

# SERIE ESTUDIOS PARA LA INNOVACIÓN FIA

## ESTUDIO DE VIGILANCIA TECNOLÓGICA

### SISTEMAS DE CONTROL Y MANEJO DE HELADAS

#### PARA EL SECTOR AGRÍCOLA NACIONAL

PARTE 1: ANÁLISIS DEL PANORAMA CIENTÍFICO Y TECNOLÓGICO









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**Serie Estudios para la Innovación FIA**  
**Estudio de Vigilancia Tecnológica**  
**Sistemas de Control y Manejo de Heladas para el Sector Agrícola Nacional**  
**Parte 1: Análisis del Panorama Científico y Tecnológico**

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ESTUDIO DE VIGILANCIA TECNOLÓGICA  
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PARTE 1: ANÁLISIS DEL PANORAMA CIENTÍFICO Y TECNOLÓGICO





## PRESENTACIÓN

La **Fundación para la Innovación Agraria (FIA)** es la agencia del Ministerio de Agricultura que tiene por misión fomentar una cultura de innovación en el sector agrario, agroalimentario y forestal, promoviendo y articulando iniciativas de innovación que contribuyan a mejorar las condiciones de vida de las agricultoras y agricultores, en todas las regiones del territorio nacional.

Uno de los ejes centrales de FIA es la focalización de su acción a través de los Programas de Innovación en temas, rubros y territorios, que generen o potencien plataformas de colaboración público-privadas, tanto a nivel nacional, regional como local. Los Programas de Innovación cuentan con una agenda clara que da cuenta de las prioridades específicas para fortalecer los procesos de innovación en el sector agrario, agroalimentario y forestal del país.

Como parte del trabajo desarrollado por los Programas de Innovación y en respuesta a los desafíos que enfrentan cada uno de ellos, FIA realiza estudios para difundir y transferir conocimiento e información prospectiva y estratégica a los distintos actores del sector, contribuyendo a dinamizar los procesos de innovación en los ámbitos productivos, de gestión, asociativos y de comercialización, principalmente para que tengan impacto en las unidades económicas de pequeña y mediana escala.

El presente **“Estudio de vigilancia tecnológica. Sistemas de control y manejo de heladas para el sector agrícola nacional. Parte 1: Análisis del panorama científico y tecnológico”** se realizó en el marco del trabajo prospectivo de FIA con el objetivo de identificar y analizar el desarrollo de nuevas tecnologías de control de heladas, sistemas de mitigación de daño por heladas y manejo post heladas, con potencial de aplicación a la realidad nacional.

Los resultados de este estudio proporcionan una base de información actualizada, que permite a técnicos y agricultores disponer de antecedentes sobre la investigación y desarrollo tecnológico que se están realizando en el mundo en cuanto a control de las heladas.

El documento expone el análisis de las tendencias científicas y tecnológicas, generado a partir de las publicaciones científicas identificadas en diferentes bases de datos, así como el análisis de las patentes solicitadas y concedidas a escala nacional e internacional en relación al monitoreo, control, mitigación de daños y manejo post heladas.

Un segundo documento elaborado por FIA (Parte 2: Análisis de la oferta comercial) presenta el análisis de los productos comerciales nacionales e internacionales en relación al control y monitoreo de heladas, así como mitigación de daños y manejo post heladas.

**HÉCTOR ECHEVERRÍA VÁSQUEZ**  
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# 1. INTRODUCCIÓN

En un entorno cada vez más complejo y cambiante como el actual, no basta con resolver los problemas de Investigación y Desarrollo, también es importante y prioritario innovar, es decir, convertir tales conocimientos en nuevos productos, procesos y/o servicios, con una propuesta de valor atractiva para el mercado. Asimismo, para la supervivencia de las empresas, el reto y desafío permanente consiste en detectar oportunidades y anticiparse a los cambios, lo cual exige realizar un seguimiento constante del entorno.

El aporte de la Vigilancia Tecnológica es entregar conocimiento actualizado a partir de la información que se dispone en el mundo, gracias al quehacer de la ciencia y la tecnología. Es una metodología transversal aplicable a cualquier área del conocimiento, donde las grandes bases de datos marcan la diferencia en cada tema y sus especificaciones, y que sirve, entre otras cosas, para:

- Evitar sorpresas tecnológicas y comerciales;
- Identificar competidores o socios potenciales;
- Evaluar las fortalezas y debilidades de los competidores e identificar las propias;

- Disminuir la incertidumbre sobre el futuro;
- Mejorar la planificación estratégica;
- Identificar nuevos mercados;
- Colocar competitivamente nuevos productos y servicios.

Actualmente, se dispone de suficientes antecedentes para constatar que los sistemas para el control y manejo de heladas no son del todo satisfactorios y que las pérdidas son cuantiosas en el sector agrícola nacional. Es justamente en este punto donde la puesta en valor de la información que entrega la Vigilancia Tecnológica, específicamente con respecto a cuáles son las tecnologías más usadas, cuáles son emergentes, cuáles son las instituciones y quiénes son las personas que están trabajando en determinados temas de interés, cuáles son sus redes de trabajo y cuáles son las iniciativas o proyectos que se están financiando para resolver este problema, facilita las buenas decisiones a mediano y largo plazo.

## 2. ANTECEDENTES

Al contar con esta información pueden evaluarse nuevas ideas de Investigación y Desarrollo, adaptarlas a las características de la agricultura nacional y orientar correctamente los esfuerzos de asistencia, a fin de obtener las mejores opciones técnicas para disminuir las pérdidas por heladas y mejorar la competitividad del sector. Con este objetivo, se han revisado los trabajos realizados y en ejecución en todo el mundo, analizando las ventajas, las posibles limitaciones, requerimientos técnicos y costos asociados a cada iniciativa de control de heladas, a fin de proveer a los usuarios con toda la información que les permita elegir adecuadamente y en sintonía con la realidad de cada región y según el tipo de agricultura/cultivo.

Uno de los efectos que se han presentado a raíz de los cambios del clima chileno, consecuencia de los cambios a nivel global, ha sido el aumento en la frecuencia de heladas de origen polar. Debido al alto poder destructivo de estas y la ocupación de suelos sujetos a riesgo, con especies susceptibles ante este fenómeno, las pérdidas económicas que provoca van en alza.

En Chile existen unas 196.457has de frutales caducos, 68.982has de especies persistentes y 31.625has de frutales menores, gran parte de los cuales pueden ser afectados por heladas. Las pérdidas promedio que ocasiona este fenómeno son del orden de 3 a

7% anual, lo que representa mermas que fluctúan entre los 132 y 318 millones de dólares anuales, sobre una exportación frutícola total que supera los 4.000 millones de dólares (esta cifra no considera el aporte del mercado interno de esta industria). Sólo en el 2013, una helada tardía en primavera generó una pérdida evaluada en 800 millones de dólares. En el caso de la pequeña agricultura los efectos son más dramáticos, por cuanto afectan a un tipo de productores de bajos recursos y baja resiliencia frente a estos eventos destructivos.

Las estrategias de reducción de riesgos de pérdidas por heladas deben equilibrar acciones pasivas y activas. Las primeras se traducen en generar información que permita a los agricultores hacer una mejor elección de los terrenos a cultivar, y de las especies y variedades adecuadas a los niveles de riesgo que enfrentarán en cada lugar. Siendo estas estrategias las más efectivas y de menor costo, no siempre es posible utilizarlas por considerar que no puede modificarse la localización de los predios y que la elección de especies a cultivar está limitada tanto por el potencial productivo del lugar y por las demandas que impone el mercado. Por estas razones es necesario asistir a los agricultores en la implementación de métodos activos, consistentes en tecnologías de protección frente a las heladas, lo que implica altos costos de inversión y operación. La competitividad de la agricultura, en

regiones con elevada incidencia de heladas, dependerá del éxito que tengan las políticas de fomento para adoptar estrategias de mitigación de daños frente a estos eventos extremos.

Es por esto que la Fundación para la Innovación Agraria (FIA) solicitó en el 2014 un estudio de Vigilancia Tecnológica, orientado a generar una base de información actualizada, que permita a técnicos y agricultores disponer de antecedentes sobre la investigación y desarrollo tecnológico que se está haciendo en el mundo en cuanto a control de las heladas.

El presente documento expone parte de este estudio de vigilancia, enfocado en el análisis de tendencias científicas y tecnológicas, generado a partir de las publicaciones científicas identificadas en las bases de datos Scopus y CAB Direct, así como también en el análisis de las patentes solicitadas y concedidas a escala nacional e internacional en relación al monitoreo, control, mitigación de daños y manejo post heladas.

Por otra parte, un segundo documento editado por FIA presenta el análisis de los productos comerciales nacionales e internacionales en relación al control y monitoreo de heladas, así como mitigación de daños y manejo post heladas.

El estudio de vigilancia, en su versión integral, puede ser consultado en el sitio web de la Fundación para la Innovación Agraria ([www.fia.cl](http://www.fia.cl)).



### 3. PANORAMA CIENTÍFICO

El panorama científico se construyó a partir de las publicaciones científicas de los últimos diez años, contenidas en las bases de datos Scopus y CAB Direct, que cumplieran con las palabras clave utilizadas como descriptores del área de interés.

El análisis de la base de datos Scopus arrojó resultados en términos de evolución, actores líderes y tendencias, que fueron coincidentes con los resultados obtenidos desde la base de datos Cab Direct y que son los que se muestran a continuación.

#### 3.1 RESULTADOS INTERNACIONALES

Considerando un total de 1.716 publicaciones, la Figura 3.1 muestra la distribución por años, con tendencia creciente, excepto leves bajas en los años 2007 y 2010, aunque con un crecimiento sostenido desde el 2011.

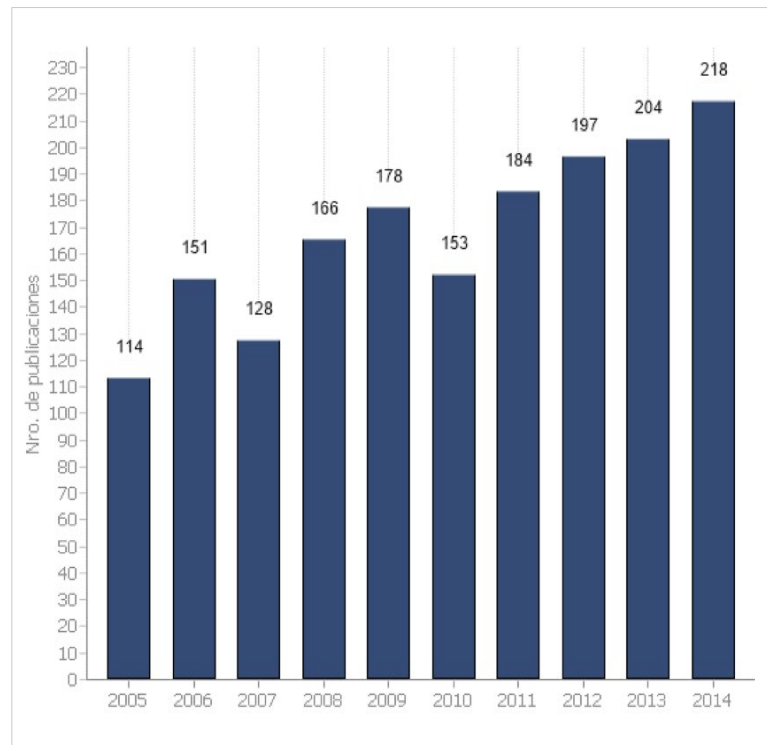
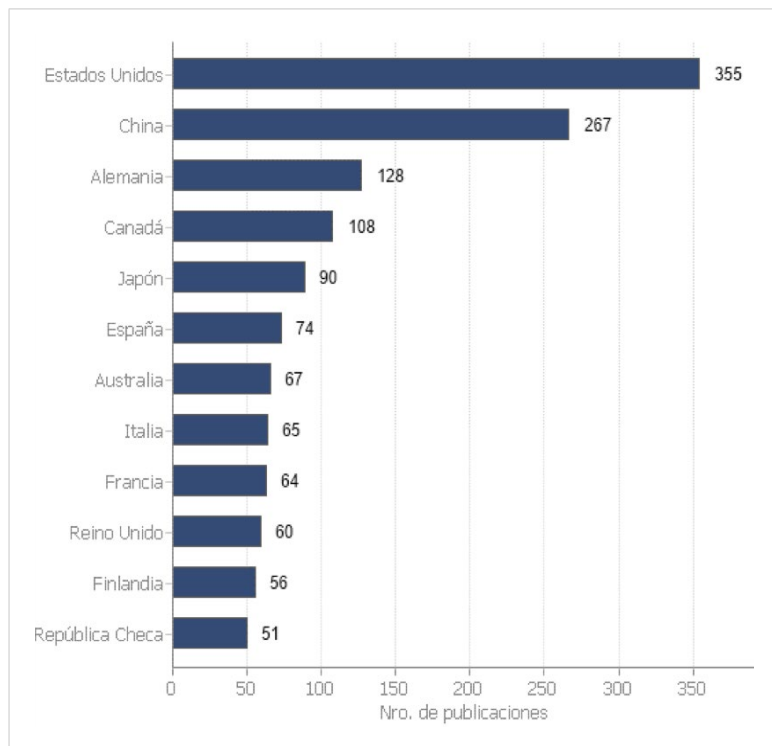


Figura 3.1. Evolución de la producción científica internacional entre 2005-2014, según la base de datos Scopus



**Figura 3.2. Países líderes, con más de 50 publicaciones**

### 3.1.1 Principales países

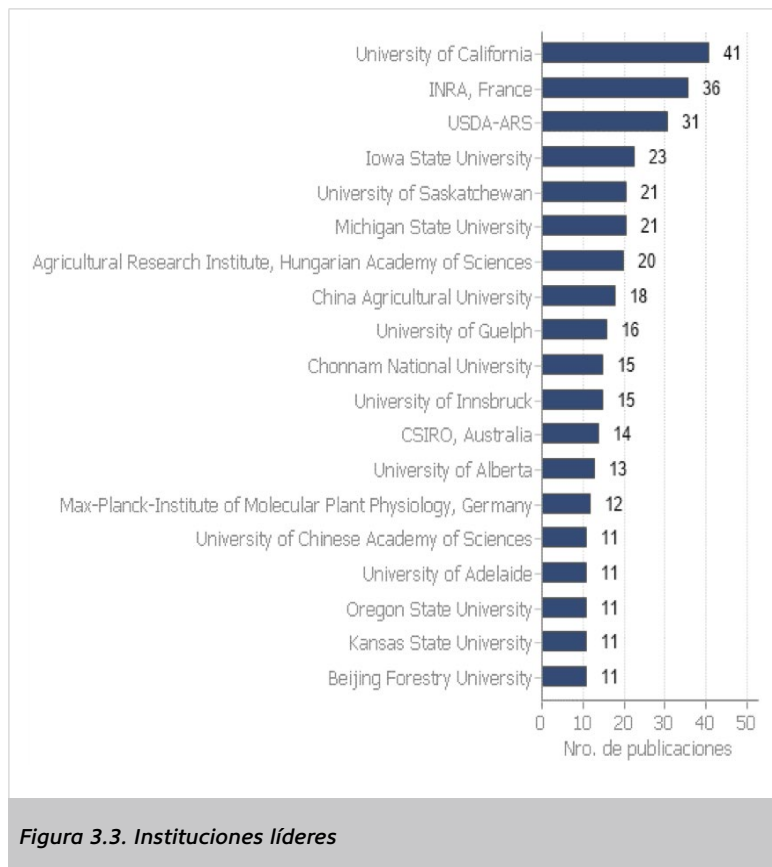
Desde el año 2005 a la fecha, el desarrollo de la producción científica mundial ha venido de la mano de 87 países, siendo Estados Unidos y China los líderes en esta materia, con más de 250 publicaciones cada uno (Figura 3.2). Estos dos países poseen cerca del 36% de las publicaciones identificadas en el área de interés, y le siguen Alemania, Canadá y Japón.

En países de Centro y Sudamérica el líder es Brasil con 31 publicaciones, seguido por Chile con 29, Argentina con 25 y México con 17.

### 3.1.2 Principales instituciones

Entre las principales instituciones de investigación, la entidad líder es la Universidad de California, seguida por el Instituto Nacional de Investigación Agronómica de Francia (INRA), y el Servicio de Investigación Agrícola del Departamento de Agricultura de Estados Unidos (USDA-ARS). En la Figura 3.3, estas aparecen ordenadas según cantidad de publicaciones.

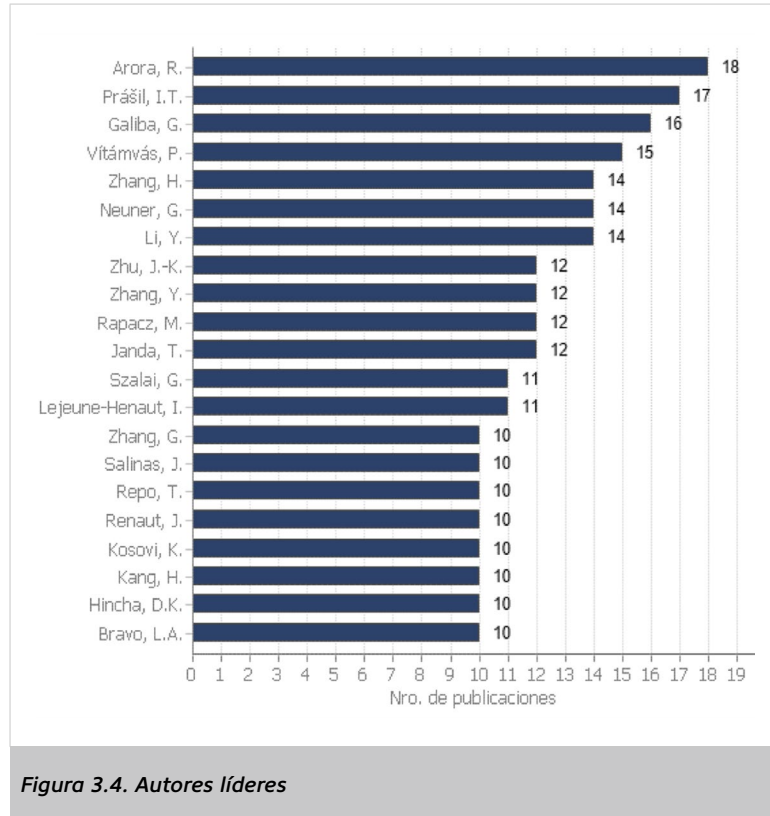
Las líneas de investigación de las instituciones líderes preferentemente están asociadas a manipulación genética de plantas, lo que se puede apreciar en la Tabla 3.1, donde se indica cuáles son las áreas de especialización de las instituciones con más de veinte publicaciones.



| INSTITUCIÓN                | Nº PUB. | PAÍS           | LÍNEAS DE INVESTIGACIÓN  |
|----------------------------|---------|----------------|--|
| University of California   | 41      | Estados Unidos | Genetics, Arabidopsis, Freezing tolerance, Wheat, Frost tolerance, Frost resistance, Frost protection, Fruit |
| INRA                       | 36      | Francia        | Genetics, Arabidopsis, Frost resistance  |
| USDA-ARS                   | 31      | Estados Unidos | Genetics, Arabidopsis, Freezing tolerance, Wheat, Crops, Frost protection, Irrigation                        |
| Iowa State University      | 23      | Estados Unidos | Genetics, Arabidopsis, Freezing tolerance, Wheat, Frost tolerance, Eucalyptus, Cryoprotection                |
| Michigan State University  | 21      | Estados Unidos | Genetics, Arabidopsis, Freezing tolerance, Frost protection  |
| University of Saskatchewan | 21      | Canadá         | Genetics, Arabidopsis, Freezing tolerance, Wheat, Crops, Frost tolerance, Frost resistance, Frost protection |

### 3.1.3 Principales autores

En referencia a los principales autores con más de diez artículos científicos publicados, destacan aquellos señalados en la Figura 3.4. Las líneas de investigación de los autores líderes están relacionadas, en su mayoría, a genética y tolerancia al frío.



El investigador Arora, R., de Iowa State University de Estados Unidos, es el autor más prolífico, con un total de 18 publicaciones referidas entre otras cosas a *Genetics*, *Arabidopsis*, *Freezing tolerance*, *Eucalyptus*. Entre sus publicaciones se encuentra una realizada en conjunto con investigadores de la Universidad de Concepción de Chile: **Isolation and characterization of three cold acclimation-responsive dehydrin genes from *Eucalyptus globulus***, (2014, Fernández, Águila, Arora, Chen), además de otras como las siguientes:

- Cold hardiness increases with age in juvenile rhododendron populations (2014, *Frontiers in Plant Science*);
- Understanding the cellular mechanism of recovery from freeze-thaw injury in spinach: Possible role of aquaporins, heat shock proteins, dehydrin and antioxidant system (2014, *Physiologia Plantarum*);
- Effect of short-term versus prolonged freezing on freeze-thaw injury and post-thaw recovery in spinach: Importance in laboratory freeze-thaw protocols T2 (2014, *Environmental and Experimental Botany*).

Por su parte, el segundo autor más activo, con 17 publicaciones, corresponde al investigador checo del Crop Research Institute, Department of Genetics and Plant Breeding de Praga, Prasil, I.T., quien ha estado trabajando en los temas: *Genetics*, *Arabidopsis*, *Freezing tolerance*, *Wheat*, *Crops*, *Frost tolerance*, *Frost resistance*. Entre sus trabajos más recientes se encuentran los siguientes títulos:

- Dynamics of cold acclimation and complex phytohormone responses in *Triticum monococcum* lines G3116 and DV92 differing in vernalization and frost tolerance level (2014, *Environmental and Experimental Botany*);



- Proteome analysis of cold response in spring and winter wheat (*Triticum aestivum*) crowns reveals similarities in stress adaptation and differences in regulatory processes between the growth habits (2013, Journal of Proteome Research);
- Accumulation of WCS120 and DHN5 proteins in differently frost-tolerant wheat and barley cultivars grown under a broad temperature scale (2013, Biologia Plantarum).

### 3.1.4 Análisis de tendencias

El análisis de tendencias temáticas analiza aquellos descriptores que tienen mayor frecuencia de aparición, debido al elevado interés que han generado en la comunidad científica a lo largo del período de estudio, y aquellos con una aparición incipiente en los últimos años porque podrían ser temas emergentes y representar áreas de trabajo a futuro, para determinados grupos científicos. Entre los **temas más publicados** en el período 2005-2014 predominan aquellos correspondientes a *freezing* y, en menor medida, a *frost*, seguido de *metabolism*, *physiology genetics*, *proteins* y *Arabidopsis*, los que se vinculan principalmente con ingeniería genética y plantas transgénicas. Como especies destacan trigo (*Triticum aestivum*) y *Arabidopsis thaliana*. Otros descriptores de interés son *Climate change* y *Abiotic stresses* (ver Figura 3.5).



Figura 3.5 Descriptores con mayor frecuencia de aparición

En cuanto a los **temas de investigación emergentes**, es decir, aquellos que recién aparecen en los últimos años, y que es aconsejable identificar ante una posible consolidación en el tiempo, se han identificado trabajos vinculados a *ingeniería metabólica* y *micro RNAs*, que se muestran a continuación:

|                        |   |
|------------------------|---|
| <b>TÍTULO</b>          | Identification of conserved and novel cold-responsive MicroRNAs in trifoliolate orange ( <i>Poncirus trifoliata</i> (L.) Raf.) using high-throughput sequencing |
| <b>Autor(es)</b>       | Zhang, X.-N., Li, X., Liu, J.-H.  |
| <b>Año publicación</b> | 2014  |
| <b>Fuente</b>          | Plant Molecular Biology Reporter  |

Pre-exposure to a period of low but non-freezing temperature, a phenomenon known as cold acclimation, can enhance freezing tolerance. However, the genetic regulatory network controlling cold acclimation of this plant remains unclear. MicroRNAs (MiRNAs) have been shown to play an important role in abiotic stress responses. To have a deeper understanding on the cold acclimation, the MiRNAs before and after low temperature treatment in trifoliolate orange (*Poncirus trifoliata* (L.) Raf.) were characterized using deep sequencing. A total of 107 conserved MiRNAs, belonging to 44 miRNA families, and 5 potential novel MiRNAs were identified. Bioinformatics analysis indicated that 36 conserved and 5 novel MiRNAs were either up-or downregulated by cold, respectively; ten of these cold-responsive MiRNAs were confirmed by quantitative RT-PCR assay. Time-course expression analysis further validated cold responsiveness of the five selected conserved MiRNAs. The predicted target genes of cold-responsive MiRNAs encode a variety of proteins implicated in abiotic stress responses, including the well-characterized transcription factors. In addition, expression patterns of two target genes were inversely related to the relevant MiRNAs. The present study gains insight into molecular mechanisms underlying the cold acclimation-mediated freezing tolerance at posttranscriptional level and unravels cold-responsive MiRNAs of significant value for stress-oriented genetic engineering.

|                        |  |
|------------------------|--|
| <b>TÍTULO</b>          | Over-expression of miR397 improves plant tolerance to cold stress in <i>Arabidopsis thaliana</i> |
| <b>Autor(es)</b>       | Dong, C.-H., Pei, H.   |
| <b>Año publicación</b> | 2014   |
| <b>Fuente</b>          | Journal of Plant Biology   |

Cold stress is an environmental factor that limits the geographical distribution and growing season of plants. The plant response to cold involves different metabolic pathways and gene regulation. Although the expression of some MiRNAs is significantly altered in cold stress, little is known about the regulatory function of MiRNAs in plant resistance to cold. In this study, we generated transgenic *Arabidopsis* plants which harbor an over-expression construct of 35S::miR397a. Analysis by RNA blotting revealed high levels of miR397a transcripts in the transformed plants. Plant cold tolerance assays demonstrated that over-expression of miR397a improved plant tolerance to chilling and freezing stresses. In addition, this study showed that the freezing tolerance of miR397a-ov plants was improved by cold acclimation at 4°C. Northern blot analysis revealed that overexpression of miR397a affected the expression of cold regulated CBF genes and downstream COR genes. These findings provide evidence demonstrating a regulatory role of miR397 in the cold signaling pathway and plant tolerance to chilling and freezing stresses.

