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SUSTAINABLE PLANT PROTECTION IN AUTONOMOUS CULTIVATION SYSTEMS: STRATEGIES FOR CONTROLLED ENVIRONMENTS

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ABSTRACT

Autonomous cultivation systems, including modern phytotrons as highly controlled plant growth environments, are becoming key tools in precision agriculture, plant research, and sustainable food production in closed systems. Their applications extend beyond academic and experimental laboratories to urban farming, gastronomy, and residential environments, enabling the cultivation of fresh crops directly at the point of consumption. These systems allow precise control of temperature, humidity, light, CO_2 concentration, and ventilation, providing optimal growth conditions but also introducing new challenges in biosafety, hygiene, environmental stability, and product quality. This paper analyzes abiotic and biotic factors affecting plant health in controlled environments and evaluates the risks associated with fungal, bacterial, viral pathogens, and insect pests. Special attention is given to contamination pathways such as water, substrates, air, and human handling and their interactions with microclimatic parameters. The persistence of pesticides in closed systems is discussed, as it may lead to higher residue levels than under field conditions. Based on current knowledge, the study proposes integrated preventive and protective strategies (IPM) combining physical, biological, and, where necessary, chemical measures. Emphasis is placed on environmental monitoring, automation, sensor technologies, and microclimate management for early risk detection and targeted intervention. The selection of resistant genotypes and optimization of microbial environments are highlighted as key elements of long-term sustainability. From a regulatory perspective, the paper underscores the need to update European GAP and HACCP systems to address the specificities of hydroponic, aquaponic, and phytotronic technologies, providing a foundation for safe, efficient, and environmentally sustainable plant production in controlled environments.

Keywords: Phytotron, Controlled environment agriculture, Autonomous cultivation, Plant health management, Biological control, Sustainable production, Vertical farming

INTRODUCTION

Growth chambers and phytotrons represent sophisticated tools that enable researchers and growers to precisely control environmental conditions such as temperature, humidity, light intensity and spectrum, photoperiod, CO_2 concentration, and ventilation, in order to study plant growth, development, and physiological responses in isolation from external influences **(Abdelouhahid *et al.*, 2020)**. Their primary advantage lies in the ability to conduct reproducible research, which is significantly limited in open-field agriculture by factors such as weather variability, contamination, and soil heterogeneity **(Downs, 1980)**.

Historically, phytotrons have been regarded as the most complex form of controlled environment systems. These systems typically combine greenhouses, controlled growth chambers, incubators, photoperiodic rooms, and precisely calibrated systems for light, humidity, and temperature. As early as the 1950s and 1960s, the first standardization efforts aimed at methodical calibration of growth conditions emerged, leading to the development of institutions such as the American Society for Horticultural Sciences **(Tibbitts & Krizek, 1997)**. Specialized personnel ensured continuous operation, minimizing downtime and reducing the risk of damage to experimental material **(Downs, 1980)**.

Today, growth chambers are evolving into highly digitized systems. The integration of Internet of Things (IoT) technologies, sensor networks, and open-source automation platforms (Arduino, Raspberry Pi, Python) allows researchers and growers to continuously monitor and adjust growth parameters such as pH, electrical conductivity (EC), light intensity, and CO_2 concentration **(Abdelouhahid *et al.*, 2020)**. A leading example of this technology is CERES 2010 a system inspired by NASA's ASTROCULTURE™ unit, integrating LED lighting, atmospheric control, humidity, and nutrient management **(Ignatius *et al.*, 1997)**.

These systems are now applied not only in research but also in vertical farming, particularly in urban areas. Vertical farms use layered growth chambers with LED lighting, hydroponic or aeroponic systems, and precise environmental control, producing 5- to 10-fold higher yields per unit area than traditional agriculture **(Asseng *et al.*, 2020)**, while consuming up to 90% less water and requiring no arable soil **(Sharma *et al.*,2024)**. Their use is expanding to gastronomy as well, with "kitchen phytotrons" enabling the cultivation of fresh vegetables and herbs directly in restaurants, reducing logistics and preserving product quality **(Olle & Viršile, 2013; Laužikė *et al.*, 2023)**.

From a research perspective, these systems are particularly effective for short-cycle species, including alternative crops like quinoa (*Chenopodium quinoa*) **(Austin *et al.*, 2016)** as well as common leafy and fruiting crops such as lettuce (*Lactuca sativa*), mustard (*Brassica juncea*), kale (*Brassica oleracea*), tomatoes (*Solanum lycopersicum*), peppers (*Capsicum annuum*), and cucumbers (*Cucumis sativus*).

Although these technologies represent the pinnacle of precision agriculture, their closed and intensive nature also introduces new risks associated with plant pathogens and pests. High planting density, elevated humidity, limited ventilation, and the absence of natural predators create favorable conditions for fungi, bacteria, viruses, and pests **(Spomer, 1981)**. Furthermore, uneven lighting and the formation of microclimatic zones may lead to non-uniform growth and reduced crop quality **(Delepoulle *et al.*, 2009; Minanda & Idris, 2022)**.

Therefore, the aim of this paper is to comprehensively evaluate the risk factors associated with plant cultivation in controlled growth chambers and phytotrons. Special attention is given to the biological, physical, and technological aspects of pest and disease emergence and spread. Subsequent sections address preventive and protective strategies, as well as approaches for optimizing the microenvironment to minimize yield losses while supporting reproducibility and ecological sustainability in cultivation systems.

Impact of Abiotic Factors on Plant Health in Controlled Cultivation Systems

Growth chambers and phytotrons allow precise control of environmental conditions, creating optimal settings for plant growth. However, this very enclosure also presents a potential risk for the accumulation of factors promoting disease and pest outbreaks.

Light, as the primary growth stimulus, plays a key role not only in photosynthesis but also in regulating plant resistance. Uneven lighting caused, for example, by improper LED placement or insufficient spectral coverage can lead to non-uniform growth and increased susceptibility to infections. Experiments have shown that even minor differences in light intensity result in variations in plant morphology and biomass quality **(Delepoulle *et al.*, 2009; Xu *et al.*, 2018)**. For instance, plants exposed only to red-blue spectra exhibited reduced photosynthesis, whereas a broader spectrum including infrared and UV radiation significantly improved vitality, as observed in peppers **(Minanda & Idris, 2022)**.

Water management is another critical factor, serving both as a nutrient carrier and a potential pathogen vector. Soil moisture fluctuations without precise monitoring, as well as inadequate filtration or water quality, disrupt growth and promote disease development **(Ariella *et al.*, 2023)**. Improving water quality, specifically increasing dissolved oxygen, enhanced lettuce photosynthesis by 164% while reducing nitrate content **(Ouyang *et al.*, 2020)**. Conversely, contaminated or recycled water led to toxin accumulation, reduced biomass, and diminished enzymatic activity **(Sadaf *et al.*, 2024)**.

Uneven temperature distribution, even a 2 °C difference between upper and lower chamber zones, can cause variation in plant size and quality **(Measures *et al.*, 1973)**. Temperature fluctuations due to lighting or insufficient ventilation increase disorders such as tip-burn **(Kumazaki, 2022)**. Even modern systems with ±0.5 °C precision may exhibit local deviations, particularly if root-zone temperature is not regulated, which critically affects plant metabolism **(Katagiri *et al.*, 2015; Liu & Walker, 1997)**.

Ventilation, integral to microclimate management, ensures uniform distribution of heat, humidity, and gases. Airflow affects both photosynthesis and leaf health. While historical guidelines suggested velocities around 0.75 m/s **(O'Leary & Knecht, 1974)**, recent studies indicate optimal photosynthetic saturation at 0.2–0.4 m/s **(Kitaya *et al.*, 2004)**, with uniformity being critical. Uneven airflow caused significant growth differences, later resolved through CFD optimization **(Peiro *et al.*, 2020)**.

Gaseous microclimate, particularly CO_2 and ethylene, is also crucial for plant health. While CO_2 is essential for photosynthesis, inaccurate regulation can reduce yields. Ethylene at 50 nmol·mol^{-1} decreased wheat yield by 36% and rice by 63% **(Klassen & Bugbee, 2002)**, and in tomatoes, levels ≥20 nmol·mol^{-1} inhibited fruit development **(Hudelson *et al.*, 2023)**. Chamber construction materials **(Knight, 1992)** and CO_2-binding filters **(Hoehn *et al.*, 2000)** can further destabilize the atmosphere.

Substrate composition and nutrition are also vital. Substrates like coconut fiber or peat provide higher yields than inert mats **(Bantis & Koukounaras, 2024)** but require proper EC management. A 1 dS·m^{-1} increase in EC reduced tomato biomass by up to 7.3% **(Schwarz *et al.*, 2014)**, limiting water uptake and nutrient availability **(Heinen *et al.*, 2002)**. Ion-exchange fiber substrates require even finer adjustments **(Berkovich *et al.*, 2003)**.

