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AGRONOMIC AND QUALITATIVE ASPECTS OF ORGANIC COTTON CULTIVATION

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Research highlights

- Organic practices can revitalize Sicilian cotton, meeting ecological demand.
- Recycled cotton cuts fertilizer, pesticides, and water use, offering a sustainable alternative.
- Mycorrhizae boost cotton growth and P/Zn uptake, offering a green alternative to fertilizers.
- Drip irrigation enhances yield and water use, despite uneven germination and salt risk.
- Armonia genotype thrives under drought, fitting for dry regions.
- Clay soils increase water productivity, reducing irrigation demand.
- Moderate water deficit keeps cotton yield economically viable.
- Pelargonic acid at 18 L ha⁻¹ achieves full cotton defoliation matching synthetic standards.

Abstract

Cotton, the world's leading natural fiber, underpins an industry valued at 5.68 billion USD. However, its cultivation faces major sustainability challenges due to high water and energy demands, intensive chemical use, and the lack of effective organic defoliants for mechanical harvesting, which currently limits organic cotton to only 0.5% of global production. A literature review identified key agronomic innovations supporting sustainability, including inoculation with arbuscular mycorrhizal fungi, crop rotation, cover cropping, and low-input irrigation strategies. A second review on Life Cycle Assessment in the cotton supply chain revealed that most environmental impacts occur during cultivation - mainly linked to water use, fertilizers, and pesticides - and in the product use phase. Based on these findings, two complementary field studies were carried out in Sicily (Southern Italy). The first, conducted at two sites (Catania and Palermo), assessed two cultivars (Armonia and ST-318) under three irrigation regimes (100%, 70%, and 30% of reference evapotranspiration, ETC), with and without mycorrhizal inoculation. Results showed that microorganism inoculation increased fiber yield (up to 0.99 t ha^{-1}), boll number, and plant height, with stronger effects in soils richer in organic matter. Moderate water deficit (70% ETC) preserved 75–79% of maximum yield while improving water use efficiency (up to 1.43 kg m^{-3}). Armonia displayed greater drought tolerance, whereas ST-318 performed best under full irrigation. The second study, aimed at overcoming the limitation posed by the widespread use of synthetic defoliants, compared pelargonic acid, a rapidly biodegradable compound, with the synthetic defoliant pyraflufen-ethyl and a water control, each applied at three different rates. The experiment, conducted over the 2023–2024 biennium at the Catania University experimental farm, was arranged in a randomized complete block design with three replications. Parameters measured included defoliation efficacy, root diameter, boll number, boll weight, and yield components. Pelargonic acid at 16 L ha^{-1} achieved the

highest boll number per plant in 2024, surpassing pyraflufen-ethyl at label rate, while 12 L ha⁻¹ improved root diameter over the chemical control. Although pyraflufen-ethyl at 1.5 L ha⁻¹ gave the highest boll weight in 2024, pelargonic acid at 18 L ha⁻¹ provided comparable defoliation without adverse yield effects. Together, these studies show that combining biologically based soil fertility management, optimized irrigation, and environmentally safe defoliation can foster the revival of cotton cultivation in southern Italy. This low-input, high-efficiency approach aligns with agroecological principles and provides a viable pathway for reintroducing sustainable cotton systems in the Mediterranean environments.

Keywords: Sustainable cotton cultivation; life cycle assessment (LCA); water management; arbuscular mycorrhizal fungi; sustainable defoliants; pre-harvest management.

Riassunto

Il cotone, principale fibra naturale a livello mondiale, alimenta un settore dal valore di 5,68 miliardi di USD. Tuttavia, la sua coltivazione presenta rilevanti criticità di sostenibilità, legate all'elevata domanda idrica, all'uso intensivo di input chimici e all'assenza di defoglianti organici efficaci per la raccolta meccanica, fattore che limita la produzione di cotone biologico a livello mondiale.

Una *review* approfondita della letteratura scientifica ha individuato le principali innovazioni agronomiche orientate alla sostenibilità, tra cui l'inoculo di micorrize, la rotazione colturale, le tecniche di inerbimento e i metodi irrigui a basso input.

Una seconda *review*, focalizzata sull'applicazione del *Life Cycle Assessment* alla filiera del cotone, ha evidenziato che i principali impatti ambientali si manifestano nella fase colturale, principalmente a causa del consumo idrico, fertilizzanti e fitofarmaci, oltre che nella fase di utilizzo del prodotto.

Sulla base di queste evidenze, sono stati condotti due studi sperimentali in Sicilia. Il primo, condotto in due località (Catania e Palermo), ha valutato le performance di due cultivar di cotone (Armonia e ST-318) sottoposte a tre regimi irrigui (100%, 70% e 30% dell'evapotraspirazione di riferimento, ETc), con e senza inoculo di micorrize. I risultati hanno evidenziato come le micorrize abbiano incrementato significativamente la resa in fibra (fino a 0,99 t ha⁻¹), il numero di capsule e l'altezza delle piante, con effetti più marcati nei suoli ricchi di sostanza organica. Un moderato deficit idrico (70% ETc) ha consentito di preservare il 75–79% della resa massima riscontrata, migliorando l'efficienza nell'uso dell'acqua fino a 1,43 kg m⁻³. Armonia ha manifestato una maggiore tolleranza allo stress idrico, mentre ST-318 ha registrato performance superiori in condizioni irrigue ottimali.

Il secondo studio, concepito per ovviare alle criticità legate all'uso intensivo di defoglianti sintetici, ha confrontato l'acido pelargonico, un composto rapidamente biodegradabile, con il

defogliante sintetico piraflufen-etile e un controllo (acqua), applicati ciascuno a tre diversi dosaggi. La sperimentazione è stata svolta nel biennio 2023-2024, presso l'Azienda Agraria dell'Università di Catania, con un disegno a blocchi randomizzati con tre repliche. L'applicazione di acido pelargonico a 16 L ha^{-1} ha determinato il maggior numero di capsule nel 2024, superando il piraflufen-etile alla dose raccomandata in etichetta. Sebbene il piraflufen-etile a $1,5 \text{ L ha}^{-1}$ abbia garantito un peso medio della capsula più alto, l'acido pelargonico a 18 L ha^{-1} ha garantito un'efficacia defogliante comparabile, senza penalizzare la resa.

In sintesi, i risultati indicano come un approccio sostenibile che valorizzi la simbiosi microbica, l'efficienza idrica e l'uso di defolianti a basso impatto ambientale, possa rilanciare la coltivazione del cotone nel bacino del Mediterraneo, offrendo un sistema colturale a bassi input e ad alta efficienza, conforme ai principi agroecologici.

Parole chiave: Coltivazione sostenibile del cotone; analisi del ciclo di vita (LCA); gestione delle risorse idriche; irrigazione deficitaria; funghi micorrizici arbuscolari; defolianti sostenibili; gestione agronomica pre-raccolta.

Preface

Cotton (*Gossypium* spp.), which belongs to the Malvaceae family, is one of the most important industrial crops in the world and the most widely used natural fibre. It is cultivated on approximately 31.92 million hectares across more than 80 countries (Wakelyn and Chaudhry 2009a), with an estimated annual fiber production of 25.51 million tons and a global market value of around USD 78.6 billion (FAOSTAT 2023). As such, cotton holds a central role in the global agricultural economy. However, its cultivation in Italy is currently almost nonexistent. Over the past forty years, cotton production has steadily declined until it virtually disappeared from the national agricultural landscape (Tuttolomondo et al. 2020). At present, despite the existence of a few hundred hectares still devoted to this crop, no official data are available in Italian statistical repositories (Istat 2025). The decline of cotton production can be attributed to several factors, the most critical of which is water availability. Cotton is a highly water-demanding crop: it is estimated that cotton cultivation alone accounts for 2.6% of global freshwater use, with approximately 70% of cotton-growing areas experiencing water stress (Chapagain et al. 2006). Under pedoclimatic conditions similar to those of southern Italy, cotton requires between 700 and 1,200 mm of water throughout its growing season (Evetts et al. 2012; Grismer 2002; Koudahe et al. 2021a). Meeting such irrigation demands is particularly challenging in regions like Sicily, where water availability is increasingly constrained due to the impacts of climate change, especially during critical growth stages. Allocating such significant volumes of water to this crop could exacerbate the already fragile water balance in the Sicilian territory.

This situation is further compounded by the widespread use of low-efficiency irrigation methods, such as furrow and sprinkler systems, which result in substantial water losses compared to more advanced and efficient techniques like drip irrigation (Barnes et al. 2020; Mai, Xue, and Azeem 2022; Vitale et al. 2024).

Moreover, cotton is often cited as one of the most environmentally impactful crops. Although it accounts for only 2.4% of the world's arable land (Bevilacqua et al. 2014), it consumes approximately 11% of all pesticides used globally—a figure that can rise to 50% in developing countries (La Rosa and Grammatikos 2019). According to recent data from the United States Department of Agriculture (USDA 2025), cotton ranks as the third-highest pesticide-consuming commodity crop in the U.S., with an average application rate of around 4 kg of active ingredients per hectare annually. The extensive use of synthetic fertilizers is also a critical concern. In the United States, in 2022, farmers applied an average of 270 kg ha⁻¹ of synthetic fertilizers, with nitrogen used on 71% of cotton-growing areas (USDA 2025). Global estimates for nitrogen use in cotton cultivation range between 300 and 450 kg ha⁻¹. Such high levels of fertilization pose serious sustainability challenges, contributing to environmental pollution and greenhouse gas emissions. Pre-harvest management presents additional sustainability challenges. Effective defoliation - the pre-harvest removal of leaves - is critical to enable mechanical harvesting, reduce trash content in fibers, and maintain cotton quality (Xie et al. 2025). Conventional systems rely heavily on synthetic defoliant (e.g., Tribufos, Dimethipin, Pyraflufen-ethyl, and Ethephon) (Liao et al. 2023; Neupane et al. 2023; Yu et al. 2023), which accelerate leaf senescence but raise concerns about environmental persistence, residual phytotoxicity, and potential risks to human health (Trakulsrichai et al. 2025; Wei et al. 2023). The lack of effective natural defoliant remains a major barrier to scaling sustainable cotton production. Without viable alternatives, growers avoiding synthetic solutions must resort to labour-intensive manual harvesting, severely limiting scalability (Lakshmanan et al. 2025). These issues- combined with rising input costs (Lawton et al. 2023) and relatively stagnant cotton prices, currently around \$1.86 kg⁻¹ (USDA 2025) - represent significant obstacles to the potential reintroduction of cotton cultivation in the Italian agricultural context.

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State of the Art

The reintroduction of cotton cultivation in Southern Italy is associated with significant sustainability challenges, particularly related to high water consumption, intensive pesticide use, and soil depletion.

Sustainability, in its most widely accepted definition, involves the use of current resources without compromising the ability of future generations to meet their own needs (Ruggerio 2021). Addressing these challenges is crucial for the future of this crop. To bridge the numerous gaps this crop presents, a holistic approach that considers the various aspects of sustainability is essential.

Life Cycle Assessment (LCA) is a standardized methodology (ISO 14040:2006) that allows the evaluation of the environmental impact of a product, process, or service throughout all stages of its life: from raw material production to final disposal (cradle-to-grave). The application of LCA to cotton is therefore an essential tool for identifying critical points along the agricultural and textile supply chains (Bevilacqua et al. 2014). Conventional cotton cultivation, in particular, has been shown to require heavy use of nitrogen fertilizers and pesticides worldwide - especially in India and China - where these inputs account for 44–61% of GHG emissions, and represent the main contributors to the Carbon Footprint (CF) of cotton in China, accounting for 35.54% from 2004 to 2018, followed by electricity for irrigation and agricultural film (Huang et al. 2022; Singh, Singh, and Sodhi 2022). Although organic cotton reduces GHG emissions by 60% per hectare (La Rosa and Grammatikos 2019), it yields less than conventional cotton and thus requires a greater cultivated area to produce the same amount of fiber, limiting its scalability (Avadí et al. 2020). Regarding water footprint, sustainable solutions converge on the adoption of high-efficiency irrigation systems, such as Drip Irrigation (DI) and Mulch Drip Irrigation (MDI). DI has proven effective in water use and yield improvement compared to traditional systems (Cetin and Bilgel 2002; Ibragimov et al. 2007), and also

promotes better uptake of nutrients like phosphorus (Mai et al. 2022). MDI, which combines DI with plastic film mulching, can further increase soil moisture and yield (Wang et al. 2020; Zong et al. 2020), although some authors have expressed concerns, particularly regarding increased CO₂ emissions (Zong et al. 2020). Moreover, DI systems have some limitations, such as potentially lower moisture uniformity during germination and salt accumulation (Çetin and Kara 2019). Another sustainability strategy is early irrigation termination, which has gained increasing attention in recent years and is also used as an alternative to chemical defoliants. When properly applied especially in mild climates such as humid subtropical and Mediterranean regions - this practice can reduce water inputs without significantly affecting yield (Karam et al. 2006; Reba et al. 2014). However, its effectiveness varies depending on specific factors such as climate, soil type, and genotype (Buttar et al. 2007; Grimes and Dickens 1974; Masasi et al. 2019; Silvertooth et al. 1989).

Arbuscular mycorrhizal fungi (AMF) are among the most widespread and functional mutualistic symbioses in sustainable agriculture. It is estimated that over 80% of higher plants can establish mycorrhizal associations, including cotton, which is considered a highly mycorrhiza-dependent plant (Smith et al. 2011; Thompson, Seymour, and Clewett 2012a). In the context of reducing chemical fertilizer use and increasing resilience to abiotic stress, mycorrhizae significantly contribute to the sustainability of cotton cultivation by improving nutrient uptake, water-use efficiency, and resistance to unfavourable soil and climate conditions (Mai et al. 2018; Ortas and Iqbal 2019). Phosphorus (P) is an essential element but poorly mobile in soils, especially those with extreme pH, where it tends to become immobilized through adsorption onto iron, aluminium, or calcium cations (Smith, Smith, and Jakobsen 2003). AMF, through their extraradical hyphae, expand the soil exploration volume and facilitate P uptake even from areas otherwise inaccessible to roots (Zhou et al. 2020). Some studies show that species such as *Funneliformis mosseae* and *Gigaspora margarita* can increase P uptake by up to 110% and

Zn uptake by up to 69% compared to uninoculated plants (Ortas and Iqbal 2019; Salgado et al. 2017). Furthermore, combinations of *Glomus* species (e.g., *G. intraradices*, *G. viscosum*, *G. mosseae*) have demonstrated improved uptake of other essential nutrients such as nitrogen (N), potassium (K), calcium (Ca), and micronutrients (Cely et al. 2016a).

Beyond this well-known "physical" mechanism, AMF enhance nutrient availability through key biochemical processes such as hydrolysis and mineralization (Hodge and Storer 2014). The mycorrhizal hyphae can exude enzymes like phosphatases and phytases that hydrolyze organic phosphorus compounds (e.g., inositol phosphates) in the soil, releasing plant-available inorganic phosphate. This enzymatic hydrolysis is a critical pathway for accessing the large pool of organic P that is otherwise unavailable to plants (Koide and Kabir 2000; Zhang et al. 2016). Furthermore, AMF play a significant role in the mineralization of organic nitrogen and sulfur. By decomposing organic matter, they convert complex organic forms of these nutrients into simpler, inorganic forms (ammonium, nitrate, sulfate) that can be readily absorbed by both the fungus and the host plant (Hodge and Storer 2014; Leigh, Hodge, and Fitter 2009). This synergistic relationship between the plant and the AMF creates a "mycorrhizal pathway" for nutrient uptake that is more efficient than the direct root pathway alone, especially in nutrient-deficient soils (Hodge and Storer 2014).

N fertilization is another crucial aspect; while essential for yield, conventional practices often involve high application rates. Recent studies show that average N applications can reach 402.5 kg ha⁻¹, with some cases exceeding 500 kg ha⁻¹ (Chen et al. 2024). However, other research indicates that efficient production is achievable with moderate doses of 300–350 kg ha⁻¹ (Guo-Zheng et al. 2013; Wang et al. 2024). Recently, Tian et al. (2023) demonstrated that a reduced N application of only 240 kg ha⁻¹ can be sufficient when strategically timed to critical phenological stages. The most effective partitioning was 0% at squaring, 60% from flowering to late peak boll,

and 40% at late peak boll. This targeted approach not only improved nutrient uptake but also increased boll biomass by up to 35.8% compared to early applications (Tian et al. 2023), highlighting optimized N management as a viable pathway for sustainable production.

Another strategic node for supply-chain sustainability is pre-harvest defoliation, a critical step for mechanization and fiber quality. The lack of sustainable defoliant has prompted research into agronomic solutions, such as irrigation termination, which has proven promising in promoting natural defoliation (Buttar et al. 2007; Karam et al. 2006; Reba, Teague, and Vories 2014; Vitale et al. 2024). Various organic compounds, such as vinegar and sodium chloride, have also been investigated (Barth and Mowrer 2022; Lakshmanan et al. 2025), but none match the efficacy of synthetic options. Recent attention has turned to pelargonic acid- a naturally occurring, rapidly degradable fatty acid with proven defoliant properties in crops (e.g., *Salix matsudana* L., *Phaseolus vulgaris* L.) (Arbolea et al. 2005; Greer and Dole 2005) - as a potential biodegradable solution. However, information on its effectiveness as a cotton defoliant is currently very limited (Greer and Dole 2005), highlighting a critical research gap.

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Aim of the thesis

This work aimed to address the main challenges related to the reintroduction of cotton cultivation in Sicily (Southern Italy), with a specific focus on sustainability through efficient water resource management, the use of mycorrhizae, and sustainable pre-harvest strategies.

The main objectives were:

- To investigate the key agronomic factors limiting cotton productivity, with particular attention to irrigation methods and schedule to accelerate phylloptosis, use of cover crops, and crop rotation, and to analyse the primary mitigation strategies.
- To evaluate the environmental impacts of cotton cultivation through a literature review employing the LCA methodology, identifying the main environmental hotspots across the supply chain and recommending mitigation strategies in line with the latest international guidelines (ISO 14040 and ISO 14044).
- To conduct experimental field trials on two cotton cultivars widely grown in the Mediterranean basin, by using sustainable agricultural practices, mycorrhizal formulations, and high-efficiency irrigation systems, to evaluate cotton plant responses to different water requirements, while reducing the environmental impact associated with fertilizers and pesticides thereby ensuring the sustainability of water and soil resources in line with organic farming principles. To evaluate pelargonic acid as a sustainable defoliation strategy by assessing its optimal dosage and its impact on yield and key agronomic parameters.
- To provide experimental data and operational guidelines for the sustainable reintroduction of cotton cultivation in Sicily, considering the region's specific pedoclimatic conditions and the needs of a traceable, low-impact textile supply chain.

Thesis outline

Chapter 1: Agronomic Strategies for Sustainable Cotton Production: A Systematic Literature Review

Vitale et al. 2024. <https://doi.org/10.3390/agriculture14091597>

The review examined the key agronomic practices for sustainable cotton cultivation. In addition to the use of beneficial microorganisms and soil conservation techniques, it explored the role of efficient water management, including irrigation termination, as strategies to optimize water use and reduce environmental inputs, to promote an integrated approach for more resilient and sustainable cotton production. This paper provided farmers with a practical guide for eco-friendly cotton management without compromising productivity.

Chapter 2: Environmental Sustainability of Cotton: a Systematic Literature Review of Life Cycle Assessments

Vitale et al. 2025. <https://doi.org/10.1016/j.jafr.2025.102069>.

The review explored the environmental impact of cotton cultivation, highlighting its high consumption of water, energy, fertilizers and pesticides, which negatively affects the environment and human health. It also pointed out that post-consumer activities, such as washing and ironing, are further contributors to cotton's ecological footprint. Finally, the study proposed strategies to improve the sustainability of the supply chain by promoting more efficient and conscious agricultural practices.

Chapter 3: Sustainable Cotton Production in Sicily: Yield Optimization Through Varietal Selection, Mycorrhizae, and Efficient Water Management

Vitale et al. 2025 <https://doi.org/10.3390/agriculture14091597>

This study highlighted sustainable strategies to revitalize cotton cultivation in Sicily by integrating organic farming, the use of mycorrhizal fungi and efficient drip irrigation. Two varieties (Armonia and ST-318), both mycorrhizal-inoculated and non-inoculated, were tested under varying water conditions and on contrasting soils. The study highlighted how factors such as varietal choices and mycorrhization significantly influence cotton plant's development, phenology and productivity. The site with sandy soil and rich in organic matter showed greater water use efficiency, while Armonia stood out for productive stability under stress conditions. The study emphasized the importance of adaptive and soil-specific approaches for a resilient and sustainable Mediterranean cotton supply chain.

Chapter 4: Evaluation of Pelargonic Acid as a Sustainable Defoliant in Cotton (*Gossypium hirsutum* L.) Production

Vitale et al. 2024. <https://doi.org/10.3390/agriculture15202134>

This paper explored sustainable strategies to address the lack of effective defoliants for mechanical cotton harvesting. In a two-year field trial in Sicily, pelargonic acid, a rapidly biodegradable compound, was tested at three application rates and compared with pyraflufen-ethyl and a water control. Results showed that pelargonic acid at 18 L ha⁻¹ delivered similar defoliation performance to the synthetic product without compromising root growth or yield. These findings suggest pelargonic acid as a viable, environmentally friendly alternative for sustainable cotton production.



Agronomic Strategies for Sustainable Cotton Production: A Systematic Literature Review

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Gaetano Pandino ¹, Sara Lombardo ^{1,*}, Umberto Anastasi ¹ and Paolo Guarnaccia ¹

1 Agronomic Strategies for Sustainable Cotton Production: A Systematic Literature Review

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Abstract

Cotton, with a cultivated area of $31.92 \times 10^6 \text{ ha}^{-1}$ across 80 countries and an estimated annual turnover of USD 5.68 billion, is the world's leading natural textile fiber. However, many cotton-producing countries have neglected to improve production practices, adversely affecting the environment and society. A systematic review of the sustainable cotton cultivation literature was performed for the first time to identify and suggest context-specific agricultural strategies that can be applied within different agroecosystems. The key aspects include (1) inoculation with arbuscular mycorrhizal species such as *Gigaspora margarita*, *Funneliformis mosseae*, and *Acaulospora scrobiculata* to enhance root exploration, biomass, and nutrient uptake; (2) using grass, legume, and brassica cover crops as a valid alternative to monoculture and fallow crop rotations to reduce resource depletion and increase the sustainability of cotton production; (3) adopting drip and mulched drip irrigation systems over traditional furrow and sprinkler systems for water conservation; (4) exploring the feasibility of prematurely terminating irrigation in humid subtropical and Mediterranean climates as an alternative to chemical defoliation without affecting cotton yield. This paper, which describes various farming practices adopted in different climates, provides farmers a guide for eco-friendly cotton agronomic management without sacrificing productivity.

1.1 Introduction

Cotton (*Gossypium* spp.) is the world's most important natural textile fiber, which is grown in ~80 subtropical and tropical countries (Wakelyn and Chaudhry 2009); due to the wide global distribution of the crop, it is advisable to be cautious about its sustainability. The concept of sustainability itself has undergone various definitions over the years, and the most widely used and accepted one is that proposed by the United States (US) Environmental Protection Agency, which states that the use of resources for present needs should not compromise the ability of future generations to meet their own needs (U.S. Environmental Protection Agency 2008).). According to the latest data provided by the USA Department of Agriculture (USDA 2023), herbicides have been used in 96% of cotton-growing areas in the USA. In particular, glyphosate isopropylamine salt is used on 45% of these acres, glyphosate potassium salt is used on 38%, paraquat on 27%, the dicamba diglycolamine salt on 25%, and diuron on 22%. Other chemicals are used in 68% of these areas. Insecticides are “only” used on 39% of these areas (USDA 2023). The reasons behind the lower use of insecticides can be attributed to the widespread use of genetically modified (GM) varieties, such as Bt (*Bacillus thuringiensis*) cotton, which were designed to control cotton bollworm (*Helicoverpa armigera*) (Reisig et al. 2018). However, the massive use of Bt cotton has resulted in strong resistance to the two families of Bt toxin (Cry1A and Cry2A) all over the world, as highlighted in China (Liu et al. 2010; Zhang et al. 2012), India (Nair et al. 2016; Mathew et al. 2018), South Africa (Kruger et al. 2011; Van den Berg et al. 2013), Argentina (Grimi et al. 2018), and the United States (Cry2Ab) (Tabashnik and Carrière 2017).

Additionally, broad-spectrum herbicides such as glyphosate

doubled on cotton in the USA territory from 2010 to 2019, according to the data reported by the USDA National Agricultural Statistics Service (USDA 2023). The phenomena of weed resistance (Huff et al. 2010), growth inhibition of some soil bacterial species (Gimsing et al. 2004), and the risks to the environment and humans (Rolando et al. 2017) are just some of the reasons for moving towards eco-friendly alternatives. Another factor that should be considered is the nutritional requirements of cotton. According to the USDA, in the 2021 growing season, farmers applied nitrogen to 71% of the growing areas at an average rate of 100 kg ha^{-1} , for a total of $3.15 \times 10^6 \text{ Mg}$. This was accompanied by phosphate, potassium, and sulfur, which had a total contribution of 0.11, 0.13, and $0.02 \times 10^6 \text{ Mg}$, respectively (USDA 2023). Nevertheless, the extensive use of mineral fertilizers has strong environmental impacts, particularly on soil microorganisms and organic matter turnover, which are key factors for soil fertility (Altieri 1999). Some nutrients, such as phosphorous (P), which is considered a non-renewable resource, are decreasing globally, resulting in a rise in prices over the recent years (Cordell et al. 2009). Moreover, most P fertilizers are immobile in soils since they are strongly adsorbed to iron and aluminum cations at low soil pHs and calcium under alkaline conditions (Smith et al. 2003). A possible solution to this issue may be offered by soil microorganisms such as arbuscular mycorrhizal fungi (AMF), which play an important role in maximizing nutrient uptake due to their ability to effectively explore a higher soil volume, increasing the plant's nutrient uptake, mainly of P (Mai et al. 2019).

Cotton is a summer crop that leaves the soil bare during winter. Indeed, a notable concern in cotton production is the limited quantity of crop residues remaining on the soil surface after harvest compared to other crops (Agricultural Resources and Environmental Indicators 2012). This deficiency has implications for the content of soil organic

matter (Adeli et al. 2021) and for cotton productivity (Ashworth et al. 2016).

Another aspect covered in this review article is water use. Agriculture is the world's largest user in terms of consumption and water withdrawal. Recent estimates show that the global crop consumptive use stands at $8053.6 \text{ km}^3 \text{ year}^{-1}$, accounting for 87% of the global water consumption (Wu et al. 2022). Cotton is an irrigated crop but it requires modest water inputs compared to other crops with similar growing cycles. Currently, the most widely used irrigation system in cotton cultivation is furrow irrigation (Roth et al. 2013; Kebede et al. 2014; Barnes et al. 2020), which has low efficiency and significantly increases waste.

There is abundant scientific literature on the sustainable agronomic practices of cotton cultivation concerning fertilization, weed control, and proper water resource management. However, to the best of our knowledge, a comprehensive review that encompasses all these aspects is still lacking. For this reason, in this review, we analyzed, for the first time, the studies on sustainable cotton cultivation from different production areas around the world in order to disseminate information and guide farmers toward environmentally friendly management. In particular, we focused on the role of microorganisms in reducing the agronomic inputs and increasing soil fertility, as well as the adoption of conservation practices such as crop rotation and cover cropping to restore the soil's natural balance. Furthermore, the review identifies the most recent agronomic strategies and tactics for water reduction, irrigation systems, and irrigation termination.

1.1 Materials and Methods

This systematic review was conducted following the PRISMA guidelines (Moher et al. 2009).

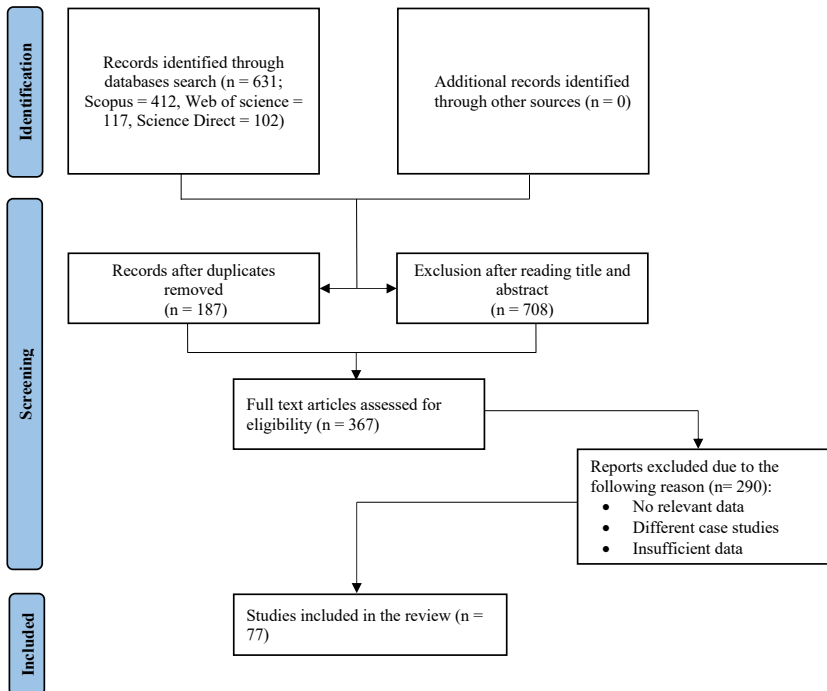
The first step was the collection of a corpus of articles related to the research topic by using relevant keywords such as “sustainable cotton cultivation” or synonyms (“responsible”, “ethical”, and “green”), “cotton water-saving irrigation strategies” or synonyms (“innovative”, “modern”, “latest”, and “emerging”), and “arbuscular mycorrhizal fungi on cotton” or synonyms (“AMF species”, “root-associated mycorrhizae”, and “mycorrhizal symbionts”). These keywords were selected as they were deemed the most representative of the research objectives. To achieve a high scientific impact for this review, the previous keywords were searched on three databases (Scopus, Web of Science, and Science Direct), and the following inclusion criteria were adopted:

- peer-reviewed articles written in English were included;
- conference papers, conference reviews, and book chapters were not reviewed;
- the most recent and informative papers from the same experimental topic were given priority.

In the second step, the articles were screened by reading their abstracts to identify those that addressed the research question. Articles that were not related to the topic, despite being identified by the search query, were discarded. The articles that reported quantitative data on the use of sustainable practices in cotton cultivation were selected, while those that did not quantify the effects of the case studies or had insufficient or unrepresentative data were not considered. Among the studies published up to August 2024 (the date of the last search), the 119 searches across the three databases

returned a total of 1262 results. Of these, 187 duplicates were removed from the dataset and 708 were excluded due to irrelevant content. The full texts of the remaining 367 articles were then reviewed, and 77 eligible articles were included in the qualitative summary. Full details of the screening process can be found in the PRISMA flowchart in Figure 1.1. In addition, the PRISMA checklist was included in the supplementary material.

Figure 1.1 - Flowchart of the results of the literature search.



1.3 Results and Discussion

This section presents the results of the literature review on sustainable cotton cultivation around the world. Within it, various topics falling under the theme of sustainability are addressed. Starting with plant–microorganism relationships, through to the use of polycultural systems and reducing water inputs. Finally, the last section is reserved for final remarks based on the results of the review.

1.3.1 Cotton Production and Literature Database

The latest estimates from 2020 indicate that the global cotton production stands at 24.2×10^6 t, which had more than doubled compared to earlier data from the 1960s. Moreover, with a production of 6 million tonnes, China is the world's largest producer, followed by India and the United States (Figure 1.2 a,b) (FAOSTAT 2023).

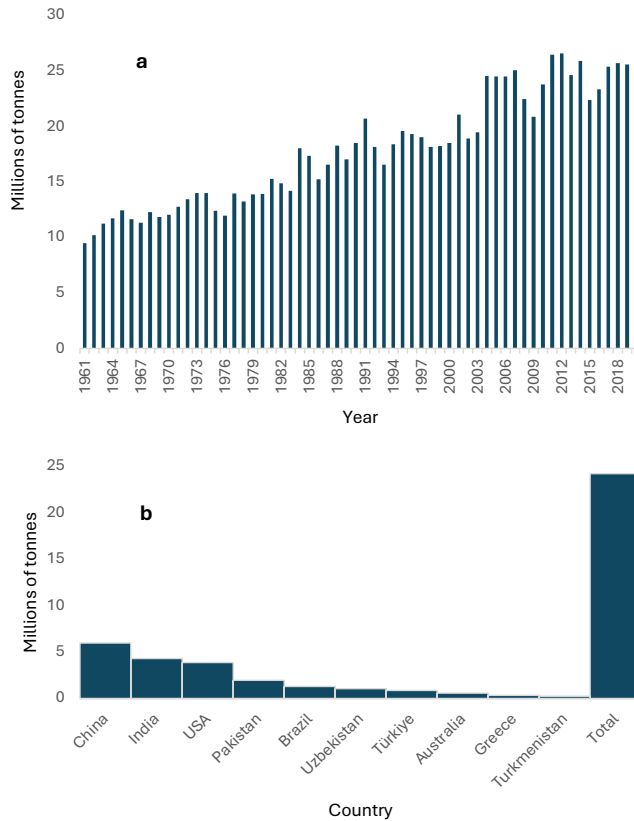


Figure 1.2 a,b - World cotton production: (a) production of ginned cotton lint around the world (1961–2021); (b) top 10 producers of cotton lint (average for 1961–2021) (FAOSTAT 2023).

Similarly, scientific research on sustainable cotton production has also seen a notable expansion in recent decades, from the first article published in 1981 to 22,800 in 2022, for a total of 111,000

document results, when using the keyword “sustainable cotton” within the Scopus® database (Figure 1.3).

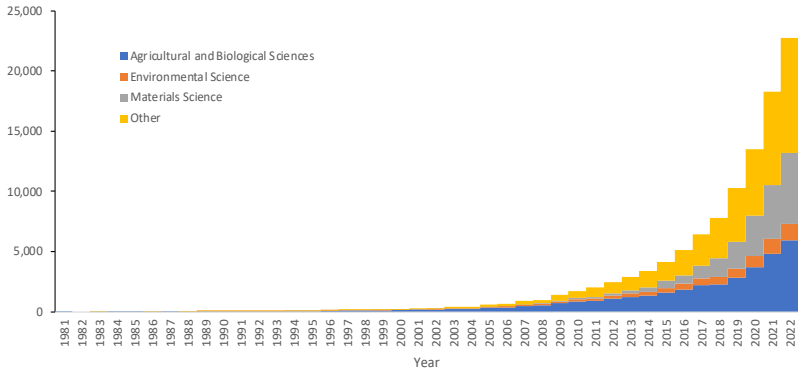


Figure 1.3 - Global scientific publications on “sustainable cotton”, 1981–2023, on Scopus®.

The publication of articles on cotton cultivation significantly increased after the 2010s in terms of annual number of articles, countries, and institutes publishing on sustainable cotton topics. Furthermore, the areas of research, indicated in Figure 1.3 as “other”, have also increased, which represents the numerical difference between the total number of articles and those present in the subject areas of “Agricultural and Biological Sciences”, “Environmental Science”, and “Materials Science” within the Scopus® database. This is emblematic of the fact that over the years, sustainable cotton cultivation has gained the interest of other scientific areas, not just purely agronomic fields.

1.3.2 Mineral Nutrients and Plant–Microorganism Relationships

The large use of mineral fertilizers has a serious impact on the environment and soil (Tilman et al. 2002), and their long-term practice has contributed to reductions in the soil organic matter content (Tong et al. 2014), increased soil acidification (Tao et al. 2019), and environmental pollution (Martínez-Dalmau et al. 2021). Therefore, the challenge for modern agriculture is to switch to sustainable fertilization practices and find alternatives to increase the amount of available nutrients for plants.