### 3.2 RESULTADOS NACIONALES

En lo que respecta a aquellas publicaciones que cuentan con la participación de uno o más investigadores nacionales, se identificaron treinta durante el período 2005-2014. En general, en la comunidad científica nacional no se aprecia la existencia de una línea de investigación específica en cuanto al manejo y control de heladas para la agricultura, sino una incipiente línea asociada a la resistencia al frío de especies forestales, especialmente *Eucalyptus*, y, en segunda medida, de especies andinas y antárticas.

A nivel de trabajo colaborativo, las universidades regionales como la Universidad de Concepción, UFRO y la Universidad Austral, han investigado en conjunto con empresas forestales como Genómica Forestal S.A. y Forestal Mininco S.A., respecto a la tolerancia del *Eucalyptus globulus* a las bajas temperaturas.

La Universidad de Concepción, a pesar de contar con la mayor cantidad de publicaciones a nivel nacional, refiere sus estudios principalmente a la tolerancia al frío de especies alpinas, andinas y antárticas, a excepción de la publicación asociada a la especie agrícola *Chenopodium quinoa*, **Plant responses of quinoa (*Chenopodium quinoa* Willd.) to frost at various phenological stages** (2005, Jacobsen, S.-E., Monteros, C., Christiansen, J.L., Bravo, L.A., Corcuera, L.J., Mujica, A.), desarrollada en conjunto con instituciones peruanas y danesas.

En términos estrictamente agrícolas, el Instituto de Investigación Agropecuaria (INIA) es quien lidera esta línea de investigación, con las siguientes publicaciones:

- **Use of a stress inducible promoter to drive ectopic AtCBF expression improves potato freezing tolerance while minimizing negative effects on tuber yield** (2007, Pino, M.-T., Skinner, J.S., Park, E.-J., Jekniak, Z., Hayes, P.M., Thomashow, M.F., Chen, T.H.H.),
- **Ectopic AtCBF1 over-expression enhances freezing tolerance and induces cold acclimation-associated physiological modifications in potato** (2008, Pino, M.-T., Skinner, J.S., Jekniak, Z., Hayes, P.M., Soeldner, A.H., Thomashow, M.F., Chen, T.H.H.).

Del mismo modo, la Universidad Andrés Bello cuenta con una publicación asociada a la manipulación genética de duraznos, **Isolation and functional characterization of cold-regulated promoters, by digitally identifying peach fruit cold-induced genes from a large EST dataset** (2009, Tittarelli, A., Santiago, M., Morales, A., Meisel, L.A., Silva, H.) en conjunto con Millennium Nucleus in Plant Cell Biotechnology (MN-PCB).

La Universidad de Chile junto a la Pontificia Universidad Católica de Chile y el EIB (Escuela de Ingeniería Bioquímica de la Universidad Católica de Valparaíso) han publicado **Decoupled evolution of foliar freezing resistance, temperature niche and morphological leaf traits in Chilean *Myrceugenia*** (2014, Pérez, F., Hinojosa, L.F., Ossa, C.G., Campano, F., Orrego, F.).

## 4. PANORAMA TECNOLÓGICO

Siguiendo el ejercicio de Vigilancia Tecnológica, una vez analizada la situación en cuanto a investigación (publicaciones científicas), se estudia el estado en que se encuentra el desarrollo de las tecnologías a nivel global. Se lleva a cabo un análisis de las patentes solicitadas y concedidas a escala internacional y nacional en relación al control, mitigación de daños y manejo post heladas.

### 4.1 RESULTADOS INTERNACIONALES

Las principales bases de datos de patentes a nivel mundial son:

- USPTO: base de datos de Patentes de la Oficina de Patentes y Marcas de Estados Unidos.
- Esp@cenet: colección de bases de datos de patentes nacionales e internacionales hospedada y gestionada en la Oficina Europea de Patentes (EPO).
- Patentscope: base de datos de la Oficina Mundial de la Propiedad Industrial (OMPI).

Se recuperan las patentes publicadas en el período 2005–2014, obteniendo un total de 436 registros entre patentes solicitadas y concedidas.

En la Figura 4.1 es posible observar que la evolución del número

de solicitudes por año tiene una tendencia relativa decreciente, mostrando un punto alto en el año 2011.

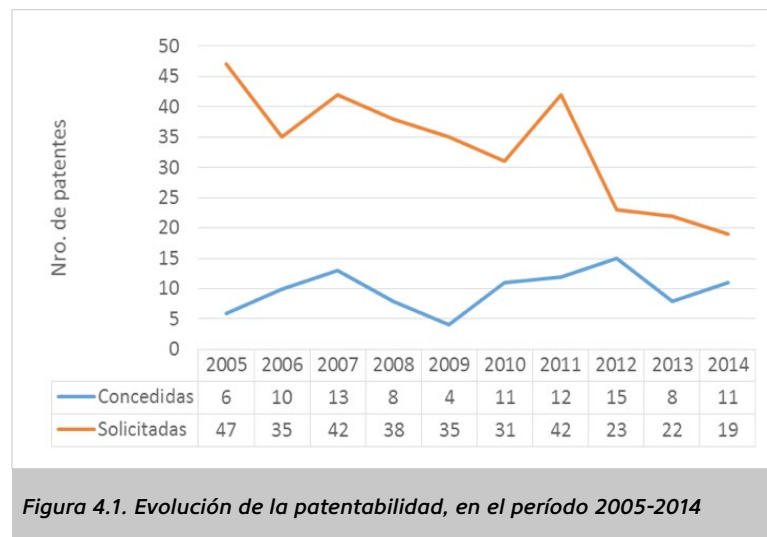


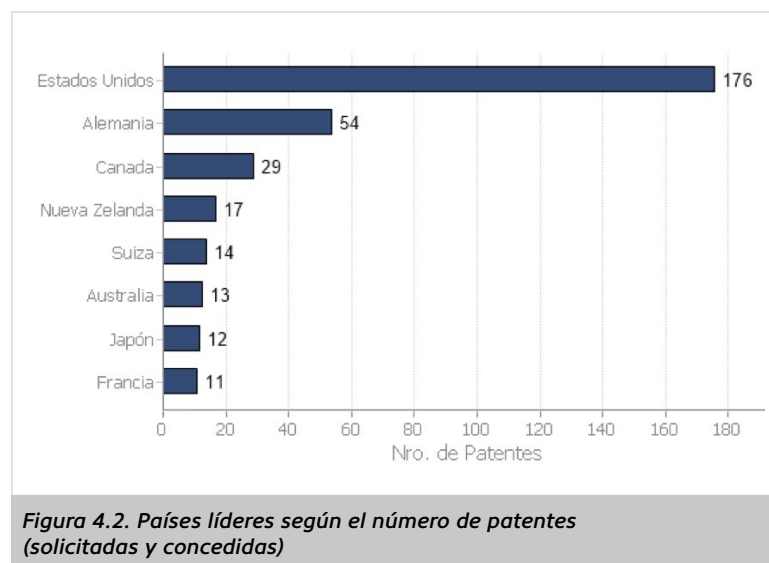
Figura 4.1. Evolución de la patentabilidad, en el período 2005-2014

### 4.1.1 Principales países

Las innovaciones e invenciones patentadas sobre sistemas de control, mitigación de daños y manejo post heladas han sido presentadas por 25 países, según afiliación de los titulares de las patentes.

Estados Unidos posee el liderazgo en términos de la producción de patentes en el área, con 176 documentos de patentes, seguido por Alemania, Canadá y Nueva Zelanda, entre otros<sup>1</sup> (ver Figura 4.2).

En términos de países líderes no se aprecia la participación de países de Centro y Sudamérica, a excepción de Argentina, que posee cinco documentos de patentes. Las áreas tecnológicas que prevalecen en este país corresponden a procesos de obtención de nuevas plantas tolerantes al estrés abiótico, principalmente mediante técnicas de mutación o ingeniería genética.



Al hacer un análisis de la evolución de las solicitudes de patentes por país, es posible destacar a Suiza quien, a partir del 2009, se asoma al panorama tecnológico con una creciente actividad patentadora; y también a la República Checa, cuyo desarrollo tecnológico se vincula con manejo y control de heladas, con una solicitud de patente presentada el año 2014.

1. Aproximadamente 61 documentos de patentes no indican el país.

#### 4.1.2 Principales empresas y entidades tecnológicas

Se han identificado un total de 365 titulares de patentes en el período analizado, entre personas, empresas, universidades y centros de investigación; con una cartera de diez o más documentos de patentes, los siguientes se sitúan como líderes:

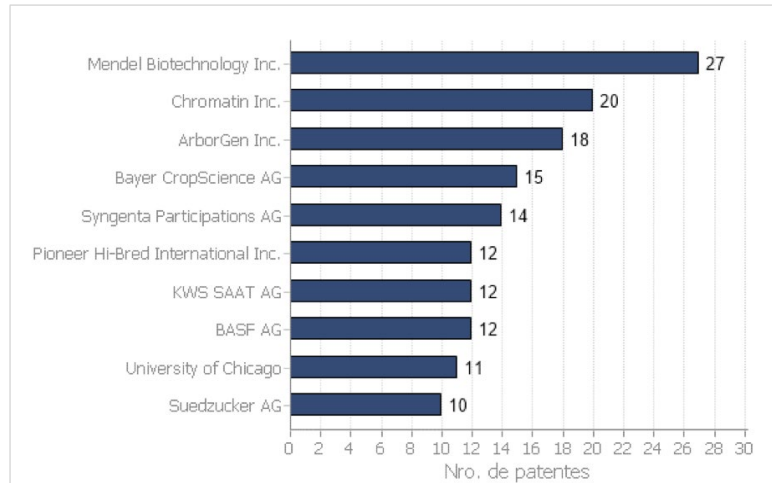


Figura 4.3. Principales organizaciones titulares de patentes

En correspondencia con el liderazgo de países, la mayoría de las empresas líder a nivel mundial tiene su casa matriz en Estados Unidos y Alemania, y corresponden a grandes multinacionales del sector biotecnológico y químico: Bayer CropScience, Syngenta,

Pioneer, BASF y Mendel Biotechnology; esta última desde diciembre del 2014 opera como subsidiaria de Koch Agronomic Services, LLC.

Adicionalmente a los líderes, aparecen nuevos actores con actividad patentadora incipiente hacia finales del período, entre los que destacan:

• CROP MICROCLIMATE MANAGEMENT INC.



Empresa estadounidense fundada con el objetivo de ofrecer soluciones a problemas de estrés abiótico, centrándose en reducir las pérdidas por exceso de calor, luz, frío y sequía, entre otros. El desarrollo tecnológico de la empresa se enfoca en todo lo relacionado con la horticultura y el tratamiento de árboles o plantas durante su crecimiento, a través de biocidas o productos que atraen o repelen a animales perjudiciales, o reguladores del crecimiento, donde el documento de patente publicado más recientemente corresponde a la concesión [US8846573B2](#), referida a un método para incrementar la tolerancia al estrés abiótico y/o reducir las consecuencias de éste, a través de una composición que comprende un ácido dicarboxílico (I) o sus derivados. El método es aplicable, entre otras cosas, a las plantas de manzano, mango y tomate.

La empresa ya posee productos comerciales, distribuidos y comercializados de manera exclusiva, tanto en Chile como en Perú, por la empresa Agrosupport Crop Protection.

• PLANT SENSORY SYSTEMS LLC



Empresa estadounidense de biotecnología agrícola que centra su trabajo en el desarrollo de tecnologías para mejorar el rendimiento agrícola y aminorar los impactos negativos del ambiente. En este sentido, el último documento de patente publicado corresponde a la solicitud [US20140082761A1](#), la cual consiste, entre otras cosas, en un método para mejorar las características agronómicas mediante la inserción de un gen que codifica SAD (descarboxilasa funcional sulfinoalanine), incrementando el crecimiento de las plantas, el rendimiento, o la tolerancia al estrés biótico y/o abiótico.

• AGRA GROUP A.S.



Empresa especializada en la producción y comercialización de fertilizantes y productos que ayudan a la nutrición y estimulación de las plantas. En este ámbito, dentro de su contribución al desarrollo tecnológico mundial, se encuentra la solicitud de patente [EP2772137A1](#), la cual se refiere a una composición para la protección de plantas contra las heladas que contiene *dimethylbetaine acylamidopropyl*, y un método para su aplicación.

• HEAT RANGER LIMITED



Bajo la premisa que los métodos de control de heladas existentes son caros y de dudosa eficacia, Heat Ranger Limited nace para desarrollar y comercializar una máquina que proporciona aire caliente direccional para proteger los cultivos hortícolas de las heladas. (Solicitud de patente: [WO2014200367A1](#)).

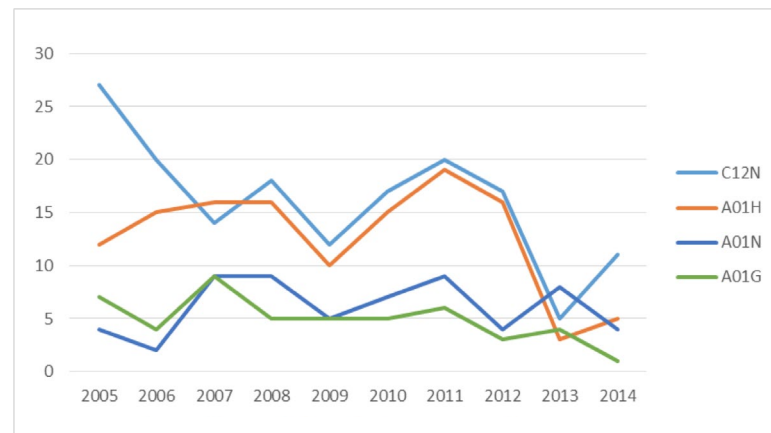
#### 4.1.3 Análisis de tendencias

Para el análisis de tendencias se hace una revisión del Sistema de Clasificación Internacional de Patentes (CIP) desde dos puntos de vista: el primero, toma en consideración aquellas áreas tecnológicas de mayor aparición en los resultados obtenidos; y, el segundo, se basa en aquellas áreas tecnológicas emergentes, entendidas como áreas que aparecieron recién el último año.

En este sentido, en cuanto a las áreas tecnológicas más patentadas, es posible distinguir tres grupos o áreas diferenciadas dentro de lo que se refiere a sistemas de control, mitigación de daños y manejo post heladas:

- **Grupo 1 “Mejoramiento genético de plantas”**, vinculado en gran parte a las subclases de la Clasificación Internacional de Patentes C12N y A01H, tiene relación con técnicas de mutación o de ingeniería genética, y novedades vegetales o procedimientos para su obtención.
- **Grupo 2 “Crioprotectores para la protección contra descensos de temperatura”**, se relaciona con sustancias para disminuir la adherencia del hielo, niebla o agua. Específicamente se trata de invenciones vinculadas con biocidas, productos que repelen o atraen animales perjudiciales, o reguladores del crecimiento de los vegetales (A01N).
- **Grupo 3 “Aparatos mecánicos para la protección de vegetales”**, se relaciona con dispositivos para la protección de vegetales en general: cubiertas protectoras, campanas, dispositivos generadores de humo, niebla, o de circulación del aire, equipos de riego, entre otros (A01G).

Si bien los tres grupos mencionados muestran una tendencia irregular en el período analizado (ver Figura 4.4), se observa que el grupo “Mejoramiento genético de plantas”, a pesar de contar con un mayor volumen de información, tiene una fuerte caída a partir del año 2011. Asimismo, llama la atención el punto de inflexión producido en el año 2007, donde se aprecia un cambio de enfoque tecnológico al pasar de lo que son los aparatos mecánicos para control de heladas, a las sustancias protectoras y reguladoras de crecimiento, como los biocidas, pesticidas, fertilizantes, etc.



**Figura 4.4. Evolución de los CIP según grupos de interés, considerando solicitudes de patentes**

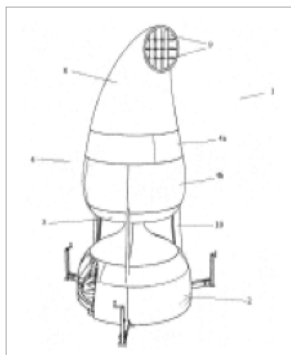
En cuanto a las **áreas tecnológicas emergentes**, que son aquellas surgidas en los últimos años, y que es aconsejable identificar ante una posible consolidación y/o fortalecimiento en el tiempo, se han identificado las siguientes:

· **Calentadores de aire con circulación forzada, estando el aire en contacto directo con el medio de calentamiento**

La tecnología identificada corresponde básicamente a un dispositivo de calentamiento adaptado para modificar las condiciones climáticas. Aspira aire a través de su base, luego sigue una trayectoria ascendente y sale a través de la boquilla superior una vez que ha sido calentado. Entre sus ventajas destaca el control de volumen y temperatura del aire distribuido; si bien su aplicación principal es la protección contra heladas, no se descartan otros usos tales como secado de cultivos y promoción de polinización de insectos.



|             |  |
|-------------|--|
| N° Patente  | <u>WO2014200367A1</u>                  |
| Año         | 2014                                   |
| Título      | Heat distribution apparatus and method |
| Solicitante | Heat Ranger Limited                    |



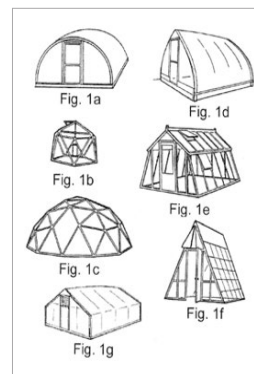
In one aspect the invention provides a heating apparatus adapted to modify local climatic conditions which includes a base arranged to support the heating apparatus on a support surface. An inlet stage is associated with the base and a mixer stage connected on top of the inlet stage. At least one outlet nozzle is connected to the mixer stage, with the outlet nozzle or nozzles being adapted to rotate relative to the base. At least one heating element is located inside the mixer stage, and an impeller is arranged to drive air through the inlet stage into the mixer stage and out at least one outlet nozzle, where the mixer stage defines an air flow path which elevates air drawn through the mixer stage.

At least one heating element is located inside the mixer stage, and an impeller is arranged to drive air through the inlet stage into the mixer stage and out at least one outlet nozzle, where the mixer stage defines an air flow path which elevates air drawn through the mixer stage.

#### · Invernaderos

La patente de la empresa Guardian Industries Corp. se identifica con un recubrimiento de vidrio, cuya composición permite generar formas planas o dobladas, adecuadas para su uso en invernaderos. Las ventanas de vidrio están cubiertas en su superficie por un revestimiento antireflectante que permite disipar la luz en dos direcciones, alcanzando mejores rendimientos en los cultivos.

|             |  |
|-------------|--|
| N° Patente  | <u>US20150013217A1</u>   |
| Año         | 2015   |
| Título      | Cross-functional architectural greenhouse glass, greenhouses including same, and/or associated methods |
| Solicitante | Guardian Industries Corp.  |



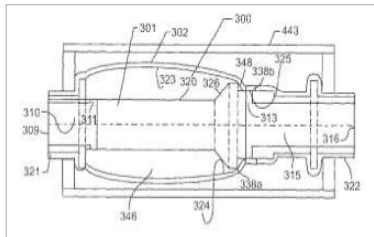
Certain example embodiments of this invention relate to cross-functional architectural greenhouse glass, greenhouses including cross-functional architectural greenhouse glass, and/or associated methods. Certain example embodiments involve combining sensor, functional coating, glass patterning, and/or glass composition selection technologies to produce flat or bent substrates suitable for use in such example applications. It advantageously becomes possible in certain example instances to give growers more control over their individual greenhouses (including portions thereof), while also switching from a more qualitative, to a more quantitative, growing operational approach. The customization afforded by certain example embodiments also advantageously enables new efficiencies to be reached, promotes better crop yields, increases profitability for the grower, and/or reduces crop yield cycle time, potentially in ways that are inconsistent with mainstream growing patterns and/or hobbyist or other pre-conceptions about the standard way(s) in which plants should be grown.

while also switching from a more qualitative, to a more quantitative, growing operational approach. The customization afforded by certain example embodiments also advantageously enables new efficiencies to be reached, promotes better crop yields, increases profitability for the grower, and/or reduces crop yield cycle time, potentially in ways that are inconsistent with mainstream growing patterns and/or hobbyist or other pre-conceptions about the standard way(s) in which plants should be grown.

· **Dispositivos de naturaleza pulsatoria para descarga de líquidos**

Esta tecnología corresponde a un dispositivo capaz de convertir un flujo bajo de agua en un flujo pulsante o intermitente, y puede operar con una o más líneas de goteo, aspersores, rociadores u otros dispositivos de riego, pudiendo ser utilizado también en un sistema antihelada.

|             |   |
|-------------|---|
| Nº Patente  | <u>WO2014068536A1</u>   |
| Año         | 2014  |
| Título      | Pulsating device with two preset pressure-responding normally-closed valves |
| Solicitante | Developed Research For Irrigation Products Inc.                             |



A pulsating device with two preset pressure-responding normally-closed valves is disclosed. The first valve is used for accumulating fluid. The second valve is used for creating resistance so as to force the first valve to open widely. The second valve

may be configured so it creates little to no resistance once opened. In some embodiments, the pulsating device converts a low controlled and/or continuous flow of fluid, such as water and/or air, to a high pulsating and/or intermittent flow. A pulsating device may operate, for example, one or more drip lines, pop-ups, sprinklers, misters and/or other irrigation devices.

· **Biocidas o reguladores del crecimiento de los vegetales que contienen boro**

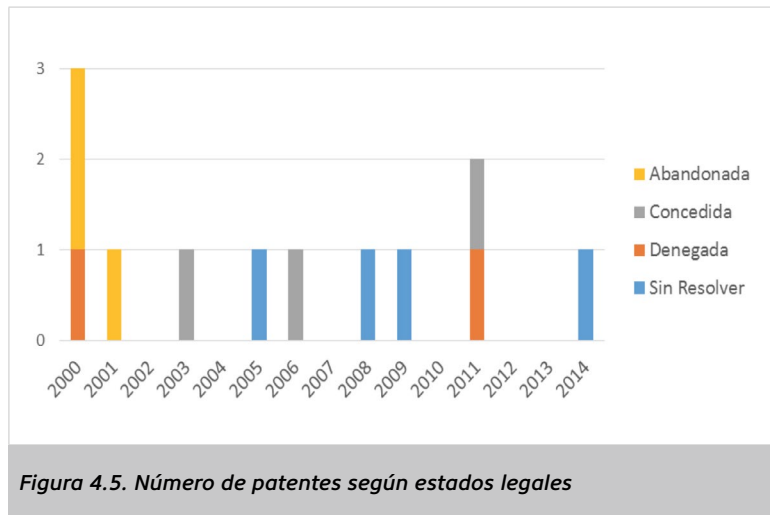
Método para identificar compuestos que contribuyen a aumentar la tolerancia de las plantas al estrés abiótico, el cual implica (a) el tratamiento de las semillas con un protector elegido entre isoxadifen-etilo y 4-ciclopropilaminocarbonil-N- (2-metoxibenzoil) bencenosulfonamida; o (b) el tratamiento de las plantas por pulverización foliar o la aplicación de un protector de suelo.

|             |  |
|-------------|--|
| Nº Patente  | <u>US20140349849A1</u>   |
| Año         | 2014   |
| Título      | Active substances for increasing the stress defense in plants to abiotic stress, and methods of finding them   |
| Solicitante | Schulz Arno, Eppstein-Bremthal<br>Bartsch Klaus, Königstein<br>Krähmer Hansjörg, Hofheim<br>Hills Martin, Idstein<br>Hacker Erwin, Hochheim<br>Rosinger Chris, Hofheim am Taunus |

The invention relates to a method of finding compounds which increase the tolerance of plants to abiotic stress factors acting on this plant, such as, for example, temperature (such as chill, frost or heat), water (such as dryness, drought or anoxia), or the chemical load (such as lack of or excess of mineral salts, heavy metals, gaseous noxious substances) by increasing the expression of plant-endogenous proteins, and to the use of these compounds for increasing the tolerance in plants to abiotic stress factors.

## 4.2 RESULTADOS NACIONALES

De acuerdo a la búsqueda realizada en la base de datos de patentes del Instituto Nacional de Propiedad Industrial (INAPI), entre el año 2000 y 2014, se identificó un total de doce solicitudes de patentes referidas al control y manejo de heladas, en diversos estados legales dentro del proceso de solicitud y concesión, según se muestra en la siguiente figura:



En cuanto al país de origen de los solicitantes de patentes en la oficina nacional, se encuentran Chile, Estados Unidos, Nueva Zelanda, España, Uruguay, Reino Unido y Francia. En estos países hay un total de 19 titulares de patentes entre empresas y personas, destacando, en correspondencia con el panorama internacional, empresas como Pioneer, Vialactia Biosciences (NZ) Limited, Elicityl y Viña Solorca S.L. Otras empresas que están presentes en los resultados son:

- S.S. Steiner, Inc.
- Biotex, S.A.
- Universidad de Chile
- Extenday IP Limited
- Engelhard Corporation

El detalle de las patentes identificadas, en estado concedidas y sin resolver, se presenta a continuación.