Finally, the "chamber effect" where nominally identical chambers exhibit different microclimatic parameters can arise from technical faults or subtle environmental interactions, compromising reproducibility and increasing local pest risk **(Porter et al., 2015)**.

Deviations in light, humidity, temperature, airflow, and nutrition interact and may trigger cascading effects that weaken plants and increase vulnerability. Examples of such interactions are given in (table 1). Effective disease and pest prevention, therefore, requires systemic thinking, precise technology, continuous monitoring, and rigorous experimental design. A well-managed environment is not an end in itself but a tool to influence plant interactions with potential pathogens and pests. The following sections focus on biotic factors threatening plant health fungal diseases, bacterial pathogens, viral infections, and animal pests whose occurrence, aggressiveness, and epidemiology are directly conditioned by the quality and stability of the growth environment.

Table 1 Examples of two-factor and three-factor interactions affecting plant vitality and resistance

Factor 1	**Factor 2**	**Factor 3**	**Mechanism of Influence**
Water	Nutrition		Water acts as a carrier for nutrients, influencing EC, pH, availability, and toxicity
Light	CO_2 (Gases)		Their combined effect influences photosynthesis, growth, and immune responses
Temperature	Ventilation		Together, they determine heat distribution and prevent stress hotspots
Water	Substrate		The substrate regulates water retention, aeration, and the microbiome
Nutrition	Substrate		Ion exchange and buffering capacity decide the availability of nitrogen, potassium, magnesium, etc.
Water	Temperature		Evaporation, irrigation needs, and the risk of drought or waterlogging are affected
CO_2	Ventilation		Proper ventilation prevents the accumulation of toxic gases and stabilizes photosynthesis
Light	Temperature		LED lighting can locally increase temperature, affecting growth and water balance
Light	Nutrition		Light alters metabolic activity and increases the demand for certain nutrients
Temperature	Substrate		Substrate temperature affects root metabolism and the uptake of water and nutrients
Ventilation	Water		It also influences leaf humidity and the risk of fungal infections, while affecting water loss from the substrate
Gases	Substrate		Some substrates absorb CO_2 or produce ethylene, for example, through the decomposition of organic matter
Light	Ventilation		Microclimatic contrasts occur within the growing environment
Water	Substrate	Nutrition	Substrates determine the flow, retention, and availability of nutrients, which is critical for osmotic balance
Light	CO_2	Temperature	Temperature regulates the speed of photosynthesis and metabolism, influencing growth and defense responses
Temperature	Ventilation	Water	It also affects evaporation, microclimate, and stress factors such as drought or waterlogging
Ventilation	CO_2	Light	Even distribution of CO_2 and light maximizes photosynthesis and reduces stress
Substrate	Nutrition	Temperature	Substrate temperature changes nutrient uptake by roots, especially nitrogen and potassium
Light	Temperature	Water	Uneven lighting increases evaporation, altering water balance and leaf stress
Gases	Substrate	Ventilation	Substrates can emit or retain gases like ethylene, which ventilation disperses
Light	Nutrition	Substrate	Light intensity affects nutrient demand and mobility across different substrates
Temperature	CO_2	Nutrition	Higher temperatures increase CO_2 consumption and nutrient requirements, particularly nitrogen
Water	Gases	Substrate	Wet, anaerobic conditions promote ethylene production and inhibit root growth

Legend: EC – electrical conductivity

Biotic Stress Factors in Controlled Environments

Fungal Pathogens

Fungal pathogens represent one of the most significant threats in controlled environments. High humidity, stable temperatures, dense vegetation, and limited ventilation create favorable conditions for the proliferation and spread of these microorganisms, necessitating targeted and integrated plant protection strategies. Diseases caused by fungi are among the most widespread in such systems and are currently estimated to account for over 70% of all plant diseases, leading to substantial yield losses and reduced quality of final products **(Jennings *et al.*, 2024; Mekapogu *et al.*, 2021)**.

The most common causal agents belong to the phyla Ascomycota and Basidiomycota, infecting various plant parts and causing symptoms such as wilting, rot, necrosis, and tissue deformation **(Manathunga *et al.*, 2024)**. Powdery mildew, caused by members of the order Erysiphales (genera *Erysiphe*, *Leveillula*, *Oidium*), is among the most prevalent diseases. It produces characteristic white coatings on leaves, disrupts photosynthesis, and leads to premature leaf senescence, thereby reducing overall yield **(Zhang *et al.*, 2022a)**. Secondary effects of powdery mildew include not only compromised product quality but also disruption of the natural microbial balance on leaf surfaces **(Yu *et al.*, 2022; Zhang *et al.*, 2018)**.

Downy mildew is another serious disease. For example, *Pseudoperonospora cubensis* causes severe damage to cucumbers under high air humidity, while *Peronosclerospora phillipinensis* attacks maize, significantly limiting growth and photosynthetic activity **(Najamuddin *et al.*, 2023)**. Anthracnose, caused by fungi of the genus *Colletotrichum*, is particularly critical; late detection can lead to up to 100% crop loss, especially in sensitive species such as chili peppers **(Jayawardena, 2016; Zakaria, 2021)**. Typical symptoms include dark lesions on fruits, necrosis, and overall reduced photosynthetic efficiency.

Gray mold (*Botrytis cinerea*) and leaf spot diseases also warrant attention, frequently occurring in tomato and lettuce cultivation. These pathogens spread primarily under high relative humidity and lower temperatures conditions commonly present in controlled environments during winter months or under intensive irrigation regimes **(Manathunga *et al.*, 2024)**.

Bacterial Pathogens

Bacterial pathogens pose a serious challenge in protected cultivation systems such as greenhouses, growth chambers, and phytotrons for two main reasons: first, they can cause plant infections and diseases, directly threatening yields and the economic efficiency of cultivation; second, they can contaminate edible plant products, posing significant risks to food safety and public health. In closed and recirculating systems, these risks are amplified by high humidity, stable temperatures, limited air circulation, and reduced opportunities for chemical disinfection.

In plant pathology, bacterial pathogens are comparably important to fungi. Controlled agricultural environments provide ideal conditions for their development and spread, while their rapid multiplication, frequent latent phase, and resistance to common chemical treatments make their control particularly challenging. Some species can cause yield losses ranging from 30% to complete crop destruction, depending on host sensitivity and environmental conditions **(Wassie & Yemata, 2025; Jibrin *et al.*, 2022)**.

The most problematic phytopathogenic bacteria include *Ralstonia solanacearum*, *Clavibacter michiganensis*, *Pseudomonas syringae*, and species of the genus *Xanthomonas*. They spread primarily through contaminated seeds, water, soil, tools, and plant debris **(Hayes *et al.*, 2022)**.

Ralstonia solanacearum causes bacterial wilt, especially in solanaceous crops like tomatoes and peppers. The pathogen enters through wounds or natural openings in the roots, rapidly colonizes the xylem vessels, and blocks water transport, leading to sudden wilting and gradual plant death **(Elsayed *et al.*, 2020; Xue *et al.*, 2020)**. Its longevity is particularly problematic, as it can survive in soil for more than 10 years in the absence of a host **(Chu *et al.*, 2022)**.

Clavibacter michiganensis subsp. *michiganensis* (Cmm), a quarantined organism, causes bacterial canker of tomatoes. Infection results in lesions on leaves, stems, and fruits, vascular system impairment, and severe yield losses of up to 84%. A dramatic example occurred in Mexico in 2006, when a 200-ha production area was destroyed, causing over $40 million in damages **(Pereyra-Bistraín *et al.*, 2021)**. The pathogen spreads primarily via seeds, wounded tissues, stomata, or hydathodes **(Yokotani *et al.*, 2021)**. Biofilms and polysaccharide structures formed in infected plants lead to asymmetric wilting, dehydration, and characteristic fruit spotting **(Carezzano *et al.*, 2023)**. It can survive on plant residues, soil, and tools for over two years, while effective control methods are very limited, and resistant cultivars are rare **(Ally *et al.*, 2023)**.

Pseudomonas syringae is a highly variable species complex infecting a wide range of hosts, including fruit, vegetables, and ornamentals. The pathogen enters through stomata, trichomes, or wounds, often remaining latent until stress conditions trigger necrosis, leaf spotting, or tumor-like growths **(Peňázová *et al.*, 2020; Jibrin *et al.*, 2022)**.

Species of *Xanthomonas* are primarily associated with bacterial leaf spot in tomatoes and peppers. These pathogens thrive under the high humidity and temperatures typical of greenhouse conditions. Infections cause numerous leaf lesions, leading to defoliation, reduced photosynthetic capacity, and, in severe cases, complete crop collapse **(Thomas *et al.*, 2024; Ally *et al.*, 2023)**.

