in water at 50°C for 45 minutes), emerging data indicates that prolonged submersion induces severe physiological shock especially in metabolically active, non-dormant species. The laboratory-scale prototype used for the HVLT trials is shown in Figure 21.

Future research must prioritize the development and optimization of alternative thermal transfer media, most notably steam-based thermotherapy (HVLT). Preliminary trials conducted by CRSFA in 2026 utilizing a prototype adapted to produce hot water vapor demonstrated highly promising results for evergreen ornamental shrubs. Specifically, for *Nerium oleander*, immersion in hot water at 53°C for 45 minutes caused severe stress, reducing budburst to 47.7% and average shoot length to 5.0 cm, compared to the 88.6% budburst and 7.2 cm growth in untreated controls. Conversely, the application of steam at the same temperature and duration maintained a budburst rate of 75.0% and normal shoot growth, indicating that steam provides a far less traumatic heat transfer mechanism for sensitive, non-dormant tissues. Scaling up HVLT infrastructure and precisely mapping the thermodynamics of vapor penetration into different types of wood represents an urgent research priority to ensure the survival of delicate Mediterranean germplasm and all evergreen species. Furthermore, deep investigations into lethal cold treatments (CALT) are required, as in vitro analyses have demonstrated bacteriostatic effects at low temperatures (4°C to 10°C). Consequently, extreme cold conditions may offer an alternative, low-energy sanitation pathway for completely dormant deciduous and cold-resistant stock, such as *Prunus* spp.

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Figure 21. Laboratory-scale prototype used for the hot vapour lethal treatment (HVLT) trials, with bundled propagation material inside the treatment chamber.

9.2. Managing the biodiversity of European nursery germplasm

A primary structural challenge identified during the project stems from the vast host range of *Xylella fastidiosa,* 665 known host plants in Milestone 12, then extended to 728 species of last EFSA update, systematically categorized according to their habitus, propagation methods, nursery/trade interest and cold/heat hardiness/tolerance. This wide biodiversity dictates that a singular, standardized treatment schedule is biologically unfeasible.

Future perspectives require a massive expansion of empirical testing across the priority species identified as representatives of the totality of interesting species grouped for similar needs/requirements. While robust data exists for *Vitis vinifera* and few other woody fruit crops, research must address specifically the specific thermal thresholds of high-interest evergreen ornamentals and forestry plants such as *Quercus ilex*, *Laurus nobilis*, *Magnolia grandiflora*, and *Viburnum tinus*. The data confirms that responses are strictly genotype-dependent: within the *Olea europaea*, cultivars such as 'Arbequina' and 'Lecciana' demonstrate relative tolerance to temperatures up to 46°C, whereas cultivars like 'Arbosana', 'Koroneiki', and 'Picual' suffer sharp reductions in rooting capacity and elevated mortality under the exact same parameters. Future research must construct a comprehensive, cultivar-specific thermal tolerance database, evaluating whether these differing responses are dictated by internal cellular architecture, water content, or biochemical/physiological profiles.

9.3. Thermodynamic complexity of material dimensions

As explicitly outlined in the operational conditions drafted by M13, the physical size and diameter of the propagation material represent absolute limiting factors for heat penetration. Because *X. fastidiosa* colonizes deep xylem tissues, the target lethal temperature must reach the exact center of the woody cylinder to guarantee eradication.

Future engineering and thermodynamic research must advance the Predictive Calculation Tools currently under development by CNR and Politecnico di Bari in Task 3.4 to be validated, on the base of lab experiments, with the wood of an higher number of species. Experimental measurements utilizing internal wire thermocouples have proven that the time required for internal tissues to reach 50°C varies drastically based on stem diameter, intrinsic wood density and hydration. Researchers categorized species into distinct thermodynamic profiles based on

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the time-fold required compared to a 1 cm reference diameter: High time (≥10x) for dense species like *Prunus avium*, *Juglans regia*, and *Prunus dulcis*; Medium time (7–10x) for *Ficus carica*; and Low time (<7x) for *Ulmus minor* and *Cercis siliquastrum*. The integration of these thermodynamic variables into automated, data-driven optimization matrices is feasible and in the future industrial thermotherapy applications the equipment could have software capable of calculating the exact exposure time required based on inputting the species and the maximum batch diameter. This precision approach will eliminate energy waste associated with over-treatment while neutralizing the risk of incomplete sanitation in unusually thick planting materials.

9.4. Pre-conditioning, agronomic management and physiological state

The efficacy and safety of large-scale treatments do not depend solely on the machinery, but heavily on the agronomic history of the plant. As highlighted in Milestone 13, the management of mother plant blocks (nutrition, protection), the harvesting time of cuttings, and the precise level of dormancy, are variables that dictate the ultimate success of the sanitation process.