### Patentes concedidas

En el período consultado (2000-2014) se identificaron tres concesiones de patentes, las que abordan principalmente áreas temáticas referidas a la protección de vegetales mediante:

- Aparatos mecánicos para la circulación del aire
- Dispositivos generadores de calor, humo o niebla
- Biocidas y/o productos reguladores del crecimiento de los vegetales

|   |  |
|---|--|
| <b>TÍTULO</b>   | Producto para el lavado de plantas que comprende una mezcla de aceite invernal y extracto de lúpulo; método para el control de pestes, bacterias y hongos en plantas; método para la protección de plantas frente a las heladas. |
| Nº Solicitud  | 201101375  |
| Fecha de solicitud  | 08/06/2011   |
| Prioridad   | US 12/624.198 23/11/2009<br>US 61/121.102 09/12/2008   |
| Solicitante   | S.S. Steiner, INC. – Estados Unidos  |
| <p>Producto para el lavado de plantas que comprende una mezcla de aceite invernal y extracto de lúpulo; método para el control de pestes, bacterias y hongos en plantas; método para la protección de plantas frente a las heladas.</p> |  |

|   |   |
|---|---|
| <b>TÍTULO</b>   | Dispositivo para aplicar una fuerza de viento a plantas en un entorno agrícola, el cual está diseñado para identificar aquellas que pueden ser tolerantes al daño producido por el viento. El dispositivo está adaptado para ser llevado por un vehículo. Consta de un dispositivo generador, un dispositivo de dirección y un controlador en comunicación con ambos. |
| Nº Solicitud  | 200603234   |
| Fecha de solicitud  | 22/11/2006  |
| Prioridad   | US 11/549.142 13/10/2006<br>US 60/739.626 23/11/2005  |
| Solicitante   | Pioneer HI-Bred International, INC. - Estados Unidos  |
| <p>Dispositivo y método para aplicar una fuerza de viento a múltiples plantas en un entorno agrícola, adaptado para ser llevado por un vehículo. Comprende un dispositivo generador que proporciona la fuerza de viento, un dispositivo de dirección y un controlador en comunicación con ambos dispositivos.</p> |   |

|  |   |
|--|---|
| <b>TÍTULO</b>  | Método de protección de plantaciones frente a las heladas con una niebla térmica, que genera y mezcla vapor y un flujo de gas, lo mezcla con aire y descarga sobre la plantación. El aparato incluye una cámara de combustión y una cámara de evaporación con descarga de gases, vapor y eyectores de niebla. |
| Nº Solicitud   | 200301654   |
| Fecha de solicitud   | 19/08/2003  |
| Prioridad  | GB 0208290.7 10/04/2002   |
| Solicitante  | Melhm Salim Salomon Namor – Reino Unido   |
| <p>Un método y aparato para proteger las plantaciones de los daños de heladas, que comprende los siguientes pasos: (1) generación de un chorro a vapor a partir de fuentes de agua y calor; (2) generación de un flujo de gas a una temperatura más alta que la temperatura de la atmósfera ambiente; (3) mezcla del chorro de vapor con el flujo de gas para formar una niebla térmica que consiste en vapor de agua dispersado como gas, a una temperatura más alta que la de la atmósfera ambiente; y (4) descarga y distribución de la niebla térmica en dirección preferentemente horizontal, bajo la propulsión dada por la presión del mismo vapor. El aparato comprende una cámara de combustión, un tubo de humos con una o más aberturas ajustables para el ingreso de aire y su mezcla con los gases de combustión, y una cámara de generación de vapor que intercambia calor con la cámara de combustión. El tubo de humos posee además uno o más conductos de salida de gases de configuración cónico convergente, cubierto cada uno por un conducto desplazable de similar configuración tipo Venturi y quedando definida una abertura regulable para el ingreso de aire ambiental. Los conductos de salida de gases están dispuestos coaxialmente con la respectiva boquilla de salida de vapor conectada a la cámara de generación de vapor.</p> |   |

### Patentes sin resolver

En términos de patentes sin resolver se hace referencia a procedimientos de modificación genética, técnicas de mutación o de ingeniería genética, biocidas o reguladores del crecimiento de los vegetales, y cubiertas protectoras para plantas.

|                    |  |
|--------------------|--|
| <b>TÍTULO</b>      | Construcciones génicas que codifican para una proteína con actividad enzimática de licopeno beta-ciclasa, y método para transformar genéticamente plantas para que sean más eficientes y de mejor rendimiento bajo ciertas condiciones de estrés abiótico. |
| Nº Solicitud       | 201402947  |
| Fecha de solicitud | 30/10/2014   |
| Prioridad          | S/I  |
| Solicitante        | Universidad de Chile – Chile   |
| S/I                |  |

|   |  |
|---|--|
| <b>TÍTULO</b>   | Polinucleótido codificante de un polipéptido tipo ubiquitina con capacidad para modular en una planta la tolerancia a por lo menos un estrés ambiental, seleccionado entre sequía, frío, heladas y salobridad; construcción genética; vector; célula huésped; y método para producir dicha planta. |
| Nº Solicitud  | 200901293  |
| Fecha de solicitud  | 27/05/2009   |
| Prioridad   | US 61/056,583 28/05/2008   |
| Solicitante   | Vialactia Biosciences (NZ) Limited - Nueva Zelanda   |
| <p>Polinucleótido codificante de un polipéptido con capacidad para modular en una planta la tolerancia a por lo menos un estrés ambiental, seleccionado entre sequía, frío, heladas y salobridad; construcción genética; vector; célula huésped; y método para producir dicha planta.</p> |  |

|  |  |
|--|--|
| <b>TÍTULO</b>  | Material en forma de hoja reflectante para recubrir el suelo, que comprende cintas de bordado y trama, con una porosidad de al menos 2%. |
| Nº Solicitud   | 200501846  |
| Fecha de solicitud   | 22/07/2005   |
| Prioridad  | NZ 534251 22/07/2004   |
| Solicitante  | Extenday IP Limited - Nueva Zelanda  |
| <p>Material en forma de hoja, tejido o no, reflectante, para recubrir el suelo, que comprende márgenes laterales opuestos que se extienden longitudinalmente, los cuales son más fuertes que una sección central que se extiende también longitudinalmente y se sitúa entre los márgenes laterales del material.</p> |  |

Otras patentes en estado abandonadas y denegadas se detallan en el Anexo 2 del presente informe. Si bien la patente de Viña Solorca S.L. (España) ha sido denegada en el Instituto Nacional de Propiedad Industrial (INAPI) por la falta de respuesta a las correcciones de fondo, esta invención sí se encuentra concedida en España y con varias solicitudes en curso en Francia, Australia, Estados Unidos y Canadá, entre otros.

## 5. RESULTADOS CIENTÍFICO-TECNOLÓGICOS POR GRUPOS DE INTERÉS

De acuerdo con el análisis de tendencias científicas y tecnológicas, se identificaron resultados relevantes asociados tanto a métodos de protección pasiva o preventiva, como a métodos de protección activa.

### 5.1 MÉTODOS DE PROTECCIÓN PASIVA O PREVENTIVA

Los métodos de protección pasiva o preventiva corresponden a aquellos implementados con anterioridad a la helada. En el panorama científico y tecnológico se identificó un total de 346 registros de patentes, entre solicitadas y concedidas, y un total de 1.190 publicaciones científicas. Estos resultados se encuentran vinculados a lo siguiente:

- Mejoramiento genético de plantas.
- Crioprotectores o sustancias destinadas a la protección contra descensos de temperatura y control del estado nutritivo de las plantas, por ejemplo, biocidas, fertilizantes, pesticidas, etc.
- Mapas ambientales, modelos de simulación y sistemas de información.

A continuación se muestra el detalle con el análisis asociado a cada uno de los métodos mencionados y algunas referencias de interés para cada uno de ellos.

#### 5.1.1 Mejoramiento genético de plantas

##### Publicaciones

El mejoramiento genético abarca cerca del 60% del corpus de información científica analizado, con un total de 1.051 publicaciones, y en aumento constante en el tiempo (ver Figura 5.1). Hay 14 investigadores líderes con más de diez publicaciones asociadas a este tema (ver Figura 5.2). Del mismo modo, las principales instituciones provienen de Finlandia, Estados Unidos, Colombia, Francia, Noruega, Suiza, Canadá, Australia y China; en la Tabla 5.1 destacan aquellas con cuatro o más publicaciones.

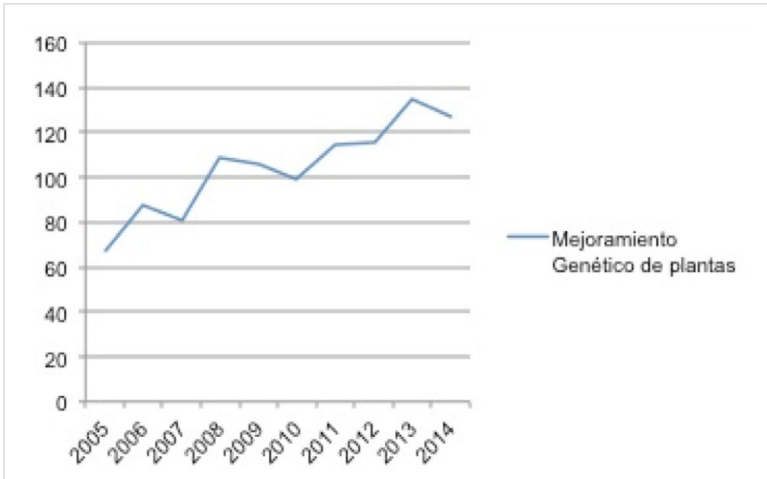


Figura 5.1. Evolución científica asociada al mejoramiento genético de plantas

| INSTITUCIONES                      | PAÍS           | Nº PUB. |
|------------------------------------|----------------|---------|
| Finnish Forest Research Institute  | Finlandia      | 5       |
| Pennsylvania State University      | Estados Unidos | 5       |
| University of Nebraska-Lincoln     | Estados Unidos | 4       |
| Colombian Oil Palm Research Center | Colombia       | 4       |
| University of Helsinki             | Finlandia      | 4       |

Tabla 5.1. Instituciones líderes asociadas a mejoramiento genético de plantas

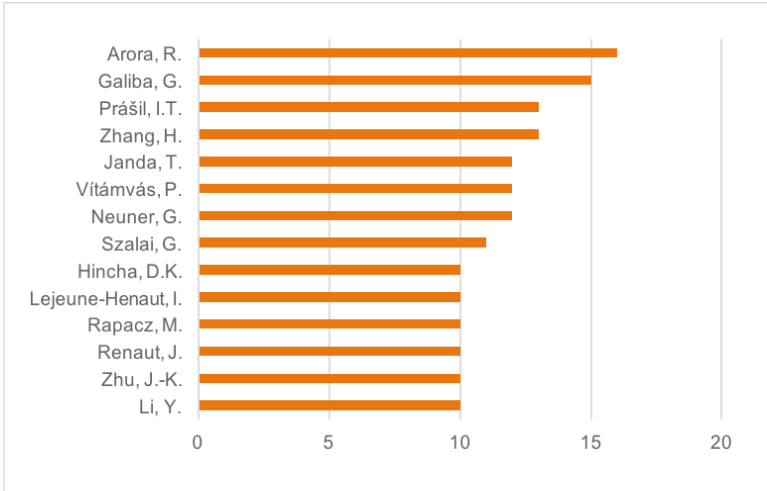


Figura 5.2. Investigadores líderes asociados al mejoramiento genético de plantas

Mediante la manipulación genética, o ingeniería genética, es posible generar variedades de plantas transgénicas resistentes al frío y las heladas. En esta área destacan los siguientes trabajos:

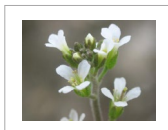
- **The use of antifreeze proteins for frost protection in sensitive crop plants** (2014, Duman, J.G., Wisniewski, M.J.) es una revisión de los estudios realizados en los últimos veinte años sobre la eficacia de las proteínas anticongelantes en plantas transgénicas, con las cuales es posible aumentar la tolerancia al frío entre uno y tres grados Celsius, pudiendo obtenerse resultados aún más significativos. En este sentido, la publicación describe orientaciones para futuros avances en este ámbito.





- **Metabolic engineering of cold tolerance in plants** (2014, Megha, S., Basu, U., Kav, N.N.V.) es una revisión del conocimiento disponible sobre ingeniería metabólica de las plantas (secuencias genómicas y transcriptómicas, datos post-transcripcionales, redes reguladoras, estados epigénicos, etc.) para el desarrollo de nuevos genotipos resistentes a las heladas.

- ***Arabidopsis* cold shock domain protein 2 influences ABA accumulation in seed and negatively regulates germination** (2015, Sasaki, K., Kim, M.-H., Kanno, Y., Seo, M., Kamiya, Y., Imai, R.) detalla los más recientes resultados de la manipulación de proteínas y genes específicos para la tolerancia al congelamiento en la planta *Arabidopsis*.



Por otro lado, diversas publicaciones detallan los resultados de ensayos genéticos para plantas específicas de cultivo; las más investigadas son los cereales, especialmente el trigo, además de maíz y arroz, y luego los *berries*, vides, y árboles frutales. Las más recientes publicaciones asociadas a manipulación genética de una planta en particular corresponden a las siguientes:

- **Identification and expression analysis of cold and freezing stress responsive genes of *Brassica oleracea*** (2015, Ahmed, N.U., Jung, H.-J., Park, J.-I., Cho, Y.-G., Hur, Y., Nou, I.-S.), la cual detalla los resultados de pruebas genéticas en una variedad de repollo.
- **The crucial role of I- and K-segments in the in vitro functionality of *Vitis vinifera* dehydrin DHN1a** (2015, Rosales, R., Romero, I., Escribano, M.I., Merodio, C., Sánchez-Ballesta, M.T.), siendo el primer estudio respecto al rol potencial de *V. vinifera* DHN1a-s en la protección contra la congelación y la deshidratación, además de inhibir el crecimiento del dañino hongo *Botrytis Cinerea*.



## Patentes

En relación a patentes vinculadas con el mejoramiento genético de plantas, hay 231 registros que se distribuyen de manera poco uniforme en el tiempo, con un importante desarrollo tecnológico al inicio del período (2005-2008) y entre los años 2010 y 2012.

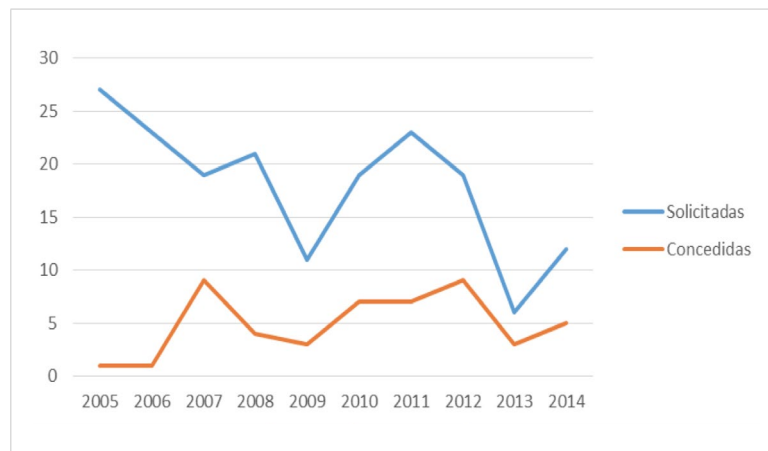


Figura 5.3. Evolución tecnológica asociada al mejoramiento genético de plantas

Los países que han contribuido al desarrollo tecnológico en esta línea son Estados Unidos, Alemania, Nueva Zelanda y Canadá, y en este liderazgo se identifican grandes instituciones de origen privado y académico, destacando en la siguiente tabla aquellas que poseen once o más registros de patentes.

| EMPRESA                            | PAÍS           | Nº DE REGISTROS DE PATENTES |
|------------------------------------|----------------|-----------------------------|
| Mendel Biotechnology Inc.          | Estados Unidos | 26                          |
| Arborgen Inc.                      | Estados Unidos | 18                          |
| Chromatin Inc.                     | Estados Unidos | 20                          |
| KWS SAAT AG                        | Alemania       | 11                          |
| Pioneer Hi Bred International Inc. | Estados Unidos | 11                          |
| University of Chicago              | Estados Unidos | 11                          |

**Tabla 5.2. Titulares de patentes con once o más registros de patentes de mejoramiento genético**

Entre las tecnologías identificadas en este grupo de interés se encuentran aquellas que, haciendo uso de técnicas convencionales de botánica, microbiología, cultivo de tejidos, biología molecular, química, bioquímica y tecnología de ADN recombinante, buscan aumentar el rendimiento de un cultivo sometido a condiciones de estrés moderado (sequía, heladas, baja temperatura, refrigeración, alta salinidad y/o patógenos invasores) o reducir su impacto negativo. Como ejemplo de esto último, destacan las siguientes patentes:

- **EP2825655A1: *Stress tolerance in plants***, de propiedad de la Universidad Nacional del Litoral (Argentina), describe un método para aumentar el rendimiento de plantas en condiciones de estrés moderado mediante un gen del factor de transcripción perteneciente a la familia ZIP HD, lo cual implica introducir y expresar secuencias específicas de ácidos nucleicos en la planta.
- **US20140075598A1: *Ice recrystallisation inhibition protein or antifreeze proteins from deschampsia, and festuca species of grass***, publicada en marzo del 2014, describe un ácido nucleico sustancialmente purificado o aislado que codifica una proteína de inhibición de la recrystalización del hielo (IRIPs), y su uso en la modificación de la respuesta de las plantas a la congelación y/o estrés por bajas temperaturas.

El detalle de estas publicaciones y patentes, así como otras de interés para el grupo de mejoramiento genético, se entrega en el Anexo 3 del presente documento.

## 5.1.2 Crioprotectores para la protección contra descensos de temperatura

### Publicaciones

Este grupo abarca un 3% del corpus de información científica analizado, con un total de 50 publicaciones, y un comportamiento errático en el tiempo (ver Figura 5.4). Estas provienen de once países, liderados por Estados Unidos y España, y de catorce instituciones (ver Figura 5.5). En la Tabla 5.3 se muestran aquellas instituciones con cuatro o más publicaciones.

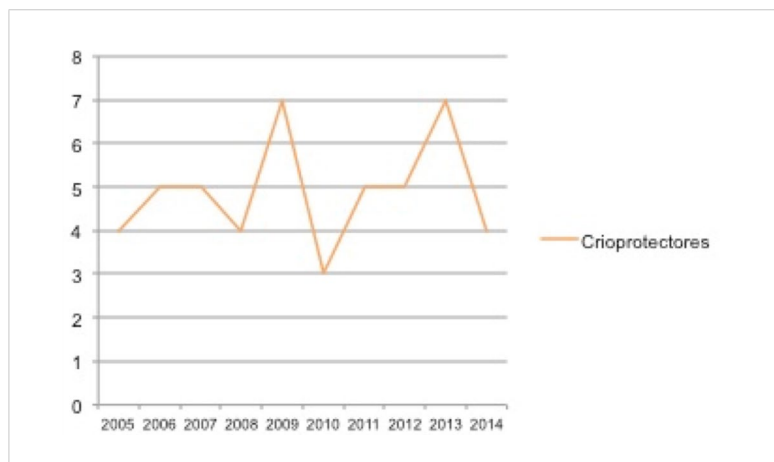


Figura 5.4. Evolución científica asociada a crioprotectores

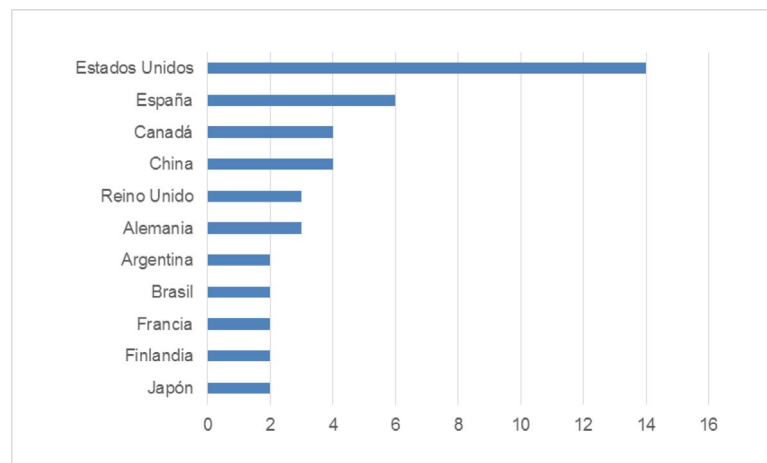


Figura 5.5. Países asociados a crioprotectores

| INSTITUCIÓN                    | PAÍS           | Nº PUB. |
|--------------------------------|----------------|---------|
| USDA-ARS                       | Estados Unidos | 6       |
| University of Nebraska-Lincoln | Estados Unidos | 4       |
| University of Karachi          | Pakistán       | 4       |

Tabla 5.3. Instituciones líderes asociadas a crioprotectores

En el mercado han aparecido infinidad de productos crioprotectores para proteger las plantas frente a las heladas, de fácil aplicación, en forma líquida o *spray*, entre ellos FrostProtect™ (ex FreezePruf); para este producto en particular, se identificó la siguiente publicación científica:

- **Does FreezePruf topical spray increase plant resistance to freezing stress?** (2012, Anderson, J.A.), evaluó su nivel de protección mediante métodos de laboratorio con especies de pimienta, tomate, celosía y Bermuda *grass*, con resultados negativos, aunque sin descartar del todo su eficacia en otras especies o con otros protocolos de medición.



Respecto a la aplicación de fertilizantes, por el contrario, varias publicaciones demuestran su eficiencia para el control de heladas, entre las cuales es posible destacar:

- **Calcium-induced freezing and salinity tolerance in evergreen oak and apple cv. 'Golden Crown'** (2008, Percival, G., Barnes, S.), demuestra que la aplicación de calcio en forma de *spray* a finales del verano puede aumentar la tolerancia al congelamiento y la salinidad de los árboles de roble y manzana durante el invierno.

Asimismo, asociado a la fertilización con nitrógeno y el uso de pesticidas, se identificaron las siguientes publicaciones:

- **The role of the fertilizing with nitrogen, calcium and sodium chloride in winter wheat leaves adaptation to freezing-thaw stress** (2011, Liu, J., Zhou, R., Zhao, M., Zhao, Y., Wang, Y.) detalla cómo estos elementos no sólo previenen la formación de hielo en las células de las plantas, sino que también mejoran las actividades de enzimas antioxidantes para prevenir la peroxidación de membranas, por lo que la fertilización en invierno, además de fortalecer su crecimiento, generará en primavera semillas más tolerantes al congelamiento.
- **Differential effects of nitrogen managements on nitrogen, dry matter accumulation and transportation in late-sowing winter wheat** (2012, Wu, G., Guo, L., Cui, Z., Li, Y., Yin, Y., Wang, Z., Jiang, G.) entrega una estrategia óptima de fertilización con nitrógeno para siembra tardía de trigo de invierno.
- **Seaweeds as an alternative to chemical pesticides for the management of root diseases of sunflower and tomato** (2011, Sultana, V., Baloch, G.N., Ara, J. - Ehteshamul-Haque, S., Tariq, R.M., Athar, M.) describe un estudio realizado con tres algas específicas en tomate y girasol, comparando los resultados con el fungicida Topsin-M; demostró que las algas, además de actuar como estimulantes del crecimiento de las plantas, también las protegen de enfermedades, sequías y heladas.

## Patentes

En las patentes vinculadas con sustancias destinadas a la protección frente a los bruscos descensos de temperatura, hay 102 registros, entre solicitadas y concedidas. Si bien la distribución en el tiempo es irregular, en la Figura 5.6 es posible apreciar una tendencia lineal creciente muy discreta en términos de concesiones de patentes.

En cuanto a las instituciones líderes vinculadas a crioprotectores, se identifican principalmente empresas de Alemania y Estados Unidos, destacando grandes multinacionales como Bayer CropScience, BASF y Syngenta, entre otras.

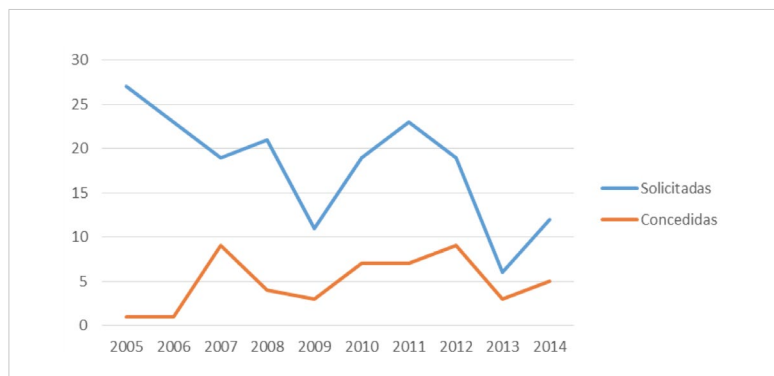


Figura 5.6. Evolución tecnológica asociada a crioprotectores

| EMPRESA                          | PAÍS           | Nº DE REGISTROS DE PATENTES |
|----------------------------------|----------------|-----------------------------|
| Bayer CropScience AG             | Alemania       | 12                          |
| BASF AG                          | Alemania       | 8                           |
| Syngenta Participations AG       | Suiza          | 7                           |
| Crop Microclimate Management Inc | Estados Unidos | 5                           |
| Agro Bio Hungary Kft             | Hungría        | 4                           |

Tabla 5.4. Titulares de patentes asociadas a crioprotectores

En términos de los resultados identificados para este grupo, se encuentran aquellos que hacen uso de diversos compuestos tales como: *ácido dicarboxílico*, *dimethylbetaine acylamidopropyl*, y *acylcyclohexanedione*, entre otros, destacando las siguientes tecnologías:

- **US8846573B2: Methods for increasing tolerance to abiotic stress in plants.** Publicada en septiembre de 2014 y solicitada por la empresa Crop Microclimate Management Inc.
- **EP2772137A1: Composition for protecting plants against frost and method of plant protection.** Publicada en septiembre de 2014 y solicitada por la empresa Agra Group, A.S.
- **US7686975B2: Use of acylcyclohexanedione derivatives for improving the tolerance of plants to cold and/or frost.** Publicada en marzo de 2010 y solicitada por la empresa BASF.
- **US20100304975A1: Process for increasing plants resistance to an abiotic stress,** de la empresa francesa Elicityl, que hace referencia

a un proceso para la adaptación de las plantas al frío o al estrés hídrico (sequía, humedad, salinidad, etc.) mediante pulverización foliar con una composición que contiene un derivado de xiloglucano, que se realiza entre una y 72 horas antes que se produzca el estrés. El proceso descrito en la patente puede utilizarse para el tratamiento de cualquier planta, en particular aquellas agrónomicamente útiles como la vid, árboles frutales (kiwi, nuez, manzana, cerezo, ciruelo, pera, etc.), cereales, oleaginosas y cultivos de hortalizas como tomates.