A second group of bacteria does not damage plants directly but can contaminate products and cause severe illnesses in consumers. In controlled systems growing leafy greens or crops for raw consumption, this risk is particularly significant. High humidity, non-circulating systems, and water handling create ideal conditions for the persistence and spread of pathogenic bacteria.

The most widespread pathogen in this category is *Salmonella*, particularly *Salmonella enterica*, frequently detected in fresh products including tomatoes and leafy vegetables. In one study, up to 2.8% of greenhouse tomatoes were contaminated **(Reis Marques *et al.*, 2018; Theodoridis *et al.*, 2021; Gómez *et al.*, 2019)**. *Salmonella* is also a common cause of outbreaks in the European Union, often linked to consumption of raw vegetables **(Jechalke *et al.*, 2019; EFSA, 2011).**

Another critical pathogen is *Escherichia coli*, particularly Shiga-toxin producing strains (STEC) such as *E. coli* O157:H7. A notable outbreak occurred in Germany in 2011 linked to hydroponically grown fenugreek sprouts, resulting in over 4,000 cases and 53 deaths **(Aquilani *et al.*, 2022)**. Significant contaminations have also been reported in the USA, especially in Yuma (Arizona) and Salinas (California), major leafy vegetable production regions **(Avgoustaki & Xydis, 2020)**.

Listeria monocytogenes is a highly resilient pathogen capable of surviving on plant surfaces and various components of closed systems, including tanks, hoses, plastic parts, and substrates such as rockwool **(Gómez *et al.*, 2019; Ilic *et al.*, 2022)**. It can then spread via nutrient solutions, increasing the risk of crop contamination.

Other potentially hazardous bacteria identified in closed systems include *Campylobacter*, *Yersinia enterocolitica*, *Shigella*, and spore-forming species such as *Bacillus cereus* and *Clostridium* spp. **(Beuchat & Ryu, 1997; Gómez *et al.*, 2019; Buscaroli *et al.*, 2021)**. These microorganisms are particularly concerning due to growing antimicrobial resistance, which reduces the effectiveness of standard decontamination procedures and increases public health risks **(Iwu & Okoh, 2019)**.

Viral Diseases

High planting density, limited ventilation, frequent handling of plants, and a homogeneous environment facilitate both mechanical and vector-mediated pathogen transmission. Viruses cause severe physiological disorders, deformities, and yield losses that, depending on the pathogen, host plant, and infection stage, can reach up to 100%, especially with highly virulent strains and sensitive cultivars **(Hilaire *et al.*, 2022)**.

Among the most susceptible crops grown in controlled environments are tomatoes, cucumbers, and peppers. The most common viral pathogens are tobamoviruses (e.g., ToBRFV, ToMV, TMV), potyviruses, and orthotospoviruses (e.g., TSWV). These viruses are primarily transmitted by insect vectors such as aphids, leafhoppers, and thrips, but also mechanically through contact, tools, hands, contaminated seeds, or vegetative propagation **(Maachi *et al.*, 2021)**. Up to 80% of known plant viruses are insect-transmitted, and globalization of seed trade and changing climate conditions facilitate their introduction even into closed cultivation systems **(Singh *et al.*, 2020)**.

One of the most significant viral pathogens in the last decade is Tomato brown rugose fruit virus (ToBRFV), first described in Israel in 2014. Since then, it has been reported in more than 35 countries **(Luria *et al.*, 2017; EPPO 2020)**. ToBRFV is highly mechanically transmissible and exceptionally stable, surviving on contaminated surfaces, tools, and hands, which leads to explosive spread in closed systems. Symptoms include leaf mosaic, necrosis, and fruit deformation, rendering the harvest unsellable **(Zhang *et al.*, 2022b)**.

Tobamoviruses such as TMV, ToMV, and CGMMV (Cucumber green mottle mosaic virus) are known for their extreme persistence, surviving months to years on surfaces, plant debris, or substrates. Since they spread exclusively mechanically, control requires rigorous sanitation and mitigation of phytosanitary risks **(Jones, 2021)**.

Emerging pathogens include Tomato mottle mosaic virus (ToMMV), first described in Mexico **(Tu *et al.*, 2021; Nagai *et al.*, 2018)**. Orthotospoviruses, such as Tomato spotted wilt virus (TSWV), are transmitted by thrips, which serve as specific vectors required for viral replication. In contrast, aphids transmit other virus groups, such as potyviruses and cucumoviruses, including the well-known Cucumber mosaic virus (CMV) **(Kil *et al.*, 2018)**. CMV induces extensive chlorosis and malformations in leaves and fruits and is commonly present in greenhouses.

In phytotron environments, human activities such as manual pollination, leaf removal, or grafting significantly increase pathogen transmission risk. Viruses can also spread via infected propagation material **(Abou Kubaa *et al.*, 2023)**, contaminated tools, surfaces, and plant debris in substrates. Diagnosis is complicated as many viruses remain latent, making regular molecular testing (e.g., ELISA, RT-PCR) essential even during the pre-cultivation stage.

Animal Pests

In greenhouses, growth chambers, and phytotrons, the most significant animal pests include aphids (*Aphididae*), thrips (*Thysanoptera*), whiteflies (*Aleyrodidae*), and mites (*Tetranychidae*). These organisms threaten crop productivity through direct feeding, virus transmission, and honeydew excretion, which promotes the growth of saprophytic fungi **(Riddick, 2017; Li *et al.*, 2023)**.

Aphids, such as *Myzus persicae* and *Aphis gossypii*, play a key role as vectors of plant viruses. *M. persicae* can transmit over 100 different viruses **(Skouras *et al.*, 2023)**, and its genetic diversity and pesticide resistance significantly complicate control. In addition to phloem feeding, aphids produce honeydew that promotes black mold growth, reduces photosynthesis, and alters the microbial balance on leaves. They also excrete toxic substances that slow tissue development and cause plant deformities **(Idris *et al.*, 2021)**.

Of the more than 4,700 known aphid species, approximately 100 have a significant impact on agricultural production. In controlled environments such as greenhouses and phytotrons, dominant species include *Aphis gossypii*, *Macrosiphum euphorbiae*, *Aulacorthum solani*, and *Myzus persicae*. These species are characterized by rapid reproduction, short life cycles, and high resistance to multiple insecticide classes, which makes control challenging **(De Backer *et al.*,**

2015). As previously noted, *M. persicae* and *A. gossypii* are particularly important as virus vectors. Molecular studies have confirmed their genetic diversity and adaptability, including resistance to neonicotinoids **(Singh *et al.,* 2021; Margaritopoulos *et al.,* 2021)**. Beyond virus transmission, aphids inflict direct physiological damage through phloem feeding, honeydew production, and secretion of toxic compounds, which reduce photosynthesis and disrupt leaf and fruit development **(Idris *et al.,* 2021)**.

Thrips, especially the western flower thrips (*Frankliniella occidentalis*), have undergone explosive global expansion since the 1980s **(Xu & Enkegaard, 2010; Jandricic *et al.,* 2024).** They feed by sucking plant cells, causing scars, deformities, and reduced fruit quality. They are also important virus vectors, transmitting Tomato spotted wilt virus (TSWV) and Impatiens necrotic spot virus **(Stuart & Funderburk, 2012)**. Other thrips species, including *Thrips tabaci*, *Thrips palmi*, *Scirtothrips dorsalis*, and *Echinothrips americanus*, similarly damage a wide range of vegetable and ornamental plants **(Ghasemzadeh *et al.,* 2017; Li *et al.,* 2014)**.

Mites can produce 9–10 generations per year under optimal conditions, typical of greenhouse environments **(Nuralieva 2023)**. Their feeding causes light spots on leaves that gradually necrotize **(El Arnaouty *et al.,* 2018)**. Economic thresholds are very low; even three mobile forms per leaf may require intervention **(El Arnaouty *et al.,* 2020)**.

The occurrence of animal pests in controlled environments is often rapid and inconspicuous, yet the consequences can be devastating. A summary of the key findings is presented in table 2.