In this scenario, plant–microorganism relationships, which occur at the level of the rhizosphere, are of key importance to improving plant growth and the product quality of field crops (Pandino et al. 2022; Lombardo et al. 2020; Badda et al. 2014; Rochester et al. 2001). It is known that some microorganisms, such as AMF, can establish symbiotic relationships with cotton (Eskandari et al. 2017; Thompson et al. 2012), thus improving the crop in terms of vigor, nutrient uptake (especially P and Zn) ability (Ortas et al. 2019), flowering, and boll maturation (Eskandari et al. 2017). Up to 90% of the length of cotton roots can be colonized by AMF (Ortas et al. 2019), which provides a significant benefit to the plant. The extra-root hyphae of AMF (Zhou et al. 2020) help the root system in exploring a larger soil surface (Lynch and Ho 2005) and increasing the plant's nutrient uptake, mainly of P (Ortas and Iqbal 2019). In particular, the growth range of cotton hyphae can extend up to 30 mm from the root surface when P fertilizers are not applied (Mai et al. 2019). In some cases, this can result in an increased P absorption range of up to 15-fold. However, greater colonization of the root system does not always mean a better plant nutritional status (Smith et al. 2011). For instance, Nagy et al. (2009) reported that plant P uptake is not necessarily related to the percentage of root length colonized by mycorrhizae; on

the contrary, they observed that with increasing soil P supply, the mycorrhiza-inducible P transporter genes were down-regulated, resulting in a decreasing proportion of colonized root length.

In accordance with the findings of Badda et al. (2014), Mai et al. (2018), observed that the Apparent Phosphorus Recovery (APR) significantly decreased with increased application rate (150–300 kg P₂O₅ ha⁻¹), demonstrating how a low P application rate (75 kg P₂O₅ ha⁻¹) not only increased root length and hyphal density but also improved the spatial distribution of cotton roots in the soil, thus increasing the APR. In contrast, Thompson et al. (2012) reported no significant change in the percentage of root length colonized in response to P fertilizer application in cotton plants.

P is not the only soil nutrient whose cotton plant uptake may undergo changes with AMF application. Mycorrhizae hyphae can contribute to between 60 and 85% of Zn uptake depending on the AMF species (Ibrahim et al. 2016; Salgado et al. 2017). It is well known that Zn is an essential micronutrient for normal healthy growth and the reproduction of cotton, playing a key role as a regulatory cofactor for a wide range of different enzymes (Korejo et al. 2015; Ortas and Iqbal 2019). However, there is an antagonistic relationship between P and Zn. Since high P fertilizer applications suppress mycorrhizae inoculation, plants cannot obtain more Zn (Ortas et al. 2016). The dynamic changes if there is a high Zn application. Ortas et al. (2019) observed that a high Zn application (10 mg Zn kg⁻¹ soil) to sterilized soils deficient in P (0 mg P₂O₅ kg⁻¹ soil intake) significantly reduced the uptake of P (-31%) in cotton plants mycorrhized by *Funneliformis mosseae*. In contrast, Zn application (10 mg Zn kg⁻¹ soil) significantly increased P uptake (+48%) in mycorrhized cotton plants grown in sterilized soil with sufficient P (100 mg P₂O₅ kg⁻¹ soil intake). Over the last few years, the research has focused on

identifying the AMF species capable of bringing the greatest benefits to cotton, especially for P acquisition. Table 1.1 shows the selected AMF species and their advantages to cotton, highlighting their potential applications beyond the different range of minerals that each analyzed AMF species provides.

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Table 1.1 - Comparison and analysis of the main arbuscular mycorrhizal fungi (AMF) species used on cotton.

AMF species	Benefits	Target parameter	Effect compared to uninoculated control	References
<i>Acaulospora scrobiculata</i>	Nutrient uptake,	Root dry matter	+75%	Salgado et al. (2017)
	increase of the surface explored by roots and shoots	P uptake	+59%	
<i>Claroideoglomus etunicatum</i>	Nutrient uptake,	Root dry matter	+56%	Salgado et al. (2017)
	increase of the surface explored by roots and shoots	P uptake	+76%	
<i>Funneliformis mosseae</i>	Zn, and P uptake (less than <i>C. etunicatum</i>)	P uptake	+110%	Ortas et al. (2019)
		Effect on the soil microbiome	abundance of Actinobacteria and Gemmatimonadetes	Zhou et al. (2020)
<i>Gigaspora margarita</i>	Nutrient uptake,	Ca, Zn, P uptake	+68% Ca, + 69%Zn, + 76% P	Salgado et al. (2017)
	increase in the surface explored by roots and shoots	Effect on the soil microbiome	abundance of Proteobacteria, Cyanobacteria, and Fusobacteria	Zhou et al. (2020)
<i>Glomus intraradices</i> , <i>G. viscosum</i> , and <i>G. mosseae</i>	Growth response of cotton - Nutrient uptake	N, P, K, Ca, Mn, Fe, Cu, Zn uptake	+65% N, +148% P, +92% K, +65% Ca, +129% Mn, +73% Fe, +91% Cu, +85% Zn	M. Ibrahim (2016)
<i>Rhizophagus clarus</i>	Increase in P and nitrogen content in inoculated plants. Root colonization	Effect on plant biomass	+81%	Cely et al. (2016)
		N shoot uptake	+75% in N uptake	Cely et al. (2016)

In fact, the best strategy lies in the careful combination of different species, promoting microbial interactions that fully meet the needs of cotton while reducing agronomic inputs and encouraging the adoption of sustainable agronomic tactics. For instance, Salgado et al. (2017) reported how the AMF species *Acaulospora scrobiculata* could produce significantly high values in root dry matter (+75% compared to uninoculated control), but at the same time, it was less efficient in P uptake capacity (+59% compared to control). On the other hand, the species *Claroideoglomus etunicatum* showed lower values in root dry matter (+56% compared to the non-inoculated control) but a high P absorption capacity of 1 mg kg⁻¹, 76% higher compared to the non-inoculated control (0.57 mg kg⁻¹) (Salgado et al. 2017). Ortas et al. (2019) reported even higher P values in soil treated with an inoculum of *C. etunicatum*, with an 80% increase in P (0.18 mg kg⁻¹) compared to the untreated control (0.1 mg kg⁻¹). Beyond *C. etunicatum*, Ortas et al. (2019) observed that the species *F. mosseae* was able to stock up P to 0.21 mg kg⁻¹, equal to 110% of that contributed by the untreated control (0.10 mg kg⁻¹) and 9% more than the species *C. etunicatum* (0.18 mg kg⁻¹ soil). *C. etunicatum*, on the other hand, performed better in Zn recovery (20.9 mg kg⁻¹ soil) than the untreated control (14.8 mg kg⁻¹ soil) and *F. mosseae* (20.1 mg kg⁻¹ soil). Furthermore, *F. mossae* has been shown to influence the taxonomic abundance of soil microbial populations. It was observed that an inoculum of *F. mosseae* resulted in a greater presence of Actinobacteria and Gemmatimonadetes, but at the same time, the soil contained Proteobacteria, Bacteroidetes, Acidobacteria, and Planctomycetes (Zhou et al. 2020). It appears that *G. margarita* also influenced soil microbial communities by promoting the colonization of Proteobacteria, Cyanobacteria, and Fusobacteria (Zhou et al. 2020).

In recent years, some authors have observed how different AMF species can supply other essential nutrients to sustain cotton production in addition to the aforementioned P and Zn. For example, Salgado et al. (2017) noted that by inoculating soil with *Gigaspora margarita*, there was an increase of 68% in Ca, as well as a greater absorption of P (+76%) and Zn (+69%) compared to non-inoculated plants.

Cely et al. (2016) found that an inoculum of *Rhizophagus clarus* was able to provide, in addition to a greater cotton biomass (+81%), a higher N shoot uptake (14 mg plant⁻¹) in comparison to the uninoculated control (7 mg plant⁻¹). In fact, Ibrahim (2016), ascertained that a mixture of *Glomus intraradices*, *G. viscosum*, and *G. mosseae* was found to have a better contribution to the cotton plants' mineral uptake in terms of N (65%), K (92%), Ca (65%), Mn (129%), Fe (73%), Cu (91%), as well as of P (148%) and Zn (85%).

1.3.3 Monoculture and Polyculture Systems

Cotton is a crop that requires large auxiliary inputs and leaves a very low content of biomass residues on the soil after harvesting, compared to corn, wheat, and soybean (Osteen et al. 2012). The lack of plant residues on the soil surface, especially under a monocropping regime (Ashworth et al. 2020), can explain the different environmental issues, including soil compaction and erosion (Ashworth et al. 2020), N gas emissions into the atmosphere (Zhang et al. 2022), loss of nutrients (Adeli et al. 2021), and a decrease in cotton yields (Zhang et al. 2022) (Figure 1.4a).

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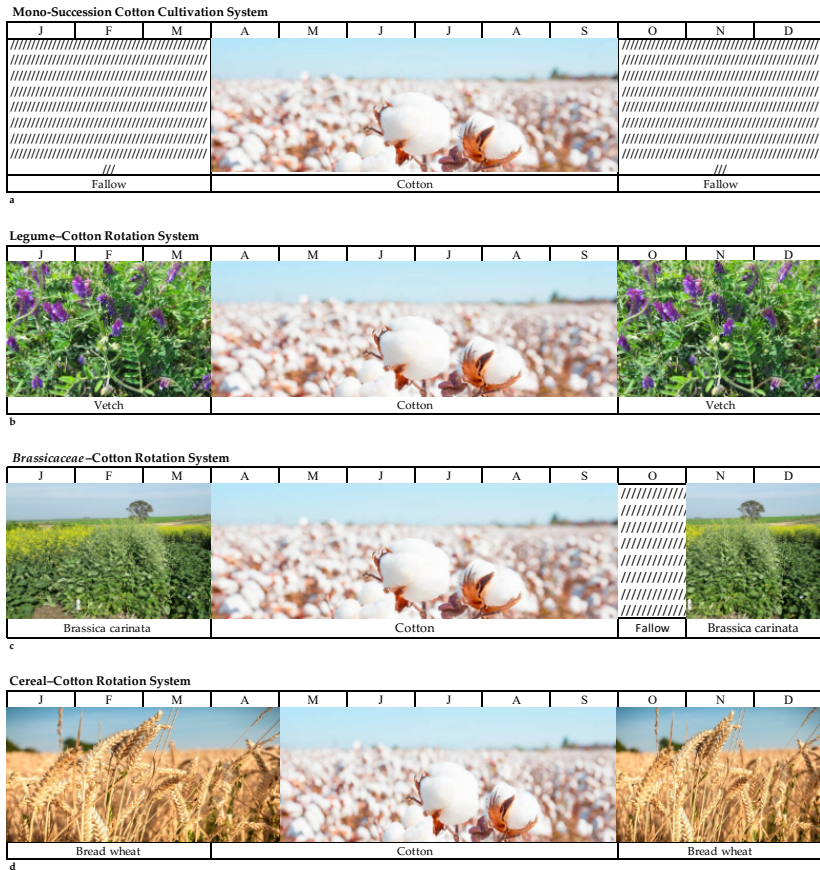


Figure 1.4 - Possible crop rotation schedules for cotton cultivation. Letters indicate the months of the year: (a) Mono–Succession cotton cultivation system.

Soil erosion, loss of nutrients, and reduced cotton yield are some of the phenomena related to the use of cotton in monoculture; (b) Legume– Cotton Rotation System. *Vicia villosa*—cotton rotation proposed by Li et al. (2021) showed that vetch is useful for increasing soil total N concentration and making it more available to cotton, potentially reducing the need for nutritional inputs during cotton cultivation.; (c) *Brassicaceae*–Cotton rotation system. Tiwari et

al. (2021) observed that the use of *Brassicaceae*, specifically *Brassica carinata*, has an inhibitory effect on the weed growth, thereby reducing competition during the early growth stages of cotton.; (d) Cereal–Cotton rotation system. Adeli et al. (2021) proposed a cultivation system based on the *Poaceae*–cotton succession. *Triticum aestivum* residue after harvest, due to its high biomass quality, can significantly reduce the loss of nitrate N concentrations by leaching compared to conventional winter fallow.

Soil conservation polyculture systems, in particular cover cropping (CC), can assist in reducing soil erosion (Chatterje et al. 2017), enhancing the soil water content, stimulating beneficial soil microbial communities (Scavo et al. 2022; Zhou et al. 2020), controlling weeds (Scavo et al. 2021; Restuccia et al. 2020; Lemessa et al. 2015; Korres and Norsworthy 2015), and reducing the use of herbicides, particularly in the early weeks after sowing (Korres and Norsworthy 2015). Recently, several authors have focused on cereals, legumes, and brassicas and their mixes as a cover crop option to enhance both weed and nutritional management of crops.

1.3.3.1 Legumes

Legume cover crops can provide significant benefits, including an improved soil structure and reduced N losses by preventing runoff and leaching during the off-season (Abdalla et al. 2019). For instance, Rochester et al. (2001) evaluated the rotational effects of winter and summer legumes grown before cotton to increase both the crop nutritional intake and soil structure. The experiments were conducted in Narrabri (NSW, Australia) on moderately fertile soil.

On the one hand, the cultivation of grain-manured legumes was evaluated using *Vicia faba* L. and *Glycine max* L., which fixed 453 and 488 kg N ha⁻¹ in the soil, respectively. It was evaluated how green-

manured legumes, using *Pisum sativum* and *Lablab purpureus*, were able to fix up to 209 and 240 kg N ha⁻¹, respectively, before the crops were mowed. These results evidenced how both strategies markedly influenced N fertilization rates, with a reduction of 99% for grain-manured and 244% for green-manured legumes, compared to cotton in a non-legume-based cropping system. Li et al. (2021) evaluated the influence of another green-manured legume, *V. villosa*, in a field trial conducted at the West Tennessee Research and Education Center in Jackson (Jackson, TN, USA) on silt loam soil with 0–2% slopes. The vetch cover crop increased the soil total N concentration by 18% and microbial transformation rate by 168% compared to the untreated control. Moreover, it also enhanced soil labile N concentrations by 21, 79, and 57% for nitrate, ammonium, and extractable organic N, respectively. These results prove that *V. villosa*, in addition to fixing N, could keep it available to the plant, reducing the dose of N fertilizer needed (Figure 1.4b). Liang et al. 2020 evaluated the benefits of intercropping cotton with mung bean (*Vigna radiata* L.) in an arid region of northwest China. The results showed that the legume intercropping system significantly increased the total soil N content (27.9–45.3%), total yield (16.6–19.8%), and water use efficiency (WUE) compared to the monoculture system.

However, there are also some drawbacks to using green-manured legumes. In fact, legume cover crops do not have a good persistence on the soil surface due to their low biomass production and low C/N ratio (Touchton et al. 1984), thus limiting their effectiveness as a soil conservation tactic. Therefore, it is necessary to assess other plant species in combination with legumes to mitigate this weakness.

1.3.3.2 Brassicas

Planting *Brassicaceae* cover crops before cotton sowing can be a valid practice to reduce the summer weed seedbanks and support crop establishment. In a study in Florida (USA) on sandy loam soil, Tiwari et al. (2021) found that after planting *Brassica carinata*, the number of *Amaranthus hybridus* (−27%) and *Senna obtusifolia* (−25%) plants decreased. This suggests that cotton can benefit from less competition with weeds during its early stages of growth when preceded by *B. carinata* (Figure 1.4c).

Zhang et al. (2021), in a study conducted in Henan Province (China) on sandy loam soil, the authors found that intercropping cotton with *Orychophragmus violaceus* increased the soil organic matter (SOM) content by 15.6% and 36.7% at 0–20 and 20–40 cm soil depths, respectively, compared to a cotton–fallow cropping system. Additionally, the use of *Brassicaceae* increased the total N content and available N by 2% and 13.1% compared to the cotton–fallow system, respectively. In fact, although legumes can fix atmospheric N, they have a lower capacity to be incorporated into the soil than brassicas (Zhang et al. 2016; Zhang et al. 2021). This can have a direct impact on cotton production in terms of an increase in the boll number m^{−2} (+5.6%), grain yield (+10.3%), and lint yield (+12.6%) compared to the cotton–fallow system. It is important to underline the ability of *Brassicaceae* to produce glucosinolates, secondary metabolites of N- and S-containing anions whose hydrolysis by myrosinase gives several compounds, including isothiocyanate, nitrile, and thiocyanate (Kliebenstein et al. 2005), which have allelopathic activities on weeds, and their use could strongly reduce weed competition for cotton (Scavo and Mauromicale 2021). Moreover, glucosinolates are also harmful to herbivorous insects, such as the cotton bollworm (*Helicoverpa armigera*), one of the main pests of cotton.

Li et al. (2022) evaluated the impact of glucosinolates on the growth and development of cotton bollworm larvae and observed that the survival rate of *H. armigera* pupae fed with 4 ppm glucosinolate was decreased to 32% compared to the control, whose pupae survival rate was 56%. Furthermore, the development duration of *H. armigera* larvae increased (+25.0%) when they were fed 160 ppm glucosinolate compared to the control. These findings suggest that the use of *Brassicaceae* cover crops may have potential for pest control in cotton crops.

However, the results obtained by Decker et al. (2022) in Headland (AL, USA) regarding the use of Brassica plants in cotton cultivation are controversial. A radish (*Raphanus sativus* L.) cover crop before cotton sowing showed a smaller increase in the soil organic carbon (SOC) content compared to using different combinations of cover crops in the region's humid subtropical climate. The radish monoculture cover crop treatments resulted in SOC values of 6.57 g kg⁻¹ soil in the top 5 cm of soil, while the rye–clover–radish, rye–radish, and clover–radish cover crop combinations showed increases in the SOC content of 13, 8.5, and 7.7%, respectively.

1.3.3.3 Cereals

The use of cereal cover crops in cotton cultivation is well documented. Petra et al. (2022) carried out a 40-year field experiment in a continuous cotton system to evaluate the use of two cover crop treatments (*Vicia villosa*, *Triticum aestivum*) compared to a control treatment as a conservation management approach to improve the SOC content in subsoil layers. The trials were conducted on well-drained clay soils in Jackson (TN, USA) under a temperate climate. Unexpectedly, the use of *T. aestivum* was found to be the most suitable

crop in terms of SOC stock of 33.7 Mg ha⁻¹ at a depth of 0–60 cm compared to *V. villosa* at 29.1 Mg ha⁻¹ and the untreated control at 24.4 Mg ha⁻¹. Specifically, *T. aestivum* resulted in 23% and 34% higher SOC content than *V. villosa* and the control, respectively, at depths of 10–30 cm and 30–60 cm.

Finney et al. (2016) argued that this is due to the low C/N ratio in *Brassicaceae* and *Fabaceae*, making these crops prone to mineralization processes and making them sources of available nutrients in the short term, especially nitrogen. Conversely, *Poaceae*, due to their high lignin content and soil persistence, improve physical characteristics and increase the ion exchange capacity (Novara et al 2021).

Zhou et al. (2021) showed that cotton rotated with soybeans (twice in 4 years) reduced the cotton lint yields (1.12 Mg ha⁻¹) more than the continuous cotton crop system (1.20 Mg ha⁻¹), while cotton rotated (twice in 4 years) with corn (1.22 Mg ha⁻¹) did not show any appreciable differences in lint yields compared to continuous cotton cultivation. This contrasts with the results obtained in Ashworth et al. (2016), where it was demonstrated that including soybeans twice within a 4-year rotation decreased the cotton yield by 16% more than the continuous cotton monoculture over the entire study period and including corn (once in 4 years) also decreased the cotton yields compared to the untreated control (2.7 and 3.1 Mg ha⁻¹, respectively).

The field study conducted by Cetin et al. (2002) in eastern Arkansas (USA) investigated the effects of cereal rye (*Secale cereale* L.) cover crops and no cover crops over several years on the properties of the soil used in cotton production. Soil samples were collected from raised beds (B), wheel track (WT), and no wheel track (NWT) furrows. The results highlighted that the SOM content in the WT furrows under cereal rye cover crops was 30.7 Mg ha⁻¹, higher than

all the other treatments and position combinations. In addition, the water stable aggregate concentrations were 1.6 times higher in the WT furrows under cover crops compared to no cover crops.

Poaceae–cotton rotation also has the advantage of reducing nitrate leaching. The authors of Adeli et al. (2021) conducted a two-year study in Mississippi (USA) and reported that the presence of bread wheat during the rainy season and their residual presence after harvest can reduce nitrate N concentrations in leachate samples by 45% and 21% (9.9 and 17.2 mg L⁻¹) compared to winter fallow (18.2 and 22 mg L⁻¹) in the first and second year, respectively (Figure 1.4d).

1.3.4 Water management

Cotton can be grown under rainfed conditions only in a limited number of cropping environments, and usually, an optimal production level cannot be achieved without irrigation (Cetin and Bilgel 2002). Therefore, appropriate water resources management is essential for cotton production to achieve a high quantitative and qualitative performance for fiber and grain (Pinnamaneni et al. 2021; Bordovsky et al. 2015). The water requirements for cotton differ around the world and depend on the local climate, soil characteristics, genotypes, growing season duration, and irrigation management (Koudahe et al. 2021).

Several studies around the world (Ayars and Hutmacher, 1994; Hunsaker 1999; Farahani et al. 2008; Kumar et al. 2015) showed that the cotton water requirement varies from 700 to 1200 mm during the growing season, which depends mainly on the growing area (Grismer 2002; Evett et al. 2012; Koudahe et al. 2021).

Studies have shown that the values are comparable to each other. Grismer (2002), for example, using the Parlier lysimeters in the

San Joaquin Valley, California (USA), reported crop evapotranspiration (ET_c) values of 710 and 845 mm in 1998 and 1999, respectively. Likewise, Likewise, Howell et al. (1984) in California (USA), using a water balance model, reported that the water use ranged from 594 to 778 mm depending on the irrigation method. In Syria, Farahani et al. (2008) measured cotton evapotranspiration using a water balance model and found an average seasonal ET_c of cotton equal to 878 mm over 3 years. But, as expected, the highest ET_c values were found under arid and semi-arid climatic conditions. For instance, in 1998 and 1999, in the semiarid regions of Lapaz, Yuma, and Mohave (AZ, USA), Grismer (2002) reported cotton mean seasonal ET_c values of 1362, 1035, and 1034 mm, respectively. Consequently, cotton requires a significant water supply during the cropping season to ensure satisfactory productivity and product quality results. For this reason, in recent years, researchers from all over the world have turned their attention towards irrigation management strategies capable of both satisfying crop water requirements, preserving such a precious natural resource, and reducing the economic impact of this practice.

1.3.4.1 Irrigation Systems

A study conducted by Cetin and Bilgel (2002) in southeastern Anatolia (Turkey) showed that cotton yields were significantly higher when using drip irrigation (DI) (4380 kg ha⁻¹) compared to furrow irrigation systems (FI) (3630 kg ha⁻¹) and sprinkler irrigation (SI) (3380 kg ha⁻¹). In addition, the averaged values over 4 years indicated that DI produced 30% more grain than SI and 21% more than FI. These data were also confirmed by the WUE, with values of 4.87, 3.87, and 3.36 kg ha⁻¹ mm⁻¹, respectively, for the DI, FI, and SI methods (Cetin and Bilgel 2002). In Uzbekistan, Ibragimov et al. (2007) observed that, over three seasons, by combining DI with

programmed irrigation set at three levels (germination, squaring, and boll maturation, respectively, at 70–70–60% of the field capacity [FC]), it was possible to increase the water use efficiency (WUE) by 71% on average compared to that of FI. This was also a determinant for grain cotton production, which increased by 14% compared to FI. Furthermore, using DI at 70% of the FC resulted in a 32% irrigation water savings compared to FI when following the same scheduling rule. Mchugh et al. (2008) also demonstrated that subsurface DI in cotton can reduce the off-site movement of pesticides and improve the WUE compared to FI. In southeastern Anatolia (Turkey), Yazar et al. (2002) compared DI and low-energy precision application (LEPA) systems and subjected these to different levels of ET_m restitution to achieve cotton-production goals. The results revealed that the LEPA system, thanks to the precise control of irrigation applications, was able to achieve very high seed cotton yields (4750 kg ha⁻¹, with consumption of 854 mm of water), reaching the average values of DI (5040 kg ha⁻¹ with a seasonal irrigation volume of 868 mm). Conversely, the studies conducted by Goebel and Lascano (2019) in Texas, USA, reported higher water use in LEPA systems compared to subsurface drip irrigation (SSD), thereby questioning their usefulness. In fact, it was observed that from cotton sowing to day 195, the SSD system was able to use

20 to 30 mm ha⁻¹ of water less due to the lower evaporation losses. Such a volume of water produced 25 to 50 kg ha⁻¹ of additional cotton lint yield in semi-arid environments (Wanjura et al. 2002).

In the districts of Maharashtra (India), Shroff et al. (2024) showed significant success in the adoption of DI. In fact, 91.1% of the cotton cultivated area was using DI, while only 8.9% was irrigated with non-micro or conventional sources. The authors pointed out that the adoption of DI resulted in an 80% increase in cotton yield.

Furthermore, after the implementation of this technique, the use of electricity for irrigation per hectare was reduced by 86%, with clear economic benefits.

The use of DI also seems to be useful from a nutritional point of view. Mai et al. (2022) in 2016–2017 in Urumqi (China), under arid climatic conditions, observed how DI also had an impact on nutrient uptake compared to flood irrigation (FLI). Surprisingly, the P concentrations were higher in DI than FLI in most cotton organs, demonstrating that the irrigation method positively affected nutrient uptake. In fact, the P uptake in the shoot of plants was significantly higher under DI ($157.6 \text{ mg plant}^{-1}$) compared to FLI ($140.6 \text{ mg plant}^{-1}$). Similarly, higher percentages of P were found in other plant organs under DI (1.02% in reproductive organs and 0.71% in leaves) compared to FLI (0.85% in reproductive organs and 0.64% in leaves). Furthermore, the shoot biomass (stems and leaves) under DI ($18.7 \text{ g plant}^{-1}$) was significantly higher than that under FLI ($15.6 \text{ g plant}^{-1}$). Despite the evident effectiveness of drip irrigation, in many areas of the world, it remains the least employed system for cotton crops (FAO, 2023). A study indicated that approximately 80% of the Australian cotton growing area is irrigated using gravity surface-irrigation systems, and only 3% of cotton is irrigated with subsurface drip-irrigation systems (Roth et al. 2013). The same is true in the United States, although there are significant differences depending on the geographical area. LEPA sprinkler irrigation dominates much of the southern High Plains and actually represents over 75% of the irrigated area in the region (Barnes et al. 2020). However, in the Midsouth, favored by deep alluvial soils, high annual precipitation, and long, frost-free cropping seasons, irrigation is predominantly accomplished through FI (70 to 80%) and center pivot methods (Barnes et al. 2020; Kebede et al. 2014).

Despite the positive qualitative and productive results, DI presents some gaps in cotton cultivation that must be addressed. As reported by Çetin & Kara (2019), DI also has limitations, particularly during the germination phase, since it does not ensure uniform soil moisture during the early phases of cotton growth. According to the authors, SI systems are more efficient during this critical phase. Another study conducted in Georgia (USA) by Whitaker et al. (2008) revealed that among overhead SI, DI, and rainfed conditions, there were no significant differences in terms of lint yield, indicating that all of these methods did not affect fiber quality. Additionally, unlike SI systems, subsurface DI can cause the accumulation of salts above the depth of the drip line, as it does not result in leaching (Çetin and Kara 2019).

In the arid region of Xinjiang (China), Wang et al. (2020) carried out a study on the use of mulched drip irrigation (MDI), which is achieved by combining drip irrigation and mulching with plastic film. The results highlighted that, compared to traditional FLI, the soil moisture content at a depth of 0–60 cm under MDI increased by 4.8–12.9% from the full flowering stage to the boll open stage, promoting the growth and development of fine roots at the full flowering stage and prophase full boll stage (Lu et al. 2014) and root/shoot ratio (Wang et al. 2020). Zong et al. (2020) in Xinjiang (China), a region with a typical arid continental climate, evaluated the environmental impact of MDI, in addition to its impacts on biomass and lint yield. While MDI significantly improved the biomass (61.5%) and yield of cotton (12.8%) compared to the non-mulched control, there was an increase in CO₂ emissions. Over two cotton-growing years, the total CO₂ emission increased both when MDI was applied in narrow rows (25.3%) and wide rows (28.9%) compared to the control.

The study conducted by Tan et al. 2024 explored the impact of

MDI on the stability of soil aggregates in cotton fields in northwestern China, focusing on how different durations of MDI (0, 9, 13, 15 and 23 years) affected the SOC content and salinity levels. The results indicated that as the MDI duration increased, the aggregate-associated SOC concentration in all fractions increased significantly, while the salt concentration decreased significantly. Specifically, the total SOC content increased by 0%, 11%, 17%, and 87% after 9, 13, 15, and 23 years of MDI, respectively, while the total salt content decreased by 33%, 68%, 67%, and 76% over the same periods.

While MDI offers several benefits, it is also important to consider some potential drawbacks. Recent studies from six cotton fields in Xinjiang, China, where MDI had been practiced for 5 to 19 years, reported an accumulation of residual plastic film (RPF) ranging from 121.8 to 352.4 kg ha⁻¹, with an annual increase of 15.7 kg ha⁻¹, predominantly at a depth of 0–30 cm (Ma et al. 2024).

Regarding CO₂ emissions, Wen et al. (2022) investigated the correlation between the presence of RPF from drip irrigation mulch in the soil and the release of greenhouse gases in Xinjiang, China. Surprisingly, they found a negative correlation, where increasing amounts of RPF were associated with decreasing CO₂ emissions. This phenomenon can be attributed to the fact that RPF can interfere with soil moisture content (Cao et al. 2020), reduce the ability of crops to absorb water and nutrients from the rhizosphere (Jiang et al. 2017), impair soil microbial activity (Jiang et al. 2017), and lead to a decline in crop yield and quality (Can et al. 2020). These factors likely inhibited cotton root development, leading to reduced plant biomass accumulation and diminishing the carbon sequestration potential of cotton fields (Wen et al. 2022).

Table 1.2 illustrates how scientific research has directed its focus toward the choice of irrigation method in relation to the climatic

area. It is possible to observe that in arid environments, researchers have focused on using DI to maximize the efficiency of water resources as much as possible (Mai et al. 2022). In semi-arid areas, attention was given to the use of mulching as a support for DI (Wang et al. 2020; Zong et al. 2020). Due to the better water availability in milder climatic areas, such as the Mediterranean basin, there was a greater interest in SI (Cetin and Bilgel 2002; Çetin and Kara 2019).

Table 1.2 - Geographical distribution of scientific research regarding cotton irrigation systems. DI: drip irrigation; LEPA: low-energy precision application; MDI: mulched drip irrigation; SSD: subsurface drip irrigation. Numbers in bold represent the total number of articles for each climatic condition.

Irrigation System	Effects	Climate				Country	References
		Arid	Arid continent al	Mediterranean	Semi-arid		
DI		2	1	2			
	Less efficient in the germination phase compared to SI			1		S,anliurfa, Turkey	Çetin and Kara (2019)
	+14% seed-cotton production compared to FI		1			Uzbekistan	Ibragimov et al. (2007)
	+19.8% shoot biomass compared to FLI	1				Urumqi, China	Mai et al. (2022)
	+20% cotton yield compared to FI and +29% than SI			1		Anatolia, Turkey	Çetin & Bilgel (2002b)
	+25% WUE compared to FI and +35% compared to SI	1				Urumqi, China	Mai et al. (2022)
	+30% seed-cotton production compared to FI and +21% compared to SI						
	+12.10% shoot P content vs. FLI						
LEPA	-5.75% seed cotton yields compared to DI	1					
	+1.61% D3 water consumption compared to DI						
		1				Southeastern Anatolia, Turkey	Yazar et al. (2002)
MDI					2		
	+61.49% biomass production compared to DI				1	Xinjiang, China	Zong et al. (2020)
	+12.84% cotton yield vs. DI +4.80%–12.87% soil moisture content				1	Xinjiang, China	Wang et al. (2020)

(from full flowering to the bolls open stage) vs. DI		
SSD	1 Irrigation water saving compared to LEPA	1 Texas, Goebel & United StatesLascano (2019)

1.3.4.2 Irrigation Termination Strategies

Under organic cotton farming systems, the main challenge is harvesting management. A lack of green leaf material is essential at this stage, as it reduces the moisture content, leaf trash, and other impurities and improves the fiber grade (Delate, Heller, and Shade 2021). However, there are no sustainable alternatives for defoliating cotton plants without compromising production. Besides thermal defoliation, the only option is to interrupt irrigation to promote cotton defoliation (Grimes and Dickens 1974; Fletcher et al. 2014; Pelletier et al. 2017). Several researchers have raised questions about the correct time to perform this critical practice and how it may impact cotton production and quality. Based on the data obtained by Vories et al. (2011), regarding the irrigation termination (IT) on FI systems, (Reba et al. 2014) observed that, in the humid subtropical climate of Mississippi (USA), interrupting irrigation from 5 to 17 days before reaching 350 Growing Degree Days (GDD) following crop cut-out (with a base temperature of 60 °F or 15.6 °C for cotton) allowed for an earlier harvest without any yield decrease in both furrow irrigation and pivot systems.

Karam et al. (2006) conducted a 2-year experiment in the Bekaa Valley of Lebanon under a Mediterranean climate and found that by terminating irrigation permanently at the first open boll, a higher cotton lint yield (639 kg ha⁻¹) was obtained compared to early boll

loading (577 kg ha⁻¹) and mid boll loading (547 kg ha⁻¹). Notably, cotton lint yields were reduced as the water amount increased. In fact, the amount of water supplied to the plots where IT occurred at the first open boll opening was 549 mm, compared to 633 and 692 mm for early boll and mid-boll loading, respectively. Additionally, the WUE was found to be higher at the first boll opening treatment (1.3 kg ha⁻¹ mm⁻¹), followed by early boll opening (1.1 kg ha⁻¹ mm⁻¹) and mid boll opening (1.0 kg ha⁻¹ mm⁻¹).

In a study carried out in the semi-arid climate of southwest Oklahoma (USA) by Masasi et al. (2019), the effects of IT on yield and fiber quality were assessed. The results showed that earlier IT treatments achieved average reductions of 16–28% in irrigation requirements during the three growing seasons, but this resulted in a lower production (347 kg ha⁻¹ on average) compared to the latest IT treatment. Moreover, early IT yielded significantly smaller grain than under the latest IT treatment. In this regard, Buttar et al. (2007) conducted a 2-year experiment in an arid region of Bathinda (India) and observed that a delay of the last irrigation from 130 to 170 Days After Sowing (DAS) resulted in an increase in cotton grain yield of 14%, 32%, and 8% in the first, second and third year, respectively. Cotton IT is also dependent on the soil type and genotypes. Grimes & Dickens (1974), in a five-year study conducted in the semi-arid region of the San Joaquin Valley of California (USA), found that cotton grown on clay loam soils had a high water-retention capacity. This is because a higher water availability leads to excessive vegetative growth, which make it difficult to defoliate and delays capsule maturity.

Silvertooth et al. (1989) also highlighted that the choice of the genotype heavily influenced IT management. Their field experiments conducted in Arizona (USA) showed that some genotypes responded

more to IT than others in terms of cotton yield. In fact, for the Upland cotton genotype DPL 20, an early IT (18 August) or late IT (9 September) did not cause significant differences in terms of harvest, but this was not the case for Prima cotton genotype S-6, where an early IT (12 August) resulted in a 27.6% decrease in production compared to a delayed IT (1 September).

We observed that early IT within mild climatic areas has been more successful when compared to the arid areas of Bathinda (India) and semi-arid areas of southwest Oklahoma (USA) described by authors Buttar et al. (2007) and Masasi et al. (2019), respectively. Although it has not yet been suitably noted, this represents a key aspect when it comes to growing cotton under an organic regime. The studies conducted by Reba et al. (2014) in the humid subtropical climate of Mississippi (USA) and by Karam et al. (2006) in the Mediterranean climate of Lebanon highlighted how such climatic areas are more suitable for cotton harvesting in organic systems without the use of chemical treatments, while also reducing irrigation inputs without affecting the cotton grain yield. Figure 1.5 shows the key points of the main results on this topic.

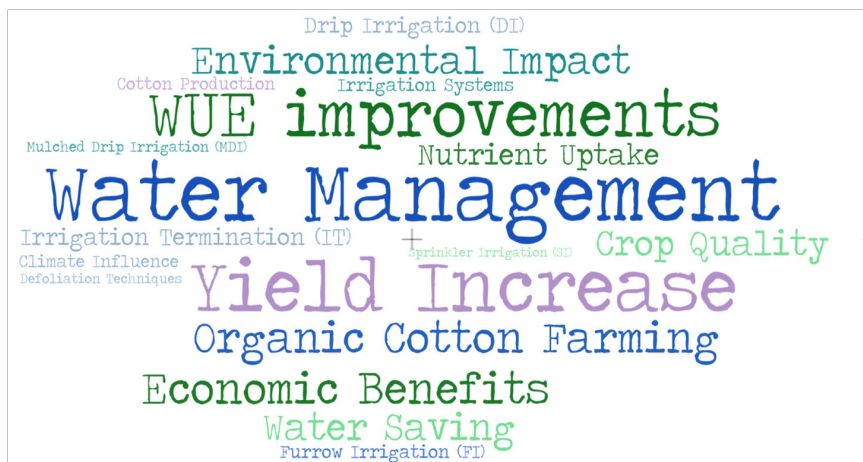


Figure 1.5 - Key findings on irrigation management.