Future deeper investigations should focus on pre-treatment agronomic interventions designed to artificially increase thermal hardiness. Trials have initiated the application of specific nutritional protocols in mother plant blocks of *Prunus avium*, *Prunus dulcis*, and *Vitis vinifera* to evaluate if targeted fertilization (e.g., altering potassium or calcium ratios to strengthen cell walls) can fortify the resulting scions against extreme thermal stress. Additionally, the specific parameters for pre-treatment hydration and conditioning require intense standardization. However, the exact water temperature, duration of submersion, and subsequent post-treatment acclimatization protocols must be meticulously experimentally tested and calibrated for different categories of plant material to prevent secondary anoxic damage. For the deciduous plants, the possibility of measure and eventually also control by hormone applications the dormancy status of the propagation material remains an essential field of research to improve heat/cold tolerance and therefore application and safety of thermal treatments.

9.5. Microbiome disruption and ecological resilience

Finally, an entirely new frontier of research concerns the ecological impact of thermal treatments on the internal plant environment. Thermotherapy is not selective and high temperatures that eradicate *Xylella fastidiosa* simultaneously could affect the plant's native endophytic microbiome. Even if long term field trials were started in BeXyl, future scientific efforts, such as the ongoing analyses by the Austrian Institute of Technology, must map the profound dysbiosis induced by thermal shock. It is imperative to understand how the treatments of vascular tissues could affect the plant's long-term resilience, nutrient uptake, and susceptibility to secondary opportunistic pathogens once transplanted into the open field. This opens the highly innovative perspective of developing post-treatment microbial re-inoculation protocols. Identifying and applying beneficial, heat-resistant endophytes, or artificially restoring a competitive microbiome immediately after the treatment phase, could vastly improve the field establishment rates of sanitized nursery stock.

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10. STRATEGIC GUIDELINES AND POLICY RECOMMENDATIONS FOR COMMERCIAL IMPLEMENTATION

Even if the extensive experimental biological validations and the large-scale industrial evaluations conducted throughout the WP3 of BeXyl have demonstrated the phytosanitary efficacy of thermal treatments for plant propagation materials of some species (as proposed in the deliverable D3.1), the transition from controlled scientific conditions to a routine commercial application in the nurseries requires also comprehensive structural and strategic adaptation. Based on the technical, operational and socio-economic realities considered during the project, this section outlines some basic points, policy recommendations, and strategic frameworks required to promote and help the sustainable and widespread adoption of HWT protocols in practice.

10.1. Fostering adoption through targeted economic incentives

The primary obstacle to the commercial spread and internalization of thermal treatments is unequivocally financial. The quantitative evaluations of the nursery sector conducted through the questionnaire (see paragraph 6) reveal that the acquisition of industrial-grade, high-precision thermotherapy equipment, coupled with the requisite handling infrastructure, represents a prohibitive capital expenditure for over 60% of operators. Furthermore, 98.3% of the sector operates under a strict price ceiling, rejecting any scenario where treatment implementation increases the final market price of the plant by more than 10%.

To secure the health of propagation materials without compromising the economic viability of the nursery sector, policy interventions should consider to provide financial support. It would be advisable and recommended that regional and national agricultural authorities could explicitly integrate investment in thermal treatments for example into National Rural Development Programs and broader European agricultural subsidy frameworks. Providing targeted financial incentives, matching grants, and subsidized credit lines specifically dedicated to the acquisition of phytosanitary equipment would certainly improve the attractiveness and the adoption of this technical innovation in the nursery production's processes. Furthermore, by using the incentive tool, it would be useful and possible to encourage cooperation, the creation of consortia or specialized companies for the supply of heat treatment services.

Simultaneously, economic incentives should be linked to the adoption of energetically efficient technological solutions. The evaluations conducted in collaboration with subcontractor Politecnico di Bari (see paragraph 5) highlight the need to reduce the carbon footprint and the operational costs associated with heating large volumes of water. Policy frameworks should prioritize funding for nurseries that implement alternative, renewable energy sources to power their thermal units, such as waste heat recovery systems, modern biomass cogeneration utilizing internal pruning residues, and solar thermal arrays. By subsidizing the integration of these green technologies, policymakers can simultaneously alleviate the operational cost burden and align the nursery sector with the sustainability objectives of the European Green Deal.

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10.2. Regulatory tools and voluntary certification schemes

The promotion of thermal treatments as a standard practice for guaranteeing the sanitary status of propagation materials requires a carefully calibrated regulatory approach. While the development of official standards, such as those currently under drafting in collaboration with the European Plant Protection Organization (EPPO), is crucial for international trade facilitation and the removal of export barriers, rigid and compulsory rules present significant risks.