Table 2 Types of biotic agents, development conditions, and main effects on plants

Pathogen type	**Species (example)**	**Critical factor**	**Main effects on plants**
FMH	*Erysiphe, Pseudoperonospora, Botrytis*	air humidity	powdery mildew, downy mildew, gray mold, reduced photosynthesis
FMH	*Fusarium, Pythium*	substrate and water	root rot, limited nutrient uptake
Bacteria	*Ralstonia solanacearum*	temperature and water	wilting, rapid crop collapse
Bacteria	*Clavibacter michiganensis*	seeds, tissue injuries	bacterial canker of tomato
Bacteria	*Pseudomonas, Xanthomonas*	humidity + leaf injuries	leaf spots, defoliation
Bacteria	*Salmonella*, *E. coli, Listeria monocytogenes*	contamination of water, substrates, and fruits	no visible symptoms on plants; risk of food contamination and public health hazard
Viruses	Tobamoviruses (*ToBRFV, TMV, ToMV, CGMMV*)	handling, contaminated surfaces	explosive spread, fruit deformities
Viruses	Orthotospoviruses (*TSWV*)	presence of thrips	chlorosis, necrosis, reduced yield
Viruses	Cucumoviruses (*CMV*)	aphids	mosaics, leaf deformities
Viruses	Potyviruses	vectors (aphids)	a wide range of symptoms, including weakening of crops
Animal pests	Aphids (*Myzus persicae, Aphis gossypii*)	planting density, microclimate	transmission of >100 viruses, deformities, and damping-off
Animal pests	Thrips (*Frankliniella occidentalis*)	microclimate, thrips as vectors	TSWV spread
Animal pests	Whiteflies (*Bemisia tabaci*)	ventilation	weakened plants, secondary infections
Animal pests	Mites (*Tetranychidae*)	drought, high temperature	leaf damage, reduced photosynthesis

Legend: FMH - Fibrous microscopic fungi, *E. coli* – *Escherichia coli*, ToBRFV – Tomato brown rugose fruit virus, TMV – Tobacco mosaic virus, ToMV – Tomato mosaic virus, CGMMV – Cucumber green mottle mosaic virus, TSWV – Tomato spotted wilt virus, CMV – Cucumber mosaic virus

Pathways of Entry

In closed cultivation systems such as hydroponic, aquaponic farms, and phytotrons, contaminated water represents one of the primary entry points for pathogens. Sources of irrigation water including municipal supplies, wells, reservoirs, and ponds may contain microorganisms hazardous to consumer health. In hydroponic systems, *Escherichia coli* is most frequently introduced through irrigation water **(Avgoustaki & Xydis, 2020)**.

Besides water, other vectors of contamination include substrates, equipment surfaces, tools, and especially personnel, if strict hygiene standards are not followed **(Ivey *et al.,* 2025)**. Pathogen dissemination in controlled environments such as phytotrons is significantly accelerated by the recirculation of nutrient solutions, a standard feature of modern cultivation systems. Once introduced, bacteria can efficiently spread among plants and colonize the entire system **(McClure *et al.,* 2023; Gómez *et al.,* 2019)**. For instance, *Stenotrophomonas maltophilia* has been detected not only in leafy greens but also directly in the nutrient solution **(Li *et al.,* 2019)**. Pathogens such as *Salmonella Typhimurium* and *Listeria monocytogenes* have demonstrated the ability to persist in the system until harvest even after a single contamination event or extreme occurrences such as flooding or backflow in the irrigation network **(Ilic *et al.,* 2022)**.

In aquaponic systems, the issue is even more complex. Pathogens may enter not only via water but also through fish feed or their gut microbiota, creating an additional risk dimension **(Joyce *et al.,* 2019)**.

Cultivation materials such as pots, trays, tanks, and substrates are also critical contamination sources; if not properly decontaminated, they can introduce both soilborne and waterborne pathogens into the system **(Koike *et al.,* 2000)**. Contaminated seeds pose a particular threat, as pathogens like *E. coli* O157:H7 and *Salmonella enterica* can survive on seed surfaces for up to two years **(Martínez-Vaz *et al.,* 2014)**. When contaminated substrates come into contact with nutrient solutions, cross-contamination of plants can occur throughout the entire cultivation cycle **(Ivey *et al.,* 2025)**.

Poor personal hygiene of staff, failure to change protective clothing or footwear between operational zones, and inconsistent use of disinfection stations enable pathogen spread even in facilities with biosecurity protocols **(Bulgari *et al.,* 2019; Dong & Feng, 2022)**. Neglecting proper cleaning and disinfection of tools and equipment can facilitate microbial transfer between system components, increasing the risk of widespread contamination **(Koike *et al.,* 2000; Ivey *et al.,* 2025)**.

Additional factors may contribute to the introduction of pathogens into phytotrons. Aerosol droplets and condensate can carry bacteria and fungal spores; vegetative planting material may harbor latent viruses and bacteria, while insects and other animal vectors can transmit pathogens directly to plants **(Tang *et al.,* 2006)** just and technical aerosols from air-conditioning systems, as well as feces from rodents and other animals, represent further contamination risks, potentially introducing enteropathogens into the environment **(Gwenzi *et al.,* 2021)**.

The development and survival of these pathogens are strongly promoted by conditions typical of phytotrons and other closed systems particularly high relative humidity (80–90%) and stable temperatures ranging from 24 to 29 °C, which create an ideal microclimate for microbial proliferation **(Ilic *et al.,* 2022)**.

Therefore, effective prevention of pathogen introduction requires a holistic approach combining strict hygiene protocols, careful control of all inputs, and monitoring of systemic vulnerabilities. The following chapter will focus on specific preventive and protective strategies aimed at minimizing these risks.

Preventive and Protective Strategies

Closed cultivation systems such as greenhouses, phytotrons, and growth chambers represent highly controlled agro-environments where parameters like temperature, humidity, light regime, and air circulation are precisely managed to optimize plant growth and productivity **(Ampim *et al.,* 2022)**. However, this cultivation approach brings not only opportunities for yield enhancement but also specific challenges in plant protection (table 3). One advantage of such systems is the possibility of implementing more sophisticated biological control strategies that would be unfeasible under open-field conditions **(Krastanova *et al.,* 2022; Pavlík *et al.,* 2016)**.

A critical factor in closed cultivation systems is the significantly limited use of chemical treatments, particularly in recirculating setups such as aquaponics. The presence of aquatic organisms (e.g., fish) makes pesticide application impossible, requiring integrated protection strategies to rely solely on non-chemical approaches such as biological control, physical barriers, adhesive and light traps, or ecological manipulation of the environment **(Kostovarova *et al.,* 2022)**. This ecological imperative simultaneously stimulates research and the development of new sustainable solutions that are fully compatible with the closed nature of these systems.

One of the most effective non-chemical strategies is environmental manipulation, which is gaining increasing importance in controlled systems such as phytotrons and greenhouses **(Yusuf *et al.,* 2025)**. Through precise control of microclimatic conditions, it is possible to create environments unfavorable for pathogen development while promoting or optimizing plant growth. Dynamic management of temperature, humidity, light spectrum, and air exchange allows physical factors to be used not only as preventive measures but also as part of curative strategies against pathogens.

Table 3 Preventive measures against pathogens in phytotrons

Factor	**Target pathogen**	**Thermal lysis of cells at 40–60 °C**	**Steam sterilization, adapted solarization**
Temperature manipulation	Soil pathogens, weeds	Reduction of condensation and leaf wetness	Nighttime ventilation, RH regulation
Humidity control	Fungal pathogens (*Botrytis*, *Peronospora*)	UV-C inactivation, disruption of pest behavior	UV-C lamps, UV-blocking films
Light management	FMH, viruses, and insects	Removal of spores/pathogens from the air	HEPA filters, positive pressure
Air filtration and exchange	Airborne pathogens, viruses	Unfavorable conditions for pathogens	Liming, acidification
Substrate/soil pH adjustment	Soil FMH, bacteria	Induction of systemic plant resistance	*Bacillus subtilis*, *Pseudomonas spp.*
Bacterial inoculants (PGPR)	Bacteria, FMH	Predator versus pest	*Encarsia formosa*, predatory mites
Biological control	Insect pests	Disruption of the pathogen life cycle	Organic mulches, crop rotation
Crop rotation and mulching	Soil pathogens, saprophytes	Timely intervention before the spread	ELISA tests, visual inspection
Monitoring and early detection	All pathogens	Limitations of pathogen entry	Disinfection, tested planting material
Hygiene and certified planting material	Viruses, bacteria	Thermal lysis of cells at 40–60 °C	Steam sterilization, adapted solarization

Legend: FMH- Fibrous microscopic fungi, RH – relative humidity, PGPR – plant growth-promoting rhizobacteria, ELISA – enzyme-linked immunosorbent assay

Among the most common physical interventions is thermal manipulation, specifically steam sterilization of substrates or soil, routinely used in greenhouse systems for decontamination of soilborne pathogens **(Abdel Farag El-Shafie, 2020)**. Another approach involves adapting solarization principles traditionally used in open-field agriculture. Although phytotrons lack direct solar radiation, the controlled environment allows for simulated thermal conditions mimicking solarization effects. Key factors for efficiency include pre-irrigation of the soil and an exposure duration of 4–6 weeks **(Kaur & Kaur, 2020)**. In phytotrons, solar heat can be substituted with soil heaters or infrared lamps, ensuring target temperatures of 40–60 °C. These temperatures cause pathogen cell lysis and reduce weed seed germination similar to conventional solarization **(Argento *et al.*, 2024)**. Moreover, the precision and repeatability of conditions in phytotrons enable detailed evaluation and optimization of such treatments, which is especially advantageous in research settings.