1.4 Final Remarks

This paper analyzed the complexities involved in implementing sustainable cultivation of cotton in different farming systems around the world. This allowed us to highlight different agronomic strategies and tactics adopted in various cropping regions, all leading towards the same goal of sustainability. Firstly, the plant–microorganism relationships were evaluated, highlighting the potential role of mycorrhizal symbiosis in sustainable cotton production. It was observed that certain combinations of AMF species of the genus *Glomus* can enhance the absorption of different key mineral nutrients for the plant, including not only P and Zn, but also N, K, Ca, Mn, Fe, and Cu. Moreover, the species *F. mosseae* and *G. margarita* were found to affect the abundance of the soil microbiome, while *R. clarus* was shown to increase biomass. Therefore, the

identification of the right species and their combinations may be crucial to reducing agronomic inputs and promoting more sustainable cotton cultivation. Secondly, the use of different plant species in farming systems with cotton was analyzed (polyculture vs. monoculture). It was observed that legume cover crops can fix nitrogen and increase labile nitrogen levels in the soil, potentially reducing the need for nitrogen fertilization. However, legumes may have low persistence due to a low C/N ratio, unlike cereals and brassicas. On one hand, cereals such as *T. aestivum* can improve the soil structure and reduce erosion, but they may not have as strong an effect on nitrogen nutrition. Brassica cover crops (*B. carinata* and *O. violaceus*) can reduce weeds and increase soil organic matter, and the total and available nitrogen content. Additionally, brassicas can release allelopathic compounds that affect cotton bollworms. Overall, using different cover crops may offer a balance between the benefits and drawbacks of each species. Third, the irrigation systems most relevant to cotton farming were examined in order to optimize water resources. It was evident that DI systems implemented in different climatic areas were more effective than the more common FI systems in terms of WUE, shoot biomass, and cotton grain yield. Even better results were reported in DI systems supported by plastic mulching films, with significant improvements in terms of soil moisture content, biomass production, and cotton yield compared to DI. Lastly, the review examined the timing of irrigation in organic cotton cultivation systems. It was highlighted that early IT in mild climatic areas, particularly in humid subtropical and Mediterranean climates, can be effectively employed to decrease irrigation inputs without affecting grain yield.

In conclusion, sustainable cotton cultivation represents a crucial option for more responsible management of natural resources. This

paper summarizes the main recent innovative results obtained by applying various sustainable farming strategies and tactics in different climates without sacrificing productivity. It is hoped that this paper can also guide farmers as, despite scientific evidence, these simple agronomic practices are not widely adopted worldwide. Mycorrhization is still not extensively practiced, the use of herbicides and pesticides often follows a set schedule, irrigation systems are outdated and inefficient, and irrigation termination is not yet seen as a valid alternative to the use of chemical defoliant in areas where climate conditions permit it. It is important to note that, despite the scientific evidence presented, the topics covered in this study require further scientific investigation and longer-term trials to reach a deeper comprehension and to fully grasp the interaction of various factors affecting sustainable cotton cultivation.

Supplementary Materials:

The following supporting information can be downloaded at:
<https://www.mdpi.com/article/10.3390/agriculture14091597/s1>.

Author Contributions:

Conceptualization, G.S.V., U.A., S.L. and P.G.; Methodology, G.S.V., S.L. and P.G.; Validation, G.S.V., S.L. and P.G.; Investigation, G.S.V. and P.G.; Resources, P.G.; Writing—original draft preparation, G.S.V.; Writing—review and editing, A.S., U.A., S.Z., T.T., C.S., G.P. and P.G.; Visualization, G.S.V., A.S. and S.L.; Supervision, T.T., C.S., S.L. and P.G.; Project administration, P.G. All authors have read and agreed to the published version of the manuscript.

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Conflicts of Interest:

The authors declare no conflicts of interest.

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Environmental sustainability of cotton: a systematic literature review of life cycle assessments

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2. Environmental Sustainability of Cotton: a Systematic Literature Review of Life Cycle Assessments

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Abstract

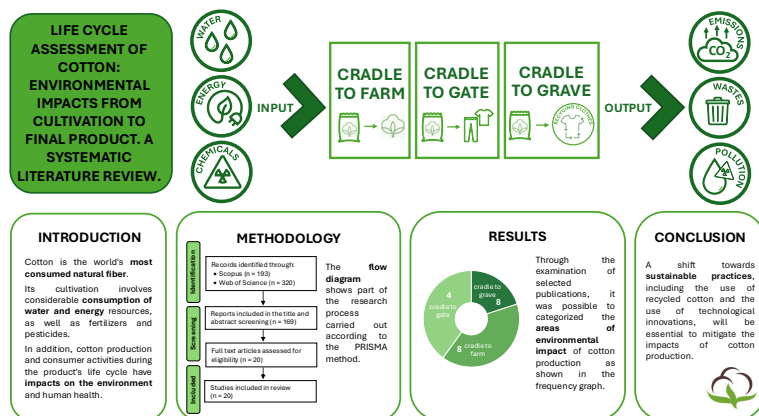
Cotton is the world's most used natural fiber, representing over 80% of global natural fiber use. However, its cultivation involves considerable consumption of water, energy, fertilizers, and pesticides, which impact both the environment and human health. Additional impacts arise from consumer activities such as washing and ironing. This review analyzes twenty peer-reviewed studies on cotton life cycle assessments, selected from Scopus and Web of Science according to PRISMA guidelines and covering the period 2010–2022. Among the inclusion criteria, studies addressing the cultivation phase were considered, while the exclusion criteria involved conference papers, book chapters and partial life cycle assessments. These studies indicate that cotton production—from cultivation to the final product—has the greatest impact on water use, energy consumption, greenhouse gas emissions and eutrophication. Organic cotton farming demonstrates lower environmental impacts per unit area compared to conventional farming yet exhibits higher impacts when evaluated on a mass basis. This discrepancy arises from the typically lower yields of organic systems relative to their conventional counterparts, emphasizing how the choice of functional unit can significantly

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influence the results and the conclusions drawn. Industrial processes like dyeing and spinning generate substantial carbon emissions, while consumer use—particularly washing and drying—accounts for over 65% of total energy consumption. The review identifies key sustainability issues in cotton production—high water use, intensive agrochemical input, and biodiversity loss—and suggests strategies to mitigate the impacts across its life cycle phases such as efficient irrigation, optimized fertilization, and adoption of organic or Bt cotton.

Keywords: cotton; life cycle assessment; water consumption; carbon footprint.

Graphical abstract



2.1 Introduction

Cotton is the most widely used natural fibre in the world,

accounting for over 82% of global natural fiber use (Şener Fidan, Kızılkaya Aydoğan, and Uzal 2023). The latest estimates put world cotton production at 24.2×10^6 t, with a cultivated area of 31.92×10^6 ha⁻¹ across 80 countries and an estimated annual turnover of \$5.68 billion (FAOSTAT 2023). China, with a production of 6 million tonnes, is currently the world's largest producer, followed by India and the United States (FAOSTAT 2023). However, while cotton is economically vital, its production and use across the entire supply chain—from cultivation to processing, consumer use, and disposal—cause substantial environmental degradation. The environmental impacts associated with cotton textiles are very complex and heterogeneous from country to country, also translating into human health problems.

For instance, cotton cultivation requires a significant amount of water, ranging from 700 to 1,200 mm during the growing season, depending mainly on the growing area (Evelt et al. 2012; Grismer 2002; Koudahe et al. 2021b). This high demand is exacerbated by the widespread use of low-efficiency irrigation methods, such as furrow and sprinkler irrigation systems, instead of more efficient alternatives like drip irrigation and mulched irrigation systems (Barnes et al. 2020; Goebel and Lascano 2019; Mai et al. 2022). Intensive irrigation for cotton in arid regions exacerbates local water scarcity and contributes to biodiversity loss, with cases like the Aral Sea disaster in central Asia illustrating the severe ecological consequences of unsustainable water management (Chapagain et al. 2006). Likewise, the extensive use of pesticides is a major environmental and health concern. Some authors estimate that around 50% of all pesticides in developing countries are used for cotton cultivation, while globally cotton accounts for 11% of total pesticide consumption (La Rosa and Grammatikos 2019). This appears even more alarming when

considering that cotton is cultivated on only 2.4% of the world's arable land (Bevilacqua et al. 2014). According to the latest data provided by the United States Department of Agriculture (USDA 2024), herbicides were used in 96% of the cotton-growing areas in the USA, in particular glyphosate isopropylamine salt, glyphosate potassium salt, paraquat, dicamba diglycolamine salt and diuron. Cases of illnesses among cotton farmers because of pesticide toxicity were reported, especially in developing countries, where there is usually considerable lack of occupational hygiene regulations (Khan and Damalas 2015). Not only that, but due to resistance phenomena, pesticides are often overused in cotton fields, with a significant portion of the applied chemicals failing to reach their intended targets. Instead, they are dispersed into surrounding ecosystems, contaminating water bodies and soil, and resulting in high eco-toxicity impacts and biodiversity loss (Claydon 2022).

Furthermore, cotton manufacturing is characterized by substantial energy requirements during spinning and textile manufacturing steps (such as weaving, cutting, and sewing), and by the consumption of water and the use of various chemicals in dyeing, including bleach, dyes, soaps, softeners, and salts (Roos et al. 2015; Yuan et al. 2013). The high amount of electricity considerably increases CO₂ emissions and the potential for acidification, whilst chemicals are of high potential concern for the environment, and human health (factory workers and consumers). Moreover, beyond exacerbating water depletion, improperly treated wastewater can enter local groundwater, and degrade the ecosystem (Esteve-Turrillas and de la Guardia 2017; Liu, Huang, Zhu, et al. 2020a).

Also, domestic usage plays a critical role in the overall environmental impact of cotton garments. For instance, the washing and drying of garments are particularly energy-intensive, often

resulting in a greater environmental impact than any other stage of the production process (Walser et al. 2011). Research indicates that energy expenditure during the consumer use phase accounts for more than 65% of the total energy (TE) consumption (Fidan, Aydoğan, and Uzal 2023). Moreover, the turnaround from production to waste is very rapid, as the lifespan of garments averages only about 3 - 3.5 years, after which they are often incinerated or sent to landfills, thereby generating additional greenhouse gas emissions and contributing to the growing burden of solid waste. While recycling initiatives are advancing, the share of recycled cotton remains limited, and the recycling process itself involves further water and energy use (Liu, Huang, Zhu, et al. 2020a).

Given these complex and far-reaching impacts, it is essential to assess cotton's sustainability from a full life cycle perspective to identify key hotspots and opportunities for improvement throughout the supply chain. Several studies have assessed the environmental impacts of cotton textile products, and the Life Cycle Assessment (LCA) methodology has been largely adopted to this purpose (Fidan et al. 2023). Indeed, LCA is unique in its ability to analyze the entire life cycle of a product or service and assess the associated environmental burdens (Mohamad et al. 2014). Initially developed for industrial production systems, LCA was later adapted to agricultural and food systems (Notarnicola et al. 2017), becoming now a key tool for the sustainable management of the agri-food sector (Pergola et al. 2023; Van Der Werf et al. 2014). The aim of an LCA study can be to compare the environmental performances of alternative products, processes, or services, and identify environmental hotspots and improvement and/or mitigation strategies (Roy et al. 2009; Zingale et al. 2022). According to the ISO 14040-44 regulation (ISO 14044 2006), an LCA study follows four interconnected phases: Goal and

Scope Definition, Life Cycle Inventory, Life Cycle Impact Assessment, and Interpretation. By identifying environmental impacts at each stage, LCA promotes the development of sustainable practices and helps enhance the competitiveness of environmentally sustainable products in the markets.

Previous reviews on cotton LCA (Chen et al. 2023; Munasinghe, Druckman, and Dissanayake 2021; Z. Zhang et al. 2023) have mainly focused on summarizing general results or categorizing studies by system boundaries, such as “cradle to farm”, “cradle to gate” and “cradle to grave”. However, a more in-depth comparative analysis of methodological choices (e.g., functional units, impact assessment methods) and contextual variables (e.g., geographic location, production methods) influence study outcomes is lacking. This review, however, not only maintains the classification by system boundaries, but also incorporates an additional level of analysis, examining how geographical and methodological variations influence reported environmental impacts. This dual-level categorization allows for a deeper understanding of LCA results and provides targeted insights into specific sustainability challenges in cotton supply chains. This approach highlights similarities and differences between studies and identifies specific areas for improvement in sustainability practices for cotton supply chains.

2.2 Material and methods

To gain new scientific insights into the environmental impact of cotton products, a systematic literature review was conducted in accordance with the PRISMA guidelines (Page et al. 2021). The search string used included the terms "Cotton" AND "Life Cycle Assessment" OR "LCA": these keywords were used as they were

deemed the most representative of the research objectives. Scopus® and Web of Science® were selected for their complementarity, which helps minimize the risk of omitting relevant literature. In addition, both databases index only peer-reviewed scientific journals, thus ensuring the reliability and quality of the sources (Page et al. 2021) included in this review. Indeed, the use of these databases increased the likelihood of capturing all relevant contributions to the central research topic of this review, thereby ensuring a high level of rigor in the search and selection process.

2.2.1 Research protocol

To ensure rigor in the literature review, the PRISMA method (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) was adopted (Page et al. 2021). Figure 2.1 presents the flowchart of the search process, which follows four steps: identification, screening, eligibility, and data extraction. The diagram details the number of studies included or excluded at each step, with ultimately 20 studies included into the qualitative review.

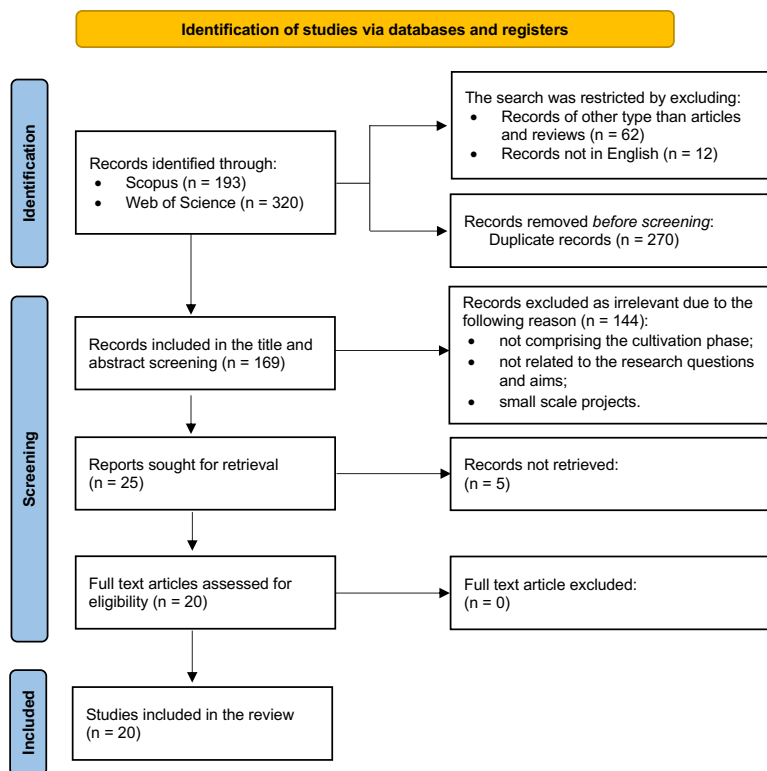


Figure 2.1 - PRISMA flowchart of the results of the literature search. Own elaboration following the PRISMA model.

2.2.2 Eligibility criteria

The selection process was carried out to include articles written in English and published in peer-reviewed journals. To ensure the inclusion of the most recent and relevant research, studies published prior to 2010 were excluded, narrowing the time range to publications between 2010 and 2022. The articles were required to present new and

relevant data, analyses, or insights on the application of LCA within cotton cultivation. In particular, they had to contribute to advancing knowledge on sustainability improvements in the cotton production process, offering either novel methodologies, case studies, or significant findings relevant to environmental impacts. Publications that were not related to the topic, despite their positive wording with the search query, were discarded to maintain a clear focus.

Specifically, the following inclusion and exclusion criteria were adopted:

- only peer-reviewed articles written in English were considered;
- conference reviews, and book chapters were not reviewed;
- gate-to-gate and gate-to-grave assessments were excluded, which means that LCAs of cotton products were considered only if they included the cultivation phase;
- preference was given to the most recent and informative studies addressing the same experimental topic, ensuring that the selected literature reflects the latest advancements and findings in the field;
- studies were excluded if they were based on simulation or hypothetical experiments and if they used non-robust methods, such as narrative analysis or overviews with no detailed discussion of a comparison;
- interventions on small-scale projects were also excluded, as they may not be representative of current production systems and may not reflect a long-term course of action.

Among the studies published until December 2022 (the date of the last research), in total, the search returned 513 results. Of these, 74 publications were removed before screening because 12 were written in other languages than English and 62 were publications of a different type than the articles. Additionally, 144 publications were excluded due to irrelevant content. The full texts of the remaining 25

publications were then reviewed, and 20 eligible publications were included in the qualitative summary. Subsequently, no publications were excluded, so that because of the screening process, a total of 20 publications were qualitatively reviewed. While our review included only 20 studies, these were selected for their quality, methodological rigor, and global relevance, and together they provide a representative overview of current LCA research on cotton. We note, however, that as the field continues to evolve, future studies will be valuable to further expand and update our findings and conclusions.

2.2.3 Synthesis of results

After the screening, the selected publications were reviewed to provide a comprehensive and critical overview of the main environmental impacts of cotton, from cultivation to fiber production, consumption and disposal. The articles were classified based on their system boundaries, as follows:

- LCAs of cotton cropping systems (from cradle to farm): this category includes all agricultural practices involved in the cultivation of cotton from planting to harvesting, with a focus on the consumption of natural resources and agricultural inputs and the main related environmental impacts;
- LCAs of cotton fiber (from cradle to gate): this group covers the industrial processes involved in the processing of cotton into fiber, highlighting the inputs at the industrial plant and the associated waste and environmental impacts;
- LCAs of cotton textile products (from cradle to grave): these studies encompass the full life cycle of cotton products, from raw material extraction to manufacturing, consumer use, and final disposal or recycling.

Besides this, they were also analyzed based on the year of publication, journals' subject area, country of origin, functional unit, life cycle impact assessment method, and main environmental impact results.

2.2.4 Bibliographic analysis

The analysis concerns the detection of the year of publication, country of origin and subject area of the publications. The selected publications were released starting in 2010 and continuing through 2022 (until December 28th). Figure 2.2 shows the trend of publications over time, showing a high level of interest in the topic starting in 2020 and an increasing trend to date.

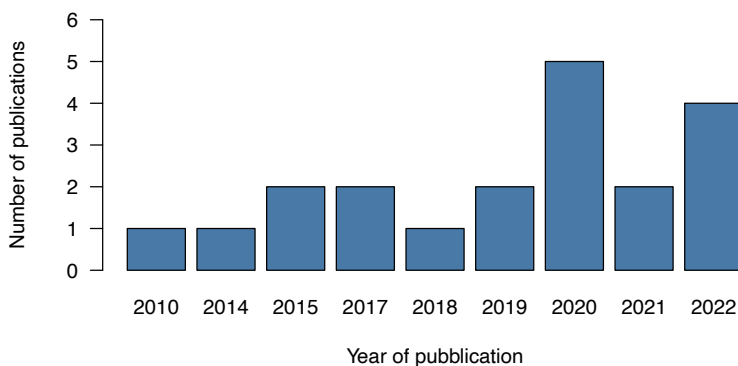


Figure 2.2 - Distribution of publications per year. Own elaboration through the R software.

The selected publications cover a wide range of subject areas. Specifically, the articles were published in a total of twelve journals,

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ranging from sustainability, agricultural science, economics and others, which are shown for completeness in Figure 2.3 The International Journal of Life Cycle Assessment was found to be the most prominent contributor, followed by the Journal of Cleaner Production.

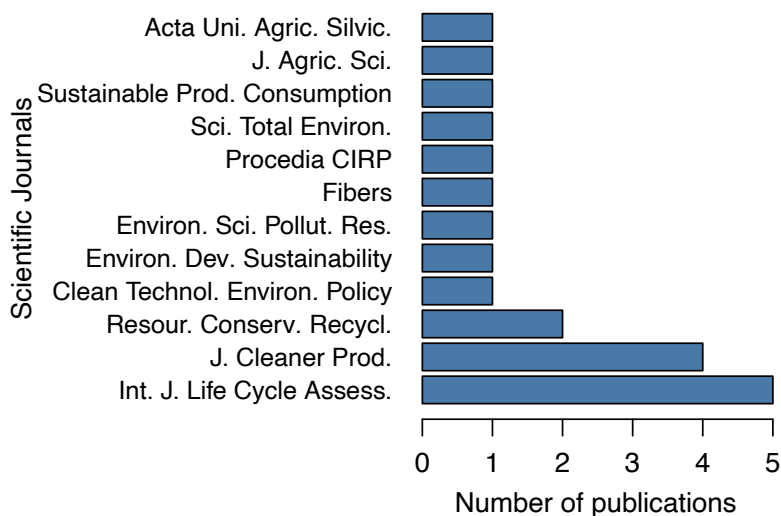


Figure 2.3 - Distribution of publications per journal. Own elaboration through the R software.

The review encompasses publications that examined cotton production across all five continents. Analyzing the papers by continent, three focus on Australia, five on America (Argentina, Brazil, Mexico, United States), twelve on Asia (China, India, Iran, Pakistan, Syria, Turkey, Turkmenistan, Uzbekistan), four on Europe (Greece, Spain), and five on Africa (Egypt, Mali). For clarity, the

distribution of selected documents by geographic area is shown in Figure 2.4.

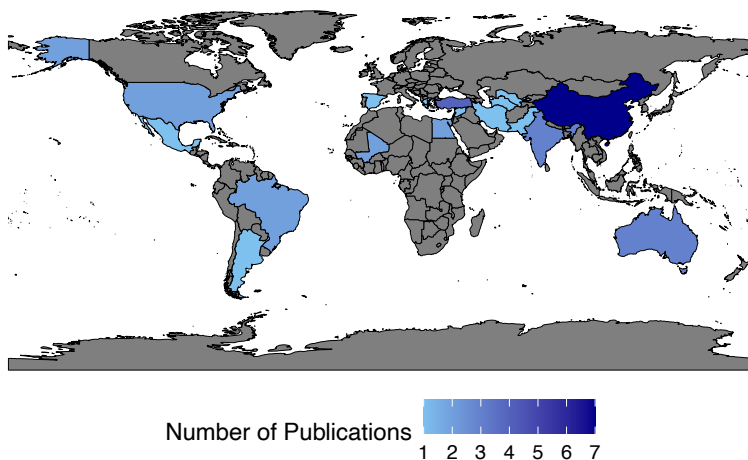


Figure 2.4 - Distribution of publications per country. Own elaboration through the R software.

To provide a clear view of the distribution of the analyzed publications in relation to the stages of the production process, figure 2.5 shows the breakdown of publications based on the system boundaries.

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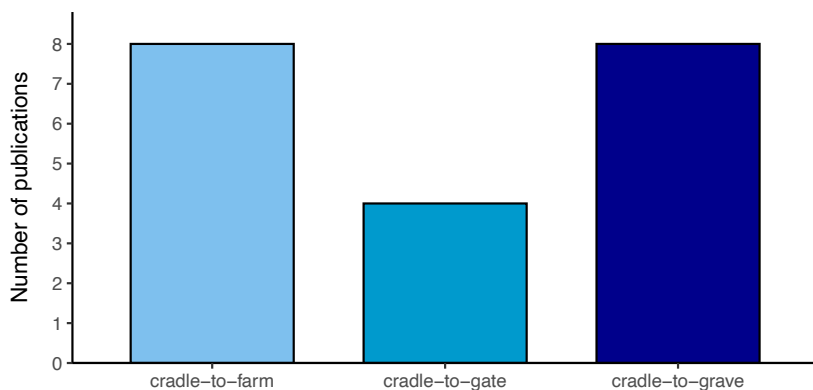


Figure 2.5 - Distribution of publications by phase of the production process.
Own elaboration through the R software.

The selected publications were also examined in terms of LCAs key methodological aspects, as shown in Table 2.1.

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Table 2.1 - LCA key methodological aspects of the reviewed studies.

Source	Country	System Boundaries	Functional Unit	Life Cycle Impact Assessment (LCIA) Method
Avadí et al. (2020)	Mali	cradle-to-farm	1 t of organic seed cotton; 1 t of conventional seed cotton; 1 ha of conventional cotton fiber; 1 ha of conventional cotton fiber	ILCD 2011 midpoint+ v1.0.9, and WF
Ridoutt et al. (2019)	World wide	cradle-to-farm	1 t seed-cotton	New method, linking land occupation to health impacts
Huang et al. (2022)	China	cradle-to-farm	1 kg of cotton	CF (CLCD 0.7, and IPCC 2019 factors)
Maraseni et al. (2010)	Australia	cradle-to-farm	1 ha of cotton planted	GWP100
Singh et al. (2022)	India	cradle-to-farm	1 ha of cotton planted	CF, GWP100, and Data Envelopment Analysis
Sami and Reyhani (2018)	Iran	cradle-to-farm	1 kg of cotton	GWP100 (IPCC, 2006)
Zhang et al. (2021)	China	cradle-to-farm	1 t of cotton	WF (WAVE+ model), ReCiPe 2016 midpoint & endpoint
Verones et al. (2017)	World wide	cradle-to-farm	1 t of cotton	New method to assess biodiversity loss
Liu et al. (2020b)	China	cradle-to-gate	1 t of the finished melange yarns	WF, GWP100 (IPCC 2001)
Liu et al. (2020a)	China	cradle-to-gate	1 t of recycled yarns and 1 t virgin cotton yarns	ReCiPe 2013 midpoint (H)

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Bevilacqua et al. (2014)	Egypt, China, India, and USA	cradle-to-gate	1 kg of dyed cotton yarn	GWP and Ecoindicator99 (H/A)
La Rosa and Grammatikos (2019)	World wide	cradle-to-gate	1 kg of cotton fiber and textile	ILCD 2011 Midpoint +, CED v 1.09, CML-IA v3.02
Luo, Wu, and Ding (2022)	China	cradle-to-grave	1 pair of cotton jeans	WF (ISO 14046), CF (PAS 2395)
Morita et al. (2020)	Brazil	cradle-to-grave	1 pair of women's jeans	GWP100 (IPCC 2013, v 1.03), CED v 1.10
Zhang et al. (2015)	China	cradle-to-grave	100 % cotton knitted dyed short-sleeved t-shirt	CLM 2001, USEtox
Şener Fidan et al. (2023)	Turkey	cradle-to-grave	1 pair of average-sized jeans manufactured	CML-IA
Baydar, Ciliz, and Mammado v (2015)	Turkey	cradle-to-grave	1000 items of knitted and dyed cotton t-shirt (200 kg)	EDIP 2003
Kazan, Akgul, and Kerc (2020)	Turkey	cradle-to-grave	100 pieces of shirt (250 kg shirt)	CML 2001
Moazzem et al. (2021)	Australia	cradle-to-grave	1 kg cotton knit dyed t-shirt	CML-IA v.4.4, ReCiPe 2008 midpoint v 1.11
Esteve-Turrillas and de la Guardia (2017)	Spain	cradle-to-grave	1 kg of colored cotton yarn, and 1 T-shirt (0.3 kg) made with 100% cotton	Multiple Impact Categories from previous published LCAs

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Country of study, system boundaries (cradle-to-farm, cradle-to-gate, cradle-to-grave), functional units (area-based or mass-based), and LCIA methods (e.g., ILCD, ReCiPe, GWP, WF, CF) used in each study.

This table provides a useful overview of key methodological aspects of various LCAs on cotton production. The diversity of system boundaries, functional units, and impact assessment methods employed across these studies highlights the challenges in comparing and synthesizing LCA results for cotton. For instance, studies consider different life cycle stages, ranging from cradle-to-farm (8), cradle-to-gate (4), to cradle-to-grave (8). The functional units also vary, including 1 ton of seed cotton, 1 hectare of cotton planted, 1 kg of cotton fiber or yarn, and 1 pair of jeans or t-shirt, and similar. Most of the authors adopted mass or product-based functional units, while only a few (Avadí et al. 2020; Maraseni et al. 2010; Singh et al. 2022) used area-based functional units. It is possible to note that no one has used a monetary-based or quality-corrected functional unit. This is common in product LCAs, and understandable considering that the cotton industry works with physical quantities as the primary basis for its operations and transactions. Using multiple and novel functional units could help improve the usefulness of LCA results for the many and diverse cotton stakeholders. For instance, the quality of cotton fiber is influenced by a multitude of factors, including climatic and soil characteristics, the selection of varieties, the timing and intensity of irrigation, plant nutrition, crop protection measures, harvesting and ginning techniques, storage methods, and numerous others.

Some articles focused on the impacts generally considered to be most relevant to the textile industry, such as global warming potential, water use and/or scarcity and energy-related impacts, while others preferred more comprehensive impact assessment methods such as

ReCiPe, ILCD, CML, EDIP and Eco-indicator 99. These latter multi-impact methods are among the most widely used globally and vary in scope, coverage and level of detail. Despite this diversity in LCIA methods, we noticed that in most cases the authors arrive at comparable conclusions. Nevertheless, the choice of LCIA method should be made carefully and studies are needed that simultaneously apply several methods to compare the results in absolute terms and to reach a consensus on the most appropriate one for the specific case of cotton. Notably, also novel methods were proposed and tested, dealing with specific issues like biodiversity loss and human health impacts (Ridoutt et al. 2019; Verones et al. 2017). A gap in the assessment phase is the underrepresentation of impact categories related to soil quality, which could be, instead, highly relevant for cotton-based cropping systems.

To offer readers a more immediate visual representation of the primary input-output flows, and main environmental impacts in cotton systems, Figure 2.6 was developed. The diagram is structured to highlight the principal interrelations between processes and environmental outcomes for system boundaries adopted.

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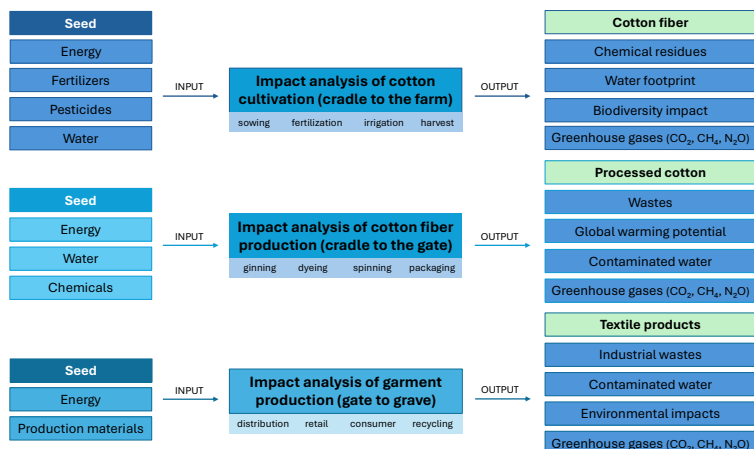


Figure 2.6 - Papers classification flowchart. Own elaboration.

2.3 Results and Discussion

2.3.1 LCAs of cotton cropping systems (cradle-to-farm gate)

To evaluate the environmental impact of cotton cultivation, articles presenting the "cradle to farm gate" system boundaries were analyzed. This involved identifying LCA studies that covered the entire agricultural phase, including resource extraction, machinery production, production and transport of agricultural inputs, sowing, base fertilization, weeding, phytosanitary treatments, fertilization until harvest and ginning. Different environmental impacts result from different cotton growing practices in different geographical contexts. For instance, depending on the climatic conditions and irrigation techniques, significant differences in water consumption could be observed (Ayars and Hutmacher 1994; Evett et al. 2012; Farahani,

Oweis, and Izzi 2008; Kumar et al. 2015). Furthermore, the application of fertilizers and pesticides exhibits substantial variation in terms of type and intensity between regions and production methods, which has the potential to result in significantly different yields and varying degrees of impact severity. Seven teams of authors have addressed these issues by focusing on cotton cropping systems (Avadí et al. 2020; Huang et al. 2022; Maraseni et al. 2010; Page et al. 2021; Ridoutt et al. 2019; Sami and Reyhani 2018; Singh et al. 2022; Zhang et al. 2021). Such studies consistently reveal that water use, irrigation energy, and agrochemical inputs are the dominant environmental hotspots during the cotton cultivation stage.

For instance, Avadí et al. (2020) conducted an LCA and WF analysis comparing conventional and organic farming systems in Mali. The authors highlight that cotton cultivation in the country is predominantly conventional, and that the pesticide use—particularly of profenofos insecticides — is the hotspot for this type of cotton production. This is mainly due to the associated toxicity impacts to humans and aquatic ecosystems. On the other hand, although organic cotton is becoming more widespread, it still represents a marginal share (0.5%) of national production. Using the production of 1 ton of cotton lint as a functional unit (FU), the study found that organic farming practices resulted in higher environmental impacts compared to conventional ones for all the analyzed impact categories, including climate change (+98% kg CO₂ eq), terrestrial eutrophication (+19% molc H⁺ eq), mineral and fossil resource depletion (+124% kg Sb eq) and acidification (+13% molc H⁺ eq). The greatest impact was observed in the WF, with an average increase of 172% in water consumption under organic cultivation compared to conventional cultivation. This is due to the comparatively lower yields of organic cotton. Conversely, the impacts of organic cotton were consistently

lower per 1 ha of cultivation, due to lower input intensity. In fact, lower emissions of greenhouse gases (-29% kg CO₂ eq), terrestrial eutrophication (-57% molc H⁺ eq), mineral and fossil resource depletion (-20% kg Sb eq) and acidification (-60% molc H⁺ eq) were observed. Considering the WF, there was an average reduction of 2% (m³ H₂O eq). Interestingly, at the endpoint level (single score), the impacts of organic cotton resulted lower than those of conventional, both per t of product and per ha of cultivation. This was explained by the authors Avadí et al. (2020) by considering the large contribution of toxicity impact categories - which feature larger impacts for conventional cotton, due to its massive use of chemical pesticides - to the single score. Such divergent results are quite common when comparing organic and conventional systems and reflect the strong influence of the choice of functional unit, as well as the good rationale for combining midpoint and endpoint assessments for the sake of a more accurate and holistic understanding of the leading environmental issues (Zingale et al. 2024). Based on these findings, some mitigation strategies were proposed by Avadí et al. (2020), including:

- adopting organic farming practices;
- reducing pesticide use; and
- adopting Bt cotton varieties to increase yield and reduce insecticide use.

Moreover, the authors acknowledged that no methods were available to assess the impacts of rainwater consumption by cotton agriculture, but it is clear that Malian rainfed cotton is in principle more sustainable, regarding water use, than irrigated cotton. Future research needs to reach a consensus on modelling rainwater management practices and characterization factors for the use of this green water.

In a manner consistent with Avadí et al. (2020), Zhang et al.

