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Cryopreservation of Insect Tissues and Developmental Stages: Sustainability and Future Perspectives for Biological Control

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Abstract

Cryopreservation is an advanced biological preservation technique that enables the long-term storage of living organisms or biological materials at ultra-low temperatures while maintaining their structural integrity and viability. In the field of entomology, this technique has gained increasing importance for the preservation of insects at different developmental stages, including eggs, embryos, larvae, and reproductive tissues. The sustainable conservation of insects, which constitute a major component of global biodiversity, has become an essential requirement from the perspectives of both agricultural pest management and biodiversity conservation.

Due to the increasing drawbacks associated with chemical control, which remains the most widely used method in pest management, biological control based on the use of natural enemies has emerged as one of the most important alternative strategies. The application of cryopreservation techniques in insects offers significant advantages for biological control programs by enabling the availability of biological control agents in the desired quantity and at the required time. Furthermore, cryopreservation represents a multidimensional technology in entomology, with a broad range of applications including the year-round propagation of insect species that are difficult to mass rear, the standardization of mass-production systems, and the long-term preservation of insect genetic resources. Future advances in cryoprotectant optimization and cryogenic protocols are expected to further expand the applicability of this technology across diverse insect taxa.

Keywords: *Biological control, Cryopreservation, Insect, Vitrification, Cryoprotectant*

Introduction

Insects represent more than half of all described animal species and play indispensable roles in ecosystem functioning, agricultural production, and biotechnological research (Denlinger & Lee, 2010; Sinclair et al., 2022). Their ecological importance extends to pollination, nutrient cycling, biological control, and the maintenance of food webs, while their economic significance is evident in agriculture, medicine, and biotechnology. Consequently, the long-term preservation of insect genetic resources is of considerable importance for pest management, conservation of beneficial insects, maintenance of laboratory colonies, and biodiversity protection. Cryopreservation has emerged as one of the most promising preservation strategies to achieve these objectives. However, the successful application of cryopreservation in insects remains challenging because of their complex physiology, diverse developmental stages, and species-specific responses to low-temperature stress.

The natural ability of insects and various other organisms to survive exposure to subzero temperatures provides valuable insights into cryopreservation strategies. Several species have evolved remarkable physiological adaptations that allow survival under extreme cold conditions. For example, the wood frog (*Rana sylvatica*) can survive while frozen throughout the winter and resume normal activity after thawing in spring. Similarly, adult fungus gnats (*Exechia nugatoria* Johannsen) have been reported to survive freezing at temperatures as low as −50°C (Sformo et al., 2009). Furthermore, some collembolans, nematode eggs, and larvae of the Antarctic midge *Belgica antarctica* survive by undergoing extensive dehydration until equilibrium is established between body fluids and the vapor pressure of surrounding ice, thereby avoiding freezing altogether (Holmstrup et al., 2002; Elnitsky et al., 2008).

Comparable cold tolerance has also been observed in nematodes such as *Trichostrongylus colubriformis* and *Panagrolaimus davidi*, as well as in nymphs of the cockroach *Periplaneta japonica*, which can survive short-term exposure to temperatures between −6°C and −8°C. These examples demonstrate that survival at extremely low temperatures is possible across a broad range of organisms and provide important biological foundations for cryopreservation research.

To cope with freezing conditions, insects and other arthropods have evolved numerous physiological and biochemical adaptations. These include the accumulation of cryoprotective compounds such as glycerol, sorbitol, trehalose, and antifreeze proteins (AFPs), which reduce the freezing point of body fluids and suppress ice crystal formation. In addition, mechanisms such as the elimination or masking of ice nucleators and controlled dehydration reduce the risk of intracellular freezing and associated cellular damage (Neven et al., 1986; Rickards et al., 1987; Lundheim & Zachariassen, 1993; Worland, 1996; Holmstrup & Sømme, 1998; Worland et al., 1998; Duman, 2001).