Humidity control is another fundamental tool for preventing fungal diseases, which often spread rapidly and aggressively in closed systems. Pathogens such as *Botrytis cinerea* **(Li *et al.*, 2023)** or *Peronospora* spp. **(Cohen & Ben-Naim, 2016)** thrive under high relative humidity, particularly when leaf surface condensation occurs. Targeted reduction of air humidity especially during nighttime can effectively interrupt the infection cycle and limit disease spread without the need for chemical control.

Light manipulation represents a remarkably versatile tool in physical plant protection. The light spectrum affects not only pest behavior but also the physiological processes of pathogens. For instance, special films and nets that block UV radiation in the 280–400 nm range have been proven to disrupt the visual orientation of many insect pests under greenhouse conditions **(El-Baky & Amara, 2021; Idris *et al.*, 2021)**. In addition to passive light filtering, the active application of UV-C radiation directly onto plants is increasingly used. This approach has proven effective, for example, in suppressing powdery mildew on strawberries and apples, with efficiency depending on the intensity, duration, and time of day of application. The best results are achieved at night, when the risk of photosynthetic photoinhibition is eliminated **(El-Baky & Amara, 2021)**. A promising research direction is the combination of blue and UV-C spectra, which not only directly inhibits pathogen growth but also stimulates the expression of plant defense genes, thereby enhancing natural resistance to reinfection **(Jazayeri *et al.*, 2024; Ramalingam *et al.*, 2024)**.

In addition to dynamic interventions, stable temperature regulation is essential. An optimal temperature around 22 °C suppresses microbial activity **(Mishra & Barolia, 2020)**, while adjustable temperature ranges prevent the formation of conditions favorable to specific pathogens (Fernandez **-Valenzuela *et al.*, 2021)**.

From a sustainability perspective, light and climate-based interventions offer valuable solutions they do not induce resistance, leave no residues, and preserve ecological balance. Their success, however, depends on detailed knowledge of pathogen–plant–environment interactions and high precision in microclimate control.

A key pillar of modern prevention is automated environmental management based on sensor data. Smart systems enable precise regulation of CO_2 concentration, humidity, temperature, and lighting, thereby eliminating stress factors that increase plant susceptibility to infections. Vertical farms with dynamic microclimate control achieve not only higher yields but also reduced disease incidence and lower energy consumption **(Duangpakdee & Sukpancharoen, 2024; Li *et al.*, 2023; Kaiser *et al.*, 2024)**. These systems also allow flexible production planning, reducing waste and improving efficiency.

An essential aspect of environmental optimization is air-flow modeling through computational fluid dynamics (CFD). This tool helps identify and eliminate air stagnation zones prone to mold and bacterial growth. Studies on crops such as basil and lettuce have shown that properly adjusted air circulation promotes uniform growth and reduces disease occurrence **(Nurmalisa *et al.*, 2021; Plas & De Paepe, 2023)**. Combining CFD simulations with sensor data enables precise microclimate control within specific cultivation zones.

However, the foundation of protection also lies in physical isolation from the external environment. Air-handling units equipped with HEPA filters effectively capture airborne pathogens and maintain sterile conditions **(Bakhsh *et al.*, 2025; Livadariu *et al.*, 2024)**. Air curtains at entry points to cultivation areas further prevent accidental contaminant intrusion **(Avgoustaki & Xydis, 2020)**.

The overall effectiveness of the protective strategy can be further strengthened through physical barriers and antimicrobial surface treatments, which significantly reduce microbial load in the environment **(Mishra & Barolia, 2020)**. The choice of substrate also plays an important role for example, mineral wool, which enters the system sterile and pathogen-free **(Van Gerrewey *et al.*, 2024)**.

A highly effective tool for preventing the spread of infections in closed cultivation systems is regular environmental hygiene aimed at reducing microbial load. Proven approaches include bioaerosol control, surface sanitation (e.g., using electrolyzed water), and air microbiome monitoring, which have shown exceptional efficacy in controlling pathogen transmission **(Kozdrój *et al.*, 2024; Bhakta *et al.*, 2023)** Despite their importance, hygienic measures are often underestimated in practice, although they form one of the key pillars of integrated plant protection. These include mechanical removal of damaged or infected plant parts such as dry, frozen, or spotted shoots, leaves, and flowers. This approach is ecologically safe, non-invasive, and does not increase the risk of pathogen resistance, though its large-scale implementation can be labor-intensive **(Sarwar, 2014)**.

The effectiveness of such manual interventions increases significantly when combined with advanced monitoring technologies that enable early detection of problematic areas. Multispectral cameras, temperature and humidity sensors, and other detection mechanisms provide real-time insight into plant health and signal environmental changes or biotic activity **(Mansoor *et al.*, 2025)**. When linked with automated data collection and analysis, these technologies support rapid decision-making, targeted actions, and proactive management, replacing traditional reactive strategies **(Khan *et al.*, 2020)**.

Another crucial aspect is substrate and circulation system hygiene, particularly in hydroponic and aquaponic systems, where the substrate is replaced by a nutrient medium. Although hydroponic systems naturally eliminate soilborne pathogens, they remain vulnerable to infection spread through recirculating water loops. Therefore, monitoring based on HACCP principles is essential identifying critical control points and regularly disinfecting circulation units **(Sela Saldinger *et al.*, 2023; Hamilton *et al.*, 2023)**. A highly promising solution is the deployment of biosensors, which allow early detection of microbial contamination before it spreads throughout the system **(Artimová *et al.*., 2023)**.

Hygiene management should also include standardized operational protocols, such as disinfection of hands, footwear, and tools before entering cultivation sections. These measures greatly reduce the risk of introducing viral diseases, which can spread rapidly in closed systems.

One of the core pillars of modern integrated plant protection is biological control, which shows exceptional efficiency in controlled environments. Thanks to the stable and regulated conditions particularly in temperature, humidity, and light beneficial microorganisms have optimal conditions for colonization, allowing them to effectively compete with pathogens **(Ally *et al.*, 2023)**.

The rhizosphere microbiome plays a particularly important role in suppressing soilborne pathogens and strengthening plant defense mechanisms. Among the most effective biological agents are filamentous fungi of the genus *Trichoderma* and bacteria of the genera *Pseudomonas* and *Bacillus*, which have demonstrated positive effects on plant health and the inhibition of a wide range of phytopathogens **(Anzalone *et al.*, 2022)**.

Bacteria of the genus *Bacillus*, particularly *B. thuringiensis* and *B. subtilis*, exhibit especially broad and effective applications. These microorganisms not only suppress pathogen growth (e.g., *Phytophthora capsici*), but also stimulate plant growth through the synthesis of growth-promoting compounds and activation of natural defense mechanisms **(Robinson *et al.*, 2024)**. Repeated applications of *B. subtilis* in greenhouse conditions have proven to be an effective biological control method without negative ecological impacts **(Abdel-Kader *et al.*, 2012)**. The success of biological strategies is further enhanced by their integration with other cultivation technologies, such as microclimate management, plant nutrition control, and regular pest and disease monitoring **(Kostovarova *et al.*, 2022)**.

In biological protection, plant growth-promoting rhizobacteria (PGPR) and arbuscular mycorrhizal fungi (AMF) play an essential role. These microorganisms enhance nutrient uptake, promote growth, and increase plant resistance to stress factors. Their application in greenhouse conditions has been shown to reduce pathogen incidence in the rhizosphere and improve plant physiological stability **(Hnini *et al.,* 2024; Fasusi *et al.,* 2023; Zeng *et al.,* 2025).** In more advanced systems, such as phytotrons, opportunities arise for the use of synthetic microbiomes or CRISPR-modified strains with specifically designed properties **(Joshi *et al.,* 2025; Du *et al.,* 2025)**.

Biological approaches include not only microorganisms but also natural enemies of pests. Well-established examples include predatory bugs of the genus *Orius* spp., which effectively regulate thrips populations, and the parasitic wasp *Encarsia formosa*, specialized in controlling whitefly larvae **(Hoddle *et al.,* 1998)**. These species are consistently used in biological control programs where pesticide use is restricted. The effectiveness of biological strategies can be further enhanced through integration with physical and behavioral methods, such as sticky and light traps exploiting pest chromatotropism and phototaxis, or protective net barriers reducing the entry of flying insects into cultivation areas. Moreover, precise microclimate control particularly of humidity, temperature, and airflow can significantly affect pest life cycles and reduce their reproductive capacity.

By combining multiple approaches biological protection, physical and environmental interventions, and precise monitoring it is possible to create a long-term, stable, and ecologically balanced system that maintains both productivity and product quality. A well-designed integrated plant protection strategy provides a sustainable alternative to repeated chemical treatments, while protecting beneficial organisms and microbiota essential for healthy crop growth in closed systems.