El detalle de las publicaciones y patentes mencionadas, así como otras de interés para el grupo de crioprotectores o sustancias destinadas a la protección contra descensos de temperatura, se entrega en el Anexo 4 del presente documento.

### 5.1.3 MAPAS AMBIENTALES, MODELOS DE SIMULACIÓN Y SISTEMAS DE INFORMACIÓN

#### Publicaciones

El uso de tecnologías de información y modelos de simulación es otro método pasivo antiheladas. Hay un 5% del corpus de información analizado que corresponde a este tipo de modelos, con un total de 89 publicaciones y una evolución creciente en el tiempo (ver Figura 5.6).

En esta línea, los trabajos provienen principalmente de Estados Unidos, China, Finlandia y Canadá (ver Figura 5.7), y de diversas instituciones de origen académico y científico. La Tabla 5.5 muestra aquellas instituciones con cuatro o más publicaciones en el período analizado.

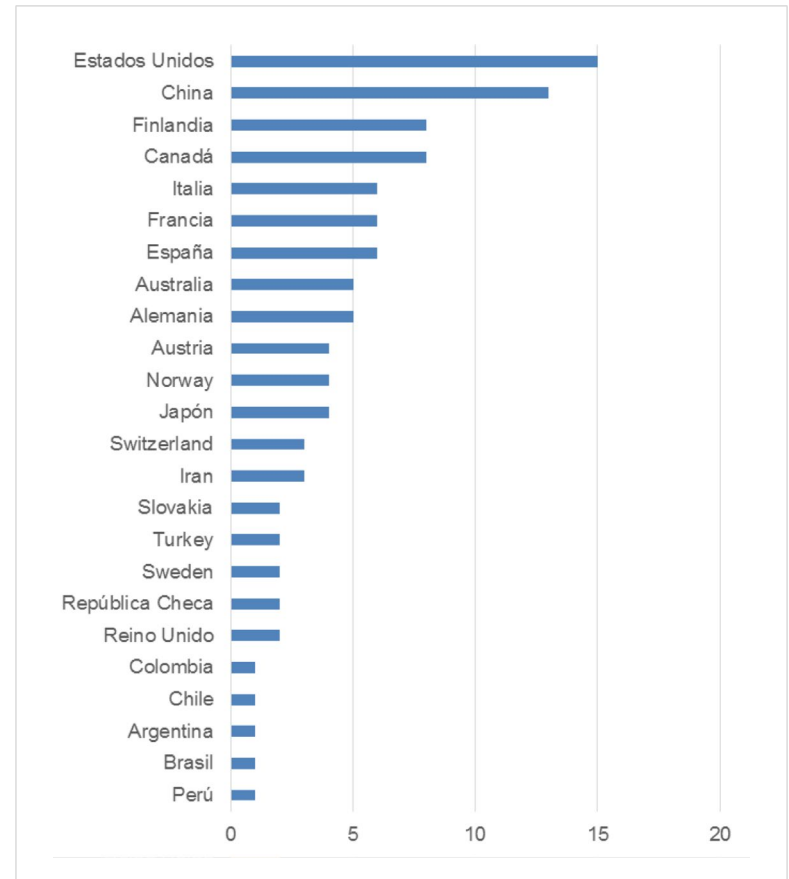


Figura 5.7. Evolución tecnológica asociada a crioprotectores

| INSTITUCIÓN                        | PAÍS           | Nº PUB. |
|------------------------------------|----------------|---------|
| Finnish Forest Research Institute  | Finlandia      | 5       |
| Pennsylvania State University      | Estados Unidos | 5       |
| Colombian Oil Palm Research Center | Colombia       | 4       |
| University of Helsinki             | Finlandia      | 4       |
| University of Nebraska-Lincoln     | Estados Unidos | 4       |

*Tabla 5.5. Instituciones asociadas a mapas ambientales, modelos de simulación y sistemas de información*

Los trabajos identificados en esta línea de interés son los siguientes:



- **Simulation of multitemporal and hyperspectral vegetation canopy bidirectional reflectance using detailed virtual 3-D canopy models** (2014, Kuester, T., Spengler, D., Barczi, J.-F., Segl, K., Hostert, P., Kaufmann, H.) describe un sistema de simulación hiperespectral desarrollado en el contexto de la misión satelital estadounidense EnMAP, con detallada información fenológica, cuyos resultados fueron validados con cultivos de tres cereales.

- **Assessing freeze injury to winter wheat with multi-temporal HJ-1 satellite imagery** (2012, Dong, Y., Chen, H., Wang, H., Gu, X., Wang, J.) detalla un algoritmo de evaluación de daños por heladas en trigo, en base a datos del mini satélite HJ-1.
- **Frost affects grain yield components in winter wheat** (2014, Wu, Y.F., Zhong, X.L., Hu, X., Ren, D.C., Lv, G.H., Wei, C.Y., Song, J.Q.) describe los resultados de un modelo de simulación numérico para seleccionar especies de trigo más resistentes, según su comportamiento en la etapa de macollaje, en condiciones de baja temperatura.

El estudio de los eventos periódicos de las plantas, o fenología, combinado con datos ambientales, permite hacer predicciones de las respuestas vegetales frente a eventos climáticos. En este sentido, se han detectado las siguientes tres publicaciones acerca de modelos fenológicos particulares.

- **Estimation of key dates and stages in rice crops using dual-polarization SAR time series and a particle filtering approach** (2014, De Bernardis, C. G., Vicente-Guijalba, F., Martínez-Marín, T., López-Sánchez, J. M.) describe un modelo numérico para estimar las etapas fenológicas de los cultivos.
- **Evaluation of frost risk in apple by modeling changes in critical temperatures with phenology** (2011, Asakura, T., Sugiura, H., Sakamoto, D., Gemma, H., Sugiura, T.) presenta un modelo fenológico más rudimentario para una especie de manzana, analizando los efectos de bajas temperaturas durante la brotación.
- **A two-step filtering approach for detecting maize and soybean phenology with time-series MODIS data** (2010, Sakamoto, T., Wardlow, B.D., Gitelson, A.A., Verma, S.B., Suyker, A.E., Arkebauer, T.J.) describe un modelo para detectar las fases fenológicas de maíz y soja.

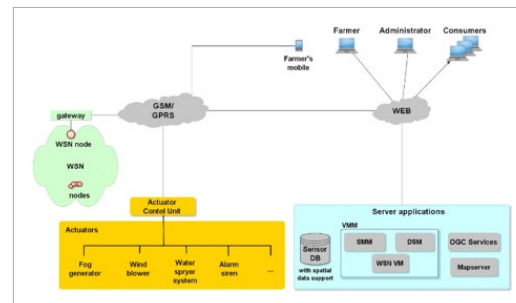
Por su parte, asociado específicamente a la viticultura, han destacado las siguientes dos publicaciones:

- **Using indicators to assess the environmental impacts of wine growing activity: The INDIGO® method** (2014, Thiollet-Scholtus, M., Bockstaller, C.) detalla la adaptación del método INDIGO® para la viticultura, sistema de evaluación ambiental, agregando nuevos indicadores asociados al rubro, entre ellos, la protección contra heladas.
- **Development and experiment of cold resistance tester for grape roots and branches** (2012, Hou, J., Dang, Y., Gao, Z., Zhai, H.) describe un aparato de alta precisión para medir, en laboratorio, la resistencia al frío de las raíces y ramas de las vides.

En términos de cambio climático se han desarrollado muchos modelos de simulación numéricos para proyectar el clima global, ejemplo de ello es la siguiente publicación:

- **Cold air intrusions over southeastern South America - GFDL model behavior regarding climate simulations in the 20th century and future projections** (2013, Cavalcanti, I.F.A., Maller, G.V., Andrade, K.M., Fernández Long, M.E.), que describe los resultados de uno de ellos específicamente para Sudamérica.

Otros trabajos asociados a este tema son:



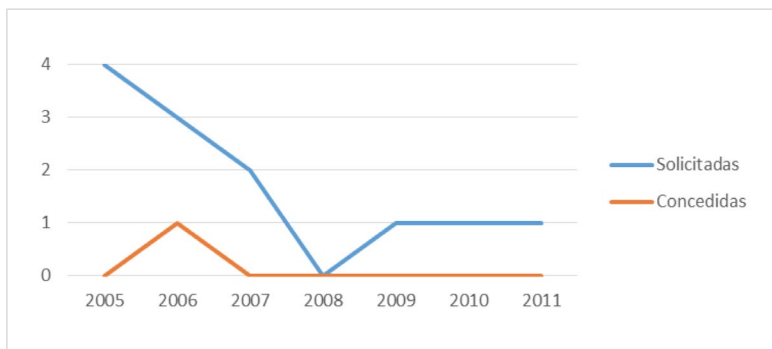
- **The possibilities to use long-range sensors for the frost protection of cranberries** (2012, Alberts, M., Brūns, P., Grīnbergs, U., Kreišmane, D., Špats, A., Tikuma, B.) describe el funcionamiento de una red de sensores en un campo de arándanos que alerta ante posibles heladas, en base a información acumulada, agregada e indexada sobre diversos factores ambientales, tales como condiciones del suelo, nubes, niebla, variaciones de temperatura y otros.
- **New wireless sensor network technology for precision agriculture** (2013, Alberts, M., Grīnbergs, U., Kreišmane, D., Kalejs, A., Dzerve, A., Jekabsons, V., Veselis, N., Zotovs, V., Brikmane, L., Tikuma, B.) detalla un interesante proyecto de investigación de la Universidad de Latvia, sobre el desarrollo de nodos en una red de sensores inalámbricos de largo alcance (más de 300m), operando bajo el sistema operativo FarmOS, sistema específico para aplicaciones agrícolas. El objetivo del proyecto es la construcción de un prototipo de red de sensores inalámbricos con 50 de estos nodos para proveer de protección automatizada a un campo de arándanos, con predicción inteligente de heladas de radiación y correspondientes formas de acción. Dependiendo de los datos reunidos en tiempo real, la inteligencia del sistema provee de información inmediata para decidir el uso ya sea de calefactores, regadores o máquinas de viento.



- **Prediction models for frost/low-temperature stress in subtropical fruit plantations** (2012, Sharma, S. K.) describe el desarrollo de un modelo predictivo para generar una carta guía, orientada a agricultores de la región del Himalaya, con información sobre intensidad y duración de períodos de temperaturas críticas para diferentes especies frutícolas.

## Patentes

A nivel de patentes, el grupo asociado a mapas ambientales, modelos de simulación y sistemas de información no ha crecido como lo ha hecho el desarrollo científico, identificándose un total de trece registros de patentes, entre solicitadas y concedidas. Esta actividad se concentra entre los años 2005 y 2011 con doce solicitudes y tan sólo una concesión (ver Figura 5.8).



**Figura 5.8. Evolución tecnológica asociada a mapas ambientales, modelos de simulación y sistemas de información**

En este ámbito, el desarrollo tecnológico ha venido de la mano de las instituciones que se indican en la Tabla 5.6, sumado a otros titulares individuales.

| EMPRESA                             | PAÍS           | Nº DE REGISTROS DE PATENTES |
|-------------------------------------|----------------|-----------------------------|
| Horticultural Asset Management Inc. | Estados Unidos | 4                           |
| Jiangsu University                  | China          | 2                           |
| Premiere Software LLC               | Estados Unidos | 1                           |
| Deere & Company                     | Estados Unidos | 1                           |

*Tabla 5.6. Instituciones vinculadas al grupo mapas ambientales, modelos de simulación y sistemas de información*

Entre los resultados identificados se encuentran las siguientes patentes:

- **US7047133B1: Method and system of evaluating performance of a crop**, publicada en mayo de 2006, describe un método y un sistema para evaluar el rendimiento de un cultivo con respecto a una o más áreas geográficas, en base a análisis de árbol de decisión de las mediciones ambientales realizadas en una región determinada.
- **US20090216594A1: Vineyard information collection and management system**, corresponde a un sistema de recolección y gestión de información de viñedos en tiempo real, permitiendo de forma fácil y rápida el acceso al análisis de información y a los informes generados, desde cualquier lugar con conexión a internet. Si bien el sistema permite gestionar información de bodega, distancia entre surcos y de protección contra heladas, también se puede ampliar para recopilar, administrar, analizar y reportar cualquier tipo de información deseada de la viña, y así dar respuesta a requerimientos particulares de cada usuario.

· **US8083481B2: Method for siting and operating a crop protection wind machine array**, publicada en diciembre del 2011. Corresponde a un sistema de control de máquinas de viento dispuestas cerca de los cultivos. El conjunto de ellas se interconecta vía satélite a través del módulo ventilador, y entran en funcionamiento al comprobarse una condición detectada de acuerdo a los criterios almacenados, protegiendo los cultivos de las condiciones meteorológicas perjudiciales, como daños por heladas. La Figura 5.9 muestra un diagrama esquemático de los elementos del sistema descrito.

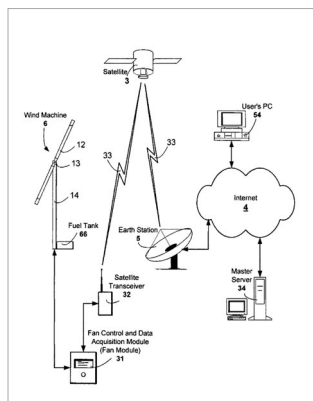


Figura 5.9. Países asociados a equipos con uso de agua

- Equipos con uso de agua, por ejemplo, riego por aspersión, generadores de niebla artificial, etc.
- Máquinas de viento, por ejemplo, ventiladores o turbinas.
- Calefactores y/o quemadores.

A continuación se muestra el detalle asociado a cada uno de los métodos mencionados y algunas referencias de interés.

### 5.2.1 Equipos con uso de agua

#### Publicaciones

Este grupo abarca un 2% del corpus de información científica analizado, con 33 publicaciones y una evolución muy irregular en el tiempo (ver Figura 5.10). Estas publicaciones provienen de diversos países, entre los que destacan Estados Unidos, China, Italia y España (ver Figura 5.11).

El detalle de las publicaciones y patentes vinculadas a mapas ambientales, modelos de simulación y sistemas de información se entrega en el Anexo 5 del presente documento.

### 5.2 MÉTODOS DE PROTECCIÓN ACTIVA

Los métodos de protección activa y sus posibles combinaciones son los que se implementan durante la noche de ocurrencia de la helada, para mitigar el efecto de las temperaturas bajo cero; se vinculan principalmente a dispositivos o aparatos mecánicos para la protección de vegetales. En este sentido, dentro del panorama científico-tecnológico mundial se ha identificado un total de 52 registros de patentes, entre solicitadas y concedidas, y 46 publicaciones científicas. Dichos resultados se vinculan con lo siguiente:

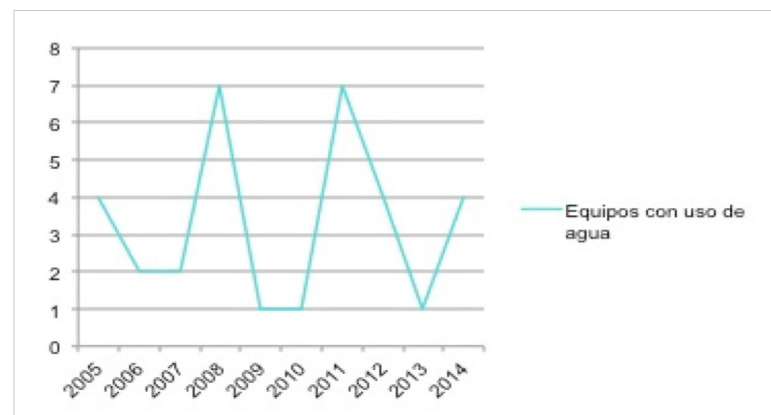


Figura 5.10. Evolución científica asociada a equipos con uso de agua

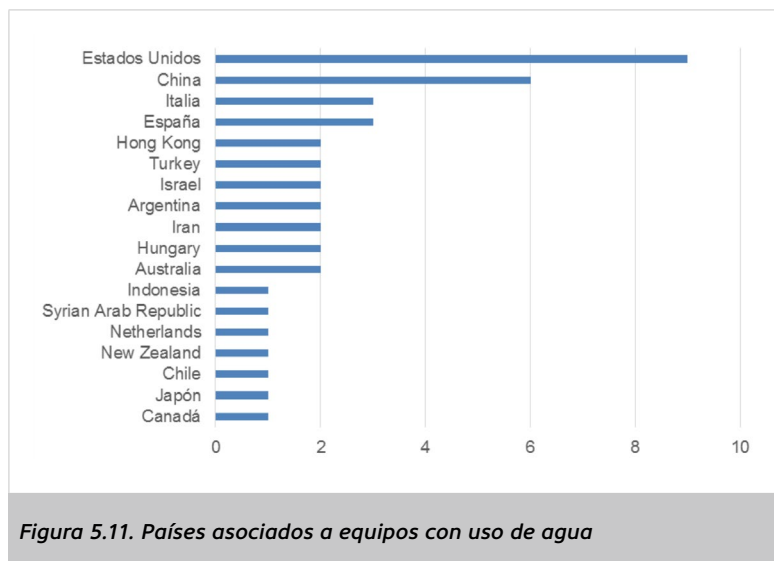


Figura 5.11. Países asociados a equipos con uso de agua

Las instituciones con cuatro o más publicaciones se presentan en la Tabla 5.7.

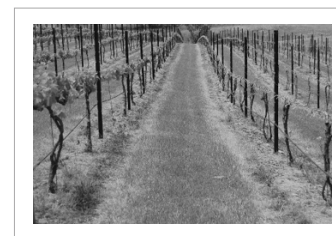
| INSTITUCIÓN                    | PAÍS           | Nº PUB. |
|--------------------------------|----------------|---------|
| INTA                           | Argentina      | 6       |
| University of Nebraska-Lincoln | Estados Unidos | 4       |
| USDA-ARS                       | Estados Unidos | 4       |

Tabla 5.7. Instituciones líderes asociadas a equipos con uso de agua

En este grupo de interés, los trabajos de investigación identificados son los siguientes:

- **Microclimate modification in pear plantation by using cooling irrigation** (2011, Lakatos, L., Dussi, M.C., Gonda, I., Soltasz, M., Nyaki, J., Szaba, Z., Zhongfu, S.) da cuenta de una experiencia en Hungría, usando aspersores de enfriamiento en un campo de perales, cuyo uso regular permite retrasar la floración y así disminuir el riesgo de daños por heladas tardías.

- **Spring cold injury to winegrapes and protection strategies and methods** (2008, Poling, E.B.) destaca el enfriamiento por evaporación con aspersores dirigidos, como uno de los mejores métodos antiheladas para los viñedos.



- **Tree-Temperature monitoring for frost protection of orchards in semi-arid regions using sprinkler irrigation** (2009, Ghaemi, A.A., Rafiee, M.R., Sepaskhah, A.R.) da cuenta de un experimento de riego por aspersión, en campos de duraznos y naranjas, como mecanismo para aumentar la temperatura de las flores, logrando aumentos de al menos dos y medio grados Celsius. Además logró un ahorro hídrico de 54% mediante la velocidad óptima de irrigación entregada por un sistema de balance energético implementado por el *software* FOSTPRO.

- **A comparison of traditional and novel methods of antifrost irrigation. Experiences in Trentino and in Emilia, Italy** (2008, Pratzoli, W., Zinoni, F., Eccel, E.) es un estudio comparativo de diferentes tipos de microaspersores, operando tanto en forma continua como alterna, contrastando el ahorro de agua con la protección ante heladas en Italia.

El uso de microaspersores es el método preferido de irrigación para árboles y viñedos, pues entrega un mayor grado de protección frente a heladas que el riego por goteo, y, a la vez, es más eficiente en el uso de agua y energía que la irrigación a ras de suelo o con aspersores comunes. Sin embargo, su uso ha sido discutido por la comunidad científica, tal como dan cuenta las siguientes dos publicaciones estadounidenses:

- **Current status of microsprinkler irrigation in the United States** (2012, Boman, B., Sanden, B., Peters, T., Parsons, L.) analiza el estado de los sistemas de microaspersores en términos de diseño, operación y mantenimiento, junto a sus beneficios y limitaciones.



- **Microsprinkler irrigation for frost protection of citrus in Florida** (2015, Parsons, L. R.), por su parte, demuestra la utilidad de los microaspersores para proteger cítricos ante las heladas, incluso en árboles maduros. Entrega recomendaciones de altura (0.6 – 0.9m) y velocidades óptimas de irrigación.

Otras interesantes publicaciones relativas a equipos con uso de agua, corresponden a:

- **Possibility for modification of microclimate in orchards by using evaporative cooling irrigation** (2012, Lakatos, L., Zyromski, A., Biniak-Pierog, M.) presenta una experiencia húngara sobre el uso de aspersores en un campo frutícola (duraznos, ciruelas, manzanas, peras), pudiendo retrasar la floración y con ello disminuir el riesgo de daños por heladas tardías.
- **Dynamics of heat exchange in strawberries in an open field at night-frost conditions** (2012, Pennar, M., Palge, V.) presenta un modelo de intercambiador de calor mediante aspersores, como

método de protección ante heladas para un cultivo de frutillas, con un bajo consumo de agua.

- **Low cost irrigation and frost protection with Centauro** (2010, Toller, G., Corradini, S., Biasi, A.) describe un sistema para hacer un uso eficiente del agua en el riego. Consiste en una serie de sensores para medir la humedad del suelo y monitorear la temperatura, de modo de alertar ante riesgo de heladas.
- **Efficient irrigation against frost uses less water** (2007, Pratzzoli, W.) da cuenta de un 75% de ahorro en el uso de agua mediante un sistema de microirrigación denominado Flipper, montado a una altura de 3m.<sup>2</sup>

### Patentes

El grupo asociado a tecnologías que hacen uso de agua para control y manejo de heladas muestra un escaso desarrollo tecnológico, identificándose diez documentos de patentes. A lo largo del tiempo, la distribución de estos resultados no es concluyente (ver Figura 5.12).

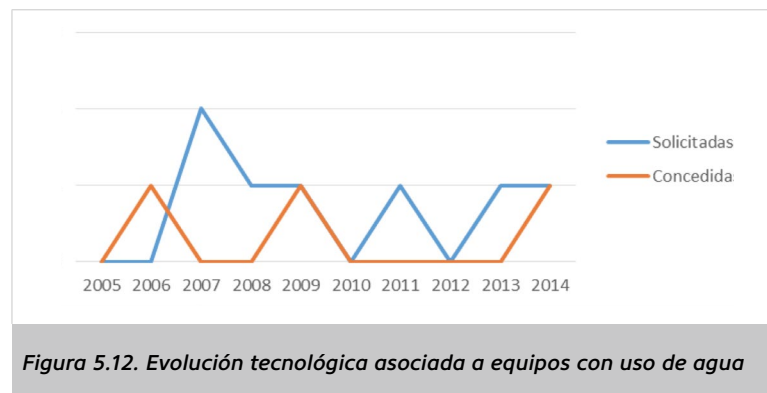


Figura 5.12. Evolución tecnológica asociada a equipos con uso de agua

### 2. FLIPPER

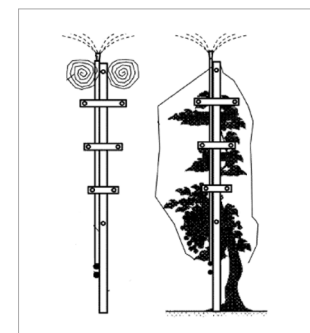
En este grupo destacan titulares de países como Estados Unidos, Francia y Alemania, y figuran entre ellos empresas y titulares individuales. La tabla siguiente muestra las empresas que han contribuido al desarrollo tecnológico en esta materia.

| EMPRESA  | PAÍS           | Nº DE REGISTROS DE PATENTES |
|--|----------------|-----------------------------|
| Kennco Manufacturing Inc.                        | Estados Unidos | 1                           |
| International Water & Energy Savers Ltd.         | Israel         | 1                           |
| Developed Research For Irrigation Products Inc., | Estados Unidos | 1                           |
| Edge Technology                                  | Estados Unidos | 1                           |

*Tabla 5.8. Instituciones vinculadas a equipos con uso de agua*

Entre los resultados para este grupo de interés se encuentran los siguientes documentos de patente:

- **US8919036B2: Trellis system for irrigation and frost prevention**, publicada en diciembre del 2014 y solicitada por la empresa EDGE Technology. Esta tecnología consiste en un sistema que combina riego por goteo para un área de terreno circundante, riego por aspersión, que proporciona agua caliente a un área que rodea el enrejado, y una red de protección (malla de tela o plástico) acoplada al enrejado, que se despliega para cubrir el cultivo (p.e. vides) y protegerlo de los efectos del clima frío (ver Figura 5.13).



*Figura 5.13. Sistema combinado de control y manejo de heladas*

- **WO2014068536A1: Pulsating device with two preset pressure-responsive normally-closed valves**, es un dispositivo de naturaleza pulsatoria para descarga de líquidos.

El detalle de las publicaciones y patentes vinculadas a equipos con uso de agua se entrega en el Anexo 6 del presente documento.