One of the most remarkable examples of insect cold tolerance is the Alaskan beetle *Cucujus clavipes puniceus* (Coleoptera: Cucujidae). During winter, its larvae can withstand temperatures as low as −58°C and enter a vitrified, glass-like state below this threshold, thereby preventing lethal ice formation. This vitrified condition enables survival at temperatures approaching −150°C. The species achieves such extraordinary freeze avoidance through extensive dehydration and the substantial accumulation of glycerol and antifreeze proteins, whose concentrations may increase up to fivefold relative to pre-dehydration levels (Bennett et al., 2005). Antifreeze proteins not only inhibit ice crystal growth but also prevent inoculative freezing initiated by external ice contacting the insect cuticle.

These naturally occurring adaptations provide valuable models for the development of cryopreservation technologies. Despite considerable advances in understanding insect cold tolerance mechanisms, reliable cryopreservation protocols remain unavailable for many species. Differences in developmental stage, membrane permeability, cryoprotectant toxicity, and freezing sensitivity often result in variable survival rates following cryogenic storage. Consequently, further research is required to optimize cryoprotective treatments, cooling and warming procedures, and post-thaw recovery protocols. A comprehensive understanding of insect cryobiology is therefore essential for expanding the application of cryopreservation in entomological research, biological control programs, and genetic resource conservation.

Benefits of Low-Temperature Storage

Low-temperature storage offers numerous advantages for insect research, mass-rearing systems, and biological control programs. One of its primary benefits is the ability to provide beneficial insect species to end users when needed, thereby improving the efficiency and flexibility of release programs. Cold storage also contributes to the sustainability of mass-production systems by enabling synchronization between insect production and field application.

In addition, low-temperature storage facilitates the production of standardized insect populations suitable for long-term ecological, physiological, genetic, and behavioral studies (Leopold, 1998; Tunca et al., 2014). By extending the storage life of insects and reducing the need for continuous colony maintenance, these techniques significantly lower production and maintenance costs.

The preservation of insect eggs and other developmental stages under refrigerated or cryogenic conditions also supports the long-term conservation of cytogenetic materials and valuable genetic resources (Branson, 1976; Sharakhova et al., 2006). Furthermore, cryopreservation enables the recovery of insect populations without substantial alterations in their genetic or phenotypic characteristics, thereby enhancing experimental reproducibility and supporting breeding programs (Leopold et al., 2001).

In biological control programs, cryopreservation of parasitoids and predators provides year-round availability and reduces the costs associated with continuous rearing operations (Pérez-Bañón et al., 2022). Moreover, the cryogenic preservation of endangered insect species represents a valuable tool for conservation biology, restoration ecology, and evolutionary studies by safeguarding genetic diversity for future research and reintroduction efforts (Lee & Costanzo, 2023).

Cryopreservation Applications in Insects

Cryopreservation is a preservation technology that enables the long-term storage of biological materials through the near-complete cessation of metabolic activity at ultra-low temperatures, typically in liquid nitrogen at −196°C (Mazur, 2004) (Figure 1). Although cryopreservation has been successfully applied to numerous mammalian and plant systems, its implementation in insects remains considerably more challenging because of the low permeability of embryonic membranes, species-specific physiological characteristics, water balance mechanisms, and sensitivity to cryoprotectant toxicity (Leopold, 2010).

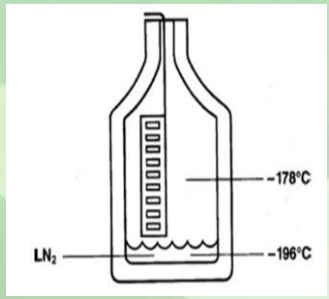

Figure 1. Temperature Ranges of Liquid Nitrogen and Vapor

The limited success achieved in insect cryopreservation can largely be attributed to substantial interspecific physiological variation, developmental-stage-specific sensitivity, cryoprotectant toxicity, and post-thaw developmental abnormalities. Consequently, the development of effective preservation protocols generally requires species-specific and stage-specific approaches rather than universal methodologies.

As an alternative to whole-embryo cryopreservation, the preservation of sperm, oocytes, and primordial germ cells has attracted increasing attention. These approaches reduce technical complexity and circumvent many of the barriers associated with embryonic structures, particularly the chorion and vitelline membranes (Ohno et al., 2020). Furthermore, germline cryopreservation provides improved reproducibility in maintaining valuable genetic lines and facilitates long-term genetic conservation efforts (Wang et al., 2024).