(2021) also combined a WF analysis and a full LCA to evaluate the environmental impacts of cotton production, in this case in China. The objective of this study was to identify the most critical geographical areas where cotton production has the greatest environmental impact, as well as the key processes within the cotton life cycle that generate the highest environmental impact. The researchers highlight that at the midpoint level, there is a water scarcity footprint of $1.44 \times 10^3 \text{ m}^3$ deprived, while consuming $2.49 \times 10^3 \text{ m}^3$ of water per 1 ton of cotton lint produced. The endpoint analysis revealed that in China, the impact on human health and ecosystems is equivalent to 1.69×10^{-3} disability-adjusted life years (DALY) and 152.83 species · yr for 1 ton of cotton lint. The impact of water alone accounts for more than 55% of the human health category, resulting in 9.36×10^{-4} DALY. This can be attributed to the excessive consumption of water resources in areas with severe water scarcity. The concentration of cotton production in the arid region of Xinjiang exacerbates the impact of water consumption on the WF. The consumption of fertilizers and pesticides has been calculated, respectively amounting to 640.2 and 1.87 kg per 1 ton of cotton lint. The production of fertilizers alone is responsible for environmental impacts, particularly in terms of cancerogenicity, freshwater ecotoxicity, and acidification categories. Overuse of fertilizers is primarily linked to contamination of soil and groundwater (Vermeulen, Campbell, and Ingram 2012). Therefore, impact categories related to freshwater ecotoxicity, aquatic eutrophication, and acidification are identified, amounting to 2.25×10^4 comparative toxic units (CTU), 2.16 kg PO_4^{3-} , and 21.6 kg SO_2 per 1 ton of cotton lint, respectively. All of this in China causes emissions of $4.59 \times 10^3 \text{ kg CO}_2$ per 1 ton of cotton, as well as emissions of ammonia into the air and water (0.0015 kg and 0.14 kg) (Verones et al. 2017). The authors suggest several mitigation strategies, such as

returning cotton crop residues to the soil to enhance soil fertility and water retention capacity. They also identified the provinces of Qinghai and Inner Mongolia as those having the most favorable meteorological conditions for cotton production, thereby potentially leading to lower environmental impacts. Promoting cotton cultivation in these regions could help alleviate the concentration of production in Xinjiang and address associated sustainability challenges. However, this recommendation is based exclusively on meteorological factors, and it does not consider other crucial aspects such as soil characteristics, infrastructure, labor availability and local agricultural policies.

In addition to its high demand for water, the cultivation of cotton also requires extensive use of land. About this, Ridoutt et al. (2019) examines the potential human health impacts related to agricultural land occupation (ALO) at 5-arc-minute spatial resolution. In particular, the authors developed a novel model focused on the impact pathway linking land occupation and protein-energy malnutrition, expressed in disability-adjusted life years (DALYs). The most alarming results were highlighted in India, where cotton cultivation occupies 8% of the arable land area; indeed, the average impact was 0.09 DALY t^{-1} of seed cotton, representing 9% of national malnutrition-related DALYs attributable to cotton production. The authors also evaluated the water equivalent human health impacts from ALO ($\text{m}^3 \text{m}^{-2} \text{year}$), indicating that tropical areas—including parts of Sub-Saharan Africa, South Asia, Southeast Asia, and Central and South America—suffer the greatest impacts from ALO in terms of potential malnutrition with values exceeding 100 $\text{m}^3 \text{m}^{-2} \text{year}$. On the other hand, China exhibits a strong duality between the north and south of the country regarding water availability. The ratio of potential human health impacts from ALO and water was found to be between 0.1-0.3 $\text{m}^3 \text{m}^{-2} \text{year}$ in the north and 10-100 $\text{m}^3 \text{m}^{-2} \text{year}$ in the south.

In view of these results, Ridoutt et al. (2019) suggested several mitigation strategies, such as:

- the sustainable intensification of agricultural practices that increase yields without expanding farmland, thus limiting land occupation; and
- the overall shift towards more sustainable production and consumption patterns to reduce pressure on natural resources.

However, the authors assume that one unit of ALO completely precludes another agricultural production activity, and they advise that this assumption may not always be true. Thereby, they highlighted that the inclusion of land-use dynamics could improve the accuracy of the model (Ridoutt et al. 2019).

Another pioneering study from a methodological perspective was that of Verones et al. (2016), who developed characterization factors for lifecycle impact assessment (LCIA) targeting biodiversity loss of various animal taxa (i.e., birds, reptiles, mammals, and amphibians) in wetlands. This study constitutes a worldwide LCA to evaluate the impact of certain crops, including cotton, on the Potentially Disappeared Fraction of Species (PDF) of species. It was found that due to the high consumption of surface water, the agronomic production of 1 ton of cotton generates an impact on animal taxa equivalent to 4.7×10^{-08} PDF global $\cdot$ year^{-t}, particularly in the USA and Australia. The impact increases if we also consider the groundwater used, which has a value of 2.0×10^{-09} PDF global $\cdot$ year^{-t}, and the countries most affected are Algeria and Tunisia.

Relevant insights on the environmental impacts at the field level were also given by Huang et al. (2022). This study shows that the Carbon Footprint (CF) for Chinese cotton production is much higher than the global average, with 3.27 kg CO₂ eq·kg⁻¹ compared to 1.95 kg CO₂ eq·kg⁻¹ (Günther et al. 2017). The main reasons are the

significant increase in the use of pesticides, fertilizers, and particularly irrigation electricity consumption. Data collected from 2004 to 2018 indicate that in China, pesticide usage increased by 55%, while diesel fuel consumption increased by 35%. Fertilizer application per hectare also increased significantly by 151.18 kg, from 177.39 kg⁻¹ in 2004 to 328.57 kg⁻¹ in 2018. This trend is particularly pronounced in the Yellow River region, where fertilizers account for over 60% of total emissions. The biggest increase was in irrigation electricity consumption, which rose from 683.73 to 1689.63 kWh·ha⁻¹ during the same period, representing a 60% increase in energy usage. Overall, these factors have led to a 35.54% increase in China's cotton CF since 2004. Thereby, the authors emphasize several mitigation strategies to reduce the CF of cotton, particularly:

- improving fertilizer efficiency—especially nitrogen—to reduce N₂O emissions;
- and optimizing the irrigation through the adoption of controlled deficit irrigation techniques.

Of similar interest is the study conducted by Maraseni et al. (2010), who highlighted the relevant role of energy in cotton cultivation, especially that used for water pumping in irrigation. This study focused on the CF of three cotton cultivation systems in the Downs region of Queensland, Australia: dryland dense planting, dryland double skip row planting, and irrigated dense planting. The aim was to quantify the GHG emissions associated with various agricultural inputs in each of these systems. The authors found that CO₂ emissions were significantly higher for irrigated cotton cultivation (4841 kg CO₂ ha⁻¹) compared to dryland dense planting (1367 kg CO₂ ha⁻¹) and dryland double skip row planting (1274 kg CO₂ ha⁻¹). This is primarily due to the high energy consumption required for irrigation, which accounts for 27.5% of the GHG

emissions in irrigated cotton. In line with Huang et al. (2022), the results also stressed that one of the main factors contributing to total emissions is the use of fertilizers. In particular, N₂O resulting from the application of nitrogen-based fertilizers, accounts for 33.8% of total emissions in irrigated cotton. The authors emphasized several improvement strategies, mainly focusing on nitrogen management. Indeed, they underscored the need to reduce the use of nitrogen fertilizers and encourage the use of cover crops that can fix nitrogen, reducing the need for fertilizers and lowering N₂O emissions. Additionally, practices such as maintaining good soil structure, improving drainage, and increasing organic matter could further reduce GHG emissions. One of the limitations identified in the study is the potential underestimation of total GHG emissions, as the authors did not account for (1) production and transportation of cotton packaging; (2) construction of buildings and building materials; (3) use of organic manures; (4) packing and overseas exportation; and (5) soil organic carbon losses. Moreover, the study discusses the potential inclusion of the cotton industry in Australia's Carbon Pollution Reduction Scheme (CPRS) but notes that most cotton farms may not meet the minimum emission thresholds for participation.

Similar findings regarding the GHG emissions associated with of cotton cultivation were reported by Singh et al. (2021) in north-western India. The aim of this study was to quantify and optimize C footprints and energy flow in cotton cultivation by integrating LCA with Data Envelopment Analysis optimization of energy use for increased C sequestration in soil, while enhancing the net ecosystem C budget of a cotton ecosystem (Singh et al. 2021). The authors detect four main environmental hotspots: irrigation, biocides, fertilizers and fuel consumption. Energy required for pumping groundwater, particularly in water-scarce areas like north-western India,

significantly contributes to high energy consumption. The irrigation-related energy input for cotton cultivation amounts to $4272.3 \text{ MJ ha}^{-1}$, or 17.8% of the technical efficiency (TE) and 20% of total CO_2 emissions. In addition, the TE input related to biocides, primarily used for controlling pests such as the cotton whitefly (*Bemisia tabaci*), contributes substantially to emissions. The energy input for biocides is estimated at $1034.2 \text{ MJ ha}^{-1}$, equivalent to 4.3% of TE and 10% of total CO_2 emissions. The study also highlights chemical fertilizers, particularly N, P_2O_5 , and K_2O -based ones, release 667.0, 25.3, and $11.4 \text{ kg CO}_2 \text{ ha}^{-1}$, respectively, contributing to 44.3% of TE consumption and 46% of total CO_2 emissions. The high energy equivalence of nitrogen fertilizer production, combined with high application rates, positions fertilizers as the primary source of emissions. Furthermore, diesel fuel consumption plays a significant role, with usage reported at $4226 \pm 116 \text{ MJ ha}^{-1}$, or 18% of TE, accounting for 13% of total CO_2 emissions. Inefficient farming practices, such as excessive tillage, cause increased fuel use. Moreover, the negative values of net ecosystem C budget revealed that cotton ecosystems act as a C source, since the crop failed to produce net biome production to offset C emissions. The overall impacts are higher when compared to those achieved by other authors (Sami and Reyhani 2018) under similar agronomic conditions. Indeed, Sami and Reyhani (2018) conducted a study with 25 cotton farmers through face-to-face interviews in the Golestan province of Iran with the aim of evaluating the impacts of cotton farming on climate changes in terms of energy and GHG emission indices. Carbon cycle in the farms was not accounted for by the authors, since the amount of C lost via harvested crops is considered to be replaced by C uptake in the following crops and there is no significant long-term accumulation of C in crop products. As a relative limitation, the study relies heavily on

energy coefficients from other countries, highlighting the need for Iran-specific data to improve the accuracy of energy consumption and emissions assessments. Results showed that fertilizers, particularly nitrogen-based ones, were the primary hotspot for both energy consumption and GHG emissions. Fertilizers accounted for 45% of TE consumption and 61.9% of the total CO₂ emissions, amounting to 1285.80 kg CO₂ ha⁻¹. Fuels, mainly used for agricultural machinery, were identified as the second largest hotspot, contributing 18.4% to TE consumption (6.35 MJ ha⁻¹) and 24.3% to total CO₂ emissions, with 504.7 kg CO₂ ha⁻¹. Additionally, the study highlights irrigation as another significant factor, contributing 17.9% to TE. Therefore, in line with previous works, even these authors suggest several mitigation strategies, particularly focusing on optimizing nitrogen fertilizer use, and also adopting conservation agriculture practices to reduce fuel consumption.

Table 2.2 presents the environmental and health impacts of cotton cultivation across different countries and sources mentioned above, covering four the following key indicators: Carbon Footprint (CF, kg CO₂ eq), Water Footprint (WF, m³ eq), Disability-Adjusted Life Years (DALY, PDF·year), and Potentially Disappeared Fraction of Species (PDF).

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Table 2.2 - Environmental impacts of cotton cultivation in different countries.

Source	Country	Functional Unit	CF (kg CO ₂ eq)	WF (m ³ of water eq)	DAL Y	PDF (PDF global · year ⁻¹)
Avadí et al. (2020)	Mali	1 kg of organic seed cotton	9.59	-	-	-
		1 kg of conventional seed cotton	4.85			
		1 ha of organic cotton fiber	2194			
		1 ha of conventional cotton fiber	1844			
Ridout et al. (2019)	Worldwide	1 t seed-cotton	-	-	90 × 10 ⁻³	-
Huang et al. (2022)	China	1 kg of cotton	3.27	-	-	-
Maraseni et al. (2010)	Australia	1 ha of cotton	4841 and 1367 in rainfed cultivation	-	-	-
Singh et al. (2022)	India	1 ha of cotton	1391			
Sami and Reyhani (2018)	Iran	1 ha of cotton	1177.7	-	-	-

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Zhang et al. (2021)	China	1 t of cotton	-	2.49×10^3	1.69×10^{-3}	-
Veronesi et al. (2017)	Worldwide	1 t of cotton	-	-	-	4.7×10^{-08}

Carbon Footprint (CF, kg CO₂ eq), Water Footprint (WF, m³ eq), Disability-Adjusted Life Years (DALY), and Potentially Disappeared Fraction of species (PDF, PDF·year) related to cotton production, differentiated by source, country, and functional unit. "-" indicates missing data.

The impacts vary by country and functional unit, reflecting any differences based on local environmental conditions and farming practices (including the irrigation system adopted, and the production method – e.g., conventional or organic). For example, among the countries listed in Table 2, Iran (Sami and Reyhani 2018) shows the lowest CF values per hectare of cotton (1177.7 kg CO₂ eq), followed by India (Singh et al. 2022) with 1391 kg CO₂ eq. Meanwhile, Australia shows similar values only when cultivated under dryland conditions (1367 kg CO₂ eq), while emissions increase by 254.13% when the cotton is irrigated (Maraseni et al. 2010). This difference illustrates a key LCA causal pathway—the shift from rainfed to irrigated agriculture drives up both energy use (mainly for water pumping) and associated emissions. Water footprint numbers further highlight the effect of geographic and management contexts. For example, Chinese cotton production exhibits a WF of 2.49×10^3 m³/t, much of which is sourced from heavily exploited irrigation systems, exacerbating regional water scarcity and contributing to high human health impacts as expressed by DALY (1.69×10^{-3} per ton) (Zhang et al. 2021).

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In general, the above-mentioned studies provide a comprehensive analysis of the environmental impacts of cotton cultivation, emphasizing the huge contribution of:

- freshwater consumption;
- land occupation and use;
- pesticide-related toxicity; and
- GHG emissions.

The differences in water consumption between regions, driven by climate conditions, and the inefficiency of common irrigation methods like furrow irrigation, are major contributors to the environmental footprint. Additionally, the reliance on fertilizers and pesticides, particularly in conventional farming, exacerbates emissions and energy use, as highlighted in studies from China, India, and Iran. Although organic farming practices offer some advantages, such as reduced pesticide use, they still face challenges related to water and land efficiency due to lower yields. Nevertheless, this farming method was proposed by several authors among the mitigation strategies. Others were the adoption of more efficient irrigation systems, and the optimization of nitrogen management. Besides, the need for region-specific approaches and improved data accuracy remains crucial for optimizing cotton cultivation's sustainability.

Based on the reviewed studies, key strategies to prevent environmental pollution during cotton cultivation include implementing efficient irrigation technologies such as DI and LEPA systems; optimizing fertilizer and pesticide use through precision agriculture and integrated pest management; and promoting crop rotation, cover crops, and the adoption of organic or Bt cotton to reduce chemical inputs and emissions. These targeted interventions together address major environmental hotspots and support more

sustainable cotton cultivation.

2.3.2 LCAs of cotton fiber (cradle-to-gate)

The "cradle to gate" approach encompasses the process starting from the ginning of raw cotton and obtaining baled cotton fiber, through the manufacturing phase (including dyeing, washing, and spinning), up to the packaging of the finished product. Studies showed that cotton fiber processing intensifies the product's carbon and water footprints, largely through high energy, water and chemical demands.

Among the cradle-to-gate LCAs reviewed, Liu et al. (2020b) conducted a WF and the CF of mélange yarns in Zhejiang province, China. They found that producing mélange yarn consumes a significant amount of water, averaging $3.1 \times 10^3 \text{ m}^3$ per 1 ton of finished yarn. Cotton cultivation alone accounts for $2.8 \times 10^3 \text{ m}^3$, representing 91.2% of the total WF. This water is mainly green water, i.e. rainwater, which is the main source for cotton production in this region of China. The second largest contributor to the total WF is the spinning stage, which accounts for 3% of the WF, significantly less than the cotton cultivation stage. However, when analyzing CF, cotton cultivation contributes only 4.0% to the overall carbon emissions of cotton yarns. The greatest impact comes from the electricity used during raw fiber processing, which represents 61.7% of the total carbon emissions. Specifically, the spinning stage alone accounts for 83% of total electricity consumption, followed by steam (13.61%) and sodium sulfate (Na_2SO_4) (11.21%), both used in the dyeing process. Overall, the production of 1 ton of finished mélange yarns results in the emission of $7.9 \times 10^3 \text{ kg}$ of CO_2 .

Liu et al. (2020a) investigated in a new full LCA study the potential of using recycled yarns as a substitute for virgin cotton yarns.

They found that using recycled yarns significantly reduces WF, requiring only 583 m³ of water to produce 1 ton of recycled yarns, compared to 3514 m³ for virgin cotton yarns. The most significant savings come from the cotton cultivation stage, where water use is reduced by 79.1%, and from the washing process before fabric conversion into fiber, where water consumption is cut by 40.3%. In terms of CF, the study estimates that growing the cotton needed to produce 1 ton of virgin cotton yarns generates 6.6×10^3 kg of CO₂ equivalent emissions. Yarn production contributes further to carbon emissions, mainly due to electricity usage. The total CO₂ emissions for producing 1 ton of virgin cotton yarns are estimated to be 1.1×10^4 kg CO₂. Recycling cotton—which includes collecting, sorting, washing, and processing textile waste—also generates CO₂ emissions; however, the total emissions for producing 1 ton of recycled yarns are significantly lower, estimated at 4.4×10^3 kg CO₂. Overall, producing recycled yarn results in 60.2% fewer CO₂ emissions compared to virgin cotton yarn production, largely due to the elimination of the resource-intensive cotton cultivation stage.

In a study conducted by Bevilacqua et al. (2014), the authors performed an LCA to assess and compare the impacts of cotton dyed yarn in four different countries, namely Egypt, China, India, and USA. Based on the results related to the cultivation scenario, India had the highest impact in terms of GHG emissions, with 0.89 kg of CO₂ per kg of cotton dyed yarn. This was mainly due to fuel consumption and artificial fertilizers, which together account for approximately 47% and 31% of total CO₂ emissions in Indian companies. These values were significantly higher compared to the other countries analyzed. China ranked second with 0.72 kg of CO₂, while Egypt and the USA had emissions equivalent to 0.62 kg of CO₂. The high levels of GHG emissions in India and China are also attributed to extensive land use,

inefficient irrigation systems (such as flood irrigation), and the lack of crop rotation with less profitable crops. The authors also highlight that the dyeing stage has the greatest impact in terms of GHG emissions (44% of the total) and resource consumption (51.5% of the total). This high impact is due to the consumption of natural gas for steam and heat production, the use of chemical reagents, and the significant consumption of water and electricity. Additionally, the spinning process consumes the most electricity and significantly contributes to GHG emissions (23% of the total). The authors suggest several mitigation strategies that could be applied across all regions studied. In particular, they recommend the use of GMO pest-resistant varieties to reduce the heavy use of pesticides. At the industrial level, they propose the recovery and reuse of cooling water and improving the efficiency of heat exchangers. However, the study analyzes data collected from a limited number of cotton suppliers (one per country), so there is a risk that the results may not be representative of the entire cotton supply chain.

La Rosa and Grammatikos (2019), in a comparative LCA of natural fibers, investigated the GHG emissions associated with cotton production in comparison to other textile fibers such as hemp, kenaf, and jute. The study shows that to produce 1 ton of cotton fiber, the average emission is 2446 kg of CO₂, significantly higher than that of jute fiber (294 kg CO₂) and kenaf fiber (360 kg CO₂). However, when analyzing the CO₂ emissions for organically produced cotton, emissions drop to 978 kg CO₂, representing a 60% reduction in this impact category. Regarding WD, the researchers reported a consumption of 1.74×10^3 m³ of water to produce 1 ton of cotton textile (yarn), which is substantially higher than the values for jute and kenaf textiles (188 m³ and 257 m³, respectively). In fact, the researchers identified cotton as having the highest global warming

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potential (GWP) among the crops studied, and they suggest adopting alternatives such as hemp, kenaf, and jute, which are natural fibers that require less water and fewer pesticides compared to cotton.

Table 2.3 provides a comparative overview of CF and WF associated with various cotton-based products across different countries and production systems, from raw fiber to finished yarns.

Table 2.3 - Environmental impacts of cotton production, from cradle to gate, in different countries.

Source	Country	Functional Unit	CF (kg CO ₂ eq)	WF (m ³ of water eq)
Liu et al. (2020b)	China	1 t of the finished melange yarns	7.9×10^3	2.8×10^3
Liu et al. (2020a)	China	1 t of recycled yarns	4.4×10^3	0.58×10^3
Bevilacqua et al. (2014)	Egypt, China, India, and USA	1 t virgin cotton yarns	6.6×10^3	3.5×10^3
		1 t of dyed cotton yarn	2.1×10^3	-
La Rosa and Grammatikos (2019)	World wide	1 ton of conventional and organic cotton fiber	2.45×10^3 (conventional) 0.98×10^3 (organic).	1.74×10^3

Carbon Footprint (CF, kg CO₂ eq), Water Footprint (WF, m³ eq). "-" indicates missing data. Units originally expressed in kilograms (kg) have been converted to metric tons for consistency.

CF varies widely depending on the cotton type and processing technology, and energy source. For example, finished melange yarns in China (Liu et al. 2020b) have the highest reported emissions (7.9×10^3 kg CO₂ eq/t), which can be directly attributed to the country's heavy reliance on coal-powered electricity for spinning and dyeing processes, as well as the high input intensity of Chinese cotton farming—including fertilization and irrigation. In contrast, the production of recycled cotton yarns in China results in a substantially lower CF (Liu et al. 2020a) (4.4×10^3 kg CO₂ eq/t), indicating environmental advantages of eliminating the resource (and thus, the emission-intensive cultivation stage by substituting virgin cotton with recycled fibers). Virgin cotton yarns across Egypt, China, India, and the USA (Liu et al. 2020b) show intermediate CF values (6.6×10^3 kg CO₂ eq/t), while dyed cotton yarns report lower CF (2.1×10^3 kg CO₂ eq/t), again reflecting differences in agricultural practices, energy mix used in processing, and efficiency of production technologies. Globally (La Rosa and Grammatikos 2019), conventional cotton fiber results in a CF of 2.45×10^3 kg CO₂ eq/t, which is more than 150% higher than that of organic cotton fiber, emphasizing the environmental benefits of organic cultivation methods. WF also varies greatly depending on the type of cotton and its production process and mirrors these trends. For example, in China, the WF for finished melange yarns is 2.8×10^3 m³ of water eq, which is approximately 382.8% higher than the WF for recycled yarns. This marked difference confirms that the use of recycled materials in yarn production can lead to substantial water savings. In fact, virgin cotton production requires large amounts of water on average, both for irrigation and industrial processes (Liu et al. 2020a), while recycled yarns, by reusing existing fibers, significantly reduce overall water requirements. Together, the quantitative results in Table 3 (and previously Table 2) clearly

illustrate other key causal pathways captured by LCA theory: regions and practices with greater reliance on fossil fuels, intensive irrigation, and agrochemical use consistently show higher CF and WF; conversely, organic practices or adoption of recycled input streams break these links and results in markedly lower environmental burdens.

Based on the above, cotton cultivation is the largest contributor to water consumption and pesticide use, exacerbating water scarcity, toxicity impacts and GHG emissions. On the industrial side, spinning, dyeing, and washing are the main sources of GHG emissions, with dyeing being the most energy- and resource-intensive stage. Optimizing these processes is crucial to reducing GWP impacts. Mitigation strategies include improving water and energy efficiency, using GMO pest-resistant cotton, and favoring natural fibers like hemp, kenaf, and jute, which require fewer resources. Recycling cotton yarns also offers a promising solution to lower both water consumption and carbon emissions compared to virgin cotton. These findings reinforce the critical importance of strategic interventions—such as optimizing water use, transitioning to renewable energy, and increasing recycling rates—in reducing the overall environmental impact of cotton production globally.

2.3.3. LCAs of cotton textile products (cradle-to-grave)

To obtain a comprehensive estimate of the environmental impact associated with the entire life cycle of cotton, LCA studies with a "cradle to cradle" system boundary were finally examined. This allows for a step-by-step description of every step in the life of the product, from cultivation to manufacturing, distribution, consumer use (including laundering, drying, and ironing), and finally to end-of-life

disposal (recycling or waste disposal). Cradle-to-grave assessments are very recommended for textiles and clothing products, since the use phase plays a significant role in terms of impacts, and clothing products that are usually meant to be used for a long span are nowadays used for shorter periods as a consequence of fast fashion. The environmental impact of cotton products from a full life cycle perspective was addressed in eight of the articles reviewed.

For instance, Luo et al. (2021) performed a footprint analysis comprising CF, water scarcity footprint, water eutrophication footprint and water ecotoxicity footprint under a full life cycle perspective. The results showed that the total impacts of a pair of conventional 100% cotton jeans were 90.37 kg CO₂ eq in terms of carbon footprint, 13.74 m³ H₂O eq for water scarcity footprint, 1.67×10^{-2} kg PO₄³⁻ eq for water eutrophication and 112.41 m³ H₂O eq for water ecotoxicity. Finishing, cotton cultivation and laundering processes were main contributors to these environmental impacts. Within the industrial manufacturing stage, finishing has the highest contribution (32.2%), followed by denim washing (25.1%) and weaving (21.8%). On the other hand, the authors emphasized that during cotton cultivation, there is a net positive impact because the plants absorb CO₂ from the atmosphere and store it as biomass. Indeed, they estimated that the carbon storage of cotton plants is about 18.04 kg CO₂ eq/pair of jeans. This figure was far greater than the total carbon emissions (3.63 kg CO₂ eq/pair of jeans), leading to the CF in the cotton cultivation process negative (-14.41 kg CO₂ eq/pair of jeans). However, this seems to be only a short-term C balance, not considering that crop residues left in the field will be decomposed within a few months or years, so that there is no or only a small sequestration effect. The authors also observed that incinerating cotton during waste disposal can generate an average of 0.85 kW h pair of

jeans⁻¹ of energy, equivalent to reducing about 0.82 kg of CO₂ eq. Still, it should be argued that while the study accounts for the sequestered carbon, it does not explicitly define the type of biomass (which could be fiber only, or also the other parts of the cotton plants like leaves, stems or roots). Furthermore, it does not uniquely indicate the lifespan of the jeans, as it states the fiber could be stored for a long time, but at the same time it assumes only 2 years at the consumer use stage for the washing processes. In addition, the CF results do not include the emissions from the incineration process, while they only show the benefit associated with the energy produced from the heat generated during incineration. In contrast, the latter process would release the CO₂ temporarily stored in the cotton fibers. We therefore recommend greater clarity and transparency in the description of the carbon sequestration accounting methodology, and in the presentation of the related results. In their analysis of water scarcity footprint, the authors Luo et al. (2022) also noted a total consumption of 13.7 m³ H₂O pair of jeans⁻¹, 88% of which is used during the cultivation phase, amounting to 12.27 m³ H₂O pair of jeans⁻¹. The second highest water consumption factor is the washing process during the consumer use phase, with repeated washing using about 1.37 m³ H₂O. Washing is also the main contributor to water eutrophication (33.7%), with phosphorus from detergents being the leading cause (Günther et al. 2017), followed by cotton cultivation (18.6%), where the eutrophication effect is mainly due to leaching of nitrogen fertilizers into water. The authors identified several mitigation strategies to lower the environmental impact during the use phase, including: reducing the frequency of washing and ironing; and opting for front-loading washing machines over top-loading ones. These actions can significantly lessen the environmental footprint of cotton jeans during their use.

Similar results were observed by Morita et al. (2020), who carried out a cradle-to-grave analysis of the impacts of GWP and primary energy demand in Brazil, using a pair of jeans as a FU. This highlighted how the production of cotton has an impact on climate change equal to 7.8 kg CO₂ jeans⁻¹. Most of the emissions, however, concern the confection/finishing stages, which contribute 51% of emissions (4.0 kg CO₂ jeans⁻¹), followed by the weaving stage with 21% of emissions (1.62 kg CO₂ jeans⁻¹). Cotton cultivation contributes 17% of total emissions (1.37 kg CO₂ jeans⁻¹), the causes of which are due to CO₂ emissions deriving from liming (359g eq), handling operations (195 g eq), N-fertilizer application (50.5 g eq) and dinitrogen oxide N₂O through degradation of the N-fertilizer in the soil (0.83 g eq). Furthermore, the study compared the energy sources used during the production processes in Brazil and the USA and highlighted that the lower CF impact (4.44 kg CO₂ eq) is associated with the production of jeans in Brazil, where wood is used as a heat source. The authors propose replacing natural gas with wood as a heat source for industrial processes as the main mitigation strategy. This is justified by the carbon neutrality of biomass, as the CO₂ released during combustion is balanced by the CO₂ absorbed during tree growth. Wood thus emerges as the optimal energy source in terms of performance (124 MJ eq).

Zhang et al. (2015) agrees that the main factor of GWP is given by the industrial manufacturing stage. Through a cradle to the grave LCA conducted in China, with a 100 % cotton knitted dyed short-sleeved t-shirt as FU, they observe that only the dyeing stage represents 34.8% of the total emissions due to the on-side combustion of hard coal to produce steam. The second contributor to GWP is made by making-up stage (31.96 %), due to indirect CO₂ emissions given on-site electricity consumption. On the other hand, the discussion

concerning water consumption is different. Water resources used for irrigation account for 78.9% of water consumption, followed by the use stage and dyeing stage. The study reveals that this high-water consumption is primarily due to the washing habits of Chinese consumers, who prefer handwashing and sun-drying. These habits lead to significantly lower energy and water consumption compared to Germany (-73%) and the USA (-84%), where machine washing and electric drying are more common. Therefore, the authors suggest promoting water-efficient washing practices, such as:

- washing clothes less frequently.
- using shorter wash cycles; and
- opting for hand washing whenever possible.

To confirm that the environmental impact of cotton cultivation is negligible compared to industrial processes and domestic consumption, Fidan et al. (2022) conducted a study comparing the differences in environmental impact between organic and conventional cotton, using an end-of-life analysis approach. The study utilized a pair of average-sized jeans manufactured in Turkey as the FU, for a lifespan of 45.5 cycles. From the analyses, it was noted that organic cotton only results in a small improvement in terms of GWP and Acidification Potential (AP), with reductions of only 3.1% and 0.9%, respectively. This demonstrates that the greatest environmental impact is due to industrial processes and domestic consumption.

Similar conclusions were obtained from the LCA of cotton, conducted in Turkey by Baydar et al. (2015). The researchers found that the production of organic cotton does not differ significantly in terms of GWP from conventional cotton, as the main impact comes from the use phase, and highlighted how the use phase, due to the electricity consumed in the washing processes, causes 4140.4 kg of CO₂ emissions for the production of 1000 cotton pieces weighing 200

kg, which is four times more than the cultivation stage. Regarding AP, a 97% reduction in water eutrophication is observed in organic cotton cultivation compared to conventional cotton. This is due to the elimination of nitrogen and phosphorus-containing chemical fertilizers in the cotton cultivation stage.

Kazan et al. (2020), on the other hand, claim that organic cotton leads to enormous improvements in terms of GWP. Through a cradle-to-grave study conducted in Turkey, using 100 pieces of shirt (250 kg shirt) as the FU, it was shown that organic cotton cultivation reduces GHG emissions by 71% (385 kg CO₂ eq) compared to conventional production (1341 kg CO₂ eq) during the agronomic process. The savings are due to field operations such as fertilizing, using pesticides, herbicides, insecticides, fungicides, machinery, and diesel fuel use in seeding and plowing, which account for 84% of emissions produced in cotton cultivation, on top of fertilizer production (13%) and energy supply for the ginning process (3%). The authors also highlight the recycling of cotton as an essential tool for reducing GHG emissions. In fact, by reusing cotton, there are no climate-altering gas emissions during the agronomic process, as this phase is eliminated. In addition, consumption related to the dyeing phase would be eliminated. These measures would guarantee an overall reduction of GWP by 47% compared to conventional cotton production.

In the LCA analysis conducted by Moazzem et al. (2021) in Australia, the authors focused on the use of organic and recycled cotton as a system to reduce the environmental impact of the textile industry. The researchers highlighted how the production of organic cotton shirts led to a reduction of 10.56% in GWP and a decrease of 88-89% in ALO. The study also revealed that organic cotton requires only 605 m³ ha⁻¹ of irrigation water, compared to 1,080 m³ ha⁻¹ for conventional cotton, resulting in a reduction of WD between 79 and

83%, as also reported by Shah, Bansal, and Singh (2018). This difference is attributed to the higher moisture retention of organically managed soils, which benefit from increased organic matter that acts like a sponge, effectively absorbing and retaining water. For this reason, the authors also examined the use of recycled cotton, finding that it can achieve an impact reduction of about 34.93% AP, 62.55% for ALO 62.97% for WD compared to virgin cotton. However, they warn that the quality of recycled cotton may be inferior to that of virgin cotton, particularly regarding fiber length and uniformity. To improve quality, recycled cotton is often blended with virgin fibers, which reduces its environmental benefits (Moazzem et al. 2021).

On the same research topic, Esteve-Turrillas and de la Guardia (2017) conducted an LCA study in Spain with a FU of 1 kg of colored cotton yarn. The researchers highlighted, on one hand, organic cotton, which, thanks to the reduction in the use of pesticides and chemicals, allows for a significant reduction in environmental impacts. Indeed, the authors estimated, for FU, a reduction of 0.53 kg CO₂ eq for GWP (26%), 0.022 kg SO₂ eq for AP (79%), 0.028 kg PO₄⁻³ eq for Eutrophication Potential (EP) (93%), and 4332 kg of water for WD (79%), which are notably lower than the results reported by Moazzem et al. (2021). However, organic cotton cannot disregard the industrial stages related to ginning (whose GWP impact is estimated to be from 0.128 to 0.173 kg CO₂ eq) and dyeing steps (where GWP data ranges from 7.0 to 17.3 kg CO₂). In fact, the best results were achieved with recycled cotton, resulting in savings of 13.98 kg CO₂ eq for GWP, 0.32 kg SO₂ eq for AP, 0.033 kg PO₄⁻³ eq for EP, and 5594 kg water for WD impact category. The authors emphasize that using recycled cotton eliminates the need for new cotton cultivation, thereby reducing water, fertilizer, and pesticide consumption. Additionally, dyeing is often unnecessary since the final color of the cotton fiber can be

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derived from the raw materials used, further minimizing the use of water, dyes, wetting agents, softeners, and other related products.

Table 2.4 illustrates significant variability in key environmental indicators – CF and WF – which can be attributed to multiple interrelated factors across the product life cycle, including production location, energy sources, manufacturing technology, and cotton type.

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Table 2.4 - Environmental impacts of cotton production, from cradle to grave, in different countries.

Source	Country	Functional Unit	CF (kg CO ₂ eq)	WF (m ³ of water eq)
Luo et al. (2022)	China	1 pair of cotton jeans	90.37	13.74
Morita et al. (2020)	Brazil	1 pair of women's jeans	7.8	-
Zhang et al. (2015)	China	100 % cotton knitted dyed short-sleeved t-shirt	6.05	1.77
Şener Fidan et al. (2023)	Turkey	1 pair of average-sized jeans manufactured	from 20.11 to 19.48	-
Baydar et al. (2015)	Turkey	1000 items of knitted and dyed cotton t-shirt (200 kg)	from 2420.7 to 1872.2	-
Kazan et al. (2020)	Turkey	100 shirts made of conventional and organic cotton (250 kg of shirts).	Organic production: 385 (cultivation) and 1221 (industrial phase). Conventional production: 1341 (cultivation) and 2772 (industrial phase)	Cotton cultivation: 1036. Industrial processes: 1.3
Kazan et al. (2020)	Australia	1 kg cotton knit dyed t-shirt	43.15	1.5
Moazze m et al.	Spain	1 kg of colored cotton yarn, and 1	13.98	5.59

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(2021) T-shirt (0.3 kg)
made with 100%
cotton

Carbon Footprint (CF, kg CO₂ eq), Water Footprint (WF, m³ eq). "-" indicates missing data.

The highest CF for a single pair of jeans is reported in China (Luo et al. 2022) (90.37 kg CO₂ eq), while much lower values are observed in Turkey (Şener Fidan et al. 2023) (ranging from 20.11 to 19.48 kg CO₂ eq) and Brazil (Morita et al. 2020) (7.8 kg CO₂ eq). Notably, garments made from organic cotton in Turkey show reductions of up to 70% in the cultivation phase and 56% in the industrial phase compared to conventional cotton garments (Kazan et al. 2020). Similarly, Morita et al. (2020) attributes Brazil's lower CF to the increased use of renewable biomass (wood) as an energy source for industrial processes. Also, the WF varies considerably, with higher values for jeans (up to 13.74 m³) in China (Luo et al. 2022) and considerably lower values for t-shirts in other contexts (1.5–1.77 m³) (Moazzem et al. 2021; Zhang et al. 2015). These outcomes are deeply influenced by the specific geographical context, reflecting differences in agricultural practices, energy sources and mixes, production efficiency, and water availability. Also in this case, organic and recycled cotton products demonstrated major CF and WF reductions compared to conventional, and virgin alternatives.