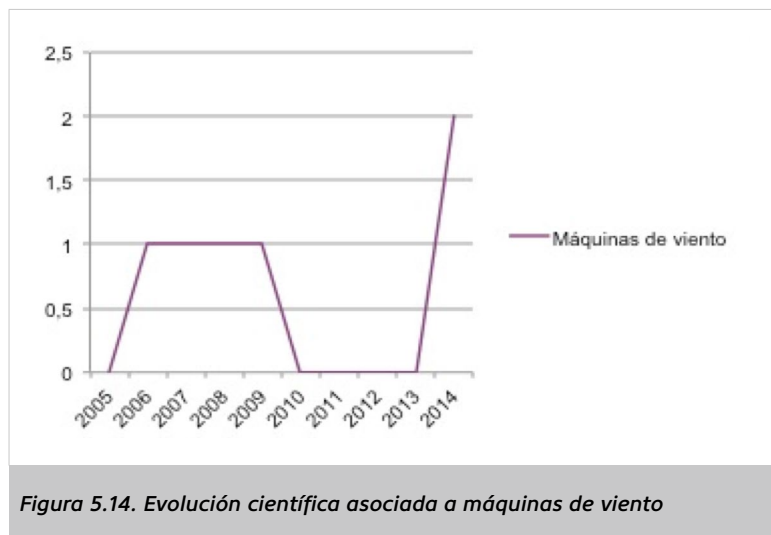
### 5.2.2 Máquinas de viento

#### Publicaciones

Este grupo abarca apenas un 0.3% del corpus de información científica analizado, donde destacan sólo seis publicaciones.

Las instituciones asociadas a éstas pertenecen a Estados Unidos, China, Portugal y Japón, liderando el ranking con dos publicaciones la USDA-ARS Northern Plains Agricultural Research Laboratory.

Uno de los métodos activos antiheladas más utilizado es el equipamiento de ventiladores; a este respecto se detectaron las siguientes publicaciones:



- **An improved design on suction-exhaust duct for frost protection in tea fields** (2014, Wu, W., Hu, Y., Zhang, H., Sun, H.) describe un nuevo diseño para una máquina de viento con soplado de aire hacia arriba. El flujo de aire aspirado a través del conducto es reforzado en la parte superior y utilizado como fuerza motriz para la convección de inversión térmica desde la parte superior, cubriendo un área mayor de protección. Este diseño permite aumentar la temperatura hasta en tres grados Celsius y tiene bajo costo de operación, además de fácil instalación y mantenimiento.

- **Effective experiment of elevated wind machine for frost protection with long blade in large apple orchards** (2014, Yin, X., Wang, Y., Ding, R., Fu, S., Zhang, F., Chen, Q.) da cuenta de la efectividad de una máquina de viento, de 8.5m de altura, un aspa de 6m de diámetro y 12kw de potencia, para proteger árboles frutales altos. Mediante sensores de temperatura y humedad, en una helada de tres días en octubre de 2013, se pudo comprobar el aumento de temperatura y disminución de la humedad relativa, protegiendo un área de 1.73-3.07hm<sup>2</sup>.
- **Frost protection experiment in tea fields using an unmanned helicopters** (2013, Hu Yong Guang, Liu Sheng Zhong, Shen Jian Wen) describe los resultados de experimentos en campos de té chinos con helicópteros no tripulados, como método de protección contra heladas, demostrando su utilidad para ser aplicados a gran escala. La temperatura de la copa de los árboles aumentó hasta en 3.8 grados Celsius.
- **Operation effects of wind machines for frost protection of tea trees on different time scales** (2013, Hu Yong Guang, Zhu Xiao Lan, Zhao Meng Long, Snyder, R. L., Li Ping Ping) se refiere a la importancia de la programación de las máquinas de viento para su real efecto. En el caso específico para proteger campos de té ante probables heladas, sugiere aplicarlas con un mínimo de siete días antes de la floración, comenzando una hora antes de la probable helada y hasta una hora después del amanecer.
- **Vineyard frost protection with upward-blowing wind machines** (2012, Battany, M. C.) presenta resultados comparativos entre máquinas de viento convencionales y otras con soplado de aire hacia arriba para protección de heladas usadas en viñedos; las segundas mostraron un bajo rendimiento cuando son de baja potencia (6.3kw).



- **Selective inverted sink efficiency for spring frost protection in almond orchards northwest of Isfahan, Iran** (2011, Yazdanpanah, H., Stigter, C. J.) demuestra el uso del dispositivo SIS (Selective Inverted Sink) en un campo de almendras de 20has en Irán, con una zona de influencia significativa de tan sólo 500m. SIS<sup>3</sup> es una invención uruguaya de 1990 que drena en forma selectiva el aire más frío y lo lanza hacia arriba en forma de chorro vertical, expulsándolo fuera de la zona de cultivo.

### Patentes

Si bien el volumen de información tecnológica referida a máquinas de viento para el control y manejo de heladas no es preponderante respecto del volumen total de información analizada, se aprecia una tendencia lineal constante en el tiempo, con un atractivo aumento en las concesiones de patentes hacia finales del período analizado (ver Figura 5.15).

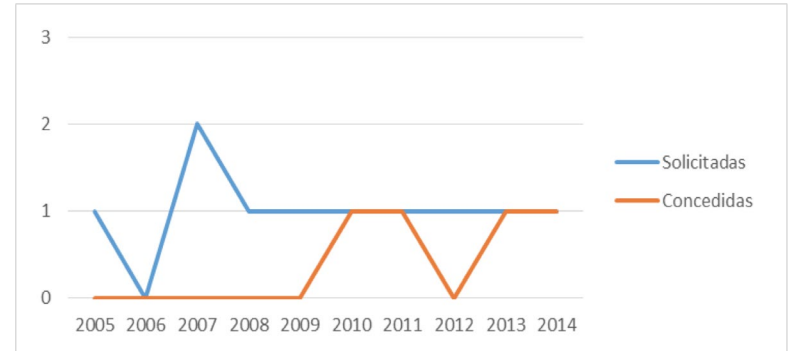


Figura 5.15. Evolución científica asociada a máquinas de viento

Entre los actores líderes predominan como países titulares Estados Unidos, China, Corea, Reino Unido y Canadá. Asimismo, se identifican mayoritariamente titulares individuales, y en menor medida instituciones académicas y de investigación como las siguientes:

| EMPRESA  | PAÍS  | Nº DE REGISTROS DE PATENTES |
|--|-------|-----------------------------|
| Jiangsu University                                 | China | 3                           |
| Korea Meteorological Administration                | Korea | 2                           |
| Jiangsu Yinchunbiya Tea Reseach Institute Co. Ltd. | China | 2                           |

Tabla 5.9. Instituciones vinculadas a máquinas de viento

3. SIS Technologies

Como sistema de control y manejo de heladas por movimiento de aire, están las siguientes patentes:

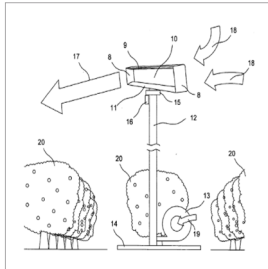


Figura 5.16. Ventilador para el control de heladas

- **US20150024674A1: Agricultural frost protection using induction fans**, corresponde a un ventilador sin aspas. Posee un dispositivo impulsor de aire compuesto por un motor eléctrico (o de combustión) y un ventilador dispuesto en la base. Están conectados a un conducto en el cual fluye el aire a alta presión hasta un punto donde se libera y se combina con un flujo de aire caliente, provocando el calentamiento de las zonas más frías y la consecuente protección de los cultivos agrícolas (naranjas, uvas y frutillas, entre otros).

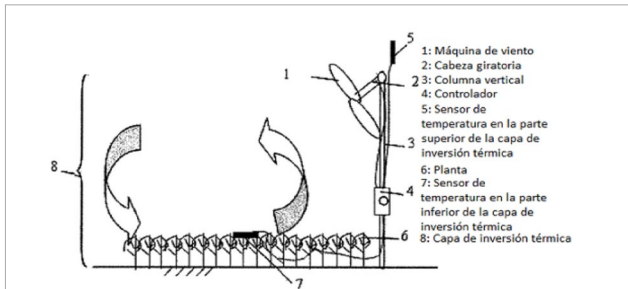


Figura 5.17. Vista esquemática del sistema de control

- **US8701341B2: Control method and apparatus of wind machine for plant frost protection**, es un método para controlar los ventiladores, en función de la información recogida a través de sensores ubicados tanto en la parte superior como inferior de la capa de inversión térmica. Esta invención resulta interesante ya que las máquinas de

viento existentes para el control y manejo de heladas, generalmente dependen sólo de la temperatura crítica o de la velocidad del viento para comenzar a funcionar; sin embargo, al no considerar como condición de funcionamiento la inversión térmica, el funcionamiento de la máquina podría agravar el daño por congelación.

- **US7654035B2: Device for the creation of containment barriers for cold air in atmospheric conditions corresponding to radiation frosts**, publicada en febrero de 2010, corresponde a un dispositivo mecánico que mueve el aire frío tomado desde las proximidades del suelo durante el período de helada y lo expulsa en forma de chorro con un eje aproximadamente horizontal y circular.

El detalle de las publicaciones y patentes vinculadas a máquinas de viento se presenta en el Anexo 7.

### 5.2.3 Calefactores y/o quemadores

#### Publicaciones

Este grupo abarca apenas un 0.4% del corpus de información científica analizado, con sólo siete publicaciones distribuidas en el tiempo según lo muestra la Figura 5.18.

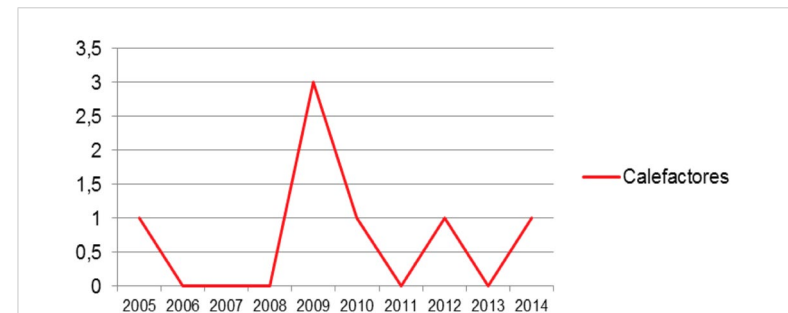


Figura 5.18. Evolución científica asociada a calefactores y/o quemadores



Coincidentemente con lo señalado en el grupo máquinas de viento, el USDA-ARS Northern Plains Agricultural Research Laboratory lidera el ranking con cuatro publicaciones asociadas a calefactores y/o quemadores.

En este sentido, el uso de calor como método activo contra las heladas ha sido investigado en esta comunidad científica por medio de cables eléctricos y sistemas de calefacción del tipo pulsorreactor. Como ejemplo de ello se encuentran las siguientes publicaciones:

- **Electrically heated cables protect vines from frost damage at early flowering** (2009, Lamb, D.W.) es un sistema de cables calefactores eléctricos para proteger las inflorescencias de los viñedos contra las heladas. Experimentos en el norte de Australia bajo una helada de  $-3^{\circ}\text{C}$ , colocando diferentes niveles de calor, demostraron que un cableado de 43kW/ha es suficiente como método de protección para las heladas en viñedos pequeños.



- **Pulse jet orchard heater system development: Part I. Design, construction, and optimization & Part II. System scaling and application** (2009, Alshami, A.S., Evans, R.G.) son el resultado de un proyecto de seis años para el desarrollo de un calefactor con motor pulsorreactor, operado con propano líquido o gaseoso, cuyas principales ventajas respecto a los calefactores convencionales son su bajo costo, portabilidad, autorregulación, escalabilidad y bajo nivel de ruido. Utiliza la tecnología de eyector de aire para reducir

la temperatura de la corriente de combustión a alta velocidad, al mezclarla en forma horizontal con el aire frío circundante, produciendo un aumento de la temperatura efectiva neta de dos a cinco grados Celsius.

- **Efficiency of air heating trailed machine for protection against spring frost in apple orchard** (2012, Rabcewicz, J., Biakowski, P., Konopacki, P.) presenta los resultados del uso de un calefactor portátil antiheladas en un campo de manzanos, llegando a observar fluctuaciones de temperatura en las hojas de los árboles hasta una distancia de 25m de la máquina. En promedio, las diferencias fueron de hasta medio grado Celsius.
- **Pistachio frost damage in Iran and new methods of frost protection** (2010, Hokmabadi, H.) describe los resultados de dos nuevos métodos antiheladas en un campo menor de pistachos en Irán; el primero, es el anteriormente mencionado SIS, pudiendo aumentar la temperatura en dos grados Celsius, y, el segundo, un quemador que logró aumentarla en 1.9.
- **Frostguard: a new technique of frost control in small orchards?** (2006, Deckers, T., Schoofs, H.) es una pequeña máquina calefactora desarrollada en Bélgica, con resultados exitosos en predios de 2has de manzanas y frutillas. Este producto se encuentra actualmente disponible en el mercado<sup>4</sup>.



4. FrostGuard

## Patentes

Para el grupo de calefactores y/o quemadores se ha identificado un total de 27 documentos de patentes, convirtiéndose en el grupo con mayor volumen de información de los métodos activos considerados. En términos de la distribución por año, se observa un punto máximo de solicitudes el año 2009, para luego continuar con una tendencia lineal a la baja (ver Figura 5.19).

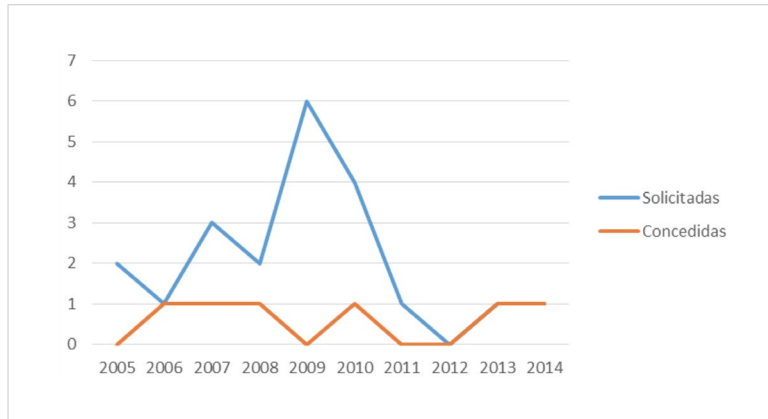


Figura 5.19. Evolución tecnológica asociada a calefactores y/o quemadores

Los países líderes de este grupo son Estados Unidos, Francia y Bélgica. En término de titulares de patentes, la mayoría de las empresas identificadas posee uno o dos documentos de patentes, entre solicitadas y concedidas, entre ellas: Lazo Europ NV, Agrofrost NV y Heat Ranger Ltd.

En cuanto a los resultados identificados para el grupo de calefactores y/o quemadores, se encuentran las siguientes invenciones:

- **WO2014200367A1: Heat distribution apparatus and method**, de la empresa neozelandesa Heat Ranger, proporciona aire caliente direccional para proteger los cultivos hortícolas de las heladas.

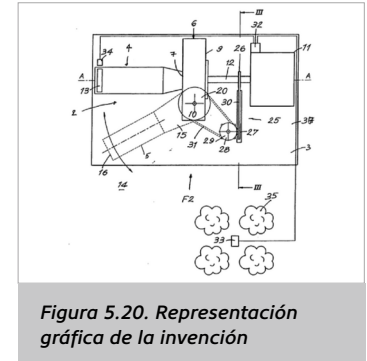


Figura 5.20. Representación gráfica de la invención

- **EP1834520B1: Method for avoiding frost damage in crops and/or for improving the fructification at low temperatures and device applied with such a method**, distribuye aire caliente en los cultivos para calentar y enfriar el aire periódicamente, con una variación de 0.2 grados Celsius. De acuerdo a la descripción de la tecnología, es ideal para superficies pequeñas, de aproximadamente 0.5ha (ver Figura 5.20).

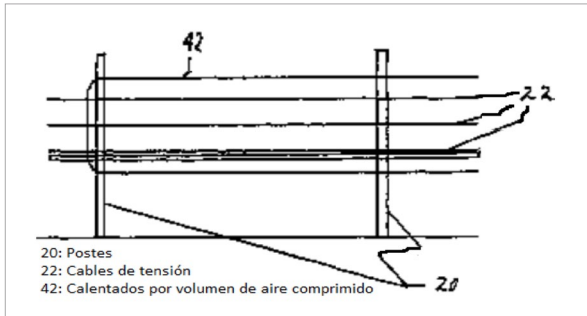


Figura 5.21. Método de protección contra heladas por calefacción de gas

- **WO2009103488A1: Device and method for the frost protection of grape vines**, solicitada por la empresa suiza Integrated Solutions GMBH, corresponde a un método mediante el cual se calienta un gas que luego es transportado por un sistema de distribución, y es liberado a través de aberturas de salida dispuestas alrededor de las vides. En el Anexo 8 es posible encontrar el detalle de las publicaciones y patentes vinculadas a calefactores y/o quemadores.

#### 5.2.4. Cubiertas protectoras

Este grupo contiene publicaciones aisladas, detectadas en la base de datos Cab Direct no del todo recientes que, sin embargo, respaldan la práctica siempre utilizada de protectores plásticos.

- **Study on using shade net for protection of frost damage for longan** (2008, Huang JiangLiu, Zhu JianHua, Luo MuLin, Zhu AnDing, Li GuiFen) expone la importancia de cubrir la totalidad de la copa de los árboles para protegerlos de las heladas. Describe un hecho de heladas con 87% de daño por cubrir sólo la punta.
- **Covering of peach (*Prunus persica*) flowers for early spring frost protection** (2006, Drogoudi, P., Tsipouridis, C., Thomidis, T., Terzis, T.) presenta los resultados exitosos de cubrir las florescencias de durazno con bolsas de doble capa de lámina de polietileno, para protegerlas de daños por heladas tardías en primavera y contra el ataque de la bacteria *Pseudomonas syringae*.
- **Evaluation of containers and plastic covering for coffee seedlings, for protection against low temperatures** (2004, Morais, H., Carneiro Filho, F., Caramori, P. H., Mariot, E. J., Ribeiro, A. M. de A.) muestra los excelentes resultados del uso de protectores plásticos de una y dos capas de poliuretano blanco y negro para proteger almácigos de café en Brasil.

En el Anexo 9 se encuentra el detalle de las publicaciones identificadas para el grupo de cubiertas protectoras.

## 6. COMENTARIOS FINALES

El presente documento expone los principales resultados obtenidos del Estudio de Vigilancia Tecnológica en Sistemas de Control y Manejo de Heladas para el Sector Agrario Nacional, en el que se analizó el panorama científico-tecnológico y la oferta disponible a nivel nacional e internacional, entre los años 2000 y 2014.

El panorama científico en torno a este tema muestra un gran número de publicaciones (1.173 en total), las cuales se distribuyen de manera creciente a partir del año 2010, liderando países como Estados Unidos con 355 publicaciones, seguido por China con 267 y Alemania con 128.

En el ámbito científico nacional no se aprecia la existencia de una línea de investigación específica en cuanto al manejo y control de heladas para la agricultura, aunque sí hay una incipiente línea asociada a la resistencia al frío de especies forestales, especialmente eucaliptus, y, en segundo lugar, de especies andinas y antárticas.

La búsqueda de patentes asociadas al área tecnológica arrojó un total de 436 documentos (solicitadas y concedidas), los que, a diferencia de lo ocurrido en el ámbito científico, muestran una tendencia decreciente en el tiempo, a excepción del punto alto alcanzado el año 2011. Entre los países líderes en esta área, nuevamente están Estados Unidos y Canadá con 176 y 29 documentos de patente respectivamente y Alemania con 54, entre otros.

A nivel tecnológico es interesante destacar el quiebre que se produjo el año 2007, donde se aprecia un cambio de enfoque al pasar de los aparatos mecánicos para control de heladas, a las sustancias protectoras y reguladoras del crecimiento, como son los biocidas, pesticidas, fertilizantes, etc. No obstante este cambio de enfoque y la proliferación de productos para aumentar la resistencia a las heladas, los resultados son discretos en su eficacia y son escasas las evaluaciones científicas que avalan el real efecto protector de estos productos.

Se identificaron grupos de interés científico-tecnológico en torno a métodos de prevención pasiva y activa:

(i) Mejoramiento genético de plantas es el grupo que concentra la mayor actividad científica y tecnológica, dada la cantidad de resultados obtenidos. Entre ellos destacan los de pruebas genéticas con la planta de laboratorio *Arabidopsis*, además de otros resultados específicos en el uso de proteínas y genes de variedades particulares para aumentar su tolerancia a bajas temperaturas.

(ii) Crioprotectores o sustancias destinadas a la protección contra descensos de temperatura, donde varias patentes dan cuenta de la diversidad de productos crioprotectores en base a diferentes compuestos (xiloglucano, ácido dicarboxílico, dimethyl betaine acylamidopropyl, acylcyclohexanedione), y donde la comunidad científica ha demostrado la eficacia de los fertilizantes y pesticidas como método pasivo contra las heladas, sobre todo aquellos en base a nitrógeno, para el trigo, y calcio, para los frutales.

(iii) Mapas ambientales, modelos de simulación y sistemas de información, donde se hallaron diversos modelos numéricos de simulación para variedades específicas, en base a datos ambientales y/o fenológicos, además de sistemas de alerta mediante modelos de predicción, redes de sensores, imágenes satelitales, entre otros.

(iv) Equipos con uso de agua, donde destaca el uso de microaspersores dirigidos para aumentar la eficiencia hídrica, y aspersores de naturaleza pulsatoria (pulsadores) para convertir un flujo bajo de agua en un flujo pulsante o intermitente. También destacó como sistema para control y manejo de heladas, el uso combinado de riego por goteo, riego por aspersión y malla protectora de cultivos.

(v) Máquinas de viento, grupo para el cual se hallaron diversas tecnologías o máquinas de viento, con o sin aspa, muchas de las cuales ya están disponibles en el mercado.

(vi) Calefactores y/o quemadores, donde también se identificaron diversos desarrollos con presencia en el mercado, entre ellos un motor pulsorreactor y tecnologías de calefacción a gas o cableado eléctrico específicas para vides.

(vii) Cubiertas protectoras, si bien no hay publicaciones recientes, su identificación respalda la práctica siempre utilizada de protectores plásticos.

En términos generales, si bien los métodos de protección activa presentan un importante desarrollo a nivel comercial y ofrecen un mayor rango de protección contra heladas, alcanzando en algunos casos aumentos de varios grados Celsius en la temperatura, también se aprecia un auspicioso panorama por el volumen de información existente en torno a la manipulación genética de los cultivos para aumentar su tolerancia a condiciones de estrés, especialmente la resistencia al frío, lo que representa una promesa de largo plazo que debería tender a la resistencia a las heladas.

## 7. BIBLIOGRAFÍA

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## 8. ANEXOS

### ANEXO 1: PUBLICACIONES NACIONALES

| TÍTULO          | Transcriptome profile in response to frost tolerance in <i>Eucalyptus globulus</i> |
|-----------------|--|
| Autor(es)       | Fernández, M., Troncoso, V., Valenzuela, S.  |
| Año publicación | 2015   |
| Fuente          | Plant Molecular Biology Reporter   |

The genetic improvement of trees for freezing tolerance is one of the most important goals to extend the plantations to colder areas. RNA-Seq technology has become a key tool in transcriptome studies. It can quantify overall expression levels for each gene simultaneously with high efficiency and speed through in silico gene expression, where differentially expressed genes can be identified by measuring the reads mapped for each transcript. In this study, the results of ESTs libraries from two *Eucalyptus globulus* genotypes showing contrasting differences in frost tolerance after cold acclimation using mRNA-Seq and in silico gene expression are discussed. A total of 14,265 non-redundant transcripts were predicted, where 163 corresponded to upregulated and 537 to downregulated genes. Pathway analyses of upregulated transcripts indicated that differences in frost tolerance might be regulated by the tree response to chemical and osmotic stimulus and organic substances, principally by overexpressing proteins that respond to hormone stress. These results suggest that genes coding for dehydrins, outer envelope, and voltage-dependent anion channel proteins are likely to participate in the regulation of the cold acclimation process and may have an important role in frost tolerance. The transcription factor analysis allowed identifying that those most differentially expressed in a resistant genotype were participating in the regulation of transcription, hormone regulation, photosynthesis, and response to stress. Additionally, the screening of polymorphic EST-SSR in silico and the validation of these markers in a reference population lead to identify a polymorphic EST-SSR with potential use for plant breeding and genotype discrimination.

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| TÍTULO          | Decoupled evolution of foliar freezing resistance, temperature niche and morphological leaf traits in Chilean <i>Myrceugenia</i> |
| Autor(es)       | Pérez, F., Hinojosa, L.F., Ossa, C.G., Campano, F., Orrego, F.   |
| Año publicación | 2014   |
| Fuente          | Journal of Ecology   |

Phylogenetic conservatism of tolerance to freezing temperatures has been cited to explain the tendency of plant lineages to grow in similar climates. However, there is little information about whether or not freezing resistance is conserved across phylogenies, and whether conservatism of physiological traits could explain conservatism of realized climatic niches. Here, we compared the phylogenetical lability of realized climatic niche, foliar freezing resistance and four morphological leaf traits that are generally considered to be adaptations to frost resistance in the Chilean species of *Myrceugenia*, which grows in a wide range of habitats. We estimated the predicted niche occupancy profiles with respect to minimum temperature (minT) of all species. We measured foliar freezing resistance (using chlorophyll fluorescence), leaf size, leaf mass per area (LMA), stomatal and trichome densities of 10 individuals per species. Finally, we estimated phylogenetic signal, and we performed independent contrast analyses among all variables. We found that both foliar freezing resistance and minT were subject to a significant phylogenetic signal, but the former had a stronger signal. We also detected a significant, but weak correlation between them ( $r = 0.49$ ,  $P_{one\ tail} = 0.04$ ). Morphological traits evolved independent of any phylogenetic effect. Synthesis. Our results show that freezing resistance evolved in association with temperature niche, but with some delay that could result from phylogenetic inertia. Our results also show that morphological leaf traits are more labile than realized climatic niche and frost tolerance and that the formers probably evolved in association with microhabitat preferences. Our results show that freezing resistance evolved in association with temperature niche, but with some delay that could result from phylogenetic inertia. Our results also show that morphological leaf traits are more labile than realized climatic niche and frost tolerance and that the formers probably evolved in association with microhabitat preferences.