The principal causes of cryoinjury include intracellular and extracellular ice crystal formation, osmotic stress, recrystallization during warming, and cryoprotectant toxicity (Mazur, 2004; Steponkus & Wolfe, 1999). Intracellular ice crystals can mechanically disrupt cellular structures and compromise membrane integrity, whereas extracellular ice formation promotes dehydration, protein denaturation, and membrane phase transitions. Understanding these mechanisms is critical for improving cryopreservation outcomes.

Natural cold-tolerance mechanisms observed in insects provide valuable insights into cryobiological processes. Adaptations such as the accumulation of low-molecular-weight cryoprotectants, including glycerol, trehalose, and proline, the synthesis of antifreeze proteins, and modifications in membrane lipid composition contribute significantly to cellular stability under cold stress (Koštál et al., 2019; Lee & Costanzo, 2023).

During cryopreservation, differences in vapor pressure between supercooled body fluids and surrounding ice result in cellular dehydration until equilibrium is established. Although controlled dehydration helps prevent intracellular ice formation, excessive water loss may increase intracellular solute concentrations and cause mechanical and osmotic damage. Therefore, carefully designed freezing procedures and appropriate cryoprotective agents are essential for maximizing post-thaw survival and maintaining cellular integrity.

Cryoprotectants and Their Mechanisms of Action

Cryoprotectants are chemical compounds used to minimize cellular damage during freezing and thawing processes by protecting biological structures from cryoinjury (Palasz & Mapletoft, 1996). Their primary function is to reduce ice crystal formation, alleviate osmotic stress, stabilize cellular membranes, and improve post-thaw survival. Based on their ability to cross biological membranes, cryoprotectants are generally classified into two major categories: permeating and non-permeating cryoprotectants (Leopold, 2010).

In insect cryopreservation, the effective use of cryoprotectants is particularly important because insect embryos possess multilayered protective structures, including the chorion and vitelline membranes, which greatly restrict the penetration of many cryoprotective compounds. Consequently, successful cryopreservation often requires prolonged cryoprotectant exposure or the application of physical and chemical pretreatments designed to enhance membrane permeability (Li et al., 2021).

Permeating Cryoprotectants

Permeating cryoprotectants are characterized by their relatively low molecular weight and ability to penetrate cellular membranes. Common examples include dimethyl sulfoxide (DMSO), glycerol, ethylene glycol (EG), 1,2-propanediol, 2,3-butanediol, propylene glycol, and several related alcohols (Palasz & Mapletoft, 1996; Agca, 1994; Sağırkaya, 2001).

These compounds exert their protective effects by replacing intracellular water before freezing occurs. As cryoprotectants enter the cell, osmotic gradients facilitate the removal of intracellular water, thereby reducing the amount of water available for ice crystal formation. This process minimizes both intracellular ice formation and excessive changes in cell volume during cooling.

In addition to reducing ice formation, permeating cryoprotectants interact directly with water molecules. Many of these substances possess high water solubility and modify the molecular structure of water by disrupting hydrogen-bond networks. At the same time, they are capable of forming hydrogen bonds with water molecules. For example, glycerol interacts with water through its hydroxyl groups, thereby lowering the freezing point and reducing the likelihood of ice crystallization.

Another important function of permeating cryoprotectants is their ability to reduce cellular damage associated with dehydration and elevated extracellular solute concentrations during freezing. By maintaining osmotic balance and stabilizing cellular structures, these compounds contribute significantly to cell survival following cryogenic storage (Palasz & Mapletoft, 1996; Agca, 1994; Sağırkaya, 2001).

Non-Permeating Cryoprotectants

Unlike permeating cryoprotectants, non-permeating cryoprotectants remain outside the cell and exert their protective effects primarily through osmotic regulation and ice crystal modification. These compounds are generally divided into low-molecular-weight and high-molecular-weight categories.

Low-Molecular-Weight Non-Permeating Cryoprotectants

Low-molecular-weight non-permeating cryoprotectants function by promoting controlled cellular dehydration prior to freezing. Through osmotic action, they facilitate the removal of intracellular water, thereby reducing the probability of intracellular ice crystal formation during cooling.

Common representatives of this group include glucose, sucrose, trehalose, raffinose, galactose, and other sugars. These compounds play a crucial role in maintaining osmotic balance and stabilizing cellular membranes throughout freezing and thawing processes.