Modern prevention strategies in growth chambers and phytotrons increasingly move toward synergistic integration of technology, biology, hygiene, and precision management. Success depends not on isolated measures but on their coordinated implementation, which minimizes the risk of pathogen and pest spread, optimizes yields, and reduces the ecological footprint of production.

The future of controlled-environment agriculture lies in intelligent, adaptive systems capable of prediction, learning, and proactive intervention before problems occur. Major advances in this area are being driven by digital technologies such as the Internet of Things (IoT), artificial intelligence (AI), big data analytics, and environmental modeling. These tools enable continuous monitoring and real-time predictive control of growing conditions.

Using IoT sensors, key parameters such as temperature, humidity, CO_2 concentration, pH, and electrical conductivity are continuously tracked, providing an accurate picture of the microclimate and substrate conditions. The collected data are then analyzed through deep learning algorithms (e.g., CNNs, RNNs), which can detect the emergence of pathogens or environmental fluctuations before disease symptoms appear **(Walia *et al.,* 2021; Alazzai *et al.,* 2024; Palani *et al.,* 2023)**.

These intelligent systems have already been successfully implemented, for example, in the prediction of bacterial root infections in maize and in the optimization of tomato growth, where they enabled improved management of water, nutrient, and energy consumption **(Al-Otaibi *et al.,* 2024; Sivakumar, 2024)**. The integration of AI and IoT, therefore, represents more than just a reactive tool it opens the path toward preventive and sustainable cultivation with a significantly lower ecological footprint **(Kosharіya *et al.,* 2025)**. Moreover, these systems are increasingly interconnected with autonomous robotic units capable of performing interventions and monitoring fully automatically and with high precision, thereby reducing dependence on manual labor **(Barbosa *et al.,* 2024)**.

An important component of modern prevention is also the genetic selection of suitable genotypes adapted to the specific conditions of phytotron systems environments characterized by high humidity, limited air circulation, and stable microclimatic parameters. Under such conditions, it is crucial to cultivate varieties with enhanced resistance to both biotic and abiotic stressors. A particularly promising approach in this context is “speed breeding” accelerated breeding in controlled environments, which allows for rapid testing and development of resilient plant lines specifically optimized for growth chambers **(Gudi *et al.,* 2022; Schwarz *et al.,* 2014; Bhattarai *et al.,* 2025)**. Special attention is given to genotypes with enhanced expression of antioxidant enzyme genes and resistance to viral pathogens, especially potyviruses, which are a frequent issue in controlled cultivation systems.

Another key element of prevention is the selection of high-quality, pathogen-free planting material. The use of certified propagation material tested for the presence of viral pathogens forms the foundation of preventive protection, ensuring that contamination risks are minimized from the very start of the production cycle.

Residue Risks

Phytotrons, as highly controlled closed cultivation systems, create specific microclimatic conditions that significantly influence pesticide persistence compared to field cultivation. Their technical design effectively eliminates the influence of external degradative factors such as wind, rain, or direct sunlight, and the absence of precipitation prevents natural leaf washing, leading to longer pesticide retention on plant surfaces **(Yang & Choi, 2024; Noh *et al.,* 2019)**. Moreover, typical conditions such as low UV intensity, limited air exchange, and high humidity slow down key degradation processes, including photolysis, evaporation, and diffusion. As a result, chemical residues persist longer in plant tissues, leading to higher pesticide residue levels a phenomenon confirmed, for example, in greenhouse-grown tomatoes **(Nakhungu *et al.,* 2021; Song *et al.,* 2021)**.

The extended persistence of pesticides is also reflected in longer half-lives. For instance, cis-fenvalerate degraded in a closed environment such as a phytotron over 9.8 days, compared to 5.7 days under field conditions **(Song *et al.,* 2021)**. Similarly, prolonged half-lives were observed for penthiopyrad in cucumbers and tomatoes cultivated in greenhouses **(Alminderej *et al.,* 2024; Lee *et al.,* 2024).**

In closed systems, the risk of pesticide accumulation also increases. Poor ventilation promotes the deposition of active substances on plant surfaces, raising the likelihood of secondary contamination. Repeated applications without sufficient degradation time can result in up to a twofold increase in residue levels in crops **(Qi *et al.,* 2023)**.

Accurate pesticide residue monitoring in phytotrons requires reliable and highly sensitive analytical methods. The standard procedure involves sample fortification at the limit of quantification and its tenfold level to ensure precision even at very low concentrations **(Kim *et al.,* 2020; An *et al.,* 2023)**.

The QuEChERS method is most commonly used a quick, easy, cheap, effective, rugged, and safe extraction and cleanup technique suitable for multi-residue analysis **(Lim *et al.,* 2016; An *et al.,* 2023)**. In analytical practice, liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) predominates, achieving quantification limits as low as 0.01 mg/kg or below **(Kim et al., 2020; Kwak *et al.,* 2023)**.

However, for certain persistent organochlorine pesticides, gas chromatography with electron capture detection (GC-ECD) remains in use, providing detection limits in the range of 0.6–6.0 μg/kg and recoveries between 74.4–115.6% **(Lim *et al.,* 2016)**.

Protection

Even with strict adherence to preventive measures, outbreaks of infections or pest infestations may still occur in closed cultivation systems such as greenhouses and phytotrons. In such cases, it is essential to respond promptly and comprehensively to halt pathogen spread, minimize crop damage, and maintain the stability of the microclimate, substrate, and beneficial microflora. Effective protection requires a multidisciplinary approach that integrates biological, physical, and when necessary, chemical strategies, all aligned with the principles of Integrated Pest Management (IPM) **(Zhou *et al.,* 2024)**.

Critical factors for successful intervention include speed of response, targeted application, and environmental compatibility. Key elements of sustainable protection comprise the combination of biological and physical methods, minimization of chemical inputs, strict sanitation, regular monitoring of crop health, and the selection of resistant varieties (table 4). According to IPM principles, protective methods should not be applied in isolation but in a coordinated combination tailored to the specific environmental and biological context. Precise microclimate regulation and the use of resistant or tolerant genotypes are also integral components of this approach.

In response to these challenges, an integrated strategy combining biological and physical methods with well-timed chemical interventions is gaining prominence. Studies have shown that combining entomopathogenic fungi such as *Beauveria bassiana* and *Verticillium lecanii* with selected fungicides can produce synergistic effects, reducing the need for high pesticide doses while maintaining the vitality and efficacy of biological agents **(Ablazova *et al.,* 2023; Zuparova *et al.,* 2023)**.

A decisive factor for success is application timing. Applying chemical treatments without regard for microbial activity can negatively impact beneficial organisms. Therefore, the principle of temporal compatibility has proven essential for preserving long-term program effectiveness and reducing resistance risk **(Singh & Kaur, 2020)**.

Conversely, unilateral and repeated chemical use, though it may yield short-term results, has long-term negative consequences. It creates selective pressure for resistant pest and pathogen strains, reduces agroecosystem biodiversity, and disrupts the delicate ecological balance, especially in closed systems. Thus, it is crucial to prefer chemicals compatible with biological agents, particularly entomopathogenic fungi. Proven fungicides that do not negatively affect microbial viability or colonization include Bayleton 25% (triadimefon) and Fundazol 50% (benomyl). These products, tested in combined biological protection systems, showed minimal interference with fungal efficacy **(Ablazova *et al.,* 2023; Laktionov *et al.,* 2020*)***.

In situations where chemical treatments are undesirable or ineffective, physical methods become a key protection tool. Upon pest outbreak, they represent one of the most effective and immediately available intervention options. Such measures act as a rapid containment barrier, reducing pest populations without disturbing biological balance or microflora, and without creating resistance pressure. Thanks to their non-invasive nature and repeatability, they are well-suited as the first line of defense following infestation detection.