Overall, the cradle-to-grave analysis highlights the significant environmental impacts associated with each stage of the cotton lifecycle. Especially industrial processes, like manufacturing and consumer use, severely contribute to GHG emissions and water consumption. However, even under a full life cycle perspective, cotton cultivation remains a critical phase where a high margin of improvement can be achieved by switching to sustainable agricultural

practices, including organic farming. Regarding GHG emissions, we also found an inconsistency in the consideration of biogenic carbon storage between LCAs and CF analyses. In fact, the majority of authors did not consider carbon sequestration by biomass, while Singh et al. (2021) and Luo et al. (2021) addressed this aspect. Both authors did not follow a traditional LCA approach but performed CF analyses under other protocols (Lal 2004). This partly explains the reason, as LCA generally does not consider this temporary carbon storage/sequestration, especially in the case of products such as clothing/garments that are only used for a few years. Increased durability, leading to a longer life of the garment, would keep the sequestered CO₂ out of the environment for longer, but this is still a debated issue, and no consensus has yet been reached. Caution should be exercised in communicating these types of results as they are highly dependent on the methodology used and the assumptions made. Adopting strategies such as choosing organic and recycled cotton, as highlighted by Vitale et al. (2024), promoting efficient washing practices, and utilizing sustainable energy sources can significantly reduce the environmental footprint of cotton products. However, challenges remain, particularly the limited adoption of organic cotton due to higher costs and lower yields, as well as the lower technological quality of recycled cotton.

2.4 Final remarks

Regarding the cradle-to-farm impact analysis publications analyzed, one of the recurring themes is the water use associated with cotton production. This crop requires a high-water input due to significant evapotranspiration, which ranges from 700 to 1,200 mm during the growing season, depending mainly on the cultivation area,

as it generally exceeds the precipitation levels in these regions (Evelt et al. 2012; Grismer 2002; Koudahe et al. 2021b; Vitale et al. 2024). In addition, there is a significant energy input associated with irrigation for pumping irrigation water, ranging from 4272.3 MJ ha⁻¹ to 4374.7 MJ ha⁻¹, representing 18%-22.4% of the TE required for cotton production (Singh et al. 2022). To effectively reduce water and energy use in cotton production, two readily achievable options are (i) the use of low-input irrigation techniques, such as DI and LEPA systems, compared to conventional FI; (ii) the relocation of cotton production to areas where frequent rainfall and low evapotranspiration allow cotton to be grown with low irrigation volumes. The massive utilization of chemical pesticides contributes significantly to the toxicity impact categories and the overall environmental profile, particularly in the context of conventional cotton. Similarly, nitrogen fertilizers constitute the primary source of GHG emissions, thus confirming the necessity for strategies that aim to reduce and/or optimize their efficiency. Added to this is the widespread use of pesticides. Studies indicate that they are a major environmental hotspot, particularly in regions such as Mali and China, where they exacerbate freshwater ecotoxicity and contribute to substantial biodiversity loss.

In terms of cradle-to-gate LCA, significant challenges and opportunities for improvement emerge. The results underline the critical role of cultivation for water consumption, particularly in regions facing severe water scarcity, accounting for a staggering 91.2% of the total WF in yarn production (Liu et al. 2020b). Moreover, data indicates that the production processes, especially spinning and dyeing, contribute substantially to GHG emissions and ecological degradation with figures reaching up to 1.1×10^4 kg CO₂ per ton of virgin cotton yarn, underscoring the urgent need for sustainable

practices in the industry (Liu et al. 2020a).

Regarding the cradle-to-grave LCA analysis, significant environmental impacts are highlighted, particularly during industrial processes and the consumer use phase. It is evident that the industrial manufacturing stage alone contributes 95.5% of the total CF, with finishing (32.2%), denim washing (25.1%), and weaving (21.8%) being the largest contributors. Organic cotton significantly reduces the use of pesticides and chemical fertilizers, with a 97% reduction in EP compared to conventional cotton (Baydar et al. 2015). However, as also highlighted by cradle-to-gate analyses, it has lower yields, requiring approximately 10-20% more area to obtain the same amount of cotton (Avadí et al. 2020). Recycled cotton, on the other hand, allows a 79% reduction in water consumption and a 60% reduction in CO₂ emissions compared to virgin cotton (Liu et al. 2020a). Despite these advantages, it represents only a small part of global production, with challenges related to the quality of the final product. In terms of water usage, cotton cultivation accounts for 88% of the total life cycle consumption (12.27 m³ H₂O per pair of jeans), while repeated laundering during the consumer phase consumes 1.37 m³ H₂O, contributing significantly to water eutrophication (33.7%) (Günther et al. 2017; Luo et al. 2022). Recycled cotton offers more substantial environmental benefits, with a 34.9% reduction in AP, 62.5% in ALO, and 62.9% in WD compared to virgin cotton (Moazzem et al. 2021).

Furthermore, the review highlights some gaps in assessing and managing the environmental impacts associated with cotton cultivation, industrial production and consumer use. For example, most studies do not adequately address local variables that influence the sustainability of cotton production, such as soil characteristics, local agricultural policies and labor availability. Also, some authors suggest relocating cotton production to areas with more favorable

climatic conditions, but do not adequately consider socio-economic factors such as infrastructure availability, local agricultural policies and access to labor. In addition, many LCAs focus on primary processes (spinning, dyeing, washing) but lack detailed data on sub-processes such as equipment maintenance, intermediate transport of materials, or the use of specific chemicals at certain stages of production. For instance, the contribution of chemicals such as surfactants or mordants used in dyeing is often neglected. Moreover, while many studies emphasize the importance of domestic use (washing and ironing) in the environmental impact of cotton, they do not sufficiently analyze the impact of new technologies such as water and energy efficient washing machines or the use of environmentally friendly detergents (Moazzem et al. 2021; Zhang et al. 2015). From a methodological perspective, an absence of congruence was identified in the consideration of temporal carbon storage between LCAs and CF analyses. In addition, the necessity for further research on cotton, employing multiple LCIA multi-impact methods in a concurrent manner to facilitate comparative analysis of the results in absolute terms was identified. Similarly, we advocate for the adoption of more and more diverse functional units to improve the usefulness of LCA results for the many diverse cotton stakeholders.

In future, the challenges inherent to cotton cultivation and its environmental sustainability are likely to be centered on the reduction of water and energy consumption, particularly in regions where water scarcity is prevalent, and irrigation plays a pivotal role. Additionally, optimization of chemical pesticides and fertilizers will be imperative to mitigate associated toxicity impacts and greenhouse gas emissions. Implementing low-input irrigation technologies, adopting organic farming practices, and recycling cotton fibers offer promising solutions. Nevertheless, the industrial processes of the textile industry,

particularly manufacturing, finishing and laundering, remain the significant contributors to carbon emissions and environmental degradation. Therefore, a wider shift towards sustainable practices, including increased use of recycled cotton and technological innovations in organic production, will be essential to mitigate these impacts and meet global sustainability goals. Overall, the review's findings highlight the need for simultaneously targeted interventions throughout the cotton life cycle. Accordingly, the following evidence-based recommendations are proposed to address the major environmental challenges associated with cotton cultivation, processing, use, and disposal:

Cultivation: Promote efficient irrigation systems as DI and LEPA irrigation, which, despite high initial costs, are reusable and can be amortized over time; optimize fertilizer and pesticide application through precision agriculture and integrated pest management, encourage organic and regenerative practices, and prioritize cultivation in regions less vulnerable to water scarcity and biodiversity loss.

Processing and Manufacturing: Adopt the best available technologies to enhance water and energy efficiency, transition to renewable energy sources, utilize less hazardous chemicals during dyeing and finishing, and ensure rigorous wastewater treatment to prevent water contamination.

Consumer Use: Support public awareness initiatives on sustainable garment care, such as reducing washing frequency, using environmentally friendly detergents, and opting for air drying. Encourage practices that extend product life, including repair, reuse, and responsible consumption.

End-of-Life: Expand textile recycling infrastructure and capacity, develop effective collection and sorting systems, advance

recycling and upcycling technologies, and implement policies that discourage landfill and incineration of textile waste.

Policy and Collaboration: Integrate life cycle assessment (LCA) into sector-wide decision making, set and enforce regulatory standards for chemicals, water, and emissions, and foster cross-sector collaboration to facilitate knowledge transfer and innovation.

Implementing these strategies at each stage of the cotton value chain is essential for minimizing environmental degradation, promoting resource efficiency, and advancing the sustainability of cotton products.

As future research step of this team of authors, it is envisaged to further contribute to the LCA cotton field by developing a multifunctional LCA study (Zingale et al. 2024) on organic and regenerative cotton cropping systems in the Mediterranean region.

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CRedit authorship contribution statement

Giuseppe Salvatore Vitale: Writing – original draft, Writing – review & editing, Methodology, Conceptualization, Investigation, Formal analysis, Data curation, Software, Visualization. **Silvia Zingale:** Conceptualization, Methodology, Writing – review & editing, Validation. **Nicolò Iacuzzi:** Data curation, Supervision. **Sara Lombardo:** Conceptualization, Supervision. **Teresa Tuttolomondo:** Validation, Supervision. **Paolo Guarnaccia:** Resources, Conceptualization, Supervision, Project administration.

Declaration of Competing Interest

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The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Article

Sustainable Cotton Production in Sicily: Yield Optimization Through Varietal Selection, Mycorrhizae, and Efficient Water Management

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Abstract

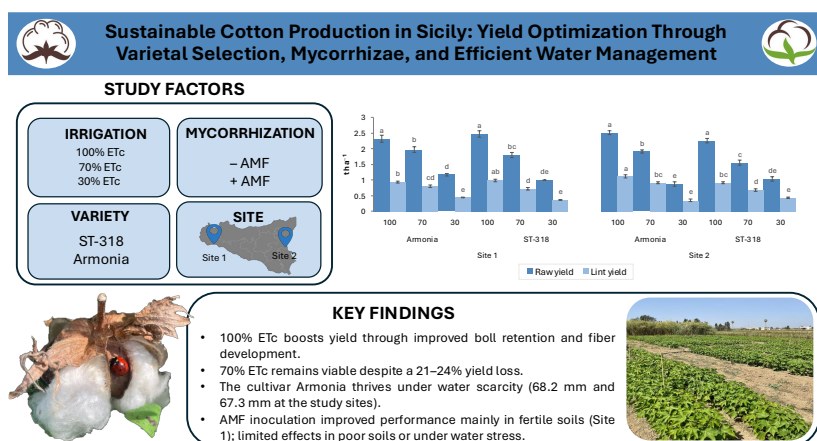
This study explores the revival of cotton (*Gossypium* spp. L.) farming in Italy through sustainable practices, addressing economic and water-related challenges by integrating cultivar selection, arbuscular mycorrhizal fungi (AMF) inoculation, and deficit irrigation under organic farming. Field trials evaluated two widely grown Mediterranean cultivars (Armonia and ST-318) under three irrigation levels (I-100: 100% crop water requirement; I-70: 70%; I-30: 30%) across two Sicilian soil types (sandy loam vs. clay-rich). Under I-100, lint yields reached 0.99 t ha⁻¹, while severe deficit (I-30) yielded only 0.40 t ha⁻¹. However, moderate deficit (I-70) maintained 75–79% of full yields, proving a viable strategy. AMF inoculation significantly enhanced plant height (68.52 cm vs. 65.85 cm), boll number (+22.1%), and seed yield (+12.5%) ($p < 0.001$). Cultivar responses differed: Armonia performed better under water stress, while ST-318 thrived with full irrigation. Site 1, with higher organic matter, required 31–38% less water and achieved superior irrigation water productivity (1.43 kg m⁻³). Water stress also shortened phenological stages,

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allowing earlier harvests—important for avoiding autumn rains. These results highlight the potential of combining adaptive irrigation, resilient cultivars, and AMF to restore sustainable cotton production in the Mediterranean, emphasizing the importance of soil-specific management.

Keywords: Sustainable cotton cultivation, water management, mycorrhizal fungi, organic; Mediterranean cotton

Graphical abstract



3.1 Introduction

Cotton (*Gossypium* spp. L.) is an annual open-field crop belonging to the *Malvaceae* family and represents one of the most important industrial crops in the world. With a cultivated area of 31.92 million ha⁻¹ across 80 countries and an estimated annual production of 25.51 million tons of fiber, cotton plays a crucial role in global

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agriculture (FAOSTAT 2025). Despite its economic significance, cotton cultivation faces major sustainability challenges, primarily due to high water consumption, intensive pesticide use, and soil degradation. The increasing global demand for cotton, coupled with environmental concerns, underscores the need for efficient water management and innovative agricultural practices. Cotton cultivation is highly water-intensive, requiring between 700 and 1200 mm of water during the growing season, depending on the growing region (Hunsaker et al. 2015; Koudahe et al. 2021b). Despite its drought tolerance, water remains critical for cotton growth, and seed cotton yield can be significantly enhanced through effective irrigation management (Bordovsky et al. 2015). It is estimated that global agricultural water consumption amounts to 8053.6 km³ year⁻¹, representing 87% of total global water consumption. Cotton production alone accounts for 2.6% of total freshwater use (Chapagain et al. 2006), and approximately 70% of the world's cotton-growing areas experience water stress (Wu et al. 2022).

In Mediterranean regions—including Sicily, Andalusia, Greece, and Turkey—cotton cultivation occurs under hot, and arid summers (Engonopoulos et al. 2025). Among these regions, Sicily emerges as a critical case study due to its compounding hydroclimatic stressors. Climate models project a 10–17% increase in reference evapotranspiration (ET₀) by 2091, with inland areas (e.g., Enna, Caltanissetta) facing the most severe intensification (+16.71%) (Di Nunno and Granata 2023). These projections suggest reduced water availability coinciding with peak irrigation demand.

This issue is further exacerbated by the use of low-efficiency irrigation techniques. Currently, the most widely used irrigation systems in cotton cultivation are furrow and sprinkler irrigation, which have low efficiency and significantly increase water waste compared

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to more advanced systems such as drip irrigation (Barnes et al. 2020; Mai et al. 2022; Vitale et al. 2024).

The growing awareness of sustainability has led to an increase in the demand for organic cotton, driven by consumer requests for products made with sustainable production practices (Eyupoglu 2019). Although cotton accounts for only 2.4% of the world's arable land (Bevilacqua et al. 2014), it consumes 11% of total pesticide use, a figure that reaches 50% in developing countries (La Rosa and Grammatikos 2019). According to the latest data provided by the United States Department of Agriculture (USDA - National Agricultural Statistics Service - Surveys - Agricultural Chemical Use Program 2025), cotton ranks as the third-highest pesticide-consuming commodity crop in the United States, with about 4 kg ha⁻¹ of pesticides applied annually. Herbicides are utilized in 96% of cotton-growing areas, with glyphosate being the predominant herbicide used (USDA - National Agricultural Statistics Service - Surveys - Agricultural Chemical Use Program 2025). Notably, the use of glyphosate in cotton production has doubled over the past decade (USDA - National Agricultural Statistics Service - Surveys - Agricultural Chemical Use Program 2025). This trend raises critical concerns due to several factors, particularly weed resistance (Vulchi, Bagavathiannan, and Nolte 2022), growth inhibition of some soil microbiome species (Huff et al. 2010; Langarica-Fuentes et al. 2024), and potential risks to the environment and human health (Vasseur et al. 2024).

According to the latest USDA estimates, farmers applied an average of 270 kg ha⁻¹ of synthetic fertilizer to cotton in the 2022 growing season. Nitrogen in particular was applied in 71% of the cultivated areas (USDA - National Agricultural Statistics Service - Surveys - Agricultural Chemical Use Program 2025). Globally,

nitrogen consumption in cotton is estimated to range from 300–450 kg ha⁻¹ (Ballester et al. 2021; Luo et al. 2018). This high level of fertilizers application poses a serious threat to crop sustainability and contributes to both direct and indirect greenhouse gas emissions (Salehin et al. 2025). Phosphorus (P), a nutrient classified as a non-renewable resource, is experiencing global depletion, leading to increased prices in recent years (Cordell, Drangert, and White 2009). Moreover, most P fertilizers have low mobility in soils due to their strong adsorption to iron and aluminum cations in acidic soils and to calcium in alkaline conditions (Smith et al. 2003). Sicilian soils (Typic Xerorthents/Calcixererts) differ significantly from those in other Mediterranean cotton-producing areas, being more alkaline (pH 7.5–8.2), poorer in organic matter (<1.5%), and richer in calcium carbonate (>25%) (Costantini and Lorenzetti 2013). These characteristics reduce phosphorus availability compared to the alluvial soils of Çukurova (Turkey) or the Vertisols of Andalusia (Spain) (Acar et al. 2025; Ortega et al. 2016).

A promising solution to this challenge lies in soil microorganisms, particularly arbuscular mycorrhizal fungi (AMF). Cotton is a mycorrhizal-responsive plant (Thompson, Seymour, and Clewett 2012b) and up to 90% of cotton root length can be colonized by AMF (Smith et al. 2011). These fungi play a crucial role in enhancing nutrient acquisition by effectively exploring larger volumes of soil, thereby significantly improving plant uptake of nutrients, especially P (Mai et al. 2018). Among these, certain species such as *Funneliformis mosseae* are particularly effective in nutrient uptake, showing increases in P uptake of up to 110% (Li 2024; Ortas and Iqbal 2019; Wang et al. 2020; Zhou et al. 2020). Moreover, mycorrhizae exhibit a highly favorable response within the Sicilian production area, as extensively documented in the literature (Pandino et al. 2022; Vitale

et al. 2024).

In Italy, the cultivation of cotton has gradually disappeared from the landscape. Today, despite the presence of only a few hundred hectares dedicated to this crop, there is no official data on its cultivation from Italian statistical sources (INSTAT 2025). The decline in cotton production can be attributed to several factors. First and foremost, agronomic problems, such as lower yields in non-irrigated conditions or with inefficient irrigation systems, together with high fertilizer and pesticide costs, have played a significant role. Economic challenges also contribute to this decline, including the low cash value of cotton production - around 1.86 € kg⁻¹ (Bevilacqua et al. 2014) - as well as labor shortages and rising labor costs (Tuttolomondo et al. 2020).

Based on the above considerations, this study aims to examine specific aspects of agronomic techniques for the restoration of cotton cultivation in Sicily (Southern Italy). To achieve this objective, two upland cotton cultivars (*Gossypium hirsutum* L.), currently cultivated in the Mediterranean basin, were grown in two Sicilian environments. The study implemented organic farming principles and utilized mycorrhizal formulations containing *Funneliformis mosseae*, along with high-efficiency irrigation systems. Consequently, the purpose of this preliminary work was to understand the bio-agronomic behavior of cotton in Sicily, subjected to three different levels of crop water requirement, while ensuring the sustainability of water and soil resources, in accordance with organic farming principles.

3.2 Materials and Methods

3.2.1 Experimental Site

The experimental trial was conducted in 2023 at two sites in Sicily, Italy. The first site was the experimental farm “Campo Carboj”

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(Site 1) owned by the Agricultural Development Authority (Ente di Sviluppo Agricolo—ESA) of the Sicilian Region, located in Castelvetro (TP) in the Belice area (37°35'18.0" N, 12°53'44.0" E, 65 a.s.l.). The second site was the experimental farm of the University of Catania (Site 2), situated in Primosole (CT) (37°24' N, 15°03' E, 10 m a.s.l.). Chemical and physical characteristics of the two soils are shown in Table 3.1.

Table 3.1 – Soil Characteristics of the Upper Layer (0–50 cm) at Site 1 and Site 2.

Soil Characteristics	Unit	Value		Method
		Site 1	Site 2	
Sand	%	59	16.6	(Lotti 2023)
Loam	%	13	27.8	(Lotti 2023)
Clay	%	28	55.6	(Lotti 2023)
N total	g kg ⁻¹	1.3	1	Kjeldahl (Kjeldahl 1883)
P	mg kg ⁻¹	9.16	2.18	Ferrari (Helrich 1990)
K	mg kg ⁻¹	112.9	203.3	Dirks and Scheffer (Helrich 1990)
Organic matter	%	1.46	1.1	Walkley and Black (Helrich 1990)
Electrical Conductivity	mS/cm	0.8643	0.15	(Dane et al. 2018)
Cation Exchange Capacity (CEC)	meq/%	27.05	14.8	(Sumner and Miller 2018)
pH		7.4	7.6	In water solution
Bulk density	t m ³	1.16	1.2	(Blake and Hartge 2018)
Field capacity at –0.03 MPa	%	27.5	27	(Klute 2018)
Wilting point at –1.5 MPa	%	16.7	11	(Flint and Flint 2018)

3.2.2 Experimental Design and Crop Management

The experimental trials at Sites 1 and 2 followed a split-plot design with three replications. The main plot factor consisted of three irrigation regimes: full irrigation (I-100), moderate deficit irrigation (I-70), and severe deficit irrigation (I-30). The subplot factor involved the application of a microbial biostimulant (Rizoplant, Biogard®, Grassobbio, Italy) during two phenological phases (BBCH-13 and BBCH-16) using a fertigation system. The sub-subplot factor included the evaluation of two *Gossypium hirsutum* L. varieties, ST-318 and Armonia. Each plot measured 16 m² (4 m × 4 m).

3.2.3 Irrigation Management

The irrigation system was designed with P5[®] dripline (Irritec S.p.A., Capo d'Orlando (ME), 98071, Italy) with emitters characterized by nominal flow rates of 2.1 L h⁻¹ at a pressure of 100 kPa. A self-compensating drip irrigation system was implemented along each row. The emitters were spaced 0.20 m apart and had a flow rate of 2 L h⁻¹. Additionally, volumetric flow meters were used to monitor the volumes distributed for each irrigation scheduling method.

Differentiated irrigation began upon reaching the sixth true leaf (BBCH-16) to ensure that all factors successfully passed the emergence stage.

To determine the hydrological constants, each plot was sampled using a random sampling method at a depth ranging from 20 to 50 cm. The soil water content corresponding to field capacity (FC) was measured using pressure plate extractors (Flint and Flint 2018) at a tension of -1 m. For each treatment, three samples were prepared by

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compacting the soil fraction through a 2 mm sieve into 5×1 cm samplers, based on the bulk density values measured from undisturbed soil (Blake and Hartge 2018). Equilibrium with the applied tension was assumed when the samples ceased to drain for at least 24 h. The volumetric water content at equilibrium was determined using the thermogravimetric method after drying the samples in an oven at 105 °C for 24 h. All measurements were conducted under controlled conditions, with the temperature set to 22 ± 1 °C. After establishing the hydrological constants for the experimental sites indicated in Table 1, daily reference evapotranspiration (ET_0) was calculated using the Penman–Monteith equation. Crop water consumption was estimated, for each phenological stage, by multiplying ET_0 by crop coefficients (K_c) defined in FAO Irrigation and Drainage Paper No. 56 (Allen et al. 1998). Additionally, the soil depth explored by the roots was considered in the calculation of available water quantity (Table 3.2).

Table 3.2 - Phenological phases and K_c values for cotton according to Allen et al. (Allen et al. 1998).

Phase	Description	K_c	Depth of Soil Explored by Roots (cm)
Initial	Germination: from dry seed (00) to emergence of hypocotyl with cotyledons (09)	0.4–0.5	30
Development	Leaf development: from cotyledons completely unfolded (10) to canopy closure (39)	0.7–0.8	50
Mid-season	Inflorescence emergence: from the first detectable bud (51) to about 90% of capsules having reached their final size (79)	1.05–1.25	50
End-season	Senescence: from about 10% of discoloured or abscessed leaves (91) to above-ground parts of dead plants	0.65–0.70	50

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Irrigation water productivity (IWP, kg ha⁻¹ mm⁻¹) was calculated following the methodology described by (Lai et al. 2022; Zhangjin et al. 2025), using the following equations (Equation (1)):

$$IWP = \frac{\text{Yield}}{I} \quad (1)$$

where “Yield” represents the crop production, and “I” denotes the total amount of irrigation water applied.

3.2.4 Microbial Biostimulants Management

The application of the microbial biostimulant was carried out at both sites using a fertigation system during two phenological phases. The first application occurred upon reaching the third true leaf stage (BBCH-13), while the second application was made at the sixth true leaf stage (BBCH-16).

The microbial biostimulant used was a commercial product (Rizoplant, Biogard®, CBC Europe S.p.A., Grassobbio, Bergamo, Italy), formulated on an inert carrier based on clay and peat. It contains 1.35×10^3 propagules g⁻¹ of arbuscular mycorrhizal fungi, specifically *Funneliformis mosseae*, *Funneliformis caledonium*, and *Rhizoglyphus irregularis*. The formulation also includes rhizosphere bacteria at a concentration of 10^4 CFU g⁻¹. The product was applied at a rate of 0.25 kg ha⁻¹ per application.

3.2.5 Variety Selection

Two varieties of *Gossypium hirsutum* L., ST-318 and Armonia, were evaluated at the two experimental sites. These cultivars were selected as non-GMO varieties widely cultivated in the Mediterranean

region (Filintas et al. 2022b; Moussouraki et al. 2019).

3.2.6 Agronomic Management

The trial was conducted under organic management, and the soil at both experimental sites had undergone a fallow period in the previous year. Soil was ploughed to a depth of 35 cm and tilled twice before sowing. In Site 2, seeding was done on 29 May 2023, while in Site 1 it was done on 9 June 2023. This shift in sowing was due to rainfall events that caused a delay in sowing at Site 1. The adopted plant density was 12 plants m⁻² (in-row spacing: 0.08 m; inter-row spacing: 1.0 m). Weed control in the inter-row areas was carried out with a mower during the leaf development phase. Harvesting was conducted when approximately 90% of the bolls had opened (BBCH 89) and was performed manually. At Site 1, harvesting occurred on 10 November 2023, while at Site 2, it took place on 3 November 2023. Fertilization was carried out before sowing and again just before the stem elongation phase, with a total application of 110 kg ha⁻¹ of nitrogen (N), 24 kg ha⁻¹ of phosphorus (P) and 46 kg ha⁻¹ of potassium (K) as organic fertilizer. The base fertilization was applied in the form of pellets, containing 10% N, 5% P₂O₅ and 5% K₂O (ORGA-KEM, Biolchim®). For surface fertilization, two applications of liquid organic nitrogen were administered during the phenological phases corresponding to the appearance of the first true leaves and flowering, using a liquid fertilizer with a concentration of 8.7% N (Tamarack, Growan Italia®, Faenza (RA), Italy). For both sites, no pest or disease control measures were applied during the trials.

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3.2.7 Weather Data

Rainfall and temperature data were collected from meteorological stations belonging to the Sicilian Agrometeorological Information Service 2024, located near the two experimental sites. The stations were equipped with an MTX datalogger (model WST1800) and various climatic sensors. Specifically, an MTX temperature sensor (TAM platinum PT100 heat-resistant model with radiation shield) and an MTX rainfall sensor (PPR model with tipping bucket rain gauge) provided data on daily average maximum and minimum air temperatures ($^{\circ}\text{C}$) and total daily rainfall frequency (mm). The thermo-pluviometric data are shown in Figure 3.1.

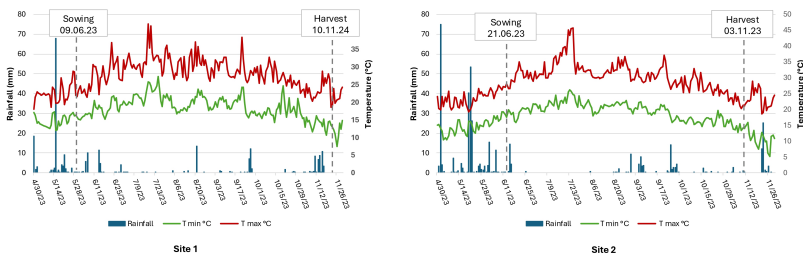


Figure 3.1 - Temperature and precipitation trends at the two experimental sites of Site 1 (Campo Carboj) and Site 2 (Primosole) from April 2023 to November 2023.

3.2.8 Data Collection

3.2.8.1 Growth Stage Data

The main growth stages of the two cultivars were determined according to the extended BBCH scale (LANCASHIRE et al. 1991): emergence (70% of plants with fully unfolded cotyledons), beginning of flowering (50% of plants with at least one open flower), and beginning of boll opening (50% of plants with at least one open boll).

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Growth degree days (GDD) were also determined for each phenological stage of the two cultivars according to Equation (2):

$$GDD = \frac{(T_{min} + T_{max})}{2} - T_b \quad (2)$$

where: T_{min} and T_{max} are the daily minimum and maximum air temperatures, and T_b is the baseline temperature below which development ceases. The basal temperature for the identified cotton plant is 15.6 °C (Beegum et al. 2024; Gowda et al. 2007). The accumulated GDDs for each phenological stage were calculated by summing the daily GDDs of each stage (Shah et al. 2017).

3.2.8.2 Morphological Data

At harvest, within a delimited area of 2.0 m² within each subplot, these parameters were determined: height of the first fruiting branch (cm), plant height (cm), number of open bolls per plant and average weight of bolls after oven drying (g). These morphological measurements were determined at harvest (BBCH-89) at 154 DAS in Site 1 and 135 DAS in Site 2.

3.2.8.3 Yield Data

Cotton lint was separated from the seeds of the raw material harvested from the parcel unit of each subplot using a laboratory ginning machine. Next, lint and seed yields (t ha⁻¹) were determined.

3.2.9 Statistical Analysis

Statistical analyses were performed using the package MINITAB 19 (State College, PA, USA) for Windows. All collected

data were subjected to the analysis of variance (ANOVA). The difference between means was analyzed using Tukey's test ($p \leq 0.05$). To ensure compliance with ANOVA assumptions, all data were tested for sphericity, normality and variance homogeneity through the Mauchly's test ($\alpha = 0.05$), Ryan-Joiner test ($\alpha = 0.05$) and Levene's test ($\alpha = 0.05$). The graphs were created using the Excel application of the Office 365 package for Windows.

3.3 Results

3.3.1 Irrigation Data

Table 3.3 presents the water volumes applied for the different irrigation levels at the two sites. Rainfall data indicate that Site 1 received a total of 68.34 mm (equivalent to $683.4 \text{ m}^3 \text{ ha}^{-1}$), whereas Site 2 received $990 \text{ m}^3 \text{ ha}^{-1}$ over the entire vegetative period—a difference of approximately 31% less rainfall at Site 1.

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Table 3.3 - Water volume supplied (Rainfall + Irrigation) for Irrigation level and phenological stage.

Irrigation Level	Phenological Phase	Site 1		Site 2	
		Rainfall (m ³ ha ⁻¹)	Irrigation (m ³ ha ⁻¹)	Rainfall (m ³ ha ⁻¹)	Irrigation (m ³ ha ⁻¹)
I-30	Initial (BBCH 00–09)	163.4	58.1	589	95
I-30	Development (BBCH 10–50)	92.3	0	17	0
I-30	Mid-season (BBCH 51–79)	365.3	0	335	0
I-30	End-season (BBCH 80–89)	62.4	0	49	0
Total water supplied (m ³ ha ⁻¹)		741.5		1085	
I-70	Initial (BBCH 00–09)	163.4	58.1	589	95
I-70	Development (BBCH 10–50)	92.3	92.2	17	108.9
I-70	Mid-season (BBCH 51–79)	365.3	90.7	335	115
I-70	End-season (BBCH 80–89)	62.4	0	49	0
Total water supplied (m ³ ha ⁻¹)		924.4		1308.9	
I-100	Initial (BBCH 00–09)	163.4	58.1	589	83.2
I-100	Development (BBCH 10–50)	92.3	127.3	17	154.6
I-100	Mid-season (BBCH 51–79)	365.3	141.7	335	250
I-100	End-season (BBCH 80–89)	62.4	0	49	0
Total water supplied (m ³ ha ⁻¹)		1010.5		1477.8	

I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc. Site 1 = Campo Carboj and Site 2 = Primosole.

Despite the lower rainfall, Site 1 required a reduced supplemental water input across all observed phenological phases. Under the I-30 treatment, for example, Site 1 received an additional 58.1 m³ ha⁻¹ exclusively during the initial phenological phase (BBCH 00–09), compared to 95 m³ ha⁻¹ at Site 2. This represents nearly 38.8%

lower water input in Site 1 under the I-30 regime. Consequently, the total water resources applied (rainfall plus irrigation) amounted to $741.5 \text{ m}^3 \text{ ha}^{-1}$ at Site 1 and $1085 \text{ m}^3 \text{ ha}^{-1}$ at Site 2—about 31.7% less at Site 1. Under the I-70 treatment, Site 1 received $241 \text{ m}^3 \text{ ha}^{-1}$ of supplemental water distributed from the initial phase up to mid-season (BBCH 79), while Site 2 was supplied with $318.9 \text{ m}^3 \text{ ha}^{-1}$ —approximately 32.3% more water in Site 2 than in Site 1. Finally, under the I-100 treatment, supplemental irrigation was $327.1 \text{ m}^3 \text{ ha}^{-1}$ for Site 1 and $487.8 \text{ m}^3 \text{ ha}^{-1}$ for Site 2, indicating that Site 1 required roughly 33% less water than Site 2 under full water restoration conditions.

3.3.2. Agronomic and Yield Data

Analysis of variance (ANOVA) revealed that the factors site (S), genotype (G), irrigation (I), mycorrhization (M), and their interactions showed significant effects in several cases, as observed in Table 3.4. In particular, ANOVA indicated a significant effect of G, M, I, and S on plant height, the height of the first fruiting branch, and the average number of open capsules per hectare. Additionally, the $G \times I \times E$ interaction had a significant effect on average capsule weight, raw yield, and lint yield. The $M \times I$ interaction was significant for only seed yield.

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Table 3.4 - ANOVA for main effects and interactions on plant height, first fruiting branch eight, average number of open capsules per hectare, raw yield, lint yield and seed yield.

Source of Variation	df	Parameters						
		Plant Height	First Fruiting Branch Eight	Number of Capsule ha ⁻¹ × 10 ⁻⁶	Average Capsule Weight	Raw Yield	Lint Yield	Seed Yield
G	1	0.01 ns	12.17 ***	1.16 ns	10.77 **	22.77 ***	41.18 ***	6.75 *
M	1	54.91 ***	2.3 ns	27.15 ***	18 ***	46.35 ***	42.9 ***	29.98 ***
I	2	591.55 ***	74.6 ***	52.4 ***	205.68 ***	612.07 ***	533.75 ***	418.65 ***
S	1	5.81 *	425.62 ***	5.44 *	6.34 *	10.53 **	3.25 ns	35.48 ***
G × M	1	0.06 ns	12.92 ***	2.05 ns	0.04 ns	0.04 ns	0.24 ns	0 ns
G × I	2	5.91 **	3.49 *	7.66 ***	1.63 ns	6.01 **	10.39 ***	1.95 ns
G × S	1	0.05 ns	8.78 **	2.56 ns	7.42 **	0.03 ns	1.91 ns	1.46 ns
M × I	2	0.42 ns	2.57 ns	1.24 ns	1.26 ns	3.94 *	2.21 ns	3.56 *
M × S	1	25.97 ***	5.42 *	13.57 ***	0.07 ns	0.02 ns	1.86 ns	1.22 ns
I × S	2	28.16 ***	10.24 ***	7.09 **	9.71 ***	1.8 ns	1.37 ns	2.64 ns
G × M × I	2	15.6 ***	3.88 *	3.06 ns	5.82 **	1.02 ns	2.34 ns	0.39 ns
G × M × S	1	8.93 **	12.04 ***	3.89 ns	0.07 ns	0.42 ns	0.48 ns	0.22 ns
G × I × S	2	3.29 *	9.45 ***	9.16 ***	4.92*	6.27**	11.86 ***	1.98 ns
M × I × S	2	2.15 ns	1.46 ns	9.3 ***	2.41 ns	1.8 ns	0.32 ns	3.09 ns
G × M × I × S	2	4.89 *	5.74 **	3.74 *	2.49 ns	0.16 ns	1 ns	0.34 ns

Values are given as F of Fisher. ***, ** and * indicate significant at $p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively. ns = not significant; df = Degrees of Freedom; I = Irrigation; G = Genotype; S = Site; M = Mycorrhizae.