High-Molecular-Weight Non-Permeating Cryoprotectants

High-molecular-weight cryoprotectants protect cells by modifying the formation, morphology, and growth of ice crystals during freezing and thawing (Palasz & Mapletoft, 1996). Rather than entering cells, these compounds act within the extracellular environment, reducing mechanical damage associated with ice recrystallization.

Examples include polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP), albumin (Pugh et al., 1998), Ficoll (Begin et al., 2003), and antifreeze proteins (AFP) (Wang, 2000) (Palasz & Mapletoft, 1996; Agca, 1994; Bağış et al., 2002). Antifreeze proteins are of particular interest because they inhibit ice crystal growth and recrystallization, thereby improving cellular survival under cryogenic conditions.

Factors Influencing Cryoprotectant Efficiency

The effectiveness of cryoprotectants depends on several factors, including cell type, cooling and warming rates, sample volume, cryoprotectant concentration, and duration of exposure. Because cryoprotectants occupy intracellular and extracellular spaces, successful cryopreservation requires careful regulation of water and solute movement, as well as close monitoring of changes in cell volume throughout the freezing and thawing process.

Among currently available cryoprotectants, DMSO remains one of the most widely used due to its high membrane permeability and strong protective capacity. However, prolonged exposure may result in cytotoxic effects. Exposure of cells to DMSO for extended periods, particularly at elevated temperatures, has been associated with increased cellular toxicity. Therefore, cryopreservation protocols generally recommend preparing cryoprotectant solutions separately and gradually introducing cells into the mixture to minimize toxic effects.

Functions of Cryoprotectants During Freezing

Cryoprotectants contribute to successful cryopreservation through several complementary mechanisms. Their major functions include:

- Prevention of intracellular and extracellular ice crystal formation;
- Reduction of electrolyte concentration effects and pH fluctuations;
- Prevention of protein denaturation;
- Protection against dehydration-induced cellular damage;
- Depression of the freezing point of cellular fluids;
- Maintenance of water in a non-crystalline state during slow cooling;
- Enhancement of long-term storage stability at temperatures below −130°C by limiting ice recrystallization;
- Improvement of post-thaw recovery through the stabilization of cellular structures during warming.

Collectively, these mechanisms increase the survival and functional recovery of cells, tissues, and embryos following cryogenic preservation and form the foundation of modern cryopreservation technologies.

Cryopreservation Techniques

Two principal techniques have been developed for the cryopreservation of cells, tissues, and embryos: controlled slow cooling and vitrification. Both methods aim to prevent lethal cellular damage during freezing and long-term storage; however, they differ considerably in their underlying mechanisms and operational procedures.

Slow Cooling (Controlled-Rate Freezing)

Controlled slow cooling was the first cryopreservation technique successfully applied to human cells and remains one of the most extensively used methods in cryobiology. In this approach, biological materials are gradually cooled at rates ranging from approximately 0.1 to 4°C min^{-1} until temperatures between −30°C and −40°C are reached, followed by rapid immersion into liquid nitrogen (Engelmann, 2004).

The primary objective of slow cooling is to avoid intracellular ice formation through controlled osmotic dehydration. As extracellular ice begins to form during cooling, the osmotic gradient created between the intracellular and extracellular environments promotes the movement of water out of the cell. Consequently, the amount of intracellular water available for crystallization is reduced, thereby decreasing the risk of lethal intracellular ice formation.

When cooling conditions are appropriately controlled, cells may become supercooled and lose water because of osmotic imbalances between extracellular frozen regions and intracellular unfrozen compartments. The ability of cells or organisms to survive this process depends on several factors, including cooling capacity, cooling rate, membrane permeability, and the composition of the surrounding medium.

Cryoprotective mixtures commonly used in controlled-rate freezing include combinations of glycerol and sucrose or dimethyl sulfoxide (DMSO) and sorbitol (Sakai et al., 1990). These compounds facilitate controlled dehydration and help maintain cellular integrity during cooling.

Insect eggs are generally small and, depending on the species, can often be cooled to temperatures ranging from −25°C to −40°C before spontaneous freezing occurs (Somme, 1964). Although this characteristic appears favorable for dehydration-based cryopreservation, embryo mortality remains high in many insect species subjected to slow-freezing protocols (Heacox et al., 1985; Mazur et al., 1992; Miles & Bale, 1995). One of the primary limitations is the relatively low permeability of insect embryonic membranes, which restricts water movement and cryoprotectant penetration (Leopold, 2010).