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| TÍTULO          | Drought effects on water use efficiency, freezing tolerance and survival of <i>Eucalyptus globulus</i> and <i>Eucalyptus globulus nitens</i> cuttings |
| Autor(es)       | Navarrete-Campos, D., Bravo, L.A., Rubilar, R.A., Emhart, V., Sanhueza, R.  |
| Año publicación | 2013  |
| Fuente          | New Forests   |

Genetic improvement of *Eucalyptus* genotypes for drought and frost resistance is essential for successful intensive management of commercial plantations. Understanding the physiological mechanisms that relate water use and frost resistance for highly deployed genotypes may allow for better prediction of their future performance, genetic selection and seedling management for site specific purposes. We studied whether instantaneous water use efficiency (WUEi) may serve as drought, freezing and photoinhibition tolerance predictor by studying its response on six *E. globulus* clones (Eg1-Eg6) and four *E. globulus* × *E. nitens* hybrid seedlings (Egn1-Egn4) under drought and irrigated (control) treatments. Net photosynthesis (A) and transpiration (E) were studied using a gas exchange system in order to calculate WUEi (A/E). Simultaneous chlorophyll a fluorescence measurements were performed to assess the non photochemical quenching components. Frost tolerance of plants under control and drought treatments were evaluated by measuring temperatures that exert 50% photoinactivation of photosystem II. Finally, drought tolerance was evaluated by plant survival within each genotype after rehydration. Our results showed significant genotype variability in the rate of soil and xylem water potential decrease during drought. While most of the genotypes reached -4.0MPa in about 35 days of drought, genotypes Eg6 and Egn4 required 56 days of drought to reach this xylem water potential. WUEi exhibited significant differences among genotypes and irrigation treatments. Genotypes Eg5 and Egn4 increased their WUEi between 70 and 80% after drought. This was associated with a more conservative control of water loss at the stomatal level combined with maintenance of relatively higher rates of net photosynthesis than the other genotypes under drought conditions. Plants exposed to drought were more freezing tolerant than control plants, having in average 3°C lower LT50 than well irrigated ones. There was no a clear correlation between WUEi and drought tolerance or drought-induced photoinhibition, however WUEi was inversely correlated with LT50. Our results suggest that WUEi is not suitable by itself to select drought tolerant genotypes, but may provide evidence for discarding drought sensitive genotypes. In addition, it could provide valuable information to select for freezing tolerance.

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| <b>TÍTULO</b>          | <b>Frost as a limiting factor for recruitment and establishment of early development stages in an alpine glacier foreland?</b> |
| <b>Autor(es)</b>       | Marcante, S., Sierra-Almeida, A., Spindelback, J.P., Erschbamer, B., Neuner, G.  |
| <b>Año publicación</b> | 2012   |
| <b>Fuente</b>          | Journal of Vegetation Science  |

Questions: How frost resistant are the early development stages (seeds, seedlings, plantlets and juveniles) of alpine plant species? Do summer frosts impair establishment of plant species typical of different successional stages on a central alpine glacier foreland? Location: Rotmoos glacier foreland, Austrian Central Alps (Obergurgl, Tyrol, Austria). Methods: Seeds of 12 species typical of different successional stages were collected in the glacier foreland and either sown directly in the field or in a growth chamber (25/10°C, 16/8h) and grown to the investigated development stages. Frost resistance of the early development and adult stages was determined by exposing them to a set of freezing temperatures and assessing viability with the tetrazolium test (LT 50, i.e. 50% of samples being lethally frost damaged). Results: Dry seeds had the highest frost resistance (LT 50: -19°C), followed by wet seeds after imbibition (LT 50: -8°C). With the onset of germination, frost resistance decreased rapidly. While germinated seeds tolerated a mean of -3.2°C, seedlings and juveniles were less frost resistant (LT 50: -2.5°C). Along the primary succession, seedlings of pioneer species were significantly less frost resistant than early- and late-successional species. However, field grown seedlings, mainly of pioneer species, showed higher frost resistance (mean: -5°C) than the growth chamber seedlings (mean: -3°C), indicating that frost hardening (transition from a lower to a higher level of frost resistance) is already possible during these early stages of development. Conclusions: The low frost resistance during and after germination may not suffice to survive summer frosts and may at least in certain years explain the high seedling mortality rates recognized in the glacier foreland. During and after germination frost resistance of glacier foreland species may not suffice to survive summer frosts. This occasionally may explain high seedling mortality rates. Frost resistance decreased from dry (-19°C) to imbibed (-8°C) and further to germinated seeds (-3.2°C) and was lowest in seedlings and juveniles (-2.5°C). Pioneer seedlings were significantly less frost resistant than later successional species.

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| TÍTULO          | Photoperiod, temperature and water deficit differentially regulate the expression of four dehydrin genes from <i>Eucalyptus globulus</i> |
| Autor(es)       | Fernández, M., Valenzuela, S., Barraza, H., Latorre, J., Neira, V.   |
| Año publicación | 2012   |
| Fuente          | Trees - Structure and Function   |

During the last few years, considerable effort has been directed toward understanding how *Eucalyptus globulus* responds and adapts to low temperature due to its low freezing resistance, especially through gene expression analysis. A recent study has confirmed that freezing stress induces the expression of three dehydrin (DHN) genes in *E. globulus*. This work reports the identification of a new YSK-type DHN gene in *E. globulus* (EugIDHN3) and examines the responses of this gene and the three previously reported (EugIDHN1, EugIDHN2 and EugIDHN10) under low temperature (LT), short photoperiod and water deficit (WD) in one sensitive and one freezing-resistant genotype of *E. globulus*, to assign them to a class: low temperature responsive, photoperiod responsive or water deficit responsive. Results indicated that two SK n-type DHNs (EugIDHN1 and EugIDHN2) were strongly induced by LT and WD, the KS-type DHN (EugIDHN10) was strongly induced by LT and the Y nSK n-type by WD. The expression patterns under LT and WD were consistent with the regulatory elements identified in the promoter regions of the four DHN genes. The differential accumulation of EugIDHN1, EugIDHN2 and EugIDHN3 transcripts suggests a differential regulation in specific tissues under WD, and EugIDHN1 and EugIDHN10 under LT.

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| <b>TÍTULO</b>          | <b>Isolation and characterization of three cold acclimation-responsive dehydrin genes from <i>Eucalyptus globulus</i></b> |
| <b>Autor(es)</b>       | Fernández, M., Águila, S.V., Arora, R., Chen, K.  |
| <b>Año publicación</b> | 2012  |
| <b>Fuente</b>          | Tree Genetics and Genomes   |

The molecular and physiological work related to cold hardiness in *Eucalyptus globulus* and the cold-responsive dehydrins is reported. The identification and full-length gene sequence of three dehydrins of 10, 20, and 30kDa and the comparison of their promoters regarding to potential stress and hormone response elements in *E. globulus* are shown. The categorization of cold-responsive proteins as dehydrin was based on the similarity in amino acid composition with selected sequenced peptides from chilling-responsive dehydrin reported for other woody plants and the increasing of gene expression level during cold acclimation. The transcript accumulation for these three dehydrin genes increased with cold acclimation and decreased with deacclimation in leaf and stem tissues, being higher in a freezing-resistant genotype of *E. globulus* compared to a sensitive genotype. By western blot, five dehydrin peptides were identified which increased their expression, under cold stress in leaf and stem tissues. These results provide valuable information about cold acclimation and gene regulation in eucalypt genotypes that differ in their ability to tolerate frost temperature.

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| <b>TÍTULO</b>  | <b>Summer freezing resistance of high-elevation plant species changes with ontogeny</b> |
| Autor(es)  | Sierra-Almeida, A., Cavieres, L.A.  |
| Año publicación  | 2012  |
| Fuente   | Environ. Exp. Bot.  |
| <p>In high-elevation habitats, an apparent trade-off between high growth-rates and freezing resistance has led some authors to assume that seedlings are less freezing resistant than adults. However, because seedlings are exposed to lower and longer freezing events near the ground, they could be more freezing-resistant than adults. As freezing resistance is related to plant height, ontogenetic changes in the intensity of freezing resistance and the mechanism involved should differ between seedlings and adults of tall vs. short plants, with those changes being greater for tall plants. We evaluated differences between seedlings and adult plants in the ability to resist freezing temperatures in 13 high-Andean species from central Chile. In a field laboratory, we determined the low temperature damage, ice nucleation temperature and freezing resistance mechanisms in their leaves. Seedlings were on average 3.4.K less freezing resistant than adults with greater differences found for tall plant species. Six species changed their freezing resistance mechanism with ontogeny, including short and tall plants, although changes were greater for tall plant species. Ontogenetic changes in the freezing resistance of high-Andean species seem to be more related to a trade-off between plant-growth and stress resistance than to differences in thermal microhabitats between seedlings and adults.</p> |   |

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| TÍTULO          | Isolation and functional characterization of cold-regulated promoters, by digitally identifying peach fruit cold-induced genes from a large EST dataset |
| Autor(es)       | Tittarelli, A., Santiago, M., Morales, A., Meisel, L.A., Silva, H.  |
| Año publicación | 2009  |
| Fuente          | BMC Plant Biology   |

Cold acclimation is the process by which plants adapt to the low, non freezing temperatures that naturally occur during late autumn or early winter. This process enables the plants to resist the freezing temperatures of winter. Temperatures similar to those associated with cold acclimation are also used by the fruit industry to delay fruit ripening in peaches. However, peaches that are subjected to long periods of cold storage may develop chilling injury symptoms (woolliness and internal breakdown). In order to better understand the relationship between cold acclimation and chilling injury in peaches, we isolated and functionally characterized cold-regulated promoters from cold-inducible genes identified by digitally analyzing a large EST dataset. Results. Digital expression analyses of EST datasets, revealed 164 cold-induced peach genes, several of which show similarities to genes associated with cold acclimation and cold stress responses. The promoters of three of these cold-inducible genes (Ppbec1, Ppxero2 and Pptha1) were fused to the GUS reporter gene and characterized for cold-inducibility using both transient transformation assays in peach fruits (in fruta) and stable transformation in *Arabidopsis thaliana*. These assays demonstrate that the promoter Pptha1 is not cold-inducible, whereas the Ppbec1 and Ppxero2 promoter constructs are cold-inducible. Conclusion. This work demonstrates that during cold storage, peach fruits differentially express genes that are associated with cold acclimation. Functional characterization of these promoters in transient transformation assays in fruta as well as stable transformation in *Arabidopsis*, demonstrate that the isolated Ppbec1 and Ppxero2 promoters are cold-inducible promoters, whereas the isolated Pptha1 promoter is not cold-inducible. Additionally, the cold-inducible activity of the Ppbec1 and Ppxero2 promoters suggest that there is a conserved heterologous cold-inducible regulation of these promoters in peach and *Arabidopsis*. These results reveal that digital expression analyses may be used in non-model species to identify candidate genes whose promoters are differentially expressed in response to exogenous stimuli.

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| <b>TÍTULO</b>  | <b>Freezing resistance varies within the growing season and with elevation in high-Andean species of central Chile</b> |
| Autor(es)  | Sierra-Almeida, A., Cavieres, L.A., Bravo, L.A.  |
| Año publicación  | 2009   |
| Fuente   | New Phytologist  |
| <p>Predicted increases in the length of the growing season as a result of climate change may more frequently expose high-elevation plants to severe frosts. Understanding the ability of these species to resist frosts during the growing season is essential for predicting how species may respond to changes in temperature regimes. Here, we assessed the freezing resistance of 24 species from the central Chilean Andes by determining their low temperature damage (LT50), ice nucleation temperature (NT), freezing point (FP) and freezing resistance mechanism (i.e. avoidance or tolerance). The Andean species were found to resist frosts from -8.2 to -19.5°C during the growing season, and freezing tolerance was the most common resistance mechanism. Freezing resistance (LT50) varied within the growing season, decreasing towards the end of this period in most of the studied species. However, the FP showed the opposite trend. LT50 increased with elevation, whilst FP was lower in plants from lower elevations, especially late in the growing season. Andean species have the potential to withstand severe freezing conditions during the growing season, and the aridity of this high-elevation environment seems to play an important role in determining this high freezing resistance.</p> |  |

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| TÍTULO          | Ectopic AtCBF1 over-expression enhances freezing tolerance and induces cold acclimation-associated physiological modifications in potato |
| Autor(es)       | Pino, M.-T., Skinner, J.S., Jeknic, Z., Hayes, P.M., Soeldner, A.H., Thomashow, M.F., Chen, T.H.H.                                       |
| Año publicación | 2008   |
| Fuente          | Plant, Cell and Environment  |

We studied the effect of ectopic AtCBF over-expression on physiological alterations that occur during cold exposure in frost-sensitive *Solanum tuberosum* and frost-tolerant *Solanum commersonii*. Relative to wild-type plants, ectopic AtCBF1 over-expression induced expression of COR genes without a cold stimulus in both species, and imparted a significant freezing tolerance gain in both species: 2°C in *S. tuberosum* and up to 4°C in *S. commersonii*. Transgenic *S. commersonii* displayed improved cold acclimation potential, whereas transgenic *S. tuberosum* was still incapable of cold acclimation. During cold treatment, leaves of wild-type *S. commersonii* showed significant thickening resulting from palisade cell lengthening and intercellular space enlargement, whereas those of *S. tuberosum* did not. Ectopic AtCBF1 activity induced these same leaf alterations in the absence of cold in both species. In transgenic *S. commersonii*, AtCBF1 activity also mimicked cold treatment by increasing proline and total sugar contents in the absence of cold. Relative to wild type, transgenic *S. commersonii* leaves were darker green, had higher chlorophyll and lower anthocyanin levels, greater stomatal numbers, and displayed greater photosynthetic capacity, suggesting higher productivity potential. These results suggest an endogenous CBF pathway is involved in many of the structural, biochemical and physiological alterations associated with cold acclimation in these *Solanum* species.



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| <b>TÍTULO</b>          | <b>Plant responses of quinoa (<i>Chenopodium quinoa Willd.</i>) to frost at various phenological stages</b> |
| <b>Autor(es)</b>       | Jacobsen, S.-E., Monteros, C., Christiansen, J.L., Bravo, L.A., Corcuera, L.J., Mujica, A.                  |
| <b>Año publicación</b> | 2005  |
| <b>Fuente</b>          | European Journal of Agronomy  |

Frost is one of the principal limiting factors for agricultural production in the high Andean region. One of the most important grain crops in that region, quinoa (*Chenopodium quinoa Willd.*), is generally less affected by frost than most other crop species, but little is known about its specific mechanisms for frost resistance. This study was undertaken to help understand quinoa's response to various intensities and durations of frost under different levels of relative humidity (RH). The effect of frost on seed yield and plant death rate was studied, and content of soluble sugars, proteins, and free proline, was analyzed, in order to develop criteria for the selection of cultivars with improved resistance to frost. On the basis of greenhouse and phytotron experiments, it was concluded that at the two-leaf stage, cultivars from the altiplano of Peru, 3800m above sea level, tolerated -8°C for 4h, whereas a cultivar from the Andean valleys tolerated the same temperature for only 2h. At -4°C, plant death rate increased from 25% at high relative humidity to 56% at low RH. After a frost treatment of -4°C applied at the two-leaf stage, final seed yield was reduced by 9% compared to control plants not exposed to frost. For the same treatment applied at the 12-leaf and flowering stages, yield reductions were 51 and 66%, respectively, indicating that frost for 2h or more during anthesis caused significant damage to the plants. In general, an increased level of soluble sugars implied a greater tolerance to frost, resulting in higher yields.

## ANEXO 2: PATENTES SOLICITADAS EN INAPI EN ESTADO ABANDONADA Y DENEGADAS

### Patentes denegadas

| Título             | Sistema protector climatológico para cultivos. |
|--------------------|--|
| Nº Solicitud       | 201100468                                      |
| Fecha de solicitud | 03/03/2011                                     |
| Prioridad          | ES P200803636 28/11/2008                       |
| Solicitante        | Viña Solorca, S.L. – España                    |

El sistema comprende una serie de postes que forman hileras en correspondencia con las hileras de la plantación, estableciendo en la parte superior de cada poste un rulo formado por una lámina enrollada sobre un tursor al que están solidarizados cables de tensado, para que la lámina del rulo pueda estar plegada por su mitad y formar dos ramas o alas que se enrollan y desenrollan simultáneamente, de manera que al desenrollarse se establecen ramas separables para determinar una cubrición del correspondiente cultivo o plantación, realizándose el desplegado de esas ramas de cubrición o protección mediante el desplegado de los brazos de un compás articulado al propio poste.

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| <b>Título</b>      | <b>Método para el sobreenfriamiento aumentado de plantas que comprende la formación de una membrana hidrofóbica sustancialmente continua, útil para proporcionar protección contra la congelación.</b> |
| Nº Solicitud       | 200002683  |
| Fecha de solicitud | 29/09/2000   |
| Prioridad          | US 09/410,283 30/09/1999   |
| Solicitante        | Engelhard Corporation – Estados Unidos   |

La presente invención se refiere a un método para aumentar el sobreenfriamiento de una planta hasta temperaturas inferiores a aproximadamente -2°C. Su objetivo es prevenir la formación de cristales de hielo adyacentes a la planta, al formar una membrana hidrofóbica sustancialmente continua, de materiales particulados, sobre porciones de la planta capaces de soportar gotas de agua. El material particulado tiene alrededor de un, 90% del peso de las partículas con un tamaño de 100um o menos, y, la membrana hidrofóbica sustancialmente continua, tiene un espesor entre 1um y 1.000um aproximadamente.

## Patentes abandonadas

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| Título             | Aparato o máquina para el control de heladas en agricultura, que comprende una tobera por la que se absorbe aire, el cual es calentado en un quemador de gas circular y luego es impulsado por una hélice o ventilador hasta las plantaciones. |
| Nº Solicitud       | 200102480  |
| Fecha de solicitud | 12/10/2001   |
| Prioridad          | S/I  |
| Solicitante        | Aracena Pozo, Manuel Antonio - Chile   |

Aparato o máquina para el control de las heladas, conformada principalmente por una tobera de doble cilindro o cámara, una levemente mayor a la otra, para un perfecto aislamiento, apta para absorber el aire del ambiente. Para un perfecto adosamiento, en su pared posterior posee un bastidor doble para el paso de pernos capaces de unir tanto la tobera como la caja circular. El cuerpo al que se acopla está compuesto por una caja circular en cuyas dos paredes, superior e inferior, se ha prolongado su desarrollo quedando dos lados con base rectangular, que serán los alimentadores de aire caliente; el quemador, estructurado por una cañería del tipo circular, ligeramente inconclusa, con a lo menos 52 perforaciones de alimentación de gas, es alimentado por una cañería que se despliega paralelo y superficialmente a la vista respecto a las porciones del aparato, llevando cerca del acceso del aire una válvula de cierre, de gas o reguladora. Paralela a esta cañería lleva otra que llega hasta el tablero del vehículo para ser encendida a través del mismo motor. La porción más importante es la composición armónica del ventilador o hélice de aluminio, dispuesta en el interior de la caja circular, conformada principalmente por ocho aspas.

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| <b>Título</b>  | <b>Sistema para el control de heladas que consiste en distribuir agua a temperatura superior a la temperatura ambiente sobre la superficie del suelo, por medio de una red de riego establecida; el agua es calentada a través de una caldera.</b> |
| Nº Solicitud   | 200003066  |
| Fecha de solicitud   | 08/11/2000   |
| Prioridad  | S/I  |
| Solicitante  | Prieto Matte, José Joaquín, - Chile, Hudson Pérez, Sergio, - Chile, Pérez Echenique, Eduardo, - Chile  |
| <p>Sistema para el control de heladas que consiste en distribuir agua a temperatura superior a la temperatura ambiente sobre la superficie del suelo que se encuentra afectado por una helada. El agua es distribuida por una red de riego ya establecida para los propósitos de la invención y es calentada a través de una caldera eléctrica a gas, o de cualquier otro energético, en módulos fijo o móviles, ayudado por un sistema de sensores de temperatura y humedad que se asocian a un sistema inteligente, al cual se le han incorporado los parámetros predictivos de la ocurrencia de una helada.</p> |  |

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| <b>Título</b>  | <b>Calefactor para elevar la temperatura en huertos o frutales, que comprende un quemador de gas y que expulsa aire caliente.</b> |
| Nº Solicitud   | 200002107   |
| Fecha de solicitud   | 08/08/2000  |
| Prioridad  | S/I   |
| Solicitante  | Cortez López, Berardo Segundo - Chile   |
| <p>Máquina reguladora de temperatura ambiental, polinizadora y secadora de plantas, con altura de trabajo opcional, para ser aplicada en períodos lluviosos y de baja temperatura en huertos altos de mediana y menor altura. Su objetivo es mejorar el cultivo de frutos, protegiendo a estas plantaciones de potenciales heladas generadoras de enormes pérdidas en el sector agrofrutícola. Consiste en una estructura metálica que tiene incorporada una voluta intercambiable, de simple o doble salida según el huerto. En su interior, hay una turbina que absorbe aire a través de un conducto quemador de gas el cual, al estar encendido, calienta el aire y lo lanza hacia afuera a través de los conductos de voluta con dos deflectores adicionales en cada extremo, permitiendo así, regular la dirección de trabajo. Este sistema es alimentado por cuatro tubos de gas propano regulados por controladores incorporados en la máquina.</p> |   |

## ANEXO 3: PUBLICACIONES Y PATENTES REFERIDAS A MEJORAMIENTO GENÉTICO

### Publicaciones

|                 |   |
|-----------------|---|
| Título          | <i>Arabidopsis</i> cold shock domain protein 2 influences ABA accumulation in seed and negatively regulates germination |
| Autor(es)       | Sasaki, K, Kim, M.-H, Kanno, Y, Seo, M, Kamiya, Y., Imai, R.  |
| Año publicación | 2015  |
| Fuente          | Biochemical and Biophysical Research Communications   |

The cold shock domain (CSD) is the most conserved nucleic acid binding domain and is distributed from bacteria to animals and plants. CSD proteins are RNA chaperones that destabilize RNA secondary structures to regulate stress tolerance and development. AtCSP2 is one of the four CSD proteins in *Arabidopsis* and is up-regulated in response to cold. Since AtCSP2 negatively regulates freezing tolerance, it was proposed to be a modulator of freezing tolerance during cold acclimation. Here, we examined the function of AtCSP2 in seed germination. We found that AtCSP2-overexpressing lines demonstrated retarded germination as compared with the wild type, with or without stress treatments. The ABA levels in AtCSP2-overexpressing seeds were higher than those in the wild type. In addition, overexpression of AtCSP2 reduced the expression of an ABA catabolic gene (CYP707A2) and gibberellin biosynthesis genes (GA20ox and GA3ox). These results suggest that AtCSP2 negatively regulates seed germination by controlling ABA and GA levels.

|                 |   |
|-----------------|---|
| Título          | Identification and expression analysis of cold and freezing stress responsive genes of <i>Brassica oleracea</i> |
| Autor(es)       | Ahmed, N.U., Jung, H.-J., Park, J.-I., Cho, Y.-G., Hur, Y., Nou, I.-S.  |
| Año publicación | 2015  |
| Fuente          | Gene  |

Cold and freezing stress is a major environmental constraint to the production of *Brassica* crops. Enhancement of tolerance by exploiting cold and freezing tolerance related genes offers the most efficient approach to address this problem. Cold-induced transcriptional profiling is a promising approach to the identification of potential genes related to cold and freezing stress tolerance. In this study, 99 highly expressed genes were identified from a whole genome microarray dataset of *Brassica rapa*. Blast search analysis of the *Brassica oleracea* database revealed the corresponding homologous genes. To validate their expression, pre-selected cold tolerant and susceptible cabbage lines were analyzed. Out of 99 BoCRGs, 43 were differentially expressed in response to varying degrees of cold and freezing stress in the contrasting cabbage lines. Among the differentially expressed genes, 18 were highly up-regulated in the tolerant lines, which is consistent with their microarray expression. Additionally, 12 BoCRGs were expressed differentially after cold stress treatment in two contrasting cabbage lines, and BoCRG54, 56, 59, 62, 70, 72 and 99 were predicted to be involved in cold regulatory pathways. Taken together, the cold-responsive genes identified in this study provide additional direction for elucidating the regulatory network of low temperature stress tolerance and developing cold and freezing stress resistant *Brassica* crops.



|                 |   |
|-----------------|---|
| Título          | The crucial role of I- And K-segments in the in vitro functionality of <i>Vitis vinifera</i> dehydrin DHN1a |
| Autor(es)       | Rosales, R., Romero, I., Escribano, M.I., Merodio, C., Sánchez-Ballesta, M.T.                               |
| Año publicación | 2015  |
| Fuente          | Phytochemistry  |

Dehydrins (DHNs), group II LEA (Late Embryogenesis Abundant) proteins, are among the most commonly observed proteins which accumulate in plants in response to cold and any other environmental factors, causing the dehydration of cells. In previous studies, we isolated a YSK2-type VvcDHN1a gene from table grapes (*Vitis vinifera* cv. Cardinal) which presented two spliced variants (the spliced, DHN1a-s and the unspliced, DHN1a-u). Their expression was induced by low temperature storage and CO<sub>2</sub>, although with different accumulation patterns. DHN1a-u codifies for a truncated YS protein lacking  $\Phi$ - and K-segments, which might affect its functionality. In this work, we expressed both DHN1a-s and DHN1a-u recombinant proteins in *Escherichia coli*. We carried out a number of in vitro assays to analyze the implications that  $\Phi$ - and K-segments have in the protective role of VvcDHN1 against different abiotic stresses and their antifungal activity against the fungal pathogen *Botrytis cinerea*. Our results showed that unlike DHN1a-u, DHN1a-s has a potent cryoprotective effect on lactate dehydrogenase activity, protects malate dehydrogenase against dehydration and partially inhibits *B. cinerea* growth. Moreover, the DHN1a promoter presented cis-regulatory elements related to cold and drought, as well as biotic stress-related elements. We also observed that both spliced variants interact weakly with DNA, suggesting that K-segments are not involved in DNA binding. Overall, this work highlights the crucial role of  $\Phi$ - and K-segments in DHNs function in plant response to abiotic stress showing for the first time, the potential role of the *V. vinifera* DHN1a-s in the protection against freezing and dehydration as well as inhibiting *B. cinerea* growth.