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The $G \times M \times I \times S$ interaction had a significant effect ($p < 0.05$) on plant height, the height of the first fruiting branch, and the average number of open capsules per hectare. As shown in Figure 2, the tallest plants were observed in Site 1, particularly for Armonia under I-100 condition reached heights of 77.03 in +AMF and 77.83 cm in -AMF. Additionally, mycorrhized ST-318 under I-100 at Site 1 recorded an average height of 80.97 cm ($p < 0.05$).

The shortest plants were also found in Site 1, specifically in -AMF treatments. In particular, Armonia under I-70 and I-30 recorded heights of 58.3 cm and 59.1 cm, respectively, while ST-318 under I-30 reached 57.4 cm.

Regarding the first fruiting branch height, the $G \times M \times I \times S$ interaction had a significant effect ($p < 0.01$). The highest values were recorded in Site 1 for the ST-318 variety under I-100 +AMF, with an average of 42.33 cm, while the lowest values were observed in Site 2 for the Armonia variety under I-30 irrigation +AMF, showing a 47.77% reduction (Figure 3.2).

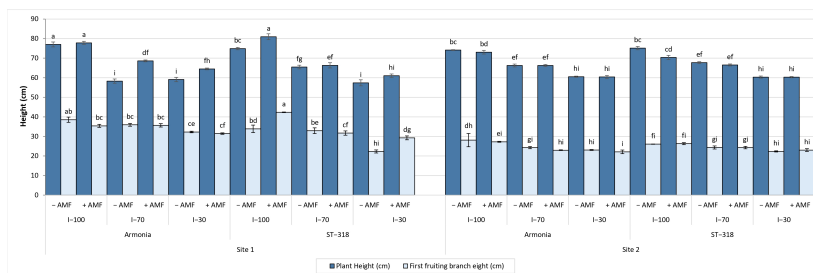


Figure 3.2 - Effect of treatments on plant height and the first fruiting branch. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-

318), irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), and mycorrhization treatment (+AMF = Mycorrhized; -AMF = Non-mycorrhized). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at $p \leq 0.05$.

Figure 3.3A illustrates the statistically significant effect ($p < 0.05$) of the $G \times M \times I \times S$ interaction on the number of capsules per hectare. The highest values were recorded at Site 1 under I-100 +AMF, with a mean of 10.13×10^5 capsules ha^{-1} , while the lowest values were observed at Site 2 under I-30 +AMF, with 3.06×10^5 capsules ha^{-1} .

The $G \times I \times S$ interaction had a significant effect on average capsule weight, raw yield, and lint yield.

Regarding average capsule weight, the highest statistically significant values ($p < 0.05$) were observed in the treatments under I-100 in both Site 1 and Site 2, with a mean of 4.0 g per capsule (Figure 3.3B). Similarly, the Armonia variety under I-70 in Site 2 showed values (3.9 g) that were not statistically different from the I-100 treatments. In contrast, the treatments under I-30 in both sites exhibited significantly lower values ($p < 0.01$) compared to the fully irrigated treatments, with an average of 2.67 g, representing a 33.1% reduction.

The $M \times I \times G$ interaction also had a significant effect on average capsule weight ($p < 0.01$). Both genotypes, Armonia and ST318, under the I100 +AMF treatment showed the highest values, each with an average capsule weight of 4.15 g. The lowest value was recorded for genotype ST318 under the I30 +AMF treatment, with an average capsule weight of 2.50 g (Figure 3.3C).

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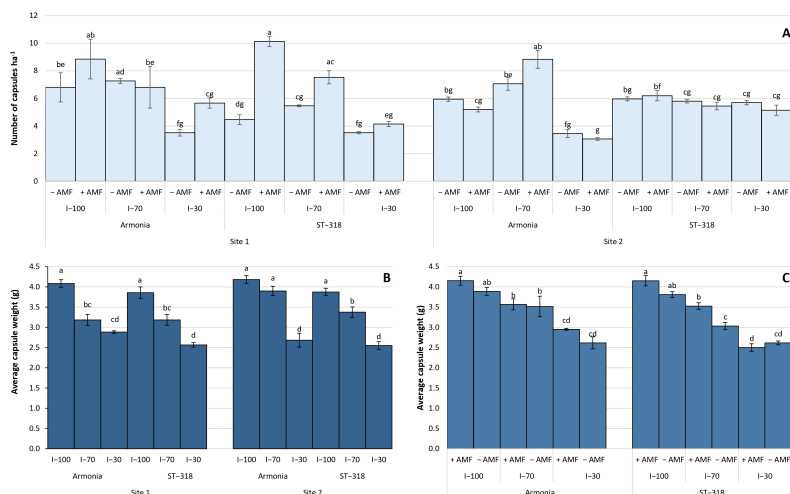


Figure 3.3 - Effect of treatments on capsule production and weight. (A) Effect of M, I, G, S on capsule number per hectare. (B) Effect of I, G, S on average capsule weight. (C) Effect of M, I, G on average capsule weight. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318), irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), and mycorrhization treatment (+AMF = Mycorrhized; -AMF = Non-mycorrhized). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at $p \leq 0.05$.

As shown in Figure 3.4A, the highest raw yield values were recorded in treatments under I-100 irrigation ($p < 0.01$). At Site 1, the ST-318 and Armonia varieties achieved average yields of 2.48 t ha⁻¹ and 2.33 t ha⁻¹, respectively. At Site 2, the highest yields were observed for Armonia and ST-318, with average values of 2.33 t ha⁻¹ and 2.48 t ha⁻¹, respectively. In contrast, the lowest yields were

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recorded under I-30 irrigation. At Site 1, the Armonia and ST-318 varieties had average yields of 2.51 t ha^{-1} and 2.26 t ha^{-1} , respectively. Regarding lint yield, as shown in Figure 3.4A, the highest values were recorded at Site 2 for the Armonia variety under I-100 irrigation, with an average yield of 1.12 t ha^{-1} ($p < 0.01$). The lowest values were observed in treatments subjected to severe water stress (I-30) at both sites, with an average yield reduction of 59.62% compared to I-100 treatments.

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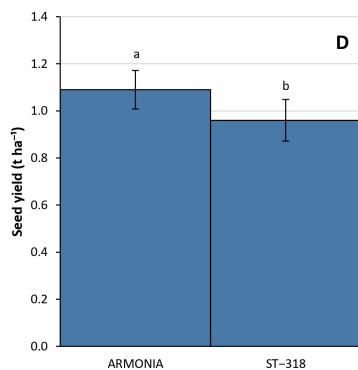
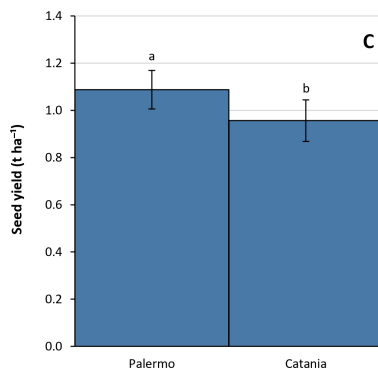
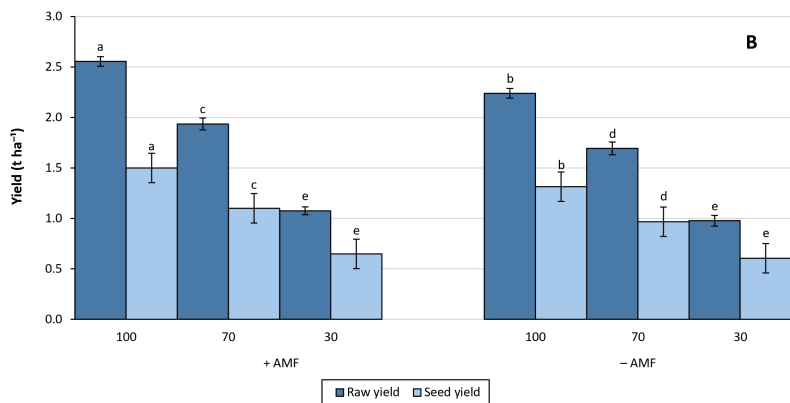
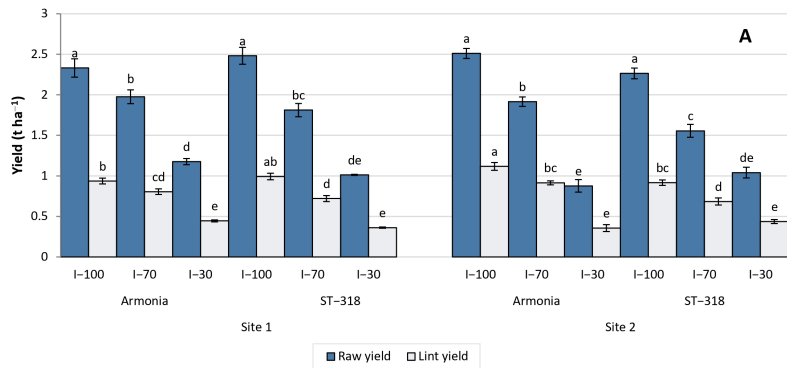


Figure 3.4 - Effect of treatments on yield components. (A) Effect of I, G and S treatments on raw and lint yield. (B) Effect of I and M on raw yield and seed yield. (C) Effect of S on seed yield. (D) Effect of G on seed yield. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318), irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), and mycorrhization treatment (+AMF = Mycorrhized; -AMF = Non-mycorrhized). Each treatment was replicated three times. Different letters indicate statistically significant differences between means at $p \leq 0.05$.

The $M \times I$ interaction had a significant effect on seed yield ($p < 0.05$), as shown in Figure 3.4B. The I-100 +AMF treatment resulted in the highest seed yield, averaging 1.50 t ha^{-1} . In contrast, treatments under I-30 showed significantly lower yields, with values of 0.65 t ha^{-1} for +AMF and 0.60 t ha^{-1} for -AMF, representing a 55.46% reduction compared to the I-100 treatment. Moreover, the $M \times I$ interaction had a significant effect on raw yield ($p < 0.05$), as shown in Figure 3.4B. The I-100 +AMF treatment resulted in the highest raw yield, averaging 2.55 t ha^{-1} . The lowest values were observed under the I-30 treatment, both with and without AMF, with mean yields of 1.07 and 0.98 t ha^{-1} , respectively, representing a reduction of 58% and 61.6% compared to the I-100 +AMF treatment.

Seed yield was also influenced by the main factor S (Figure 3.4C) ($p < 0.001$). In Site 1, seed yield was significantly higher, reaching 1.09 t ha^{-1} , which represents an increase of 13.5% compared to Site 2 (0.96 t ha^{-1}).

The main factor G also affected seed yield ($p < 0.05$). Armonia showed the highest values, with an average of 1.05 t ha^{-1} , which is 6.1% higher than ST-318 (0.99 t ha^{-1}) (Figure 3.4D).

3.3.3. Phenological Data

Table 3.5 shows the output of the analysis of variance (ANOVA) regarding the duration in days of the phenological stages of cotton. Statistical differences, for the period sowing–emergence (S–Em) were detected only by the I factor. For the duration of the phenological phases emergence–flowering (Em–F), statistical differences were detected for the I factors, S and by the interaction of the factors $G \times I$, $G \times S$ and $I \times S$. For the duration of the phenological phases flowering–capsule opening (F–Oc), statistically significant differences were found for the factors I and S and by the interaction $G \times I$, $G \times S$, $I \times S$ and $G \times I \times S$. Finally, for the duration of the phenological phases capsule opening–flowering (Oc–H), statistical differences were detected for the factors G, M, I, S and in the interactions $G \times I$, $I \times S$, $G \times M \times S$ and $G \times I \times S$.

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Table 3.5 - ANOVA for main effects and interactions on the duration of cotton phenological stages.

Source of Variation	Parameters				
	df	S-Em	Em-F	F-Oc	Oc-H
G	1	0.27 n.s.	0.39 n.s.	3.88 n.s.	8.57 **
M	1	0.12 n.s.	0.96 n.s.	1.86 n.s.	14.56 ***
I	2	4.83 *	164.94 ***	54.57 ***	237.74 ***
S	1	0.76 n.s.	38.81 ***	28.12 ***	11.98 **
G × M	1	0.76 n.s.	0.07 n.s.	1.12 n.s.	0.07 n.s.
G × I	2	0.48 n.s.	8.75 **	8.92 **	31.25 ***
G × S	1	0 n.s.	51 ***	14.8 ***	1.77 n.s.
M × I	2	1.64 n.s.	0.49 n.s.	0.28 n.s.	1.28 n.s.
M × S	1	0.27 n.s.	1.14 n.s.	0.4 n.s.	0.95 n.s.
I × S	2	0.73 n.s.	3.35 *	130.18 ***	51.05 ***
G × M × I	2	0.33 n.s.	0.29 n.s.	0.71 n.s.	0.5 n.s.
G × M × S	1	3.03 n.s.	0.03 n.s.	0.452 n.s.	4.17 *
G × I × S	2	1.3 n.s.	1.31 n.s.	7.8 **	72.31 ***
M × I × S	2	2.07 n.s.	1.43 n.s.	0.02 n.s.	0.29 n.s.
G × M × I × S	2	0.14 n.s.	0.436 n.s.	0.29 n.s.	1.43 n.s.

Values are given as F of Fisher. ***, ** and * indicate significant at $p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively; Degree of freedom = df. ns = not significant. S-Em = sowing-emergence; Em-F = emergence-flowering; F-Oc = flowering-opening capsules; Oc-H = opening capsules-harvesting. I = Irrigation; G = Genotype; S= Site; M = Mycorrhizae.

The main effect I was significant ($p < 0.05$) for the S–Em phenological phase (Table 3.5). Significantly shorter durations were observed under the I-100 regime, with a mean of 18.75 days, while treatments subjected to I-30 and I-70 required on average 17.63 and 17.75 days, respectively, to complete this phenological phase (Figure 3.5).

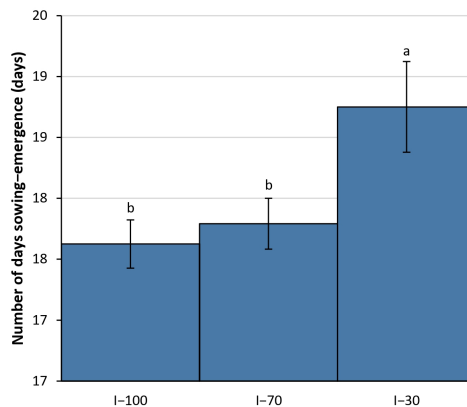


Figure 3.5 - Effect of I on the length of phenological S–Em phase. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey’ s test. Each treatment was replicated three times. Error bars represent standard error. Irrigation levels (I-100 = 100% ETc; I-70 =70% ETc; I-30 = 30% ETc).

During the Em–F phenological phase, the interaction between I $\times$ S was significant ($p < 0.05$) (Table 3.5). Under the I-30 regime, both sites showed a marked reduction in duration, averaging 38.58 days in Site 2 and 41.5 days in Site 1. Conversely, the I-100 treatment led to significantly longer phases, with means of 55.16 days in Site 1 (+32.9% vs. I-30) and 52.58 days in Site 2 (+26.6% vs. I-30) (Figure

3.6A).

The $G \times I$ interaction was also significant ($p < 0.01$) for the Em–F phenological phase. Significantly shorter durations were observed for Armonia under the I-70 regime, with a mean of 38.4 days. On the other hand, significantly longer durations were recorded under the I-100 regime for both cultivars, with means of 55.2 days for Armonia and 52.5 days for ST-318 (Figure 3.6B).

The $G \times S$ interaction was likewise significant ($p < 0.001$) for the Em–F phenological phase. ST-318, in Site 1, showed the shortest duration, with 42.9 days, while Armonia in Site 2 recorded the longest duration to complete this phenological phase, with 51.3 days, representing a 19.6% increase compared to ST-318 within the same site (Figure 3.6C).

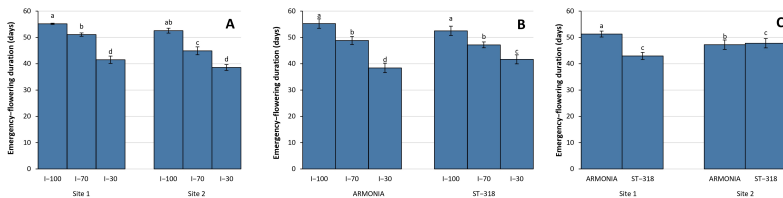


Figure 3.6 - Effect of treatments on the duration of phenological Em–F phase. (A) Effect of I and S on the length of phenological Em–F. (B) Effect of I and G on the length of phenological Em–F. (C) Effect of G and S on the length of phenological Em–F. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Each treatment was replicated three times. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318) and irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc).

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The $G \times I \times S$ interaction was significant ($p < 0.01$) for the F–Oc phenological phase (Table 5). Significantly shorter durations were observed for the Armonia in Site 2 under the I-70 regime, with a mean of 36 days. In contrast, longer durations were recorded for the ST-318 variety under I-100 in the same site (53.83 days) and for Armonia under I-70 in Site 1 (67.50 days) (Figure 3.7).

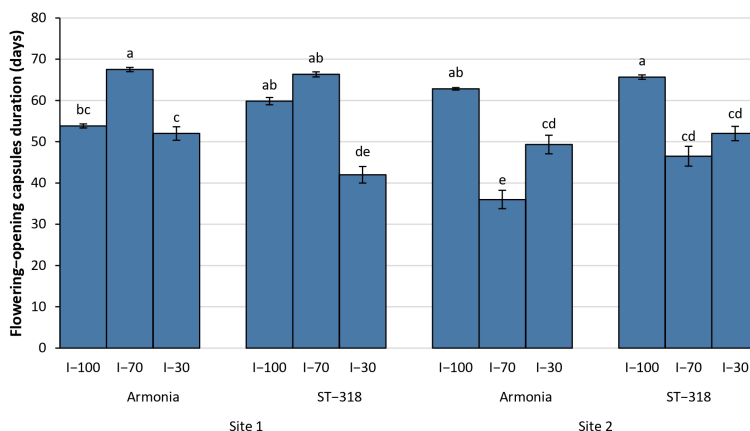


Figure 3.7 - Effect of I, G and S on the duration of the Oc–H phenological phase. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Each treatment was replicated three times. Error bars represent standard error. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318) and irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc).

The $S \times G \times I$ interaction had a significant effect ($p < 0.001$) on the Oc–H phase (Table 5). Significantly shorter durations were observed in Site 2 under I-30 for ST-318 (11.8 days) and Armonia (14.0 days). Similarly, in Site 1, the Armonia variety under I-30

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exhibited the shortest mean duration, averaging 11.5 days (Figure 3.8A).

The M factor also had a significant effect ($p < 0.001$) on the Oc–H phenological phase (Table 3.5). Plots under +AMF showed significantly longer durations compared to –AMF, with an increase of 6.7% (19.2 days vs. 18 days) (Figure 3.8B).

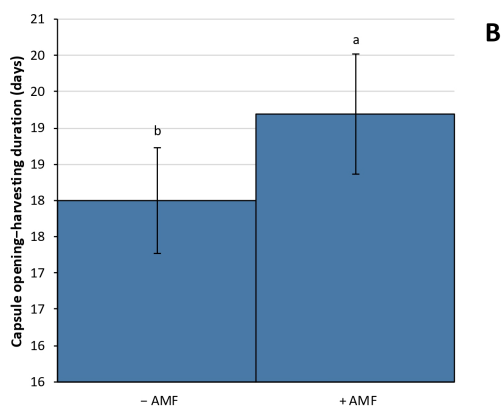
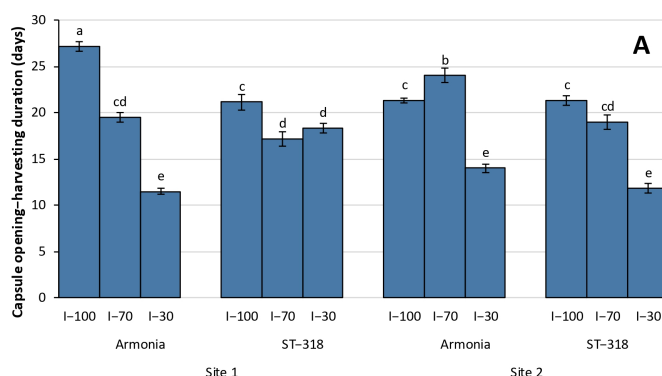


Figure 3.8 - Effect of treatments on the duration of the F–Oc phenological phase. (A) Effect of S, G and I on the duration of the Oc–H phenological phase. (B) Effect of M on the duration of the Oc–H phenological phase. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Each treatment was replicated three times. Error bars represent standard error. Irrigation levels (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), genotype (Armonia and ST-318) and mycorrhization treatment (+AMF = Mycorrhized; –AMF = Non-mycorrhized).

Figure 3.9 shows the accumulation of GDD across different phenological phases of cotton in the two experimental sites (Site 1 and 2) for the genotypes Armonia and ST-318, under different irrigation regimes (I-100, I-70, I-30). GDD accumulation was highest during the intermediate phases, such as Em–F and F–Oc. A reduction in accumulated GDD was observed under lower water availability (I-30 vs. I-100). Differences in thermal requirements were recorded between the two genotypes, with ST-318 generally requiring more GDD than Armonia in certain phases (e.g., Em–F phases). Variations in GDD accumulation were also detected between Site 1 and Site 2.

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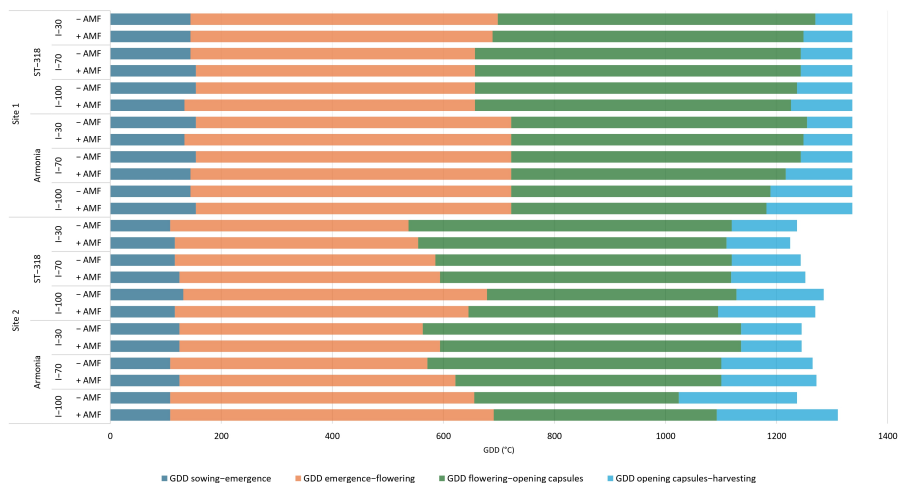


Figure 3.9 - Accumulation of Growing Degree Days (GDD) across different phenological phases of cotton. Site (Site 1 = Campo Carboj and Site 2 = Primosole), genotype (Armonia and ST-318), (I-100 = 100% ETc; I-70 = 70% ETc; I-30 = 30% ETc), and mycorrhization treatment (+AMF = Mycorrhized; -AMF = Non-mycorrhized).

3.3.4. Crop Water Productivity

Table 3.6 summarizes the IWP for seed yield and lint yield under different irrigation treatments at two experimental sites. For seed yield, the IWP was recorded under the I-100 treatment, with the highest value observed at Site 1 (1.43 kg m^{-3}). The lowest IWP value for seed yield was recorded at Site 2 under I-30, with a value of 0.52 kg m^{-3} . A similar trend was observed for IWP calculated based on lint yield, where Site 1 under I-100, recorded a higher value in IWP of 37.7% more than Site 1 under I-100. The lowest IWP value based on lint yield was recorded at Site 2 I-30, with a value of 0.37 kg m^{-3} .

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Table 3.6 - IWP of seed and lint yield under different irrigation treatments.

Site	Irrigation level	Water Supplied (m3 ha ⁻¹)	IWP (kg m ⁻³)	
			Seed Yield	Lint Yield
Site 1	I-30	741.5	0.93	0.54
	I-70	924.4	1.22	0.83
	I-100	1010.5	1.43	0.95
Site 2	I-30	1203.9	0.52	0.37
	I-70	1308.9	0.72	0.61
	I-100	1477.8	0.93	0.69

I-100 = 100% ET_c; I-70 = 70% ET_c; I-30 = 30% ET_c. Site 1 = Campo Carboj and Site 2 = Primosole.

3.4 Discussion

This study focused on reintroducing cotton cultivation in Sicily through sustainable practices to meet the growing demand for organic and “Made in Italy” cotton. For this reason, an integrated approach was developed, combining organic farming principles, efficient irrigation systems, and mycorrhizal formulations. The goal was to improve cotton yield while ensuring the sustainability of water and soil resources. The results show how the effects of E, I, M and G affect cotton growth, first fertile branch intersection, yield and phenological development, providing valuable insights for sustainable cotton production in Mediterranean environments.

3.4.1. Irrigation and Water Productivity

Despite receiving lower precipitation compared to Site 2, Site 1 required 38.4% less total water. This outcome is primarily attributable

to the soil characteristics of Site 1. In fact, it has a higher organic matter content, which increases water retention (Zhao et al. 2024), and a predominantly sandy texture that facilitates deeper root penetration, allowing plants to access water from greater depths (Guo-Zheng et al. 2013; Wang et al. 2024).

In contrast, Site 2 is characterized by a heavy soil with a high clay content. Under drought conditions, such soils tend to form a hardpan, reducing infiltration and causing rapid water loss (St Aime et al. 2021); without enough irrigation, this condition exposes plants to water stress, thereby necessitating more frequent and abundant water applications.

During BBCH 10–50 supplemental irrigation was applied to I-70 and I-100 based on the ET_c values. Notably, the total water supplied in this study was significantly lower than literature values for similar pedoclimatic conditions (7000 m³ ha⁻¹–12,000 m³ ha⁻¹ per growing season; Vitale et al. 2024)). This reduction is largely due to the use of drip irrigation, which achieves efficiencies close to 90%, compared to common cotton irrigation systems such as furrow and sprinkler methods, whose efficiencies are typically around 65% and 75%, respectively (Bryant et al. 2021; Chauhdary et al. 2023). Finally, both sites demonstrate good potential for the reintroduction of cotton in terms of water consumption, as their total water use remains well below that reported for similar pedoclimatic conditions. However, among the two, Site 1 has proven to be the most suitable.

This study also showed that IWP is affected by both site and irrigation level. Specifically, as water supply increases, the IWP for both seed yield and lint yield increases as well. This happens because higher irrigation promotes plant growth and increases leaf area index (LAI) and dry matter accumulation by improving root water uptake and transport functions, as well as deep soil moisture and nutrient

utilization (Bai et al. 2024). Site also significantly influence IWP; Site 1 showed higher values, likely because sandy soils promote deep root development, reducing irrigation needs (R. Guo et al. 2023; Wang et al. 2021a).

3.4.2 Agronomic and Yield Performance

Water stress is well-documented to suppress cotton vegetative growth by limiting cell expansion and photosynthesis [56–58]. In Site 1, the tallest plants were observed under I-100 +AMF for both cultivars, as well as in Armonia I-100 –AMF, where irrigation alone promoted growth, suggesting a threshold effect under optimal water conditions. In contrast, I-30 led to a ~25–30% reduction in plant height.

Mycorrhization significantly increased plant height. As shown by Ortas & Iqbal (Ortas and Iqbal 2019), inoculation with *Funneliformis mosseae* enhances root exploration and nutrient uptake, boosting phosphorus acquisition in cotton by up to 110%. This effect is largely driven by AMF-induced modulation of phytohormones—particularly gibberellins—which stimulate stem cell division and elongation (Evelin et al. 2019; Jajoo and Mathur 2021). AMF also influence other hormones (ethylene, abscisic acid, cytokinins, salicylic acid, jasmonic acid, and auxins), improving water status under drought by enhancing hydraulic conductivity (Dhiman et al. 2022; Jajoo and Mathur 2021).

The mycorrhizal effect was observed across all treatments in Site 1, except for Armonia I-100 –AMF and Armonia I-100 +AMF, where plant height was primarily driven by irrigation. Conversely, in Site 2, soil characteristics may have compromised mycorrhizae colonization and nutrient uptake (Mai et al. 2018; Smith et al. 2003);

however, neither colonization rate nor nutrient uptake was directly measured in this experiment.

The height of the first fruiting branch is a key indicator of earliness in cotton, reflecting the plant's ability to transition from vegetative to reproductive growth (Chen et al. 2024). Higher branch heights correlate with earlier phenological development, enabling earlier harvest—a critical trait in Mediterranean climates like Sicily, where autumn rains threaten cotton quality (Istipliler et al. 2024; Melillo et al. 2016). The tallest first-fruiting branches were observed for ST318 under I-100 +AMF in Site 1, aligning with findings that AMF inoculation, particularly with *Rhizoglyphus irregularis*, accelerates reproductive development under optimal irrigation (Gao et al. 2020).

However, under I-30 and particularly in Site 2, AMF efficacy was limited by clay-rich soils and low fertility (Mai et al. 2018; Smith et al. 2003). Cultivar choice also played a crucial role in determining the first-fruiting branch height. Some authors highlighted ST318's ability to thrive under optimal water availability (Moussouraki et al. 2019), whereas Armonia, exhibited greater stability in water-limited environments (Filintas et al. 2022b).

The number of capsules ha^{-1} was highest under I-100, particularly for the ST-318 cultivar +AMF in Site 1. This is consistent with studies showing that number of capsules ha^{-1} is a key indicator of reproductive efficiency and yield potential in cotton, reflecting proper carbohydrate translocation to developing bolls in response to water availability (Gao et al. 2021; Q. Zhang et al. 2023).

Moreover, the application of certain AMF species, may have contributed to improved boll retention and capsule development due to their ability to enhance P uptake and overall plant nutrient status (Li 2024; Ortas and Iqbal 2019).

Genotypic differences also influenced responses: ST-318 maintained higher capsule numbers even under suboptimal conditions, highlighting the importance of genotype-specific stress tolerance.

Average capsule weight reflects water status and the efficiency of resource allocation during boll development (Xu et al. 2021; Yang et al. 2024). This parameter was higher in both sites under the I-100 treatment, as adequate water supply under full irrigation supports optimal boll filling and fiber development, leading to increased capsule weight (Hunsaker et al. 2015). In contrast, I-30 reduced capsule weight, likely due to limited water availability restricting nutrient translocation and boll maturation (Hou et al. 2024). An interesting trend was observed with the Armonia cultivar, particularly under the I-70 treatment, where no significant differences were found compared to I-100. Therefore, genotypic variation was evident: Armonia not only showed the highest values under optimal conditions but also maintained stable weights under water stress, likely due to traits such as osmotic adjustment or improved resource partitioning, as highlighted by several authors (Filintas et al. 2022b; Moussouraki et al. 2019).

Raw yield and lint yield responded positively to I, G, and S. Optimal water availability led to higher yields, in line with recent findings by Zhangjin et al. (Zhangjin et al. 2025), which demonstrated that an adequate water supply enhances boll retention, carbohydrate partitioning, and fiber development, ultimately increasing yield. In contrast, water stress, particularly under severe deficit irrigation, reduces both raw and lint yield by limiting cell expansion, restricting carbohydrate translocation, and increasing boll abortion (Herritt, Thompson, and Thorp 2022; Hou et al. 2024).

A particularly noteworthy result is the yield obtained under the I-70 regime, which, despite a physiological reduction in yield,

remained economically viable. Specifically, raw yield decreased by 24.3% and lint yield by 21.1% compared to the I-100 treatment. However, a formal economic analysis comparing yield value and irrigation costs across regimes would be required to confirm this.

These findings highlight the strong adaptability of cotton, particularly the cultivars Armonia and ST-318, which maintained productivity and profitability even with a 30% reduction in water supply. This aspect is crucial, especially in regions severely affected by water scarcity, as also evidenced in Figure 1, where total rainfall during the entire vegetative period amounted to only 68.2 mm in Site 1 and 67.3 mm in Site 2.

Seed yield was significantly influenced by the $M \times I$ interaction. This parameter was primarily affected by M, as evidenced by the I-100 and I-70 treatments, which showed increases in seed yield of 14.1% and 13.7%, respectively, compared to the –AMF treatments. AMF boosts seed yield by elevating phosphorus-dependent ATP synthesis, fueling energy-intensive seed development (Ferrol et al. 2002). Additionally, they promote carbon allocation to reproductive sinks via upregulated sucrose transporter genes in mycorrhizal roots (Salmeron-santiago et al. 2021).

However, in the I-30 treatment such improvements were not observed. As documented by Madouh & Quoreshi (Madouh and Quoreshi 2023) and Pathan et al. (Pathan et al. 2023), under strong water stress, AMF encounter considerable difficulties in both colonization and executing their symbiotic functions, thereby reducing or even nullifying their benefits.

3.4.3 Phenological Performance

Deficit irrigation significantly reduced the S-Em stage at both

experimental sites. This result is in line with studies indicating that water stress reduces germination and early establishment of cotton seedlings (J. Ma et al. 2024). However, in Site 2, the I-70 treatment resulted in faster emergence compared to Site 1, likely due to differences in soil texture and thermal conditions. The higher clay content in Site 2 (55.6% vs. 28% in Site 1; Table 1) likely contributed to better soil warming, improved seed-soil contact, and greater water retention, accelerating germination (Mauget et al. 2021; Vories et al. 2007). It is important to highlight that a shorter emergence phase is critical for cotton establishment, as prolonged exposure to suboptimal soil moisture increases the number of failures (Raphael et al. 2017). The faster emergence observed at site 2 under I-70 suggests that clay soils in warmer environments may help mitigate some of the risks associated with deficit irrigation during the early growth stages.

The duration of the F–Oc phase is strongly influenced by the $S \times G \times I$ interaction, reflecting complex trade-offs between boll development and yield outcomes. It is well documented that shorter F–Oc phases often correlate with an increased number of bolls but may compromise yield due to insufficient time for fiber accumulation and boll filling (Bange et al. 2022; Luo, Bange, and Clancy 2014). In this study, the I-100 treatments in both sites exhibited the longest F–Oc durations, maximizing boll development and yield potential. Notably, the I-70 treatment in Site 1 also extended this phase, likely due to its higher organic matter and nitrogen content (Tian et al. 2024). Conversely, the nutrient-poor soil of Site 2 resulted in a significantly shorter F–Oc period.

The Oc–H phase was significantly influenced by the $S \times G \times I$ interaction. A shorter duration of this phenological phase allows for earlier harvesting, reducing the risk of exposure to autumn rainfall events, which frequently affect southern Italy and can compromise

cotton fiber quality (Istipliler et al. 2024; Melillo et al. 2016). In both sites, the Armonia variety under I-30 irrigation showed a marked reduction in the number of days to harvest, demonstrating its suitability for early maturation across different environmental conditions. This confirms its potential adaptability for cultivation in regions where early harvesting is advantageous. Meanwhile, the ST-318 variety exhibited a significant reduction in the Oc–H phase duration in Site 2, likely due to the sandy soil (Table 1) and thermopluviometric conditions (Figure 1) that better matched its growth requirements.

3.5. Conclusions

This study underscores the potential for revitalizing cotton cultivation in Sicily through integrated sustainable strategies. Optimized irrigation practices significantly enhanced yields, while moderate water reductions maintained viable productivity, achieving 75.7% and 78.9% of raw and lint yields, respectively, compared to full irrigation. The selected Mediterranean cultivars exhibited distinct adaptive responses: Armonia demonstrated stability under water-limited conditions, whereas ST-318 thrived under optimal irrigation. The inoculation of AMF contributed to many agronomic parameters analyzed, particularly in fertile soils (Site 1). However, its benefits were less pronounced under I-30 or on poor soils (Site 2). Soil properties and environmental factors played a crucial role in phenological development, with clay-rich soils (Site 1) prolonging boll maturation and sandy soils (Site 2) promoting earlier harvest readiness.

Future research should focus on refining adaptive management strategies, optimizing irrigation regimes, and developing stress-

resilient genotypes to facilitate the reintroduction of cotton cultivation in these regions.

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Conflicts of Interest: The authors declare no conflict of interest.