Vitrification

Vitrification represents an alternative cryopreservation strategy designed to eliminate ice crystal formation entirely. Unlike slow cooling, vitrification does not rely on controlled extracellular freezing. Instead, biological materials are exposed to highly concentrated cryoprotectant solutions and subsequently cooled at extremely rapid rates by direct immersion in liquid nitrogen, bypassing the preliminary cooling stages associated with conventional freezing methods (Sakai & Engelmann, 2007).

The principle of vitrification is based on transforming cellular water into an amorphous glass-like state rather than allowing crystallization. This transformation is achieved through the combined effects of high cryoprotectant concentrations and ultra-rapid cooling rates (Li et al., 2021; Ohno et al., 2020). Because ice crystals do not form during the process, vitrification effectively eliminates one of the primary sources of cryogenic injury.

The first successful vitrification protocol was developed by Rall and Fahy in 1985 for the preservation of mouse embryos (Roversi et al., 2008). Subsequently, the technique was adapted for insect embryos, including those of *Drosophila melanogaster* (Mazur et al., 1992; Steponkus & Caldwell, 1993). Since then, vitrification has been successfully applied to the preservation of eggs and embryos from several insect species (Mazur et al., 1992; Wang et al., 2000; Nunamaker & Lockwood, 2001; Leopold et al., 2001; Rajamohan & Leopold, 2007).

Beyond entomological applications, vitrification has also been employed successfully in the cryopreservation of various plant and animal cells, tissues, microorganisms, and embryos (Takahashi et al., 1986; Jutte et al., 1987; Nishizawa et al., 1993; Van Wagtendonk-De Leeuw et al., 1997; Leopold, 2007).

Despite its advantages, vitrification is not without limitations. High cryoprotectant concentrations may induce cytotoxic effects, while rapid temperature fluctuations can result in thermal stress. Furthermore, successful vitrification requires strict control of both cooling and warming procedures to prevent ice crystal formation during storage and recovery.

Comparison of Slow Cooling and Vitrification

Although both slow cooling and vitrification aim to preserve biological materials at ultra-low temperatures, they differ substantially in their mechanisms of action.

In slow cooling, cellular survival depends largely on controlled extracellular ice formation and gradual osmotic dehydration. While this approach reduces the likelihood of intracellular ice formation, it may expose cells to prolonged osmotic stress and extracellular ice-related damage. In contrast, vitrification seeks to eliminate ice formation altogether by converting cellular contents into a glass-like state.

Slow cooling generally requires specialized equipment capable of accurately controlling cooling rates, whereas vitrification often relies on direct immersion into liquid nitrogen following cryoprotectant treatment. However, vitrification typically requires higher concentrations of cryoprotectants, thereby increasing the risk of cryoprotectant toxicity.

For insect embryos, vitrification has generally demonstrated greater potential than conventional slow freezing because it circumvents many of the limitations associated with low membrane permeability and intracellular ice formation. Nevertheless, both techniques require careful optimization according to species-specific physiological characteristics and developmental stages.

Insect Embryo Cryopreservation Through Vitrification

One of the most successful vitrification protocols developed for insects involves embryos of *Cochliomyia hominivorax* (Diptera: Calliphoridae) (Berkebile et al., 2000). The protocol consists of a series of carefully controlled steps designed to increase membrane permeability, facilitate cryoprotectant penetration, achieve controlled dehydration, and maximize post-thaw survival.

Initially, embryos are subjected to chemical treatments involving sodium hydroxide, sodium hypochlorite, isopropyl alcohol, hexane, and Drosophila Ringer solution. These treatments are intended to modify embryonic barriers and enhance permeability. Following these preparatory procedures, embryos are loaded with ethylene glycol and subsequently dehydrated using a vitrification solution containing ethylene glycol, polyethylene glycol, and trehalose.

After dehydration, embryos are transferred onto polycarbonate membranes and exposed to liquid nitrogen vapor before being immersed in liquid nitrogen for long-term storage. Recovery involves rapid warming followed by the gradual removal of cryoprotectants using trehalose-containing solutions, repeated washing procedures, and incubation under conditions suitable for embryonic development and hatching.