|                 |  |
|-----------------|--|
| Título          | The use of antifreeze proteins for frost protection in sensitive crop plants |
| Autor(es)       | Duman, J.G., Wisniewski, M.J.  |
| Año publicación | 2014   |
| Fuente          | Phytochemistry   |

Antifreeze proteins (AFPs), also known as ice binding proteins (IBPs), have evolved as an important adaptation in numerous organisms exposed to subzero temperatures. Plant AFPs have only been identified in freeze tolerant species (those able to survive extracellular freezing). Consequently, plant AFPs have very low specific activities as they have not evolved to completely prevent ice formation in the plant. In contrast, fish and most insect AFPs function to prevent freezing in species that have evolved freeze avoidance mechanisms. Therefore, the activity of these AFPs, especially those of insects (as they are generally exposed to considerably lower temperatures than fish), is much greater. The ability of AFPs to non-colligatively lower the freezing point of water (thermal hysteresis) has led to the idea that frost-sensitive crop plants could avoid damage resulting from common minor frost events in late spring and early autumn by expressing high activity AFPs that permit them to remain unfrozen to temperatures of approximately -5°C. Over the past 20 years, the efficacy of this concept has been tested in a variety of studies that produced transgenic plants (including *Arabidopsis thaliana*, and several crop plants) expressing various AFPs. Initially, fish AFPs were employed in these studies but as insect AFPs, with higher levels of antifreeze activity, were discovered these have become the AFPs of choice in plant transformation studies. Some studies have produced transgenic plants that have exhibited improved cold tolerance of 1-3°C compared to the wild-type. None of the studies with transgenic plants, however, have yet attained a sufficient level of protection. Progress to this point indicates that more significant results are achievable. If so, the billions of dollars lost annually to frost damage of sensitive crops could be avoided. Geographic ranges and growing seasons could also be expanded. This review provides an overview of the studies of transgenic plants producing AFPs, and makes suggestions for future advancements in this field of study.

|  |  |
|--|--|
| <b>Título</b>  | <b>Metabolic engineering of cold tolerance in plants</b> |
| <b>Autor(es)</b>   | Megha, S., Basu, U., Kav, N.N.V.                         |
| <b>Año publicación</b>   | 2014   |
| <b>Fuente</b>  | Biocatalysis and Agricultural Biotechnology              |
| <p>Low temperature stress is one of the major abiotic stress challenging the growth and productivity of economically important crops. Both chilling and freezing temperatures have severe effects on growth of plants and have resulted in temperate plants, such as perennial rye grass and wheat to evolve mechanisms to avoid or, at the very least, minimize this damage. Accumulating osmoprotectants including glycine betaine, sugars (trehalose and fructans), polyamines, changes in lipid membrane profile, photosynthetic acclimation along with extensive reprogramming at molecular level help temperate plants acquire tolerance to low temperatures. In this review, we have focused mainly on metabolic engineering of plants by introduction of biosynthetic genes involved in various metabolic pathways. Availability of genomic, transcriptomic sequences combined with post-transcriptional data is beginning to link the gene function, regulatory networks and epigenetic states to different phenotypes. Generation of this knowledge together with our ability to manipulate genes involved in mediating tolerance to various stressors including low temperature will lead to the development of cold-resistant genotypes.</p> |  |

## Patentes

| PATENTE  | RESUMEN  |
|--|--|
| <p><b><u>EP2825655A1</u></b><br/>Stress tolerance in plants</p> <p><b>Pub. Date</b><br/>2015-01-21</p> <p><b>Applicant(s)</b><br/>Universidad Nacional del Litoral (Argentina)</p>   | <p>We provide methods for increasing yield in plants under moderate stress conditions by expression of a transcription factor gene belonging to the HD Zip family of transcription factors.</p>  |
| <p><b><u>US20150013030A1</u></b><br/>Plant transcriptional regulators</p> <p><b>Pub. Date</b><br/>2015-01-08</p> <p><b>Applicant(s)</b><br/>Mendel Biotechnology Inc.<br/>(Estados Unidos)</p>   | <p>The invention relates to plant transcription factor polypeptides, polynucleotides that encode them, homologs from a variety of plant species, and methods of using the polynucleotides and polypeptides to produce transgenic plants having improved tolerance to drought, shade, and low nitrogen conditions, as compared to wild-type or reference plants.</p>  |
| <p><b><u>US20140075598A1</u></b><br/>Ice recrystallisation inhibition protein or antifreeze proteins from <i>Deschampsia</i>, and <i>Festuca</i> species of grass</p> <p><b>Pub. Date</b><br/>2014-03-13</p> <p><b>Applicant(s)</b><br/>Agriculture Victoria Services Pty Ltd.<br/>(Australia)</p> | <p>The present invention relates to nucleic acids or nucleic acid fragments encoding amino acid sequences for polypeptides involved in tolerance to freezing and/or low temperature stress in plants. More particularly, the present invention relates to nucleic acids or nucleic acid fragments encoding amino acid sequences for ice recrystallisation inhibition proteins (IRIPs) in plants, and the use thereof for the modification of plant response to freezing and/or low temperature stress. Even more particularly, the present invention relates to polypeptides involved in tolerance to freezing and/or low temperature stress in <i>Deschampsia</i> and <i>Festuca</i> species.</p> |

## PATENTE

## RESUMEN

### US20140082761A1

Methods to increase plant productivity

#### Pub. Date

2014-03-20

#### Applicant(s)

PLANT SENSORY SYSTEMS LLC  
(Estados Unidos)

The present invention describes an approach to increase plant growth and production. The invention describes methods for the use of functional sulfinoalanine decarboxylase (SAD) or the promiscuous enzyme activity of SAD in plants or algal cells. Transgenic plants will have increased plant growth, biomass, yield, and/or tolerance to biotic and/or abiotic stresses and could be used as a pharmaceutical, nutraceutical or as a supplement in animal feed.

### US20140047583A1

Centromere sequences derived from sugar cane and minichromosomes comprising the same

#### Pub. Date

2014-02-13

#### Applicant(s)

Chromatin Inc.  
(Estados Unidos)

The invention is generally related to sugar cane mini-chromosomes containing sugar cane centromere sequences. In addition, the invention provides for methods of generating sugar cane plants transformed with these sugar cane mini-chromosomes. Sugar cane mini-chromosomes with novel compositions and structures are used to transform sugar cane cells which are in turn used to generate sugar cane plants. Methods for generating sugar cane plants include methods for delivering the sugar cane mini-chromosomes into sugar cane cell to transform the cell, methods for selecting the transformed cell, and methods for isolating sugar cane plants transformed with the sugarcane mini-chromosome.

### WO2014113605A1

Proteins involved in plant stress response

#### Pub. Date

2014-07-24

#### Applicant(s)

University of California  
(Estados Unidos)

Methods and compositions are provided for enhancing the stress tolerance of plants.

| PATENTE  | RESUMEN   |
|--|---|
| <p><b><u>US8378172B2</u></b><br/>Methods using acyl-CoA binding proteins to enhance low-temperature tolerance in genetically modified plants</p> <p><b>Pub. Date</b><br/>2013-02-19</p> <p><b>Applicant(s)</b><br/>University of Hong Kong<br/>(China)</p> | <p>ACBP6 can be used to enhance low temperature tolerance in genetically modified plants. An acbp6 T-DNA insertional mutant that lacked ACBP6 mRNA and protein, displayed increased sensitivity to freezing temperature (-8°C.), while ACBP6-overexpressing transgenic <i>Arabidopsis</i> were conferred enhanced freezing tolerance. Methods of using ACBP6 to enhance low temperature tolerance of plants are provided.</p>   |
| <p><b><u>WO2013133454A1</u></b><br/>Environmental stress-resistant plant with high seed productivity and method for constructing same</p> <p><b>Pub. Date</b><br/>2013-09-12</p> <p><b>Applicant(s)</b><br/>Kaneka Corporation<br/>(Japón)</p>             | <p>The present invention relates to a plant having a high resistance to environmental stress and a high seed productivity. More specifically, the present invention relates to a transgenic plant having an enhanced resistance to environmental stress and an increased seed productivity, said transgenic plant having been genetically modified so as to overexpress polyadenylate-binding protein (PABN) gene.</p>  |
| <p><b><u>US8222028B2</u></b><br/>Plants modified with mini-chromosomes</p> <p><b>Pub. Date</b><br/>2012-07-17</p> <p><b>Applicant(s)</b><br/>Chromatin, Inc.<br/>(Estados Unidos)</p>  | <p>The invention is generally related to methods of generating plants transformed with novel autonomous mini-chromosomes. Mini-chromosomes with novel compositions and structures are used to transform plants cells which are in turn used to generate the plant. Methods for generating the plant include methods for delivering the mini-chromosome into plant cell to transform the cell, methods for selecting the transformed cell, and methods for isolating plants transformed with the mini-chromosome. Plants generated in the present invention contain novel genes introduced into their genome by integration into existing chromosomes.</p> |

| PATENTE   | RESUMEN   |
|---|---|
| <p><b>US20120266326A1</b><br/>Freeze tolerant eucalyptus</p> <p><b>Pub. Date</b><br/>2012-10-18</p> <p><b>Applicant(s)</b><br/>Arborgen Inc.<br/>(Estados Unidos)</p> | <p>The present disclosure relates to freeze-tolerant transgenic Eucalyptus, including methodology for making and products produced there from. Following exposure to cold temperature, and compared to a control plant, the freeze-tolerant Eucalyptus displays a freeze-tolerant phenotype characterized by at least one of increased height, less leaf damage, less dieback of main stem, less crown defoliation, and/or improved tree stem form.</p> |

#### ANEXO 4: PUBLICACIONES Y PATENTES REFERIDAS A CRIOPROTECTORES

##### Publicaciones

| Título   | Does FreezePruf topical spray increase plant resistance to freezing stress? |
|--|---|
| Autor(es)  | Anderson, J.A.  |
| Año publicación  | 2012  |
| Fuente   | HortTechnology  |
| <p>One method of plant freeze protection involves the application of compounds that promote freeze avoidance or tolerance. FreezePruf, a commercially available product recently marketed to improve both freeze avoidance and tolerance, contains polyethylene glycol, potassium silicate, glycerol, silicone polyether surfactant, and a bicyclic oxazolidine antidessicant. The goal of the present study was to evaluate the protection level provided by FreezePruf using laboratory-based methods involving plants and plant parts from species capable and incapable of low-temperature acclimation. FreezePruf did not lower the freezing temperature of pepper (<i>Capsicum annuum</i>) seedlings, celosia (<i>Celosia argentea</i>) seedlings, detached tomato (<i>Solanum lycopersicum</i>) leaves, or postharvest tomato fruit. Spray application of the putative cryoprotectant did not increase the freeze tolerance of Bermuda grass (<i>Cynodon dactylon</i>) crowns or stolons. It is possible that a greater level of protection could be achieved with other species or different experimental protocols.</p> |   |

|                 |  |
|-----------------|--|
| Título          | Nursery fertilization affects the frost-tolerance and plant quality of <i>Eucalyptus globulus</i> Labill. Cuttings |
| Autor(es)       | Fernández, M., Marcos, C., Tapias, R., Ruiz, F., López, G.   |
| Año publicación | 2007   |
| Fuente          | Annals of Forest Science   |

*Eucalyptus globulus* is widely used in productive exotic plantations but the expansion of these plantations is limited by low temperatures, as its cold hardening capacity is limited (0.5 to 3.0°C). It is not well understood how nursery fertilization affects the field performance of plants. This led us to study the effect of three mineral nutrients (N, P and K) on both plant quality and frost tolerance. The experiment comprised eight growth treatments in which a high dose (H-) or a low dose (L-) of each nutrient was applied. Nitrogen was the nutrient that determined shoot growth, new root growth after transplanting (root egress), frost tolerance and field performance. Performance was better with treatment H-N than with treatment L-N, leaf nitrogen contents being 1.53 and 0.89% respectively. The effects of phosphorus and potassium were not significant between treatments for any parameter. The exception was P which, when interacting with N, favoured root egress for the H-N treatment. It was concluded that nursery fertilization offers a management tool for eucalyptus growers concerned with plant stock quality.



|                 |   |
|-----------------|---|
| Título          | Calcium-induced freezing and salinity tolerance in evergreen oak and apple cv. 'Golden Crown' |
| Autor(es)       | Percival, G., Barnes, S.  |
| Año publicación | 2008  |
| Fuente          | Arboriculture and Urban Forestry  |

Greater variability in weather patterns and later spring frosts equate to poor winter hardiness, premature spring budbreak, and greater susceptibility to low-temperature damage and concomitant deicing salt application. A field trial was undertaken to determine the influence of a range of commercially available calcium fertilizers applied as foliar sprays on the freezing and salinity tolerance of two tree species, evergreen oak (*Quercus ilex* L.) and apple (*Malus* cv. 'Golden Crown'). In all cases, application of calcium sprays increased twig, leaf, and root freezing and salt tolerance of both species as measured by leaf chlorophyll fluorescence and tissue electrolyte leakage bioassays. In the case of apple, a hardiness gain of 4.3°C (7.74°F) was recorded in twig tissue. In the case of evergreen oak, a hardiness gain of 2.1°C (3.78°F) was recorded in leaf tissue. After a -5°C (23°F) (apple) and -6.5°C (20°F) (evergreen oak) freezing stress, root electrolyte leakage values as a measure of cell membrane structural damage were 16% to 27% less in calcium-treated trees compared with noncalcium-treated controls. The salt concentration needed to cause 50% reductions in leaf chlorophyll fluorescence as a measure of photosynthetic efficiency rose by 0.2% to 1.2% in calcium-fertilized trees indicating a positive influence of calcium on enhancing leaf tissue tolerance to salt damage. Differences in the magnitude of freezing and salinity tolerance gained were noticeable between the calcium products used. In general, calcium hydroxide, calcium nitrate borate, and calcium metalosate improved twig, leaf, and root freezing and salt tolerance in both tree species to a greater degree than calcium chloride, calcium sulphate, calcium nitrate, and a calcium-magnesium complex. A significant correlation existed between increased freezing tolerance and internal tissue calcium content. Results of this study indicate that calcium sprays during late summer and fall can increase the freezing and salinity tolerance of evergreen oak and apple during the winter. This should be considered noteworthy for individuals involved in the management of trees in areas subject to subzero temperature fluctuations and/or concomitant applications of deicing salts in the form of sodium chloride.

|                 |  |
|-----------------|--|
| Título          | The role of the fertilizing with nitrogen, calcium and sodium chloride in winter wheat leaves adaptation to freezing-thaw stress |
| Autor(es)       | Liu, J., Zhou, R., Zhao, M., Zhao, Y., Wang, Y.  |
| Año publicación | 2011   |
| Fuente          | Shengtai Xuebao / Acta Ecológica Sinica  |

Warmer winters and extreme climate fluctuations induced by increasing global temperatures result in freeze-thaw cycle stress. Helping winter wheat cope with this stress is a key to maintaining stable agricultural development. The goal of this study is to reveal the role of different fertilizer applications in enhancing winter wheat tolerance to freeze-thaw as evidenced by physiological and biochemical parameters. In late fall, winter wheat was sown in pots and grown for 7 days, then fertilized with either nitrogen (60mmol/L NH<sub>4</sub>CO<sub>3</sub>), calcium (100mmol/L CaCl<sub>2</sub>) or salt (100mmol/L NaCl) for 30 days or given no fertilizer (control). After 30 days, seedlings were transferred to growth-chambers and subjected to a freeze-thaw cycle using a regimen of 3 hours at each of 15°C, 0°C, -15°C, 0°C, and 15°C. When the temperature decreased from 15°C to -15°C, the leaves froze and membrane permeability increased by 62.3% and lipid peroxidation product by 40%. When the temperature was increased from -15°C to 15°C, the leaves thawed and membrane permeability and lipid peroxidation declined. The changes in membrane permeability and lipid peroxidation of winter wheat leaves exhibited a significant negative correlation ( $R = -0.89 **$ ,  $R = -0.85 **$ ) with thaw-freeze stress. The activity of catalase (CAT) increased with the temperature drop from 15°C to -15°C, but the activities of superoxide dismutase (SOD) and peroxidase (POD) were higher at 0°C. However, compared to the controls, the membrane permeability and MDA content in the leaves of winter wheat treated with different fertilizers and salt were lower, but the activities of SOD, CAT and POD were higher, indicating that fertilizer application reduces membrane lipid peroxidation and enhances membrane antioxidant capacity. There were differences between nitrogen and calcium fertilizers and salt in regulation of osmolytes in the leaves of winter wheat under thaw-freeze-thaw stress. During thaw-freeze-thaw stress (temperature drop from 15°C to -15°C and increase from -15°C to 15°C), the leaves of winter wheat with N fertilizer had 44.3% higher proline and 23.6% higher soluble protein content but 17.3% lower soluble sugar content than controls. The leaves with calcium fertilizer had 57.5% higher soluble sugar content and leaves with NaCl had 37.1% higher soluble sugar content than controls. This indicates that nitrogen fertilizer may be increasing proline and soluble protein content through improved nitrogen metabolism, and the increase in soluble sugar content with calcium fertilizer and salt may be through improved glucose metabolism. This study suggests it may be efficient and cost-effective to provide winter wheat seedlings both kinds of fertilizers in winter. These fertilizers can not only increase several types of osmolytes to prevent formation of ice within plant cells but also enhance the activities of antioxidant enzymes to prevent membrane lipid peroxidation in response to freeze-thaw stress. The application of fertilizers in winter thus make seedlings more tolerant to freeze-thaw stress and strengthen growth the next spring.

|                 |  |
|-----------------|--|
| Título          | Differential effects of nitrogen managements on nitrogen, dry matter accumulation and transportation in late-sowing winter wheat |
| Autor(es)       | Wu, G., Guo, L., Cui, Z., Li, Y., Yin, Y., Wang, Z., Jiang, G.   |
| Año Publicación | 2012   |
| Fuente          | Shengtai Xuebao / Acta Ecológica Sinica  |

The elemental cycling of nitrogen plays a key role in agricultural ecosystem to ensure either food security or environmental sustainability. Bases on the premise of grain yield stability, cutting down proper part of N fertilizer application would largely decrease environmental pollution and significantly promote greenhouse gas emission reduction. In recent years, because of climate change, improvements of varieties, water conservation, delay of maize harvest and other factors, the area of late-sowing winter wheat increases continually. A reasonable field management of nitrogen fertilizer is therefore considered as an important measure to improve the yield of late-sowing winter wheat. Appropriate late sowing of winter wheat could extend growth period of previous maize, and reduce the consumption of water and fertilizer in winter wheat at early stage. In the Huanghuai Plain, seedtime postponing to mid-late October in winter wheat could mitigate the damage of frost on winter wheat to a certain extent. There are many researches which document the effects of temperature, light, moisture, density and other factors on carbon and nitrogen metabolism, yield and quality in late-sowing winter wheat. However, the influence of fertilizer management on nutrition absorption and dry matter accumulation in late-sowing winter wheat has been rarely reported. Meanwhile, no final conclusion has yet been reached on nitrogen application strategy in late-sowing winter wheat. Under field conditions, we here designed four treatments of nitrogen fertilizer application: 0kg/ hm<sup>2</sup>(N<sub>0</sub>), 168.75kg/hm<sup>2</sup>(N<sub>1</sub>), 225kg/hm<sup>2</sup>(N<sub>2</sub>), and 281.25kg/hm<sup>2</sup>(N<sub>3</sub>), at two topdressing stages: jointing stage (S<sub>1</sub>), jointing and anthesis stage (S<sub>2</sub>). We found that regulation effect of nitrogen application rate on dry matter accumulation changes due to nitrogen application time. The accumulation and transportation of dry matter and nitrogen, as well as nitrogen use efficiency of late-sowing winter wheat were carefully investigated. We also noted that elevating 25% nitrogen fertilizer rate on the basis of 225kg/hm<sup>2</sup>(N<sub>2</sub>) had no significant influence on either the total nitrogen accumulation at anthesis or nitrogen transportation amount in vegetative organs when nitrogen fertilizer topdressed at jointing stage (S<sub>1</sub>). However, those variables increased when nitrogen fertilizer topdressed at jointing and anthesis stages (S<sub>2</sub>). Compared with treatment S<sub>1</sub>, nitrogen accumulation amount in grain and vegetative organs, nitrogen accumulation amount after anthesis and nitrogen distribution proportion were much higher in treatment S<sub>2</sub>. At the same nitrogen fertilizer rate, the dry matter accumulation at maturity, dry matter accumulation intensity from anthesis to maturity and dry matter amount of grain were higher in treatment S<sub>2</sub> than those in S<sub>1</sub>. While at the same nitrogen fertilizer topdressing stage, grain yield has no significant difference between treatment N<sub>2</sub> and N<sub>3</sub>, but nitrogen fertilizer partial factor productivity declined after nitrogen fertilizer rate increased. Grain yield and nitrogen fertilizer recovery efficiency were higher in S<sub>2</sub> than those in S<sub>1</sub> when nitrogen fertilizer rate was identical. We therefore reasonably drew the conclusion that topdressing nitrogen fertilizer at jointing and anthesis stages with total nitrogen fertilizer application rate at 225kg/hm<sup>2</sup> was the optimal nitrogen fertilizer management mode which could not only achieve high yield but also obtain high nitrogen fertilizer use efficiency in late-sowing winter wheat.

|                 |   |
|-----------------|---|
| Título          | Seaweeds as an alternative to chemical pesticides for the management of root diseases of sunflower and tomato |
| Autor(es)       | Sultana, V., Baloch, G.N., Ara, J., Ehteshamul-Haque, S., Tariq, R.M., Athar, M.                              |
| Año publicación | 2011  |
| Fuente          | Journal of Applied Botany and Food Quality  |

With the rising popularity of organic farming, due to adverse effect of chemicals, the seaweed fertilizer industry is growing rapidly worldwide. Seaweeds act as natural plant growth stimulator and enable the plants to withstand drought, disease or frost. Root diseases of tomato and sunflower caused by root rotting fungi, *Fusarium spp.*, *Rhizoctonia solani* and *Macrophomina phaseolina*, and root knot nematode, *Meloidogyne spp.*, are the major constraints in tomato and sunflower production. In our studies, ethanol and water extracts of several seaweeds showed significant nematicidal activity against *Meloidogyne javanica*. In this study, efficacy of three seaweeds *Spatoglossum variable*, *Melanothamnus afaqhusainii* and *Halimeda tuna* was compared with a fungicide Topsin-M and a nematicide carbofuran both in screen house and under field condition. Seaweed and pesticides showed more or similar suppressive effect on root pathogens of tomato and sunflower by reducing fungal root infection and nematode's galls on roots and nematode's penetration in roots. However, mixed application of *S. variable* with carbofuran caused maximum reduction in nematode's penetration in roots and produced greater fresh shoot weight, root length and maximum yield of tomato under field condition. Seaweeds offer a non-chemical means of disease control, which would also protect our environment from the use of hazardous chemicals.

## Patentes

| PATENTE   | RESUMEN   |
|---|---|
| <p><b><u>US8901040B2</u></b><br/>Active substances for increasing the stress defense in plants to abiotic stress, and methods of finding them</p> <p><b>Pub. Date</b><br/>2014-12-02</p> <p><b>Applicant(s)</b><br/>Bayer Intellectual Property Gmbh<br/>(Alemania)</p> | <p>The invention relates to a method of finding compounds which increase the tolerance of plants to abiotic stress factors acting on this plant, such as, for example, temperature (such as chill, frost or heat), water (such as dryness, drought or anoxia), or the chemical load (such as lack of or excess of mineral salts, heavy metals, gaseous noxious substances) by increasing the expression of plant-endogenous proteins, and to the use of these compounds for increasing the tolerance in plants to abiotic stress factors.</p> |
| <p><b><u>US8846573B2</u></b><br/>Methods for increasing tolerance to abiotic stress in plants</p> <p><b>Pub. Date</b><br/>2014-09-30</p> <p><b>Applicant(s)</b><br/>Crop Microclimate Management Inc.(Estados Unidos)</p>   | <p>The present invention provides a method for increasing tolerance to abiotic stress and/or reducing the consequence of abiotic stress in a plant or part thereof comprising contacting a plant or part thereof with a composition comprising an effective amount of dicarboxylic acid or derivative thereof.</p>  |
| <p><b><u>EP2772137A1</u></b><br/>Composition for protecting plants against frost and method of plant protection</p> <p><b>Pub. Date</b><br/>2014-09-03</p> <p><b>Applicant(s)</b><br/>Agra Group, A.S. (República Checa)</p>  | <p>The invention provides a composition for plant protection against frost containing at least one acylamidopropyldimethylbetaine and at least one aminoborate. The invention also describes a method of application of the compositions.</p>   |

**PATENTE****RESUMEN****US20130079228A1**

Agricultural spray solution compositions and methods

**Pub. Date**

2013-03-28

**Applicant(s)**

Brian E. Freed  
(Estados Unidos)

An agrichemical is disclosed having increased bio-activity which is adapted for spray application in a form which offers reduced aerial drift and improved deposition on organisms, reduced agglomeration and gellation problems, is adapted for use with chemicals, pesticides and fertilizers and allows for lower agrichemical use rates. Various adjuvants and surfactants are combined with fertilizers or pesticides, such as herbicides fungicides and insecticides, to reduce application problems relating to droplet size, foaming, chemical stability, plant and area coverage, droplet drift, surface tension, suspension, incompatibility, phytotoxicity, solubility, volatilization and evaporation, while providing enhanced bio-efficacy and low aquatic toxicity. Purifying the agrichemical composition water source by any of various known processes such as oxidation, ultra-filtration, deionization, reverse osmosis or nanofiltration lowers or removes alkaline components, such as calcium, magnesium, iron, sodium, etc., and reduces water hardness, and further increases the agrichemical's bio-efficacy.