Abbreviations: The following abbreviations are used in this manuscript:

AMF	Arbuscular Mycorrhizal Fungi
FC	Field Capacity
ETc	reference crop evapotranspiration
GDD	Growth degree days
I-100	100% ETc
I-70	70% ETc
I-30	30% ETc
ET0	daily reference evapotranspiration
S	site
G	genotype
I	irrigation
M	mycorrhization
+AMF	mycorrhized plants
-AMF	non-mycorrhized plants
Kc	crop coefficients

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4. *Evaluation of Pelargonic Acid as a Sustainable Defoliant in Cotton (Gossypium hirsutum L.) Production*



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Article

Evaluation of Pelargonic Acid as a Sustainable Defoliant in Cotton (*Gossypium hirsutum* L.) Production

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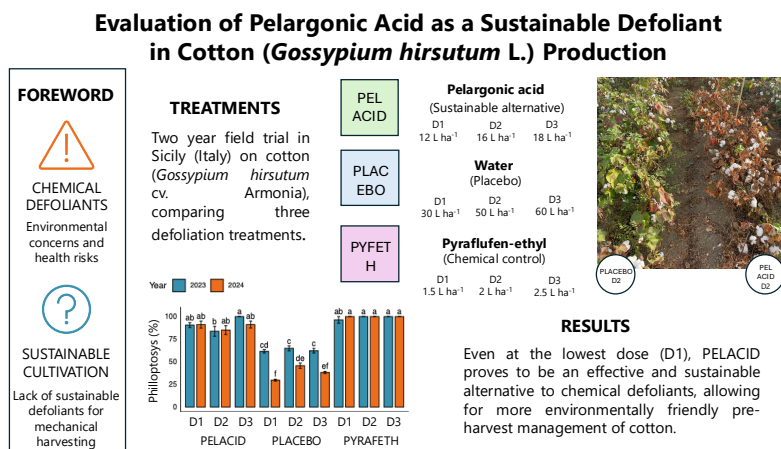
Abstract

Cotton production faces sustainability challenges due to the lack of effective sustainable defoliants for mechanical harvesting, which constrains the expansion of organic cotton (currently 0.5% of global production). In this framework, this study evaluated pelargonic acid, a rapidly biodegradable compound, as a sustainable defoliant alternative, comparing it with the synthetic pyraflufen-ethyl and a water placebo. A two-year field trial (2023–2024) in Sicily, southern Italy, tested three application rates per treatment in a randomized complete block design. Parameters assessed included defoliation efficacy, root diameter, boll number per plant, average boll weight, raw yield, lint yield, and seed yield. Results indicated significant “Year × Treatment” interaction effects on all parameters. Pelargonic acid applied at 16 L ha⁻¹ achieved the highest boll number per plant in 2024, significantly exceeding pyraflufen-ethyl at its label-recommended rate, with treatments at 12 L ha⁻¹ also producing larger root diameters than the synthetic defoliant. Pelargonic acid at 18 L ha⁻¹ in 2023 achieved complete defoliation, matching the efficacy of pyraflufen-ethyl, while the lowest pelargonic rate (12 L ha⁻¹) produced >90% leaf drop across both years. These findings position pelargonic acid as a rapidly degradable alternative to synthetic defoliants, directly addressing a key bottleneck in sustainable cotton production.

Keywords: cotton; defoliation; pelargonic acid; sustainable cultivation; pre-harvest management

4. Evaluation of Pelargonic Acid as a Sustainable Defoliant in Cotton (*Gossypium hirsutum* L.) Production

Graphical abstract



4.1 Introduction

Cotton (*Gossypium* spp.) is a globally important industrial crop, serving as the primary source of natural fiber for the textile industry (Wakelyn and Chaudhry 2009b). According to FAO (FAOSTAT n.d.), annual cotton fiber production exceeds 24.8 million tons, cultivated across more than 80 countries, with China, India, Brazil and the United States being the top producers. However, cotton cultivation faces significant challenges related to water consumption, pesticide use, and post-harvest management, necessitating the adoption of sustainable agronomic practices (Vitale et al. 2024; Vitale, Iacuzzi, Zingale, et al. 2025). One critical step in sustainable cotton production is defoliation; a pre-harvest practice aimed at removing leaves to facilitate mechanical picking and reduce trash content in the harvested fiber (Xie et al. 2025). The absence of effective organic defoliants remains a primary constraint in global organic cotton production, which constitutes merely 0.5% of total output (Wakelyn and Chaudhry

2009b). While conventional cotton production relies on synthetic defoliants to enable efficient mechanical harvesting, organic systems must depend on manual boll picking—a labor-intensive practice that significantly limits scalability (Lakshmanan et al. 2025). Traditionally, this operation is carried out through the application of chemical defoliants based on compounds such as Tribufos, Dimethipin, Pyraflufen-ethyl, and Ethephon, which are widely used (Liao et al. 2023; Neupane et al. 2023; Yu et al. 2023); some of these active substances are authorized within the European Union (e.g., pyraflufen-ethyl, ethephon), whereas thidiazuron—widely employed in other parts of the world, including the United States—was not included in Annex I of Directive 91/414/EEC (European Union Commission 1991), and authorizations for products containing this substance were withdrawn by Commission Decision 2008/296/EC (European Union Commission 2008). These substances promote leaf drop by accelerating cellular senescence (Snipes and Cathey 1992; Song et al. 2022). However, the prolonged use of these products raises concerns regarding their environmental persistence, residual phytotoxicity, and potential health risks to humans (Trakulsrichai et al. 2025; Wei et al. 2023).

In response to these issues, research is increasingly focusing on agronomic practices aimed at promoting the plant's natural senescence without resorting to chemical defoliants and without causing significant yield losses. One such approach is irrigation termination, which consists of stopping irrigation 4–5 weeks before the final harvest to stimulate natural defoliation (Buttar et al. 2007; Karam et al. 2006; Reba et al. 2014; Vitale et al. 2024).

In addition, efforts have been made to identify organic and environmentally friendly defoliants. For instance, the use of vinegar—specifically, applying 30% vinegar at a rate of 280 L ha⁻¹ before

cotton harvest—has shown some promise as an organic alternative to synthetic defoliants. However, its efficacy is lower, and it requires higher application volumes compared to conventional defoliants (Barth and Mowrer 2022). Moreover, cotton pickers and strippers often face difficulties due to incomplete defoliation, which can result in reduced lint recovery efficiency (Sk Musfiq Us Salehin 2025). Other authors have reported the use of sodium chloride (NaCl) (Lakshmanan et al. 2025) or acetic acid (Greer and Dole 2005); however, no studies have yet been conducted regarding their application on cotton. Currently, there is no universally accepted sustainable defoliant for cotton capable of delivering results comparable to synthetic products. For this reason, researchers have turned their attention to pelargonic acid, an organic compound widely used as a defoliant in other crops, such as onion (Arboleya et al. 2005) and curly willow, where defoliation is necessary for the commercial quality of the product (Greer and Dole 2005).

Pelargonic acid ($\text{CH}_3(\text{CH}_2)_7 \text{COOH}$) is a saturated fatty acid composed of nine carbon atoms (European Food Safety Authority 2013). It was first isolated from the leaves of *Pelargonium roseum* Willd and can also be found in various vegetables and fruits (such as grape, orange, apple, and potato), as well as in milk, cheese, and beef (Ciriminna et al. 2019; Torres-Pagán et al. 2024). This compound functions as a contact, non-selective, non-residual foliar bioherbicide that disrupts plant cells and rapidly desiccates green tissues, with degradation in the soil occurring in less than two days (Soltani, Willemse, and Sikkema 2024).

Although there is considerable data on the dosage of pelargonic acid as an herbicide (Ciriminna et al. 2019; López-González et al. 2024a; Muñoz et al. 2022), information on its use as a desiccant remains scarce. Greer et al. (Greer and Dole 2005) reported that 1000

to 1500 mg·L⁻¹ per plant of pelargonic acid is required to defoliate curly willow (*Salix matsudana* L.), where defoliation is necessary for commercial purposes. Other authors have observed that an application rate of 3400 g ha⁻¹ of pelargonic acid caused up to 98% desiccation in white beans (*Phaseolus vulgaris* L.) (Soltani et al. 2024).

However, no data are currently available regarding its use as a defoliant on cotton. For this reason, the study will be structured around two key objectives:

1. Evaluation of the defoliation efficacy of pelargonic acid at different concentrations, comparing its performance to that of pyraflufen-ethyl, a widely used defoliant in the cotton industry and authorized within the European Union (Brancato et al. 2018; Xie et al. 2025), and to a placebo treatment (water).
2. Analysis of physiological and yield-related parameters in response to the treatments.

The results could open new perspectives for the adoption of a rapid-acting, biodegradable, low-toxicity organic defoliant, thereby contributing to the transition toward more sustainable cotton production.

4.2 Materials and Methods

4.2.1 Experimental Site and Weather Data

The experimental trial was conducted at the experimental farm of the University of Catania, located in Primosole (Catania, Sicily, Italy) (37°24'N, 15°03'E; 10 m a.s.l.), during the 2023 and 2024 growing seasons. The chemical and physical characteristics of the soil, which is sub-alkaline clay, are presented in Table 4.1.

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Table 4. 1 – Soil Characteristics of the upper layer (0–50 cm) of the field site.

Soil Characteristics	Unit	Value	Method
Sand	%	16.6	(Lotti 2023)
Loam	%	27.8	(Lotti 2023)
Clay	%	55.6	(Lotti 2023)
N total	g kg ⁻¹	1	Kjeldahl (Kjeldahl 1883)
P	mg kg ⁻¹	2.18	Ferrari (Helrich 1990)
K	mg kg ⁻¹	203.3	Dirks and Scheffer (Helrich 1990)
Organic matter	%	1.1	Walkley and Black (Helrich 1990)
Electrical Conductivity	mS/m	15	(Dane et al. 2018)
Cation Exchange Capacity (CEC)	cmolc kg ⁻¹	14.8	(Sumner and Miller 2018)
pH		7.6	In water solution
Bulk density	kg m ³	1200	(Blake and Hartge 2018)
Field capacity at -0.03 MPa	%	27	(Klute 2018)
Wilting point at -1.5 MPa	%	11	(Flint and Flint 2018)

The local climate is typically Mediterranean, with mild winters and hot, dry summers. Meteorological data on rainfall and air temperature were obtained from stations operated by the Sicilian Agrometeorological Information Service, located in proximity to the experimental site. The stations were equipped with MTX dataloggers (model WST1800) (Campogalliano, Italy) and a range of climatic sensors. Temperature data were recorded using an MTX TAM platinum PT100 heat-resistant sensor with a radiation shield (Campogalliano, Italy), while precipitation was measured using an MTX PPR tipping bucket rain gauge (Campogalliano, Italy). These instruments provided daily records of maximum and minimum air temperatures (°C) and total daily rainfall (mm). Reference evapotranspiration (ET₀), expressed as the monthly sum of daily values, was calculated according to the FAO Irrigation and Drainage

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Paper No. 56 (Allen and Pereira 1998). The thermo-pluviometric data are shown in Figure 4.1.

A comparison of the two years showed that the summers were extremely hot, with temperature peaks exceeding 45 °C. Precipitation during the period from 1 April to 30 November 30th decreased by approximately 21.8% in 2024 (147 mm) compared to 2023 (188 mm), with a particularly sharp decline observed in May and June.

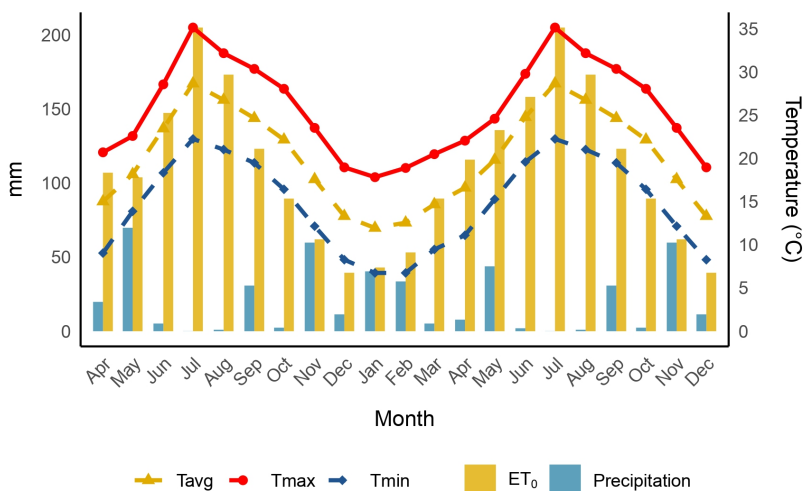


Figure 4. 1 - Temperature and precipitation trends at the experimental farm of the University of Catania (Catania, 37°24' N, 15°03' E, 10 m a.s.l.) from April 2023 to December 2024. Tmax = month average of daily maximum temperature, Tavg = month average of daily mean temperature, Tmin = month average of daily minimum temperature, ET₀ = month sum of daily reference evapotranspiration (Allen and Pereira 1998), Precipitation = monthly rainfall.

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4.2.2 Experimental Design and Treatment

The field experiments, repeated over two years (2023 and 2024), involved the comparison of three defoliants at different doses (Table 2):

- PELACID: Pure pelargonic acid (Beloukha®, Belchim, Saronno (VA), Italy)
- PLACEBO: A water-based placebo solution
- PYRAFETH: Pyraflufen-ethyl (Revolution®, Sipcam, Milan, Italy), a commonly used commercial defoliant in conventional cotton farming.

Table 4. 2 - Defoliants used for the research.

	Active Ingredient	Rate (L ha ⁻¹)
PELACID D1	Pure pelargonic acid 680 g L ⁻¹	12
PELACID D2	Pure pelargonic acid 680 g L ⁻¹	16*
PELACID D3	Pure pelargonic acid 680 g L ⁻¹	18
PLACEBO D1	Water	30**
PLACEBO D2	Water	50
PLACEBO D3	Water	60
PYRAFETH D1	Pyraflufen-ethyl 10.6 g L ⁻¹	1.5
PYRAFETH D2	Pyraflufen-ethyl 10.6 g L ⁻¹	2
PYRAFETH D3	Pyraflufen-ethyl 10.6 g L ⁻¹	2.5**

* Label-recommended dose for pre-harvest desiccation of soybean [*Glycine max* (L.) Merrill]

** Doses of PLACEBO treatments are expressed as volumes of carrier water

*** Label-recommended dose for pre-harvest desiccation of cotton

For each year, the experimental trials were arranged in a

randomized complete block design with three replications. Each plot measured 16 m² (4 m × 4 m).

Defoliantes were applied once 80% of the cotton bolls were open (BBCH 88). Application was performed using a handheld sprayer with the nozzle fully open. For both PELACID and PYRAFETH treatments, three carrier water volumes were used according to the treatment dose: 30 L ha⁻¹ for D1, 50 L ha⁻¹ for D2, and 60 L ha⁻¹ for D3. To ensure proper experimental control, three PLACEBO (water-only) treatments were included, corresponding to these carrier volumes. Defoliant treatments were applied 20 October 2023, at 144 days after sowing (DAS), and 9 October 2024 (138 DAS), respectively. Manual harvesting was carried out 21 days after defoliant treatments, on 10 November 2023, and 30 October 2024.

4.2.3 Crop Management and Irrigation

The seedbed was prepared after the winter rains, in soil that had undergone a fallow period the previous year. The soil was ploughed to a depth of 35 cm and tilled twice before sowing.

The genotype used was Armonia, a *Gossypium hirsutum* L. variety widely cultivated in the Mediterranean basin (Filintas et al. 2022a; Moussouraki et al. 2019; Vitale, Iacuzzi, Tortorici, et al. 2025). Sowing was carried out on May 29, 2023, and May 24, 2024, during the first and second growing seasons, respectively. A plant density of 12 plants m⁻² (0.08 m × 1.0 m) was adopted. Weed control in the inter-row areas was performed using a mower during the leaf development stage.

Fertilization was carried out using organic fertilizers. A basal fertilization was applied before sowing, consisting of 110 kg N ha⁻¹, 55 kg P₂O₅ ha⁻¹, and 55 kg K₂O ha⁻¹, supplied as an organic fertilizer (10 N, 5% P₂O₅, 5% K₂O; ORGA KEM[®], Biolchim S.p.A., Medicina

(BO), Italy). Additionally, a liquid fertilizer containing 8.7% N (Tamarack, Growan Italia®, Faenza (RA), Italy) was applied at a total rate of 40 kg N ha⁻¹, during the phenological stages corresponding to the appearance of the first true leaves (BBCH 11) and flowering (BBCH 51). No pest or disease control measures were applied during the trials, as no phytosanitary issues were observed.

Harvesting was conducted manually when approximately 90% of the bolls had opened (BBCH 89), specifically on 10 November 2023, and 30 October 2024.

Irrigation was scheduled when the cumulative daily crop evapotranspiration (ET_m) exceeded 70% of the available soil water content at field capacity (123.2 mm).

Based on the hydrological constants for the experimental site shown in Table 4.1, crop water consumption was estimated for each phenological stage by multiplying ET₀ by the crop coefficients (K_c) defined in FAO Irrigation and Drainage Paper No. 56 (Allen and Pereira 1998). Additionally, the soil depth explored by the roots was considered in the calculation of the available water volume (Table 4.3). The irrigation system consisted of a P5® dripline (Irritec S.p.A., Capo d'Orlando (ME), 98071, Italy) equipped with emitters with a nominal flow rate of 2.1 L h⁻¹ at a pressure of 100 kPa.

Harvesting was conducted manually to ensure accurate and reliable yield measurements, as opposed to mechanical harvesting. The procedure was carried out when most bolls had opened (BBCH 99), specifically on 10 November 2023, and 30 October 2024. Only capsules that were open at the time of harvesting were collected.

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Table 4. 3 - Phenological phases and Kc values for cotton according to Allen et al. 1998.

Phase	Description	Kc	Depth of Soil Explored by Roots (cm)
Initial	Germination: from dry seed (00) to emergence of hypocotyl with cotyledons (09)	0.4–0.5	30
Development	Leaf development: from cotyledons completely unfolded (10) to canopy closure (39)	0.7–0.8	50
Mid-season	Inflorescence emergence: from first detectable bud (51) to about 90% of capsules having reached their final size (79)	1.05–1.25	50
End-season	Senescence: from about 10% of discoloured or abscessed leaves (91) to above-ground parts of dead plants	0.65–0.70	50

4.2.5 Economic Parameters

The economic assessment was based on four parameters. The *Defoliant cost* was calculated as the product of the unit cost of the defoliant and the application dose: PELACID costs approximately €20 L⁻¹ (680 g L⁻¹) (Certis Belchim 2025; Loddo, Jagarapu, et al. 2023), whereas PYRAFETH (10.6 g L⁻¹) costs 37.5 € L⁻¹ (Certis Belchim 2025; SIPCAM 2025). Gross revenue was obtained from lint yield multiplied by the lint market price (1.315 € kg⁻¹), derived from the average upland cotton price of 64.4 cents per pound for the 2023–2024 marketing year (USDA Foreign Agricultural Service 2025). Net revenue was determined as the difference between gross revenue and the cost of the defoliant per hectare according to the applied dose. Finally, the Cost-Effectiveness Ratio (CER) was expressed as the ratio of net revenue to defoliant cost.

4.2.5 Data Collection and Analysis

The effect of defoliation was assessed by counting the number of leaves on 10 plants within a delimited area of 2.0 m² in each subplot, at two time points: before treatment application and 21 days after application (i.e., at harvest), following the methodology of (Wang et al. 2023). The defoliation rate was calculated as the percentage reduction in leaf number observed, considering only those that had abscised, at 21 days post-treatment.

At harvest, within the same 2.0 m² area of each subplot, the following parameters were measured: plant height (cm), number of open bolls per plant, and average boll weight (g) after oven drying. Additionally, root diameter (measured 1 cm below the crown) and raw cotton yield (t ha⁻¹) were recorded as the total amount of opening capsules collected at the harvest.

Cotton lint was separated from the seeds using a laboratory ginning machine. Subsequently, both lint and seed yields (t ha⁻¹) were determined for each subplot.

A two-way ANOVA was performed to assess the effects of treatments across the two growing seasons, with Year (Y) included as a random effect and Treatment (T) as a fixed effect. Tukey's HSD test was subsequently applied for mean separation. The Shapiro–Wilk test was used to verify the normality of residuals, while Bartlett's test was applied to assess homoscedasticity. All statistical analyses were conducted using R software (CRAN version 4.5.1).

4.3 Results

Analysis of variance (ANOVA) revealed that the factors Year (Y), Treatment (T), and their interaction (Y × T) had significant effects on all the parameters analyzed, as shown in Table 4.4.

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Table 4. 4 - ANOVA for main effects and interactions on root diameter, boll number plant⁻¹, average boll weight, raw yield, lint yield, seed yield and phylloptosis. The p-value is reported. *, ** and * indicate significant at $p < 0.001$, $p < 0.01$ and $p < 0.05$ respectively. ns = not significant; df = Degrees of Freedom. Y = Year; T = Treatment.**

		Parameters						
Source of Variation	df	Boll Number Plant ⁻¹	Average Boll Weight (g)	Root Diameter (mm)	Raw Yield (kg ha ⁻¹)	Lint Yield (kg ha ⁻¹)	Seed Yield (kg ha ⁻¹)	Defoliation
Y	1	3.26×10^{-4} ***	0.19ns	6.98×10^{-8} ***	5.14×10^{-5} ***	3.22×10^{-3} **	1.79×10^{-6} ***	4.33×10^{-7} ***
T	8	7.58×10^{-8} ***	2.95×10^{-8} ***	1.60×10^{-7} ***	1.09×10^{-10} ***	1.74×10^{-11} ***	1.02×10^{-10} ***	2×10^{-16} ***
Y × T	36	6.17×10^{-4} ***	1.19×10^{-2} *	1.33×10^{-3} ***	4.51×10^{-4} ***	2.01×10^{-4} ***	7.85×10^{-4} ***	6.09×10^{-7} ***

4.3.1 Morphological and Productive Traits

Boll number per plant was influenced by the Y × T interaction. Plots under PELACID D2 in 2024 exhibited the highest boll number ($p < 0.001$), with a 73.2% increase compared to PLACEBO D1 in 2023 (7.29 bolls plant⁻¹ vs. 4.21 bolls plant⁻¹) and a 71.9% increase compared to PYRAFETH D3 in 2024 (7.29 bolls plant⁻¹ vs. 4.24 bolls plant⁻¹) (Figure 4.2A).

The Y × T interaction also had a significant effect ($p < 0.05$) on average boll weight. PYRAFETH D1 in 2024 showed the highest

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value, equal to 8.45 g. The lowest value was observed in 2023 under PELACID D3, with 3.74 g, corresponding to a 55.7% reduction (Figure 4.2B).

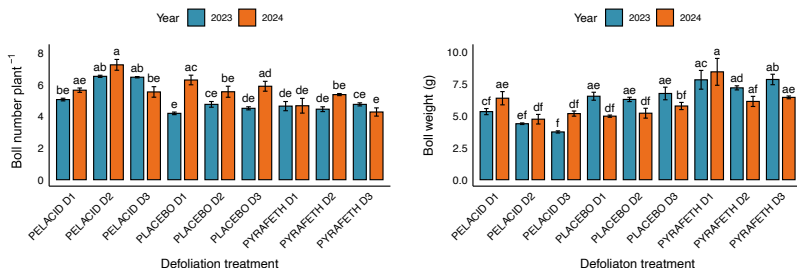


Figure 4. 2 - Effect of Y and T on boll number plant⁻¹ (A) and average boll weight (B). For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. Treatment (PELACID D1 = Pure pelargonic acid at rate 12 L ha⁻¹; PELACID D2 = Pure pelargonic acid at rate 16 L ha⁻¹; PELACID D3 = Pure pelargonic acid at rate 18 L ha⁻¹; PLACEBO D1 = water at rate at 30 L ha⁻¹; PLACEBO D2 = water at rate at 50 L ha⁻¹; PLACEBO D3 = water at rate at 60 L ha⁻¹; PYRAFETH D1 = Pyraflufen-ethyl at rate 1.5 L ha⁻¹; PYRAFETH D2 = Pyraflufen-ethyl at rate 2 L ha⁻¹; PYRAFETH D3 = Pyraflufen-ethyl at rate 2.5 L ha⁻¹).

The $Y \times T$ interaction had a significant effect ($p < 0.01$) on root diameter, with the highest values observed in PELACID D1 in both 2023 (9.67 mm) and 2024 (9.87 mm), PELACID D2 in 2024 (9.65 mm), and PLACEBO D1 in 2024 (10.01 mm). The lowest value was recorded in PYRAFETH D2 in 2023, with 6.43 mm (Figure 4.3).

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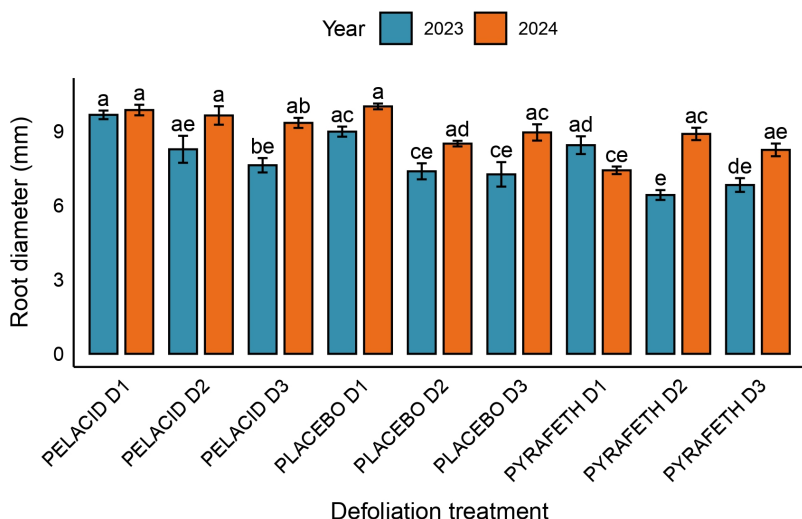


Figure 4. 3 - Effect of treatments on root diameter. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. Treatment (PELACID D1 = Pure pelargonic acid at rate 12 L ha⁻¹; PELACID D2 = Pure pelargonic acid at rate 16 L ha⁻¹; PELACID D3 = Pure pelargonic acid at rate 18 L ha⁻¹; PLACEBO D1 = water at rate at 30 L ha⁻¹; PLACEBO D2 = water at rate at 50 L ha⁻¹; PLACEBO D3 = water at rate at 60 L ha⁻¹; PYRAFETH D1 = Pyraflufen-ethyl at rate 1.5 L ha⁻¹; PYRAFETH D2 = Pyraflufen-ethyl at rate 2 L ha⁻¹; PYRAFETH D3 = Pyraflufen-ethyl at rate 2.5 L ha⁻¹).

The Y × T interaction had a significant effect ($p < 0.001$) on raw yield, lint yield, and seed yield.

As shown in Figure 4A, the highest raw yield was recorded in 2024 under PYRAFETH D2, at 2827 kg ha⁻¹, while PELACID D2 in 2024 achieved a raw yield of 2729 kg ha⁻¹, comparable to that observed in PYRAFETH D1 in 2023 and 2024.

The lowest value was observed in PLACEBO D3 in 2023, at

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1490 kg ha⁻¹, corresponding to a 47.2% reduction.

Seed yield showed higher values in plots treated with PYRAFETH D2 in 2024, at 1740 kg ha⁻¹ (Figure 4B), approximately 105% higher than PLACEBO D3 in 2023 (845 kg ha⁻¹).

PELACID D2 in 2024 reached 1597 kg ha⁻¹, comparable to the yield obtained with PYRAFETH D1 in 2023.

Regarding lint yield, the highest values were observed in PYRAFETH D1, with 1196 kg ha⁻¹ in 2024 and 1136 kg ha⁻¹ in 2023 (Figure 4C), as well as in PELACID D2 in 2024 (1131 kg ha⁻¹). The lowest yields occurred in 2023 for PELACID D3 (698 kg ha⁻¹) and PLACEBO D3 (646 kg ha⁻¹), representing reductions of 41.7% and 45.8%, respectively, when compared to the PYRAFETH D1 in 2024.

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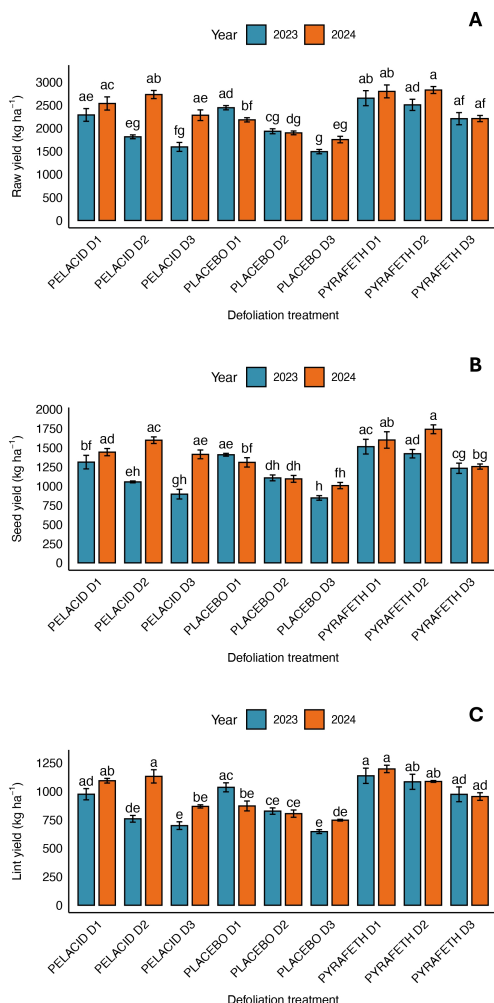


Figure 4. 4 - Effect of Y and T on raw yield (A) seed yield (B) and lint yield (C). For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test. Error bars represent standard error. Treatment (PELACID D1 = Pure pelargonic acid at rate 12 L ha⁻¹; PELACID

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D2 = Pure pelargonic acid at rate 16 L ha⁻¹; PELACID D3 = Pure pelargonic acid at rate 18 L ha⁻¹; PLACEBO D1 = water at rate at 30 L ha⁻¹; PLACEBO D2 = water at rate at 50 L ha⁻¹; PLACEBO D3 = water at rate at 60 L ha⁻¹; PYRAFETH D1 = Pyraflufen-ethyl at rate 1.5 L ha⁻¹; PYRAFETH D2 = Pyraflufen-ethyl at rate 2 L ha⁻¹; PYRAFETH D3 = Pyraflufen-ethyl at rate 2.5 L ha⁻¹). Different letters indicate statistically significant differences between means at $p \leq 0.05$.

4.3.2 Defoliation Traits

The Y $\times$ T interaction had a significant effect ($p < 0.001$) on phylloptosis (Figure 4.5).

Plots treated with PYRAFETH D2 and D3 in both years, as well as PYRAFETH D1 in 2024 and PELACID D3 in 2023, reached 100% leaf drop. The lowest level of defoliation was recorded in PLACEBO D1 in 2024, with only 28.7% phylloptosis.

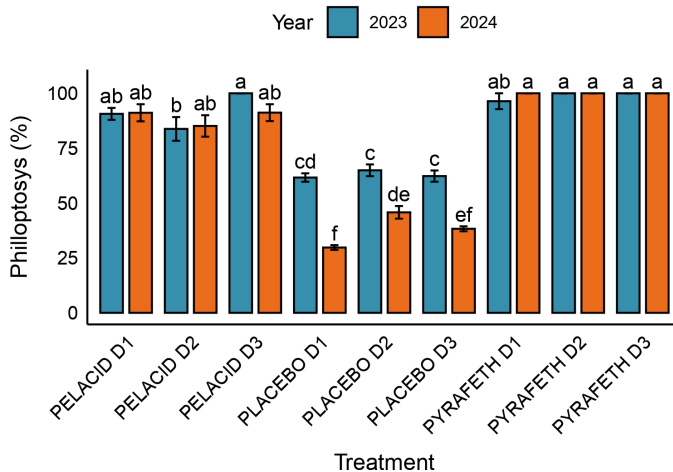


Figure 4. 5 - Effect of Y and T on defoliation. For each data series, values with different letters are significantly different at $p \leq 0.05$, according to Tukey's test.

Error bars represent standard error. Treatment (PELACID D1 = Pure

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pelargonic acid at rate 12 L ha⁻¹; PELACID D2 = Pure pelargonic acid at rate 16 L ha⁻¹; PELACID D3 = Pure pelargonic acid at rate 18 L ha⁻¹; PLACEBO D1 = water at rate at 30 L ha⁻¹; PLACEBO D2 = water at rate at 50 L ha⁻¹; PLACEBO D3 = water at rate at 60 L ha⁻¹; PYRAFETH D1 = Pyraflufen-ethyl at rate 1.5 L ha⁻¹; PYRAFETH D2 = Pyraflufen-ethyl at rate 2 L ha⁻¹; PYRAFETH D3 = Pyraflufen-ethyl at rate 2.5 L ha⁻¹).

4.4 Discussion

For this reason, we compared different application rates of a widely used chemical defoliant—pyraflufen-ethyl—with increasing concentrations of pure pelargonic acid and a water-based placebo over two growing seasons. We aimed to achieve comparable defoliation efficacy and yield performance while reducing reliance on conventional defoliants, thereby promoting more sustainable cotton production. All analyzed parameters (boll number per plant, average boll weight, root diameter, raw yield, lint yield, seed yield, and phylloptosis) were significantly affected by the Y × T interaction.

From the results, it emerged that the highest boll numbers were recorded with PELACID D2, particularly in 2024. In contrast, PYRAFETH consistently underperformed, with the recommended dose (D3) yielding the lowest boll count in 2024. Pyraflufen-ethyl, a protoporphyrinogen oxidase (PPO) inhibitor—a key enzyme in chlorophyll biosynthesis—induces the accumulation of protoporphyrin IX and reactive oxygen species (ROS), leading to rapid cellular necrosis (Barker et al. 2023). The excessive accumulation of ROS, such as H₂O₂, superoxide, singlet oxygen, and the hydroxyl radical causes cellular damage, which manifests itself through a marked increase in malondialdehyde, a reliable indicator of membrane integrity and oxidative injury under diverse stress conditions (Jin et al. 2020; Sha et al. 2019). Although the plant

attempts to respond by increasing the activity of certain antioxidant enzymes (such as superoxide dismutase and peroxidase), catalase activity decreases significantly, indicating that ROS scavenging capacity is weakened and homeostasis is disrupted (Jin et al. 2020). This oxidative damage is directly related to the abscission rate (Jin et al. 2020; Li et al. 2024).

This mechanism is exacerbated under high-temperature conditions, where it can cause severe tissue damage and significant yield reductions (Grichar, Dotray, and Baughman 2010). In 2024, prolonged water stress intensified this effect, further impairing developing cotton bolls and reducing their final number. Conversely, PELACID, which acts independently of PPO inhibition, exerts its defoliation effect by interacting with membrane phospholipids, causing cell lysis and loss of cellular compartmentalization, ultimately leading to rapid tissue desiccation (Ciriminna et al. 2019). This mode of action prevented the negative physiological impacts seen with PYRAFETH, allowing PELACID to maintain high boll numbers even under elevated temperatures.

Regarding boll weight, PYRAFETH D1 recorded the highest values in 2024, while higher doses (D2 and D3) reduced boll weight. This confirms reports by (Grichar et al. 2010) that increased PYRAFETH dosage can trigger PPO-inhibition-related phytotoxicity, resulting in yield decline. Conversely, PELACID D3 in 2023 showed boll weight values below even the PLACEBO (across all dosages and in both years). This is explained by the elevated boll number induced by PELACID D3 that year, which reduced individual capsule weight due to resource competition – consistent with the yield component compensation principle in *Gossypium hirsutum* (Constable and Bange 2015; S. Guo et al. 2023; Liu et al. 2024).

Concerning root diameter, PELACID D1 in 2023 and 2024, as

well as PELACID D2 in 2024, achieved the highest values—comparable to PLACEBO D1 in 2024. This indicates that even at increasing PELACID dosages, root morphology remains unaffected, preserving the plant's capacity to translocate water and nutrients to maturing bolls. As demonstrated in studies on herbaceous crops, a larger root diameter promotes (i) wider xylem vessels, enhancing the flow of water and nutrients (N, P, K) (Huot et al. 2025), and (ii) greater drought resistance—especially in semi-arid Mediterranean environments—while maintaining leaf photosynthetic efficiency (Ghimire et al. 2025; Guo, Bao, et al. 2024; Guo, Sun, et al. 2024; Huot et al. 2025). Therefore, even following defoliation, a well-developed root system remains fundamental for the photosynthetic activity performed by the non-foliar photosynthetic organs, which in fact contribute to boll filling during the final stages of boll maturation (Hu et al. 2014; Zhan et al. 2014).