Particular attention must be given to dehydration and cryoprotectant removal, as these stages represent critical points where osmotic injury may occur. To minimize such damage, cryoprotectants are introduced gradually, dehydration is typically performed at 0°C, and insect cell culture media are frequently used as diluents during treatment. These measures help maintain osmotic balance and improve post-thaw survival.

The importance of osmotic regulation during recovery has been demonstrated in *Cochliomyia hominivorax*, where Drosophila Ringer solution was used as an equilibration and transfer medium following hexane treatment designed to remove the lipid layer associated with the vitelline membrane (Steponkus & Caldwell, 1993; Leopold et al., 2001). Such procedures contribute significantly to successful embryonic recovery after cryogenic storage.

Successful vitrification requires that ice crystal formation be avoided not only during cooling but also throughout warming and recovery. Although vitrification itself generally causes limited cellular and molecular damage, disturbances in water balance and intracellular solute concentrations may result in severe physiological stress and mortality. Therefore, continued refinement of vitrification protocols remains essential for improving cryopreservation outcomes in insects.

Studies on Cryopreservation

Numerous studies conducted over the past several decades have demonstrated the feasibility of cryopreservation for a wide range of invertebrates and insect species. Early investigations focused primarily on evaluating the survival of organisms following exposure to ultra-low temperatures and identifying suitable preservation strategies.

One of the earliest successful applications was reported by Campbell et al. (1973), who demonstrated that larvae of the nematode *Haemonchus contortus* remained viable after storage in liquid nitrogen at −196°C for 44 weeks. This study highlighted the potential of cryogenic preservation for maintaining biological materials over extended periods

Among insects, considerable attention has been directed toward species used in biological control programs. Parasitoids belonging to the genus Trichogramma are widely utilized against numerous lepidopteran pests, making the development of efficient storage methods particularly important for large-scale rearing and release programs (Leopold, 1998).

Correa-Ferreira and De Oliveira (1998) successfully preserved eggs of Nezara viridula in liquid nitrogen for up to twelve months. Following storage, parasitization rates by Trissolcus basalis were found to be comparable to those observed in fresh eggs, demonstrating the suitability of cryopreserved host material for biological control applications.

Similarly, Greco and Stilinovic (1998) reported that cryopreservation of *Sitotroga cerealella* eggs did not significantly affect the parasitization performance of *Trichogramma pretiosum*. These findings suggested that liquid nitrogen storage could provide a practical alternative to continuous host rearing.

Albuquerque et al. (2000) further demonstrated that liquid nitrogen storage was superior to conventional freezing temperatures for the long-term preservation of *Nezara viridula* eggs. Their results emphasized the advantages of cryogenic storage for maintaining host quality over prolonged periods.

Leopold et al. (2001) investigated the cryopreservation of embryos of *Cochliomyia hominivorax*. Although a reduction in pupal weight was observed among cryopreserved individuals, these effects were not transmitted to subsequent generations, indicating that the preservation process did not result in permanent genetic or developmental alterations.

Research involving *Ephestia kuehniella* eggs revealed that prolonged low-temperature storage may negatively influence the biological performance of associated parasitoids. Özder and Sağlam (2002) reported reductions in parasitization rates, adult longevity, and reproductive capacity following extended storage periods.

Studies of naturally cold-tolerant insects have also contributed substantially to cryobiological knowledge. Sformo et al. (2010) demonstrated that larvae of Cucujus clavipes puniceus could survive exposure to temperatures approaching −100°C through vitrification-like mechanisms that prevent ice crystal formation. These findings provided important insights into naturally evolved freeze-avoidance strategies and their relevance to artificial cryopreservation protocols.

Cryopreservation has also been successfully applied to reproductive tissues. Ovarian tissues of *Harmonia axyridis* were cryopreserved and subsequently transplanted into recipient females, resulting in the production of fertile offspring capable of maintaining genetic lineages (Sformo et al., 2010). Such studies highlight the potential of germline preservation as an alternative to whole-embryo cryopreservation.