Table 4 Plant Protection Strategies in Phytotrons: Integration of Methods, Targets, and Risk

Type of Protection	Specific method	Target organism	Advantages	Limitations/risks
Physical	Fine meshes (80-mesh)	Aerial insect vectors (whiteflies, aphids, thrips)	Reduce pest ingress, without chemical load	May reduce ventilation (up to 50%)
Physical	Insecticide-treated nets	Aerial insect vectors	A combination of mechanical and chemical protection, maintained circulation	Risk of resistance development with long-term use
Physical	Surface barriers (diatomaceous earth, sand, non-woven fabrics)	Soil stages of pests (*Sciaridae, Bradysia spp.*)	Immediate deployment, interruption of reproduction	Requires regular renewal and monitoring
Physical	UV-C radiation	Pathogens, insect vectors	Sterilizing effect, possible positive impact on plant growth	Requires precise dosing, risk of photodamage
Biological	Entomopathogenic fungi (*Beauveria bassiana*, *V. lecanii*)	Insect pests	Ecological, effective with proper application	Sensitivity to unsuitable environments, compatibility with other interventions
Biological	*Trichoderma* fungi	Soil and foliar pathogens (*Pythium, Botrytis, Sclerotinia*)	Antagonistic action + growth stimulation	Requires favorable conditions for colonization
Biological	*Bacillus thuringiensis*	Insect pests (mainly caterpillars)	Targeted effect, safe for non-target organisms	Requires precise dosing, effectiveness depends on pest life stage
Biological	*Bacillus siamensis*, *Paenibacillus polymyxa*	Soil and foliar pathogens	Induction of resistance, suppression of pathogens	Variable efficacy depending on strain, requires live inoculum
Biological	PGPR bacteria (*Bacillus*, *Paenibacillus*)	Pathogens + general growth stimulation	Induction of systemic resistance, growth-promoting effect	Effectiveness depends on sanitation and seed quality
Biological	Natural enemies (*Orius spp.*, *Encarsia formosa*)	Thrips, whiteflies	Persistent regulatory effect in the system	Proper introduction and conditions for survival required
Chemical	Selective fungicides compatible with bioagents	Fungal pathogens	Precise application, synergistic effect	Risk of resistance requires correct timing
Chemical	Targeted application of insecticides	Insect pests	Rapid action against high pest populations	Multi-resistance, environmental risks

Legend: PGPR – plant growth-promoting rhizobacteria

However, the acute deployment of physical methods requires the complete sealing of the growing environment, not only the main inlets and ventilation. Pest migration through cracks, cable ducts, or frame joints is often underestimated. Therefore, after pest detection, it is critical to immediately inspect and temporarily seal all potential entry points, including maintenance and service areas. Only then can barrier systems remain functional during an outbreak and prevent cross-zone contamination **(Boiteau & Vernon, 2001)**.

Among the most effective interventions against insect pests is the installation of fine mesh screens on ventilation and service openings. In cases where pests spread through the air such as *Aleyrodes vaporariorum*, aphids, or thrips, it is recommended to switch from conventional 1–1.5 mm meshes to finer 80-mesh screens, which can significantly reduce the number of new intrusions **(Kusakari *et al.*, 2022; Wang *et al.*, 2018)**. In situations where microclimatic conditions do not allow a reduction in airflow (fine meshes can reduce ventilation by up to 50% **(Agrafioti *et al.*, 2020)**, insecticide-treated meshes can be advantageously used. These combine mechanical protection with the effects of chemical agents while maintaining better air circulation **(Dáder *et al.*, 2015)**.

For soil-associated pests, such as larvae of *Sciaridae* or *Bradysia spp.*, surface barriers made of diatomaceous earth, sand, or nonwoven fabrics have proven effective. These materials prevent adults from laying eggs in the substrate, disrupting the pest reproductive cycle and reducing the risk of secondary pathogen transmission via soil vectors. The advantage of these solutions is their immediate deployability without interfering with the plant growth cycle **(Cloyd, 2015)**.

Beyond mechanical barriers, physical technologies with sterilizing or inhibitory effects are increasingly being implemented. Methods such as UV-C radiation, ozone mist, or low-pressure plasma have demonstrated high efficiency in reducing pest populations such as aphids, without the environmental burden associated with chemical pesticides **(Zver *et al.*, 2024; Ebihara *et al.*, 2017)**. Moreover, experiments suggest that repeated exposure to low doses of UV-C radiation can not only suppress pathogenic microorganisms but also stimulate positive physiological responses in plants, including enhanced fruit formation and yield, likely through photomorphogenic signaling and hormonal regulation **(Fehrenbach *et al.*, 2023)**.

Unlike physical barriers, which act immediately and locally, biological control represents a dynamic but more time-dependent form of protection. In closed cultivation systems such as greenhouses and growth chambers, biological agents have emerged as ecologically safe and long-term sustainable solutions. The most commonly used organisms include entomopathogenic fungi (e.g., *Beauveria bassiana* **(Ferdous & Ahmmed, 2024; Barbar *et al.*, 2024)**) and bacteria such as *Bacillus thuringiensis* **(Sun, 2024)**, along with natural predators and parasitoids. These biological factors have been successfully employed mainly in greenhouse environments, but promising results are also being achieved in growth chambers, where automated distribution systems, such as drones or dosing units are increasingly used to ensure precise and repeatable application with minimal human intervention.

Particular attention within biological protection is given to fungi of the genus Trichoderma, which not only exhibit strong antagonistic activity against phytopathogens but also stimulate plant growth. For instance, *T. lignorum* achieves 100% inhibition against *Pythium spp.*, while *T. asperellum* and *T. koningii* show efficacy ranging from 83.5% to 94.1% **(Scerbacova, 2024; Rossa *et al.*, 2019)**. Other species, such as *T. harzianum*, *T. viride*, and *T. virens*, are highly effective in controlling both soilborne and foliar diseases particularly *Sclerotinia sclerotiorum* and *Botrytis cinerea* and their ability to colonize wounded plant tissues significantly reduces the risk of secondary infections **(Abdel-Kader *et al.*, 2012; Ally *et al.*, 2023)**.

Effective protection against pathogens such as downy mildew (*Pseudoperonospora cubensis*), anthracnose (*Colletotrichum spp.*), or gray mold (*Botrytis cinerea*) requires a comprehensive strategy. This involves a combination of biological agents (e.g., *Trichoderma spp.*, *Bacillus siamensis*, *Paenibacillus polymyxa*), physical measures, and targeted fungicide applications **(Abdelfatah *et al.*, 2025; Hafez *et al.*, 2018)**. These bacteria also induce systemic resistance in plants, enhancing their ability to respond to infection stress. As a complementary approach, the selective use of low doses of UV-C radiation has shown promise damaging pathogenic cells without negatively affecting host plant vitality **(Suprapta, 2022; Felek *et al.*, 2025)**.

An important part of integrated strategies is represented by plant growth-promoting rhizobacteria (PGPR), which combine protective effects with improved crop performance. Their effectiveness increases significantly when accompanied by strict sanitation practices including tool disinfection, seed quality control, and weed elimination as pathogen reservoirs. Such measures greatly reduce infection recurrence and improve overall crop health **(Singh *et al.*, 2022)**.

Beyond microorganisms, natural enemies of insect pests are increasingly used in biological control for example, *Orius spp.* (thrips predators) or *Encarsia formosa* (whitefly parasitoid) **(Hoddle *et al.*, 1998; Dai *et al.*, 2024)**. These strategies can be effectively complemented by behavioral manipulations, such as light traps or the use of chromotropic responses, and combined with physical barriers. The result is the stabilization of pest populations and the creation of ecological balance suitable for long-term sustainable cultivation **(Foster & Harris, 1997; Rhainds, 2024)**.

Chemical plant protection in closed cultivation systems continues to play an important role, but only as a carefully applied component of integrated pest management (IPM). Current recommendations suggest its use only where biological and physical methods fail to achieve sufficient control **(Lazarević-Pašti *et al.,* 2025)**. Its effectiveness depends primarily on precise timing and targeted application during the early phases of infection, allowing the extent of intervention to be minimized.

Closed systems such as greenhouses or hydroponic units offer significant advantages through precise monitoring and optimized microclimate control. These conditions make it possible to reduce both the frequency and dosage of pesticide applications, thereby directly minimizing environmental risks while increasing treatment efficiency **(Kostovarova *et al.,* 2022)**. In modern IPM, chemical control is no longer the primary tool but a supplementary, targeted intervention that must be harmonized with biological and physical methods. This integrated approach reduces the risk of resistance development, helps preserve beneficial microflora, and stabilizes production systems over the long term.

When these principles are consistently applied, it is possible to achieve effective pathogen suppression without disrupting the ecological balance of the system. Such a design represents the most sustainable and environmentally responsible strategy for plant protection in controlled environments such as phytotrons.

Successful plant protection in phytotrons requires tailoring strategies to the specific type of pest or pathogen. For fungal pathogens, such as gray mold (*Botrytis cinerea*), which thrives under high humidity and poor ventilation, the most effective approach is a combination of chemical treatments (e.g., phenylpyrroles) with microclimate adjustments. Improving airflow, reducing leaf condensation, and timely intervention create a synergistic effect that significantly limits secondary infections **(Kuwahara *et al.,* 2024; Hai *et al.,* 2025)**.

Bacterial pathogens, such as *Ralstonia solanacearum*, *Clavibacter michiganensis*, and *Xanthomonas spp.*, pose a different challenge due to their rapid vascular colonization and survival across a wide range of environmental conditions. Given their high resistance to bactericides, biocontrol strategies using antagonistic microorganisms from the genera *Bacillus*, *Streptomyces*, and *Pseudomonas* are particularly suitable. These microbes not only suppress pathogen populations but also stabilize rhizosphere microflora, enhancing the overall resilience of the crop **(Hu *et al.,* 2021; Wang *et al.,* 2017)**.