PYRAFETH D2 exhibited a sharp reduction in root diameter in 2023 despite 21.8% higher rainfall compared to 2024. This suggests that pyraflufen-ethyl-induced phytotoxicity is dose-dependent and independent of hydric conditions. The effect is particularly pronounced at higher doses (D2/D3), where PPO inhibition might have triggered ROS production, damaging root tissues (Barker et al. 2023; Grichar et al. 2010).

Regarding raw yield, PYRAFETH D2-treated plots in 2024 showed the highest values compared to PLACEBO D3 in 2023, which recorded the lowest yield (47.2% reduction). This substantial yield difference may result from incomplete defoliation in PLACEBO plots, which can prolong vegetative growth at the expense of reproductive development (Faircloth et al. 2004a, 2004b). Interestingly, across both trial years, PYRAFETH D3 consistently showed significantly lower raw yields than lower-dose treatments (D1/D2). This suggests that

pyraflufen-ethyl applications exceeding 2 L ha^{-1} ($10.6 \text{ g a.i. L}^{-1}$) cause substantial yield suppression, aligning with documented phytotoxicity thresholds in peanut (*Arachis hypogaea* L.) (Grichar et al. 2010; Manuchehri et al. 2017). Notable results emerged from PELACID formulations in 2024: both D2 and D1 yielded comparably to PYRAFETH D2, with only a 3.2% reduction in PELACID D2 and a 10.3% reduction in PELACID D1. In contrast, while PELACID D1 achieved optimal 2023 yields, higher doses (D2/D3) caused significant yield declines. This phytotoxicity is attributed to high PELACID concentrations, which induce: (i) cellular membrane leakage via fatty acid intercalation, and (ii) light-dependent singlet oxygen peroxidation, with the consequent necrosis of plant tissues (Lederer et al. 2004; Loddo, Jagarapu, et al. 2023).

Concerning seed yield, PYRAFETH D2-treated plots in 2024 showed the highest values compared to PLACEBO D3 in 2023, which recorded the lowest yield (51.1% reduction). These results indicate that PYRAFETH D2 may represent the optimal dosage for maximizing seed yield, as it enhances production without suppressing yield.

Notable results emerged from PELACID D2 in 2024, yielded comparably to PYRAFETH D2, with only an 8.0% reduction. In 2023, however, PELACID treatments demonstrated clear dose-dependent yield reductions, with yields decreasing by 19.8% from D1 to D2 (1312 to 1054 kg ha^{-1}) and by 31.3% from D1 to D3 (1312 to 895 kg ha^{-1}). This could be attributed to (i) progressive phytotoxicity due to increasing concentrations of PELACID (Lederer et al. 2004; Loddo, Jagarapu, et al. 2023; López-González et al. 2024b), along with (ii) the milder climate observed in 2023, which was characterized by higher rainfall (188 mm during the 2023 growing season compared to 147 mm in 2024). The greater soil water availability likely prolonged

vegetative growth, diverting assimilates away from reproductive development (Koudahe et al. 2021a; Mi et al. 2025).

The significant reduction in seed yield observed in both years with the high dosage of PYRAFETH D3 may be attributed to its adverse effect on the critical process of residual photosynthesis in non-leaf tissues. Following defoliation, photosynthetic activity relies heavily on remaining green structures, primarily bracts and boll walls, which contain functional chloroplasts capable of substantial CO₂ assimilation—reaching 60–72% and 20–26% of leaf rates, respectively (Hu et al. 2014; Zhan et al. 2014). Defoliation shifts the source–sink balance by removing the dominant source, forcing greater reliance on any remaining photosynthetic tissues and on remobilization of stored carbohydrates, and often reducing carbon allocation to sinks (Wang et al. 2025). Under defoliation stress, the contribution of these non-foliar sources, which normally provides over 24% of the plant's photoassimilates (Hu et al. 2014), becomes vital for yield formation. It is plausible that the high concentration of PYRAFETH D3 caused phytotoxic damage to these tissues, impairing this essential compensatory mechanism. This hypothesis is supported by shading studies showing that disrupting photosynthesis in bolls alone can reduce seed weight by 10–30% (Zhan et al. 2014). Therefore, the decrease in yield is likely a direct consequence of the failure to compromise carbon fixation, as the severity of the defoliant not only removed the leaves but also potentially damaged the tissues responsible for sustaining head development after defoliation.

Regarding lint yield, PELACID D2 in 2024 recorded the highest values, comparable to those observed with PYRAFETH D1 in both 2023 and 2024. In contrast, PELACID D3 in 2023 showed the lowest values, similar to those recorded with PLACEBO D3 in 2023.

These outcomes can be attributed to two main factors. First, the

more favorable climate conditions in 2023, particularly in terms of rainfall, which may have promoted vegetative growth at the expense of lint yield (Koudahe et al. 2021a; Mi et al. 2025). Second, the high dosage applied in D3 (18 L ha⁻¹ of pure pelargonic acid at 680 g L⁻¹), inducing cellular damage that directly compromised lint production, consistent with established herbicide mode-of-action research (Lederer et al. 2004; Loddo, Jagarapu, et al. 2023; Loddo, Kumar Jagarapu, et al. 2023; López-González et al. 2024b).

The parameter phylloptosis showed that PELACID D3 achieved 100% fallen leaves, matching the performance of PYRAFETH D1 in 2024, as well as PYRAFETH D2 and D3 in both 2023 and 2024. This result demonstrates that PELACID at D3, corresponding to 18 L ha⁻¹ of pure pelargonic acid (680 g L⁻¹) can achieve defoliation levels equivalent to those of synthetic chemical defoliants, without compromising efficacy.

This is because PELACID penetrates the leaf cuticular wax layer and integrates into plant cell membranes, causing leakage of intracellular solutes and triggering light-induced peroxidative reactions that lead to tissue necrosis (Lederer et al. 2004; López-González et al. 2024a). This phytotoxic effect becomes visible within a few hours after application and affects only the treated areas, as the product acts strictly by contact and is not translocated within the plant (Alvarez et al. 2021; Álvarez et al. 2025; Poiger et al. 2024).

Moreover, PELACID has a soil half-life (DT₅₀) of less than 1 day, resulting in a minimal risk of residues (Poiger et al. 2024). But even at dosages higher than those commonly applied—typically about 16 L ha⁻¹ of a commercial formulation, equivalent to 10,880 g a.i. ha⁻¹ for weed control (Loddo, Jagarapu, et al. 2023)—degradation in soil is very rapid, as reported in EU registration studies (DT₅₀ of 17-38 hours at a dose level of 20 mg/kg) (Álvarez et al. 2025; Poiger et al.

2024). These characteristics make it a promising candidate for sustainable cotton pre-harvest management.

Interestingly, even lower doses such as PELACID D1 proved highly effective, achieving 90.6% defoliation in 2023 and 91.1% in 2024, with results not far from those obtained with conventional chemical treatments. Such high efficacy at a low application rate—12 L ha⁻¹—could represent an optimal economic compromise. As reported in (Loddo, Jagarapu, et al. 2023) , the cost of PELACID is approximately €20 per liter, which translates to €300 ha⁻¹ at the standard 16 L ha⁻¹ dose. Reducing the dose and implementing complementary strategies such as irrigation termination (e.g., as described in (Vitale et al. 2024))—which require no additional input costs—could both lower the adoption cost of PELACID and promote more sustainable agronomic practices.

4.4.1 Limitations and Future Research Directions

Beyond the clear agronomic efficacy of PELACID, two key limitations must be acknowledged.

The first concerns its economic feasibility: its high cost per hectare, driven by the large application volumes required compared with synthetic defoliant. Nevertheless, a cost–benefit analysis that considers both input costs and economic returns from yield provides important insights. The economic analysis revealed a substantial difference in application costs. While the synthetic defoliant PYRAFETH ranged from €56.25 ha⁻¹ (D1) to €93.75 ha⁻¹ (D3), PELACID treatments were considerably higher, costing €240 ha⁻¹ at 12 L ha⁻¹, €320 ha⁻¹ at 16 L ha⁻¹, and €360 ha⁻¹ at 18 L ha⁻¹.

As shown in Table 5, at the lowest dose (PELACID D1, 12 L ha⁻¹), the treatment achieved net revenues comparable to those of

PYRAFETH in both years. However, the CER (representing the economic return per euro invested in the defoliant) was consistently lower for PELACID because of the larger product volumes required.

Although the results clearly underscore the economic limitation of PELACID—namely, that high application volumes reduce cost-effectiveness compared with PYRAFETH—it is important to recognize that the CER does not capture externalities such as national regulations, eco-labels, or market premiums for sustainable fibres. These factors could, in practice, support the adoption of PELACID despite its apparently lower CER.

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Table 4.5 - Cost-benefit comparison of T by Y.

Year	Treatment *	Dose (L ha ⁻¹)	Defoliant cost (€ ha ⁻¹)	Lint yield (kg ha ⁻¹)	Lint Yield Price (€ kg ⁻¹)	Lint Gross revenue (€ ha ⁻¹)	Net lint revenue (€ ha ⁻¹)	Cost- Effectiveness Ratio
2023	PELACID D1	12	240	975	1.315	1282.13	1042.13	4.34
2023	PELACID D2	16	320	759	1.315	998.09	678.09	2.12
2023	PELACID D3	18	360	699	1.315	919.19	559.19	1.55
2023	PYRAFETH D1	1.5	56.25	1136	1.315	1493.84	1437.59	25.56
2023	PYRAFETH D2	2	75	1083	1.315	1424.15	1349.15	17.99
2023	PYRAFETH D3	2.5	93.75	974	1.315	1280.81	1187.06	12.66
2024	PELACID D1	12	240	1093	1.315	1437.30	1197.30	4.99
2024	PELACID D2	16	320	1131	1.315	1487.27	1167.27	3.65
2024	PELACID D3	18	360	868	1.315	1141.42	781.42	2.17
2024	PYRAFETH D1	1.5	56.25	1196	1.315	1572.74	1516.49	26.96
2024	PYRAFETH D2	2	75	1087	1.315	1429.41	1354.41	18.06
2024	PYRAFETH D3	2.5	93.75	954	1.315	1254.51	1160.76	12.38

* Treatment (PELACID = Pure pelargonic acid; PYRAFETH = Pyraflufen-ethyl).

The second limitation concerns fibre quality, as the potential impact of pelargonic acid on technological parameters remains largely

unexplored. It is well established that PELACID acts as a contact desiccant, inducing rapid membrane disruption and necrosis of green tissues (Soltani et al. 2024). When such effects occur before physiological boll and fibre maturation, they may reduce carbohydrate availability for secondary cell-wall deposition, thereby negatively affecting micronaire, fibre length, and strength. This concern is supported by studies on harvest-aid timing, which indicate that prematurely applied defoliants can alter micronaire and other fiber parameters, whereas appropriately timed applications generally avoid measurable quality penalties (Snipes and Baskin 1994).

In this context, where no data are currently available on the use of PELACID as a cotton defoliant, future studies should investigate its potential influence on fibre quality. Such research should consider not only the effects of early defoliant application but also those of treatments applied at advanced phenological stages (e.g., BBCH 88). A comprehensive fibre-quality assessment—including upper-half mean length, length uniformity index, strength, elongation, micronaire, fineness, and maturity ratio—would be particularly valuable to distinguish direct phytotoxic effects of pelargonic acid from indirect quality changes associated with assimilate limitation.

4.5 Conclusions

The results of this two-year study demonstrate that PELACID represents a viable organic alternative to conventional synthetic defoliants, offering comparable efficacy with distinct environmental advantages. Field trials in Sicily, Italy, confirmed that the D3 dosage (18 L ha⁻¹) achieved complete defoliation (100%) in 2023, matching the performance of the benchmark chemical defoliant PYRAFETH. More significantly, the D1 dosage (12 L ha⁻¹) still delivered over 90%

leaf drop, revealing substantial potential for cost-efficient optimization without compromising effectiveness.

From a physiological perspective, PELACID ensures a superior safety profile. Unlike PYRAFETH, whose systemic action can impair root development, PELACID's contact-based mechanism—disrupting cellular membranes—preserves root growth and function. This was evident from the larger root diameters recorded under PELACID treatments (up to 9.87 mm), especially at moderate doses (D1–D2), maintaining the plants' water and nutrient uptake capacity—an essential trait under Mediterranean drought-prone conditions.

On the agronomic side, while high doses of PYRAFETH (D3) led to phytotoxicity and yield reductions of up to 47%, PELACID at 16 L ha⁻¹ (D2) showed remarkable performance. In 2024, a season marked by lower rainfall, it matched the fiber yield of the best conventional treatment (1.13 vs 1.20 t ha⁻¹ for PYRAFETH D1) and exceeded it in boll number per plant by 73%, underscoring its superior resilience to high thermal stress.

Environmentally, rapid soil degradation (DT₅₀ < 48 hours) eliminates toxic residue risks associated with persistent synthetic defoliants, and the compound's natural origin enables sustainable deployment. Economically, the feasibility of reducing application rates to 12 L ha⁻¹ could lower costs to approximately €240/ha, making it a competitive option, especially when combined with complementary defoliation practices.

Future research should explore integrated approaches combining reduced pelargonic acid doses with pre-harvest irrigation termination to further enhance defoliation efficiency and support the adoption of more sustainable agronomic practices for cotton cultivation.

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Abbreviations

The following abbreviations are used in this manuscript:

CER	Cost-Effectiveness Ratio
FC	Field Capacity
ET ₀	daily reference evapotranspiration
K _c	crop coefficients
PELACID	Pelargonic Acid
PLACEBO	Placebo solution (water)
PYRAFETH	Pyraflufen-ethyl
PPO	protoporphyrinogen oxidase

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5. General discussion and conclusion

Sustainability in cotton cultivation represents a global agronomic and environmental challenge. The combined analysis of sustainable agronomic strategies, LCAs and experimental studies conducted in the Mediterranean region provided an integrated understanding of the key challenges and opportunities associated with this crop.

From the analysis of the scientific literature, it emerged that, from an agronomic perspective, plant-microorganism symbioses play a central role, particularly the inoculation of AMF species, which can enhance the uptake of key nutrients such as P, zinc Zn, and N, thereby reducing the need for chemical fertilizers. Ortas and Iqbal (2019) demonstrated that *Funneliformis mosseae* can increase P uptake by 110% compared to uninoculated control, while *Rhizophagus clarus* was shown to increase plant biomass by 81% (Cely et al. 2016b). The selection of the mycorrhizal species is therefore strategic for maximizing nutrient use efficiency, with differential effects not only on the host plant but also on the composition and structure of the soil microbiome (Zhou et al. 2020).

In terms of water management, DI has proven more efficient than FI and SI, leading to yield increases of 14–30% and improvements in WUE of 25–35% (Cetin and Bilgel 2002; Ibragimov et al. 2007). Combined systems such as MDI further enhance soil moisture (+4.8–12.9%), aboveground biomass (+61.5%), and lint yield (+12.8%), although they might have caused side effects related to residual plastic accumulation and increased CO₂ emissions (Z. Ma et al. 2024; Zong et al. 2020). Moreover, early IT has been found effective in mild climates such as the Mediterranean, where interrupting irrigation at the first boll opening improved yield (Karam

et al. 2006) and enhanced water use efficiency (Reba et al. 2014).

Additionally, the adoption of polycultural systems, including crop rotations with legumes, brassicas, and cereals, has emerged as a valuable strategy to improve agroecosystem sustainability. Legumes such as *Vicia villosa* could increase total soil nitrogen (+18%) and microbial N transformation (+168%) (Li et al. 2021). However, due to their limited persistence in soil, Patra, Saha, and Jagadamma (2022) recommend intercropping with cereals such as wheat, which has a higher SOC storage capacity (+23% compared to vetch). Brassicas like *Brassica carinata* have also proven to be effective in enhancing soil organic matter and suppressing weeds, reducing populations of *Amaranthus hybridus* (−27%) and *Senna obtusifolia* (−25%) through the release of glucosinolates with allelopathic and insecticidal properties (Tiwari et al., 2021; Li et al., 2022).

Therefore, the integration of sustainable agronomic practices, such as plant-microbe symbioses, optimized irrigation strategies, and polycultural systems, was an effective approach to improving the productivity and environmental sustainability of cotton cultivation in Mediterranean environments. When adapted to specific soil and climate conditions, these strategies could significantly reduce the ecological footprint of the cropping system, improve resource use efficiency, and promote greater agroecosystem resilience.

The systematic review of LCA studies applied to the cotton supply chain highlighted that the most significant environmental impacts were in the agricultural phase, industrial processing, and domestic use of cotton products. Cotton cultivation, assessed within “cradle-to-farm gate” boundaries, showed a high contribution to water consumption, mainly due to evapotranspiration and the use of inefficient irrigation systems, alongside substantial energy requirements for water pumping, which could reach up to 4,300 MJ per hectare, especially in contexts such as India and China (Huang et

al. 2022; Singh et al. 2022). Greenhouse gas emissions, particularly nitrous oxide (N₂O) from nitrogen fertilizers, and ecotoxicological effects from pesticide use represent additional environmental hotspots (Avadí et al. 2020; Zhang et al. 2021). Although organic cotton reduces the reliance on chemical inputs, its lower yields increase the impacts per unit of product; however, when assessed per unit of cultivated area, the impacts were often lower than those of conventional systems, as demonstrated by Avadí et al. (2020).

In the industrial phase (cradle-to-gate), the highest environmental burdens originated from spinning, dyeing, and washing processes, due to their intensive use of energy and water. Cradle-to-gate assessments indicated that cotton cultivation alone can account for up to 91% of the total water footprint of yarn production (Liu, Huang, Ren, et al. 2020), while the associated carbon footprint may exceed 1.1×10^4 kg CO₂ per ton of virgin yarn (Liu, Huang, Zhu, et al. 2020). The use of recycled cotton fibers enables substantial environmental savings—up to 79% reduction in water use and 60% reduction in GHG emissions—by eliminating the cultivation phase and lowering the resource intensity of subsequent manufacturing processes (Liu, Huang, Ren, et al. 2020).

Cradle-to-grave assessments confirm that environmental impacts remain significant during the use and end-of-life stages. Notably, finishing, domestic laundering, and weaving account for over 90% of the total carbon footprint of a cotton garment (Luo et al. 2022). Water consumption throughout the entire life cycle is largely driven by the cultivation phase (88%), followed by laundering, which also contributes substantially to eutrophication, particularly due to the use of phosphate-based detergents (Zhang et al. 2015). In this perspective as well, organic cotton demonstrates reduced impacts - especially regarding eutrophication potential (Baydar et al. 2015) - while recycled cotton offers even greater environmental savings (Esteve-

Turrillas and de la Guardia 2017; Moazzem et al. 2021).

These findings indicate that improving the environmental sustainability of cotton production and processing requires an integrated and systemic approach. This includes reducing water and energy use in the agricultural phase, improving industrial process efficiency, promoting cotton fiber recycling, and fostering greater consumer awareness. The systematic integration of LCA into decision-making processes, the development of dedicated recycling infrastructure, and the widespread adoption of sustainable agricultural technologies and practices were essential steps to mitigate the environmental impacts associated with cotton along its entire life cycle.

Studies conducted in Sicily under Mediterranean conditions confirmed that the integration of mycorrhizal inoculants, combined with rational water management, can significantly enhance the sustainability and yield of cotton cultivation, provided that practices are adapted to the local pedoclimatic context. From the perspective of WUE, it was observed that in Campo Carboj (Site 1), despite receiving less rainfall than in Primosole (Site 2), required 38.4% less total water. This was attributable to the higher organic matter content and sandy soil texture, which, as reported by Zhao et al. (2024) and Guo-Zheng et al. (2013), promoted water retention and deeper root development. The use of DI also allowed for lower irrigation volumes compared to what is documented in the literature for similar pedoclimatic conditions Vitale et al. (2024), thanks to higher distribution efficiency (Bryant et al. 2021; Chauhdary et al. 2023). In this context, WP was higher in treatments with more abundant irrigation, particularly in Site 1, where the soil structure supported deeper root growth and improved use of moisture and nutrients (Bai et al. 2024; Wang et al. 2021b).

From an agronomic standpoint, irrigation positively influenced plant height, boll number and weight, as well as seed and fiber yields,

while mycorrhization showed particularly positive effects in the fertile soils of Site 1, enhancing phosphorus uptake and root development (Ortas, Akpinar, and Demirbas 2016; Smith et al. 2003). However, the effectiveness of mycorrhizae was limited under I-30 and in the poorer soils of Site 2. The cultivar Armonia demonstrated greater yield stability under water stress, maintaining high yields even under I-70, as also observed by (Filintas et al. 2022b), whereas ST-318 performed better under full irrigation conditions.

Phenological development was also significantly influenced by the interactions among E, G, and I levels. The time to emergence (S-Em) was shorter in the clayey soils of Site 2, due to more favorable thermal conditions and the higher heat retention capacity of clayey soils (Raphael et al. 2017; Vories et al. 2007). The duration of the F-Oc phase was longer under I-100 treatments, supporting boll filling (Tian et al. 2024), while the Oc-H phase was shortened under I-30, allowing for earlier harvest, which was particularly useful to avoid autumn rains (Istipliler et al. 2024; Melillo et al. 2016).

These results confirm that targeted management practices, combined with the selection of adaptable genotypes and the use of mycorrhizal inoculants, could make cotton cultivation both sustainable and productive in Southern Italy. In particular, irrigation at 70% of ETc ensured economically sustainable yields with a 30% reduction in water input, highlighting the remarkable resilience of cotton in water-scarce conditions.

Another critical aspect of sustainability concerns pre-harvest defoliation management. The use of synthetic defoliant is still widespread, but it raises environmental and toxicological concerns (Trakulsrichai et al. 2025; Wei et al. 2023). Results from our study with PELACID demonstrated that this natural compound represented a promising sustainable alternative. At rates of 12–18 L ha⁻¹, PELACID achieved over 90% defoliation, matching the efficacy of

PYRAFETH while avoiding phytotoxic side effects on roots and yield. Its rapid degradation ($DT_{50} < 38$ h) (Álvarez et al. 2025; Poiger et al. 2024) minimized residue risks, and at moderate doses ($12\text{--}16$ L ha⁻¹) it ensured boll retention, fiber yields comparable to conventional defoliants, and larger root diameters, enhancing resilience under Mediterranean drought-prone conditions. This positions PELACID as a key tool to overcome one of the main bottlenecks of sustainable cotton cultivation, mechanized harvesting, where no effective sustainable defoliants are yet available. When combined with agronomic practices such as irrigation termination, its adoption could further reduce costs and environmental impacts.

In conclusion, it is possible to affirm that a sustainable cotton cultivation system can be achieved through an integrated approach that combines agronomic and management innovations with environmental assessments based on LCA. The use of AMF, crop rotations with appropriate cover species, efficient irrigation systems, and sustainable defoliants such as pelargonic acid, supported by careful land-use planning, are key strategies for reducing environmental impact and enhancing crop performance. However, the adoption of these practices remains limited: AMF species are not yet widely utilized, DI is still scarcely implemented in many regions, and the replacement of synthetic defoliants with agronomic or bio-based alternatives is uncommon. Therefore, greater efforts are required in applied research, knowledge transfer to agricultural stakeholders and the development of supportive policies to foster the widespread adoption of sustainable practices. Only through such coordinated actions will it be possible to reconcile production goals with the protection of environmental resources.

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Zhou, Jiachao, Xiaofen Chai, Lin Zhang, Timothy S. George, Fei Wang, and Gu Feng. 2020. “Different Arbuscular Mycorrhizal Fungi Cocolonizing on a Single Plant Root System Recruit Distinct Microbiomes.” *MSystems* 5(6). doi:10.1128/MSYSTEMS.00929-20/SUPPL_FILE/MSYSTEMS.00929-20-SD001.DOCX.

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Annexes

Articles published in international journals

- **Vitale, G. S.,** Scavo, A., Zingale, S., Tuttolomondo, T., Santonoceto, C., Pandino, G., Lombardo, S., Anastasi, U., & Guarnaccia, P. (2024). Agronomic Strategies for Sustainable Cotton Production: A Systematic Literature Review. *Agriculture*, 14(9), 1597. <https://doi.org/10.3390/agriculture14091597>
- **Vitale, G. S.,** Iacuzzi, N., Zingale, S., Lombardo, S., Tuttolomondo, T., & Guarnaccia, P. (2025). Environmental sustainability of cotton: A systematic literature review of life cycle assessments. *Journal of Agriculture and Food Research*, 22, 102069. <https://doi.org/10.1016/j.jafr.2025.102069>
- **Vitale, G. S.,** Iacuzzi, N., Tortorici, N., Indovino, G., Franco, L., Mosca, C., Giovino, A., Scavo, A., Lombardo, S., Tuttolomondo, T., & Guarnaccia, P. (2025). Sustainable Cotton Production in Sicily: Yield Optimization Through Varietal Selection, Mycorrhizae, and Efficient Water Management. *Agronomy*, 15(8), 1892. <https://doi.org/10.3390/agronomy15081892>

Articles published in national journals

- **Vitale, G. S.,** Marcuzzo P., Lovat L., Toffolatti S., Forte V., Simoni S., Tomasi D., Difesa sostenibile Icaro X4 alla prova. «Corriere Viticolo» (2022) n. 17, p :13-15.

Memberships and IDs

- **ORCID:** <https://orcid.org/0009-0009-0427-0749>
- **Scopus:** <https://www.scopus.com/authid/detail.uri?authorId=59345313200&origin=recordpage#>

Refereeing scientific articles

- Wang N, Zhan J, Feng K, Qi J and Nan H (2024) Higher yield sustainability and soil quality by reducing chemical fertilizer with organic fertilizer application under a single- cotton cropping system. Front. Plant Sci. 15:1494667. doi: 10.3389/fpls.2024.1494667
- Luo Z, Tang W, Wang X, Lu H, Li C, Liang J and Kong X (2024) Effects of N application methods on cotton yield and fertilizer N recovery efficiency in salinity fields with drip irrigation under mulch film using ¹⁵N tracing technique. Front. Plant Sci. 15:1394285. doi: 10.3389/fpls.2024.1394285

Training in national or international universities and/or research institutions

From January to July 2024, research activities were conducted in collaboration with Texas A&M University in Uvalde, TX, USA. The study aimed to select water stress-resistant cotton genotypes, using a randomized block design with five replications across 40 pots. Each pot, measuring 0.2286 m (9”) diameter and 0.1524 m (6”) height, was filled with a 70% native soil and 30% sand mixture. Two key factors were investigated: irrigation regime and choice of drought-tolerant genotype. The irrigation regimes tested were 100% and 50% of ETM, applied from the sixth true leaf stage (16 BBCH). Four genotypes (PHY400, DP2020, NG4190, and ST4990) were tested.

Agronomic Management:

- Fertilization: based on cotton’s recommended nutrient doses and the soil’s fertility, 35 kg ha⁻¹ of N, 32 kg ha⁻¹ of P₂O₅, and 0 kg ha⁻¹ of K₂O were applied using “Peters Professional” fertilizer (20-20-20).

- Irrigation: initially, all plants were uniformly irrigated until they reached the sixth true leaf stage. Subsequently, irrigation volumes were differentiated based on hourly soil moisture readings, monitored using an EM-50 data logger and TEROS-12 soil moisture sensors.

Surveys and Measurements:

- Non-destructive measurements included recording key phenological dates (e.g., emergence, floral bud appearance, bloom stages) and assessing leaf gas exchange, maximum photochemical efficiency of photosystem II (Fv/Fm), and soil water content.
- Destructive measurements focused on final biomass (both belowground and aboveground), leaf water potential (LWP), leaf dry matter content (LDMC), and leaf osmotic potential (Ψ_o).

Ongoing Observations:

- These include analysing leaf carbon isotope ratios, leaf nitrogen content, root size, and root mass. These analyses are being performed at Texas A&M, College Station.

Participation in conferences, workshops, seminars and courses

First year

- **Seminar** – *Using Stated Preference Techniques to Value Landscape Impacts*. Prof. Ståle Navrud – Norwegian University of Life Sciences, Ås, Norway. October 18, 2023, at 3:00 PM – Classroom N, Via Santa Sofia 100
- **Seminar** – *For Quality Research – Artificial Intelligence and Scientific Research: Opportunity or Threat?* Prof. Sebastiano Battiato. October 3, 2023, at 4:00 PM – Palazzo dell'Etna, Via S. Nullo 5

- **English Course** – *B2 Level Academic English Course (for PhD student)*. Dr. J.M. Stuckey (native speaker) – CLA University of Catania. March 6 – May 31, 2023, at 10:00 AM – every Monday and Wednesday – Via Santa Sofia
- **Course** – *Writing a Scientific Review Article*. Prof. Gaetano Chinnici and Dr. Daniela Spina:
 - June 6, 2023, at 3:00 PM – Classroom G, Via Santa Sofia 100
 - June 13, 2023, at 3:00 PM – Classroom H, Via Santa Sofia 100
- **Course** – *Scientific Publishing in the Peer Review Era*. Prof. Antonio Biondi and Dr. Michele Ricupero
 - June 23, 2023, at 3:00 PM – Classroom G, Via Santa Sofia 100
 - June 27, 2023, at 3:00 PM – Classroom G, Via Santa Sofia 100
- **Course** – *CAD and GIS for Sustainable Agriculture*. Prof. Claudia Arcidiacono and Dr. Provvidenza D’Urso:
 - July 4, 2023, at 2:30 PM – Classroom M, Via Santa Sofia
 - July 5, 2023, at 2:30 PM – Classroom M, Via Santa Sofia
 - July 6, 2023, at 2:30 PM – Classroom M, Via Santa Sofia
- **Course** – *ICT for Participatory Methods, Citizen Science & Disseminating Research*. Prof. Teresa Graziano:
 - July 10, 2023, at 9:00 AM – Classroom Lupo, Via Valdisavoia
 - July 11, 2023, at 9:00 AM – Classroom Lupo, Via Valdisavoia
 - July 12, 2023, at 9:00 AM – Classroom Lupo, Via Valdisavoia
- **Workshop Participation** – *5th Joint Workshop of the Agriculture-Oriented PhD Programs at UNICT, UNIFG, and UNIUD*. September 25–28, 2023 – De Carlo Auditorium, Benedettini Monastery, Catania. Poster presented: *Agronomic Strategies for Sustainable Cotton Production: A Systematic Literature Review*

Second year

- **Seminar** – *Technological Innovations in Dairy Cattle Farming: Development of an Automatic Monitoring System (COWTECH)*.

- October 13, 2023, from 9:00 AM to 1:00 PM – Aula Perrotta, Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100
- **Webinar** – *Technology Transfer and Third Mission in IRCCS & Universities: Definition and General Principles*. Dr. Giuseppe Campanella – November 13, 2023, from 3:00 PM to 6:00 PM
 - **Seminar** – *Prevalence and Distribution of Antimicrobial-Resistant Bacteria in Brazilian Food Animal Production*. University of Catania, in partnership with Federal University of Viçosa, Brazil. December 11, 2023, from 9:00 AM to 11:00 AM – Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100, classroom I.
 - **Course** – *Agricultural Policies in the EU: From the Past to the Future*. Prof. Giovanni La Via
December 18, 19, and 22, 2023 – Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100.
 - **Course** – *Introduction and Recent Trends in Experimental Approaches for Agricultural Engineering* – Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100
 - December 15, 2023 (4 hrs) – *GIS Applications for Environmental Impact Assessment of Livestock Housing Systems* – Prof. Provvidenza Rita D’Urso
 - January 12, 2024 (4 hrs) – *Multispectral Indexes for Detecting Crop Features* – Dr. Daniela Vanella
 - January 19, 2024 (4 hrs) – *Hyperspectral and Thermal Data for Precision Agriculture Applications* – Dr. Juan Miguel Ramirez-Cuesta
 - **Course** – *Managing Biological Data with R: Applications for Plants and Fruit Tree Species*. Prof. Mario Di Guardo. January 22

- February 26, 2024 (20 hours) – Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100.
- **Webinar** – *Shaping the Future of Research with AI*. March 27, 2024 (2 hours) – Conducted by Dr. Jacob Wickham – Editage®.
- **Webinar** – **National Conference** – *Geopolitics and Security in Defense of the Italian Agri-Food System*. April 4, 2024. – Speakers: Prof. Biagio Pecorino, Hon. Francesco Lollobrigida.
- **Webinar** – *How to Use Paperpal's AI Academic Editing to Write and Edit Like a Pro*. April 8, 2024 (2 hours) – Conducted by Charlotte Baptista – Editage®.
- **Seminar** – *From Pasture to Field: Investigating the Effects of Sheep Grazing on Cotton Growth and Weed Suppression*. April 10, 2024 – College Station, Texas (USA) – Conducted by Prof. Matthew Stewart
- **Seminar** – *The Agroecological Paradigm: Values, Principles and Actions for the Transition to Sustainable Agri-Food Systems*. April 22, 2024. Organized by Prof. Paolo Guarnaccia – Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100
- **Webinar** – *Food Life-Cycle Assessment (LCA) Databases*. April 23, 2024. GdL DIRE, Associazione Reta Italiana LCA.
- **Seminar** – *Impact of Cotton Root Development Based on Cultivar, Soil Compaction, and Application of Mepiquat Chloride*. April 24, 2024 (2 hours) – Prof. Benjamin McKnight – College Station, Texas (USA).
- **Oral presentation at Field Day Seminar** – *Vegetal Spring Field Day*. May 2, 2024, from 8:00 AM to 5:00 PM – Texas A&M AgriLife Research & Extension Center, Uvalde, Texas (USA). Speaker slot: 9:15–9:30 AM
- **Conference** - *Corpus Christi Center: 50th anniversary of Texas A&M AgriLife Research and Extension*. Organised by Prof. Juan

Landivar, Director of the AgriLife Research and Extension Center in Corpus Christi on 23 May 2024.

- **Conference** – *Genetic and Digital Innovation in Agriculture: Today's Emergencies, Tomorrow's Solutions*. September 25, 2024, from 9:30 AM to 1:30 PM – Ortigia, Siracusa (G7 Agriculture)
- **Event** – *Inauguration of the G7 Youth Agriculture Summit*. September 25, 2024, from 3:30 PM to 5:30 PM – Ortigia, Siracusa (G7 Agriculture)
- **Workshop Participation** – *6th Joint Meeting of Agriculture-Oriented PhD Programs (UNICT, UNIFG, UNIUD)*. September 29 – October 5, 2024 – Lesina (FG), Italy. Oral presentation: *Agronomic and Qualitative Aspects of Sustainable Cotton Cultivation*

Third year

- **Seminar** – *Water Resources Management*. Prof. Anna Della Marta and Prof. Carmelo Maucieri. December 12, 2024, from 4:00 PM to 6:00 PM – Room E, University of Palermo.
- **Seminar** – *Cultivating and Preserving Sicilian Plant Biodiversity... while Enhancing Its Value*. Prof. Cosentino, Prof. Lombardo, Prof. Romano, Prof. Guarnaccia. December 19, 2024, from 9:00 AM to 1:00 PM – Aula Magna, Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100.
- **Course** – *Statistical Methodology for Agricultural Sciences – “Dario Sacco” Course: General and Generalized Linear Models*. Organized by the Italian Society of Agronomy (SIA). February 3–7, 2025 – University of Pisa.
- **Meeting** – *Institutional Visit of the Embrapa Soja Delegation (Brazil)*. February 24, 2025, from 10:00 AM to 1:30 PM (3.5 hours) – Meeting Room, Department of Agriculture, Food and

Environment, University of Catania, Via Santa Sofia 100.
Speaker from 1:00 PM to 1:15 PM

- **Oral presentation at the international conference**– *1st Mediterranean Agroecology Congress – AEMED 2025*. June 9–12, 2025 – Agrigento, Italy. Presentation title: “*Rebirth of Cotton in Sicily through a Sustainable Approach: An Integrated Assessment of Organic Practices, Deficit Irrigation, and Mycorrhizal Inoculation.*”
- **Seminar** – *Sicilian Organic Avocado: A Superfood for Enhancing the Ionian-Tyrrhenian Regions*. June 17, 2025 – Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100.
- **Conference** – *Final Conference of the “Di-Vino – Digital Innovation in Wine” Project*. June 24, 2025 – Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100.
- **Conference** – *Drought and Water Scarcity in Sicily: What Can We Learn from the Ongoing Drought?* June 20, 2025 – Aula Perrotta, Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100.
- **Conference** – *SIPROFF – Innovative Solutions for the Production and Valorization of Functional Baked Goods*. July 10, 2025 – Room G, Department of Agriculture, Food and Environment, Via Santa Sofia 100, Catania.