More recently, Krechemer and Foerster (2016) and Özder and Tayat (2018) demonstrated that cryopreserved host eggs remained suitable for parasitization by various *Trichogramma* species. These findings further support the practical application of cryopreservation technologies in biological control programs by facilitating long-term storage and year-round availability of host materials.

Collectively, these studies demonstrate that cryopreservation can successfully preserve a variety of insect developmental stages and reproductive tissues while maintaining biological functionality after recovery. However, survival rates and post-thaw performance remain highly species-dependent, emphasizing the need for continued protocol optimization.

Applications of Cryopreservation in Other Biological Systems

The successful implementation of cryopreservation extends far beyond entomological applications and has become an essential technology in human medicine, plant biotechnology, and animal reproduction.

In human reproductive medicine, cryopreservation has enabled the long-term storage of spermatozoa, embryos, and reproductive tissues. Successful pregnancies have been achieved using sperm samples preserved in liquid nitrogen for extended periods, demonstrating the effectiveness of cryogenic storage for maintaining reproductive potential and genetic integrity (Wetzels et al., 1996; Delilbaşı, 2008; Walters et al., 2009; Verheyen, 2010; Kadıoğlu, 2011).

Plant cryopreservation has likewise become an important tool for germplasm conservation. Since the 1960s, cryogenic storage techniques have been successfully applied to plant cell cultures, meristems, embryos, pollen grains, seeds, and other propagative materials (Grout & Henshaw, 1980; Towill, 1983; Withers & King, 1979, 1980; Kartha et al., 1980; Mullin & Schlegel, 1976; Sakai et al., 1978; Niwata et al., 2000). These approaches provide long-term preservation of valuable genetic resources while minimizing the risks associated with conventional storage methods.

In livestock breeding programs, cryopreservation has become a routine component of assisted reproductive technologies. The successful production of offspring from cryopreserved embryos and vitrified spermatozoa has demonstrated the reliability of cryogenic preservation for maintaining reproductive material in economically important animal species (Chemineau et al., 1986; Baril et al., 1989; Nowshari & Holtz, 1995; Guignot et al., 2006; Thibier, 2009; Kızıl et al., 2011).

The widespread adoption of cryopreservation across diverse biological systems illustrates its versatility as a preservation technology and highlights its potential for broader application in entomology and insect conservation.

Conclusion

Cryopreservation represents one of the most promising approaches for the long-term preservation of insect genetic resources and beneficial species. Advances in cryobiology have significantly improved our understanding of the physiological and biochemical mechanisms underlying cold tolerance, freeze avoidance, and cryoinjury in insects. These developments have facilitated the design of increasingly effective preservation strategies for embryos, reproductive tissues, and other developmental stages.

Despite these advances, the successful cryopreservation of insects remains considerably more challenging than that of many plant and vertebrate systems. Species-specific physiological characteristics, low membrane permeability, developmental-stage sensitivity, cryoprotectant toxicity, and osmotic stress continue to limit survival and recovery rates. Consequently, the development of universally applicable cryopreservation protocols remains unlikely, and species-specific optimization is required.

Among currently available approaches, vitrification appears particularly promising because it minimizes ice crystal formation and reduces mechanical damage during freezing. Nevertheless, further improvements in cryoprotectant formulations, permeability enhancement techniques, and post-thaw recovery procedures are necessary to maximize survival and reproductive performance following cryogenic storage.

Cryopreservation offers substantial opportunities for biological control programs by enabling year-round availability of parasitoids, predators, and host materials while reducing production costs associated with

continuous rearing. In addition, the preservation of endangered insect species and valuable laboratory strains contributes to biodiversity conservation, restoration biology, and long-term genetic resource management.

Future research should focus on improving our understanding of insect-specific cryobiological responses, identifying less toxic and more effective cryoprotectants, and integrating advances in molecular biology, physiology, and reproductive biotechnology into cryopreservation protocols. Such efforts will contribute to the development of reliable and efficient preservation strategies capable of supporting both fundamental research and practical applications in entomology.

Declarations

Author Contribution Statement

The contribution of each author to this study should be specified in terms of stage and nature of contribution. For example: O.M: Data collection, investigation, formal analysis, and original draft writing. H.Ç: Supervision, review, and editing. F.N.E: Data curation, writing.

Conflict of Interest

The authors declare that there is no conflict of interest.

Kaynaklar

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