Viral infections such as ToBRFV, CMV, and TMV are even more challenging because effective direct treatments are lacking. Upon diagnosing an infected plant, immediate removal, space decontamination, tool sterilization, and strict adherence to hygiene protocols are essential **(Ally *et al.,* 2023)**. Since most viruses spread via insect vectors, controlling these vectors is crucial, using sticky traps, insecticidal barriers, or biological control agents. Preventive measures also include certified, virus-tested planting material and rigorous hygiene protocols.

Animal pests, particularly whiteflies, mites, thrips, and aphids, are a specific problem in phytotrons because the closed environment supports rapid reproduction in the absence of natural predators. Many of these species also exhibit multi-resistance to several insecticide classes **(Cao *et al.,* 2019; Nuralieva 2023; Singh *et al.,* 2021; Margaritopoulos *et al.,* 2021; Xu & Enkegaard, 2010; De Backer *et al.,* 2015),** which severely limits the effectiveness of chemical control. A typical example is the western flower thrips (*Frankliniella occidentalis*), some populations of which show extreme resistance. In such cases, it is necessary to implement comprehensive management programs that integrate biological agents, behavioral tools, and precise environmental control.

These strategies emphasize prevention, monitoring, and rapid intervention, ensuring that pest populations are managed sustainably without disrupting the ecological balance within the controlled environment.

Legislative framework

Foods of non-animal origin are becoming an increasingly significant source of foodborne illnesses, representing a new trend in public health. While infections associated with meat, eggs, and dairy products dominated in the past, there is currently a rise in cases linked to the consumption of plant-based foods **(Moravkova *et al.,* 2019)**. The European Food Safety Authority (EFSA) has reported a significant increase in outbreaks, hospitalizations, and deaths caused by fresh plant products **(Surányi *et al.,* 2023)**. According to EFSA, the highest-risk pathogens include verotoxin-producing *Escherichia coli* (especially in seeds and sprouts), *Salmonella*, and Norovirus (in leafy vegetables, berries, tomatoes, and melons), as well as pathogen combinations (in onions, stem vegetables, and carrots). Guidelines therefore emphasize the prevention of illnesses caused by *E. coli*, hepatitis A virus, and *Listeria*, with all growers required to comply with relevant EU regulations (EC Guidance 163/2017).

Legislative and regulatory frameworks have responded to these challenges. In the United States, the foundation is the Food Safety Modernization Act (FSMA), which obliges growers to develop food safety plans, conduct risk analyses, and undergo regular audits **(Dong & Feng, 2022; Coleman *et al.,* 2017)**. However, FSMA was primarily designed for field production, which complicates its application in soilless systems particularly regarding water regulation, where the specifics of closed hydroponic systems are not considered **(Ilic *et al.,* 2022; Riggio *et al.,* 2019)**. Additionally, many small farms, including hydroponic and aquaponic operations, are not subject to these regulations **(Dong & Feng, 2022)**.

In the European Union, microbiological risks are regulated by Commission Notice (EC 163/2017), which provides a framework for hygiene requirements in the primary production of fresh fruits and vegetables. The document focuses primarily on harvesting, washing, sorting, and packaging processes and aligns with the general requirements of Regulation (EC) No. 852/2004. Its goal is to create a uniform approach for all growers, regardless of farm size.

From a practical cultivation perspective, legislation for controlled environments such as growth chambers, phytotrons, or vertical farms is often based on rules originally designed for field production. These frameworks are derived from Good Agricultural Practices (GAP), which promote hygiene, contamination prevention, and sustainable production **(Nakano, 2017)**. Expert recommendations in this area are provided by organizations such as USDA, GlobalGAP, and the Aquaponic Association **(Sawyer, 2021)**. Given the specifics of soilless systems particularly hydroponic and aquaponic production there is a growing need to develop GAP guidelines tailored to these technologies **(Sela Saldinger *et al.,* 2023)**. This also applies to phytotrons, which operate in closed environments with precisely controlled conditions, differing from traditional field and greenhouse models.

The European and Mediterranean Plant Protection Organization (EPPO) serves as an intergovernmental organization dedicated to cooperation and harmonization in plant protection across the European and Mediterranean region **(Milavec *et al.*, 2021; Brunetti *et al.*, 2022; Tovar-Pedraza *et al.*, 2024)**. EPPO functions as an intergovernmental organization with 52 member countries spanning the European and Mediterranean region **(Moss *et al.*, 2019)**. As the Regional Plant Protection Organization (RPPO) for the Euro-Mediterranean region, EPPO coordinates plant protection activities among member National Plant Protection Organizations (NPPOs), extending its coverage across the whole of Europe rather than just the European Union **(Montilon *et al.*, 2023; Hilaire *et al.*, 2022)**. EPPO operates several critical databases and information systems that serve as foundational tools for plant protection across the Euro-Mediterranean region. The organization maintains the EPPO Global Database, which serves as a comprehensive repository for pest information, including data on host plants and distribution patterns that supports plant protection research and regulatory decision-making **(Bragard *et al.*, 2020; Jeger *et al.*, 2018)**. Additionally, EPPO publishes the Reporting Service, a monthly information report covering phytosanitary events such as new geographical records, new host plants, and new pest reports **(Rosace *et al.*, 2023)**.

At a global level, the Codex Alimentarius, developed by the Codex Alimentarius Commission (CAC), established by FAO and WHO in 1963, serves as a reference framework. Codex provides a set of international standards, guidelines, and recommendations for the hygienic handling of fresh products especially in countries that do not yet have their own legislation for modern controlled-environment cultivation **(Mickov & Bauer, 2021; Faour-Klingbeil & Todd, 2018)**.

Codex defines microbiological criteria that establish maximum allowable limits for pathogens and toxins, as well as standardized methodologies for sampling and analysis **(Stavropoulou & Bezirtzoglou, 2019)**. It also includes guidelines for HACCP systems **(Pop *et al.,* 2018)**, general standards for contaminants and toxins (CXS 193-1995) **(Logrieco *et al.,* 2018; Eskola *et al.,* 2020)**, and over 5,000 pesticide residue limits **(Vermelho *et al.,* 2024)**.

For fresh products, a key reference document is the Codex Code of Hygienic Practice for Fresh Fruits and Vegetables (CAC/RCP 53-2003), which outlines recommended procedures for harvesting, handling, and distribution **(EFSA, 2011; Penteado, 2017)**.

In the area of contaminants, the EU has adopted stricter limits than Codex. Regulation (EC) No. 1881/2006 defines maximum levels for aflatoxins, ochratoxin A, DON, zearalenone, and fumonisins (**Eskola *et al.,* 2020**), while Regulation (EC) No. 396/2005 harmonizes pesticide residue limits across the EU **(Holvoet *et al.,* 2014)**.

Microbiological criteria are regulated by Regulation (EC) No. 2073/2005, which sets limits for *E. coli* and the absence of STEC in sprouted seeds **(Boqvist *et al.,* 2018)**. In practice, however, there is still a lack of a harmonized microbiological monitoring program for fresh plant products within the EU, which presents a challenge for producers of controlled-environment systems **(Holvoet *et al.,* 2014)**.

Applying these regulations to specific systems, such as greenhouses, hydroponic farms, and phytotrons, reveals several shortcomings. For example, current regulations do not include specific microbiological limits for viruses in fresh products, leaving room for broad interpretation of the rules **(EFSA, 2011)**.

At the national level in Slovakia, the State Veterinary and Food Administration of the Slovak Republic (ŠVPS SR) oversees food safety and conducts inspections in hydroponic and greenhouse operations. EU legislation is applied in practice, including Regulation (EC) No. 852/2004 on food hygiene and the obligation to implement a HACCP system.

The Central Agricultural Control and Testing Institute (ÚKSÚP) is Slovakia's main authority responsible for testing agricultural varieties and certifying seeds. It evaluates new crop varieties for yield potential, disease resistance, and adaptability to local conditions to ensure quality and performance before market entry. By maintaining national and EU standards for variety registration and seed certification, ÚKSÚP supports agricultural productivity and food security in Slovakia. ÚKSÚP's authority and responsibilities are defined by Slovakia's Act No. 597/2006, which governs the registration of plant varieties in the Slovak Republic. Under this legislation, ÚKSÚP acts as the designated state authority responsible for conducting variety testing and managing the registration process **(Psota *et al.*, 2020; Psota *et al.*, 2022)**.

CONCLUSION

Phytotrons or growing chambers as closed cultivation systems, offer exceptional opportunities for controlling growth conditions, but they also require precise and integrated approaches to plant protection. The key to success lies in combining timely monitoring, targeted regulation of abiotic factors, physical barriers, and carefully selected cultivars. The use of biological agents, environmentally friendly technologies, and rationally applied chemical protection helps maintain system balance and minimize risks. For the sustainable development of these technologies, it is also essential to update legislative frameworks to better reflect the specificities of controlled environments.

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