Therefore, especially for the more sensitive species, the regulatory tools should be designed with inherent flexibility, allowing for species-specific and cultivar-specific parameter adjustments based on continuous empirical validation. More importantly, regulatory authorities should pivot toward incentivizing quality through market-driven mechanisms. The Nursery sector surveys (see paragraph 6) demonstrate overwhelming support (77%) for the establishment of a formalized "Xylella-free" certification scheme, with a clear preference for the voluntary participation. Establishing a recognized, institutional voluntary certification framework represents the most effective regulatory tool available that could transform the thermal sanitation from a punitive administrative burden into a highly marketable asset, allowing proactive nurseries to command premium prices and recover their initial investments while supplying the agricultural sector with certified and safe propagation materials.

10.3. Addressing knowledge gaps and advancing technological solutions

The operational foundation established by BeXyl WP3 must be viewed as the starting point rather than the conclusion of the research trajectory. The empirical evidence generated has highlighted profound complexities in plant physiology and thermodynamics that dictate the success or failure of the sanitation process. A significant portion of the nursery sector (39.7%) reports an absolute lack of technical familiarity with thermal protocols, creating an immediate need to resolve existing knowledge gaps.

It is vital to continue and expand the research initiatives started under WP3 to explore a higher number of species as well new forms of thermal treatment and more efficient technological solutions (see paragraph 8). For example, while standard Hot Water Treatments (HWT) are highly effective for dormant deciduous species like *Vitis vinifera* and *Ficus carica*, prolonged submersion induces severe physiological shock in metabolically active, non-dormant evergreen species. Based on an unpublished personal communication about *Citrus sp*., preliminary trials conducted in BeXyl on *Nerium oleander* demonstrated that substituting hot water with steam (Hot Vapour Lethal Treatment) significantly reduced plant stress, maintaining high budburst rates and normal shoot growth compared to the severe suppression observed under HWT. Continued research is urgently required to map the thermodynamics of steam penetration across different wood densities and to scale up vapour-based thermotherapy infrastructure for commercial use.

Additionally, research must pursue the refinement of predictive calculation tools. Generating data-driven optimization matrices capable of calculating the exact necessary exposure time based on the specific species' thermal conductivity and the exact stem diameter of the batch is essential to prevent both energy waste and incomplete sanitation.

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10.4. Expanding research through targeted collaborative calls

The huge biodiversity managed by the European nursery industry, encompassing over 750 *Xylella* host and specified plant species, presents an unprecedented scientific challenge. Current protocols have validated responses for primary agricultural crops such as grapevines, fig, walnut and others. However, experiments have consistently demonstrated that thermal tolerance is strictly genotype-dependent, even within a single species, with responses varying drastically between tolerant and sensitive cultivars. Similarly, extreme sensitivity has been documented in the majority of the evergreen species tested (such as olive, the evergreen *Quercus suber* and other ornamentals) highlighting the unsuitability of current schedules for specific germplasm.

To address this vast biological variability, it is advisable that the European Commission and other funding bodies issue specific, targeted calls for Research and Development directly focused on plant thermotherapy. These funding mechanisms should structurally require direct collaboration between public research institutions, commercial nurseries or their representatives, equipment manufacturers. By funding participatory field trials located directly within the nursery facilities, scientists could test and calibrate time-temperature combinations across a vastly expanded number of species and varieties under real operational conditions.

Such targeted calls should also help the establishment of regional "Knowledge Transfer Hubs" located where the production is often concentrated in highly specialized nursery centres or districts. These operational hubs would serve dual purposes: executing continuous, large-scale empirical testing to update the database of varietal thermal tolerances, and providing the hands-on technical training, standardized calibration protocols, and continuous scientific support that nursery operators will require to safely implement these advanced sanitation technologies.

10.5. Information, promotion and awareness campaigns

To transform thermal treatment technology into a solid market standard, EU institutions, nursery, producer’s and trade associations should develop and launch promotional campaigns targeting end-users to stimulate natural demand of TT plants. For farmers, the campaign should reframe the safety and minimal price increase of treated plants as a highly cost-effective insurance policy that prevents early crop failures and protects long-term investments against destructive quarantine pathogens like *X. fastidiosa*. For the ornamental and forestry markets, the messaging could also highlight the eco-friendly, "pesticide-free" nature of this physical process. This sustainability appeal is significantly strengthened when treatments are powered by clean energy, such as the photovoltaic of other clean sources, which can drastically reduce the operational carbon footprint compared to traditional fossil fuels. Ultimately, educating consumers about the tangible benefits of safe, low-carbon plants will generate a strong market pull, transforming thermal sanitation from a perceived emergency cost into a powerful competitive advantage for European nurseries.

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