**US8580708B2**

Plant cryoprotectant compositions and methods of use

**Pub. Date**

2013-11-12

**Applicant(s)**

University of Alabama (Estados Unidos)

The present disclosure relates to cryoprotection of plants. The compositions and methods disclosed herein provide a means for protecting plants from frost or freeze damage or death due to sudden exposure to low temperature conditions. The present disclosure further relates to methods for providing cryoprotection to plants.

**WO2012123408A1**

Liquid herbicidal preparations

**Pub. Date**

2012-09-20

**Applicant(s)**

Bayer Cropscience Ag  
(Alemania)

The invention is directed to a liquid herbicidal emulsifiable concentrate comprising (a) the herbicidal active substance metribuzin, (b) one or more herbicidal active substances selected from the group consisting of the ACCase inhibitors (b1), (b2) and (b3): (b1) esters of fenoxaprop-P and esters of fenoxaprop, (b2) esters of clodinafop, (b3) pinoxaden, (c) optionally other active ingredients, which do not substantially interfere with the stability of the co-formulation, (d) one or more non-polar organic solvent, (e) one or more polar organic co-solvent, (f) one or more non-ionic emulsifiers, (g) one or more anionic emulsifiers, and (h) optionally further formulation auxiliaries.

## PATENTE

## RESUMEN

### US20110039699A1

cryoprotective aqueous composition and methods for implementing same

#### **Pub. Date**

2011-02-17

#### **Applicant(s)**

Cryoprotectors & Biotechnologies, S.L. (España)

The present invention provides a cryoprotective aqueous composition for removing frost from lawns and for preventing the appearance thereof, characterized in that it comprises two or more alcohols and at least one wetting compound. Likewise, the invention provides a method for removing frost from lawns, consisting of applying the cryoprotective aqueous composition, in diluted form, directly on the lawn surface by means of spraying.

### US7686975B2

Use of acylcyclohexanedione derivatives for improving the tolerance of plants to cold and/or frost

#### **Pub. Date**

2010-03-30

#### **Applicant(s)**

Basf Aktiengesellschaft (Alemania)

The invention relates to the use of acylcyclohexanedione derivatives for improving the tolerance of plants to cold and/or frost.

### US20100304975A1

Process for increasing plants resistance to an abiotic stress

#### **Pub. Date**

2010-12-02

#### **Applicant(s)**

Elicityl (Francia)

A process for adapting plants to an abiotic stress, in particular to cold or to a hydric stress, in particular drought, humidity or salinity, wherein the process includes at least a step of treatment of the plants by foliar field spraying with a composition including at least one xyloglucan derivative in particular conditions of application.

**PATENTE****RESUMEN****EP1998614A2**

Method for improving the tolerance of plants to chilling temperatures and/or frost

**Pub. Date**

2008-12-10

**Applicant(s)**

BASF SE (Alemania)

The present invention relates to the use of an active compound that inhibits the mitochondrial breathing chain at the level of the b/c1 complex for improving the tolerance of plants to low temperatures.

**EP1148781B1**

Compositions for protecting plants from frost and/or freeze and methods of application thereof

**Pub. Date**

2008-11-19

**Applicant(s)**

AGROSHIELD LLC (Estados Unidos)

Compositions and methods of applying the compositions to plants are disclosed. The compositions include a polymer component that releases heat over a range of dropping ambient temperatures beginning at about 32°F. By exhibiting a broad freezing temperature transition range, the compositions, when applied to plants, effectively reduce the threshold temperature at which substantial frost and/or freeze damage to plants will occur.

**US20070113304A1**

Antifreeze proteins isolated from forage grasses and methods for their use

**Pub. Date**

2007-05-17

**Applicant(s)**

Agrigenesis Biosciences Limited  
(Nueva Zelanda)

Isolated polynucleotides encoding antifreeze polypeptides are provided, together with expression vectors and host cells comprising such isolated polynucleotides. Methods for the use of such polynucleotides and polypeptides are also provided.



## PATENTE

## RESUMEN

### WO2007008580A1

Increasing plant drought and cold resistance: aba + triazole

#### Pub. Date

2007-01-18

#### Applicant(s)

MENDEL BIOTECHNOLOGY INC.  
(Estados Unidos)

The present application provides methods of imparting increased stress resistance on a plant by application of exogenous ABA and one or more triazole compounds, diniconazole, uniconazole and uniconazole-P.

### US7022651B1

Composition, method, and apparatus for protecting plants from injury caused by frost or freezing temperatures

#### Pub. Date

2006-04-04

#### Applicant(s)

Donald V. Lightcap, Jr.,  
Mark F. Smith (Estados Unidos)

The present invention is directed to a composition, method, and apparatus for protecting plants from injury caused by frost or freezing temperatures by applying an insulating foam to the surface of plants. Plants are sprayed with a foam formed from a pre-emulsion concentrate comprising a vegetable oil, an emulsifier, and a forming agent. The pre-emulsion concentrate is mixed with water and aerated to form a foam. The foam is then directed onto the surfaces of the plant to be covered.

## ANEXO 5: PUBLICACIONES Y PATENTES REFERIDAS A MAPAS AMBIENTALES, MODELOS DE SIMULACIÓN Y SISTEMAS DE INFORMACIÓN

### Publicaciones

|                 |  |
|-----------------|--|
| Título          | Simulation of multitemporal and hyperspectral vegetation canopy bidirectional reflectance using detailed virtual 3-D canopy models |
| Autor(es)       | Kuester, T., Spengler, D., Barczi, J.-F., Segl, K., Hostert, P., Kaufmann, H.  |
| Año publicación | 2014   |
| Fuente          | IEEE Transactions on Geoscience and Remote Sensing   |

The influence of plant and canopy architecture on canopy bidirectional reflectance and the bidirectional reflectance distribution function (BRDF) is the subject of this paper. To understand BRDF-influenced reflectance signals, this influence must be identified and quantified, which requires detailed knowledge concerning the structure and BRDF of the observed canopies. In situ BRDF measurements of canopies are time consuming and depend on the availability of a field goniometer. In contrast to field measurements, computer-based simulations of the canopy BRDF offer an alternative approach that considers parameter-driven setups of virtual canopies under constant illumination conditions. This paper presents the hyperspectral simulation of canopy reflectance (HySimCaR) system, which has been developed in the context of the EnMAP mission. This spectral, spatial, and temporal simulation system consists of detailed virtual 3-D cereal canopies of different phenological stages, whose geometries are linked to the corresponding spectral information. The system enables the simulation of realistic bidirectional reflectance spectra on the basis of virtual 3-D scenarios by incorporating any possible viewing position with ray-tracing techniques. The parameterization of a number of canopy structure parameters, such as phenological stage, row distance, and row orientation, enables the modeling of the bidirectional reflectance and, based on them, the approximation of the BRDF for many structurally different cereal canopies. HySimCaR has been validated with respect to structural and spectral accuracy using three cereal types, namely, wheat, rye, and barley, at 13 different phenological stages. The results show that the virtual cereal canopies are re-created in a realistic way, and it is possible to model their detailed canopy bidirectional reflectance and their BRDF using HySimCaR.

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| <b>Título</b>   | <b>Estimation of key dates and stages in rice crops using dual-polarization SAR time series and a particle filtering approach</b> |
| <b>Autor(es)</b>  | De Bernardis, C. G., Vicente-Guijalba, F., Martínez-Marín, T., López-Sánchez, J. M.   |
| <b>Año publicación</b>  | 2014  |
| <b>Fuente</b>   | IEEE Journal of Selected Topics in Applied Earth Observations and Remote Sensing  |
| <p>Information of crop phenology is essential for evaluating crop productivity. In a previous work, we determined phenological stages with Remote sensing data using a dynamic system framework and an extended Kalman filter (EKF) approach. In this paper, we demonstrate that the particle filter is a more reliable method to infer any phenological stage compared to the EKF. The improvements achieved with this approach are discussed. In addition, this methodology enables the estimation of key cultivation dates, thus providing a practical product for many applications. The dates of some important stages, as the sowing date and the day when the crop reaches the panicle initiation stage, have been chosen to show the potential of this technique.</p> |   |

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| <b>Título</b>  | <b>Assessing freeze injury to winter wheat with multi-temporal HJ-1 satellite imagery</b> |
| <b>Autor(es)</b>   | Dong, Y., Chen, H., Wang, H., Gu, X., Wang, J.  |
| <b>Año publicación</b>   | 2012  |
| <b>Fuente</b>  | Nongye Gongcheng Xuebao / Transactions of the Chinese Society of Agricultural Engineering |
| <p>The spatial distribution and severity level of freeze injury on winter wheat is of great importance to timely agriculture recovery. In this paper, taking central and southern Hebei province as study area, a rapid algorithm for assessing freeze injury on winter wheat was developed. Firstly, Enhanced Vegetation Index (EVI) was selected as the optimal vegetation index after sensitivity analysis of vegetation index to freeze injury. Secondly, a freeze injury assessing algorithm was developed based on multi-temporal HJ-1 satellite imagery without Ground data. Thirdly, this algorithm was validated through ground survey points. It is shown that the relative change of pre- and post-frozen EVI is linearly related to the damage severity. The new approach is proved to be able to assess winter wheat injury spatial distribution and severity level rapidly and successfully.</p> |   |

|                 |   |
|-----------------|---|
| Título          | Evaluation of frost risk in apple by modeling changes in critical temperatures with phenology |
| Autor(es)       | Asakura,T., Sugiura, H., Sakamoto, D., Gemma, H., Sugiura, T.                                 |
| Año publicación | 2011  |
| Fuente          | Acta Horticulturae  |

Frost damage is a major concern in fruit-producing regions. Quantitative assessment of the risk of frost damage is important in selecting orchard sites and in the installation and operation of frost control tools. The risk depends mainly on frost hardiness and minimum temperature. We constructed a model of spring phenology from bud break to petal fall of 'Fuji' apple, analyzing the effect of chilling and forcing temperatures on bud development. By curve-fitting the relationship between growing degree-hours (GDH) and critical temperatures of the buds, we expressed the changes in frost hardiness with development as a function of predicted GDH. We investigated the springtime changes in frost hardiness ( $T_c$ ) and daily minimum air temperature ( $T_m$ ) during 1977-2009. Taking into account the difference between plant and air temperatures and the local diversity in  $T_m$ , we predicted that frost events would occur on days when  $T_m - T_c < 3^\circ\text{C}$ . The predictions agreed well with observations: the lower the value, the severer the frost damage. These models of spring phenology and frost hardiness will be useful not only for assessing the present risk of frost damage from historical temperature records, but also for predicting the future risk from simulated temperatures.

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|------------------------|--|
| <b>Título</b>          | <b>A Two-Step filtering approach for detecting maize and soybean phenology with time-series MODIS data</b> |
| <b>Autor(es)</b>       | Sakamoto, T., Wardlow, B.D., Gitelson, A.A., Verma, S.B., Suyker, A.E., Arkebauer, T.J.                    |
| <b>Año publicación</b> | 2010   |
| <b>Fuente</b>          | Remote Sensing of Environment  |

The crop developmental stage represents essential information for irrigation scheduling/fertilizer management, understanding seasonal ecosystem carbon dioxide (CO<sub>2</sub>) exchange, and evaluating crop productivity. In this study, we devised an approach called the Two-Step Filtering (TSF) for detecting the phenological stages of maize and soybean from time-series Wide Dynamic Range Vegetation Index (WDRVI) data derived from Moderate Resolution Imaging Spectroradiometer (MODIS) 250-m observations. The TSF method consists of a Two-Step Filtering scheme that includes: (i) smoothing the temporal WDRVI data with a wavelet-based filter and (ii) deriving the optimum scaling parameters from shape-model fitting procedure. The date of key crop development stages are then estimated by using the optimum scaling parameters and an initial value of the specific phenological date on the shape model, which are preliminary defined in reference to ground-based crop growth stage observations. The shape model is a crop-specific WDRVI curve with typical seasonal features, which were defined by averaging smoothed, multi-year WDRVI profiles from MODIS 250-m data collected over irrigated maize and soybean study sites. In this study, the TSF method was applied to MODIS-derived WDRVI data over a 6-year period (2003 to 2008) for two irrigated sites and one rainfed site planted to either maize or soybean as part of the Carbon Sequestration Program (CSP) at the University of Nebraska-Lincoln. A comparison of satellite-based retrievals with ground-based crop growth stage observations collected by the CSP over the six growing seasons for these three sites showed that the TSF method can accurately estimate the date of four key phenological stages of maize (V2.5: early vegetative stage, R1: silking stage, R5: dent stage and R6: maturity) and soybean (V1: early vegetative stage, R5: beginning seed, R6: full seed and R7: beginning maturity). The root mean square error (RMSE) of phenological-stage estimation for maize ranged from 2.9 [R1] to 7.0 [R5] days and from 3.2 [R6] to 6.9 [R7] days for soybean, respectively. In addition, the TSF method was also applied for two years (2001 and 2002) over eastern Nebraska to test its ability to characterize the spatio-temporal patterns of these key phenological stages over a larger geographic area. The MODIS-derived crop phenological stage dates agreed well with the statistical crop progress data reported by the United State Department of Agriculture (USDA) National Agricultural Statistics Service (NASS) for eastern Nebraska's three crop agricultural statistic districts (ASDs). At the ASD-level, the RMSE of phenological-stage estimation ranged from 1.6 [R1] to 5.6 [R5] days for maize and from 2.5 [R7] to 5.3 [R5] days for soybean.

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| Título          | Using indicators to assess the environmental impacts of wine growing activity: The INDIGO® method |
| Autor(es)       | Thiollet-Scholtus, M., Bockstaller, C.  |
| Año publicación | 2014  |
| Fuente          | European Journal of Agronomy  |

Environmental assessment methods are needed by agronomists working on the enhancement of cropping systems to meet the demand for more sustainable farming practices. A growing number of operational methods based on a set of indicators have been designed, more for arable crops and livestock than for perennial crops like viticulture. Among them, the INDIGO® method, originally developed for arable crops, offers a compromise between feasibility and predictive quality. Here we present a modified and expanded version of INDIGO® for viticulture. The development of new indicators specific to viticulture and the adaptation of existing ones followed a five step approach: (i) preliminary definition of the objectives and identification of the end-users, (ii) construction of the indicator, (iii) selection of a reference value, (iv) sensitivity analysis and (v) validation. Stakeholders from professional institutions and winegrower organizations were closely associated with step (i) to define the framework and step (ii) to supply technical databases. We designed INDIGO® indicators with all available scientific and expert knowledge which was aggregated into expert systems associating fuzzy subsets or, when possible, quantitative equations. Four indicators; pesticides, nitrogen, energy and soil organic matter, were directly adapted from the initial INDIGO® method, whereas soil cover and frost protection management were new indicators. Potentialities of their use are highlighted by examples of implementation on different scales and for various purposes.

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|-----------------|---|
| Título          | Development and experiment of cold resistance tester for grape roots and branches         |
| Autor(es)       | Hou, J., Dang, Y., Gao, Z., Zhai, H.  |
| Año publicación | 2012  |
| Fuente          | Nongye Gongcheng Xuebao / Transactions of the Chinese Society of Agricultural Engineering |

With the rapid development of wine industry in China, wine brewing cultivation moves to the west and north on a large scale. The cold and droughty weather of northern winter frequently causes the frost even death of dormant grape plants. There exist obvious differences among different grape varieties, breeds and cold-resistance of organs. In particular, the cold-resistance of roots is the weakest. Therefore, choosing comparatively improved cold-resistant breeds becomes an important task of scientific workers. In order to accurately and conveniently measure the cold-resistance of grapes, it is necessary to develop a tester to measure the cold-resistance of grape roots and branches in the laboratory. The tester mainly consists of STM32 MCU, off-chip ADC, temperature transmitter, and thermoelectric module. It has the function of measuring the cold-resistance grape roots and in the laboratory with a precision of 0.96%.The tester controlled the temperature transmitter by MCU and measured the real-time temperature within the test box. Meanwhile, thermoelectric module is controlled to measure the heat release of tissue cells belonging to the roots and branches when they freeze and the Measurement data are transmitted to the MCU through serial port. The LT50 is obtained through the way of processing these data. Finally, the experiment result and storage data are showed by the epistatic machine system. Within the test box which can imitate the change of natural temperature, an experimental study is conducted to measure the cold-resistance of various grape roots and branches by using this apparatus. The experiment result indicates that Gloire Riparia and Vidal roots which LT50s are below -6.0°C have the stronger cold-resistance. The branch of Gloire Riparia has the strongest cold-resistance and its LT50 is between -28.1 and -30.7°C. This apparatus operates in a stable way which can meet the cold-resistance test requirement of grape roots and branches and also provide a basis for the cultivation of new cold-resistance breeds.

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| <b>Título</b>          | <b>Cold air intrusions over southeastern South America - GFDL model behavior regarding climate simulations in the 20th century and future projections</b> |
| <b>Autor(es)</b>       | Cavalcanti, I.F.A., Maller, G.V., Andrade, K.M., Fernández Long, M.E.   |
| <b>Año publicación</b> | 2013  |
| <b>Fuente</b>          | Global and Planetary Change   |

Cold air intrusions in three areas frequently affected by frosts of southeastern South America are analyzed based on GFDL-CM2.0 Coupled Atmospheric and Oceanic Global Circulation Model. The general objective is to investigate the model ability to simulate the frequency of intrusions in the present climate as well as the changes in the frequency of occurrence and atmospheric characteristics in a future climate scenario. The cold period (May to September) is analyzed for the control period 1961 to 1990 and for the period 2081 to 2100 from the CMIP3 A2 scenario, which reflects the extreme global warming. The coupled GFDL-CM2.0 overestimated the number of cold air intrusions for the present climate (control). This systematic error should be considered in the analyses of future climate results. Future projections indicated a reduction of these cases in GFDL results. As this model overestimated the number of cases, the reduction could be even greater. Composites of extreme cases for the present and future climate in the three areas indicated intensification of the temperature gradient which suggests more vigorous frontal systems, intensification of post-frontal highs and cold air extending to lower latitudes as compared to the present climate. Anomaly intensification was related to the climatological mean temperature, which is higher in the future than in the present. Therefore, even with less cold air intrusion over southeastern South America and a lower number of frost cases in the three areas, the occurrence of more intense systems would have an impact on the agriculture of these areas and such impact would extend to lower latitudes.



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|------------------------|---|
| <b>Título</b>          | <b>Frost affects grain yield components in winter wheat</b>               |
| <b>Autor(es)</b>       | Wu, Y.F., Zhong, X.L., Hu, X., Ren, D.C., Lv, G.H., Wei, C.Y., Song, J.Q. |
| <b>Año publicación</b> | 2014  |
| <b>Fuente</b>          | New Zealand Journal of Crop and Horticultural Science                     |

The influence of frost stress during the jointing stage in winter wheat was examined for original tillers (OT), regenerated tillers (RT) and the yield components of the cultivars Yumai 18, Zhoumai 18 and Yanzhan 4110. Ear number per plant, kernel number per ear, 1000-kernel weight and yield per plant were recorded under a range of subfreezing temperatures (-1, -3, -5, -7 and -9°C). The results were modelled by linear and quadratic regression. In the quadratic model, ear number per plant and yield per plant of the three cultivars were all significant ( $P < 0.001$ ) and their fitted curves showed a concave-downward parabolic trend as the subfreezing temperature decreased. By analysing the change characteristics of ear number and yield per plant of OT and RT following frost treatment, it was found that OT decreased and RT increased resulting in the concave-downward parabolic yield trend. Final ear number and yield were the result of these opposing behaviours with OT and RT partly offsetting one another and thus tending to stabilize yield loss. The resulting compensatory behaviour of the three cultivars showed obvious differences with the RT of Yumai 18 making the greatest contribution to grain yield. The work offers the potential for creating a rational basis for selecting cultivars having improved frost resistance based on recording their tillering behaviour under low-temperature conditions.

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| Título   | The possibilities to use long-range sensors for the frost protection of cranberries  |
| Autor(es)  | Alberts, M., Bruns, P., Grinbergs, U., Kreiömane, D., äpats, A., Tikuma, B.  |
| Año publicación  | 2012   |
| Fuente   | Zinatniski praktiskas konference, "Zinatne Latvijas Lauksaimniecibas Nakotnei: Partika, Lopbariba, äkiedra un Energija", Jelgava, Latvia |
| <p>There is particular interest in the world market about the cultivation of American large cranberry (<i>Vaccinium macrocarpon Ait.</i>) as a particularly healthy product. At the same time the cultivation of large cranberries is related to climate risks and damage to crop. During the last years in Europe the local radiation frost is more frequently observed as being the most significant threat to the industry. It is certain opportunity for a grower to receive an alert about frost, when using the long-range wireless sensor networks, or automatically to engage frost protection techniques. Due to the real time measurements, sensor application provides momentary response to adverse climatic conditions that means better quality of products and higher return on investment. Network performance is based on accumulated, aggregated and indexed information about different frost-causing environmental factors, such as soil conditions, clouds, fog, temperature fluctuations, etc.</p> |  |

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|------------------------|---|
| <b>Título</b>          | <b>Prediction models for frost/low-temperature stress in subtropical fruit plantations</b>            |
| <b>Autor(es)</b>       | Sharma, S. K.   |
| <b>Año publicación</b> | 2012  |
| <b>Fuente</b>          | Journal of Horticultural Sciences 7 (1) Bangalore: Society for Promotion of Horticulture, 2012, 56-61 |

During winters, frost is a phenomenon of common occurrence in subtropical lower Himalayan region. In the recent past, it has caused considerable economic losses to fruit growers. Recommendations for protection against frost do exist, but benefits to orchards are rare due to lack of information on the level of low temperature these crops may experience in a frosty event. Studies have been conducted at Regional Horticultural and Forestry Research Station, Neri, Hamirpur, Himachal Pradesh on development of prediction models for minimum temperature and temperature evolution during a frost event. Variables like sunset time temperature, temperature drop and humidity increase from sunset time until two hours, have been found to explain about 74% of the total variation observed in minimum temperature. Evolution of temperature during a frosty night showed that temperature drop after sunset was an inverse exponential function of time after sunset. It justified about 67% of the total variation in temperature-evolution trend. Thiel's inequality coefficient for predicted versus actual values indicated good to very good forecasting performance of the regression lines developed. Further decomposition of inequality into bias, variance and covariance proportions also supported fitness of these lines for future prediction. Based on the information generated, a grower-friendly frost protection guide-chart (S-chart) has been developed. The chart provides information on intensity and duration of temperature below the critical level of damage for different fruit species. It also serves as a guide for the level of protection needed and for automation of protection methods against frost and low temperature damage.

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|------------------------|--|
| <b>Título</b>          | <b>New wireless sensor network technology for precision agriculture</b>  |
| <b>Autor(es)</b>       | Alberts, M., Grinbergs, U., Kreismane, D., Kalejs, A., Dzerve, A., Jekabsons, V., Veselis, N., Zotovs, V., Brikmane, L., Tikuma, B.  |
| <b>Año publicación</b> | 2013   |
| <b>Fuente</b>          | “Applied information and communication technologies”, Proceedings of the 6th International Scientific Conference, Jelgava, Latvia, 25-26 April, 2013 Jelgava: Faculty of Information Technologies, Latvia University of Agriculture, 2013, 153-162 |

Institute of Mathematics and Computer Science (IMCS) of the University of Latvia is implementing the project “Development of Long Range Wireless sensor network for precision farming applications in Latvia” (The Project). The Project has two main directions of research. The first is development of new long range Wireless Sensor Network (WSN) nodes providing radio link in a long distances (more than 300m), the second is development of energy efficient operating system (FarmOS) for large scale WSNs with main focus on robust easy to use agricultural applications. Development of current WSN nodes is based on Texas Instrument’s hardware and AgroSeNET technology drafted by Cominfo Inc. FarmOS is being developed in cooperation with Institute of Electronics and Computer Science. In framework of the Project IMCS is implementing field trial of large scale Long Range WSN technology for automated radiation frost protection of cranberry fields. FarmOS supports mesh topology of WSN. For small networks star and multihop topologies could be used. The main goal of the Project is to build WSN prototype with newly designed 50 long range nodes running FarmOS to provide automated cranberry field protection with intelligent radiation frost prediction and decision making features. Depending on data gathered in the real time the intelligence of the system will provide immediate decision whether fog generator, water sprayer system or just wind blowers would be chosen. Farmer’s end user client software will deliver Software as a Service concept.

## Patentes

| PATENTE   | RESUMEN   |
|---|---|
| <p><b><u>US7047133B1</u></b><br/>Method and system of evaluating performance of a crop</p> <p><b>Pub. Date</b><br/>2006-05-16</p> <p><b>Applicant(s)</b><br/>Deere &amp; Company<br/>(Estados Unidos)</p>   | <p>A method and system of evaluating crop performance facilitates characterization of the environmental impact of a geographic region or areas within the region for growing plant-life. Environmental measurements are obtained. The environmental measurements are associated with a geographic region. Each environmental measurement includes at least one of soil data and weather data. Respective location data is obtained. The location data is associated with corresponding environmental measurements. An estimated performance characteristic is determined for a particular crop planted in the geographic region based on the obtained environmental measurements and respective location data. Contours are established for one or more uniform performance areas with generally uniform performance characteristic within the geographic region by applying a decision-tree analysis to the obtained environmental measurements.</p> |
| <p><b><u>US20090216594A1</u></b><br/>Vineyard Information Collection and Management System.</p> <p><b>Pub. Date</b><br/>2009-08-27</p> <p><b>Applicant(s)</b><br/>James Verhey, Alfred Buckland,<br/>Steve Matthiason, John<br/>Duckhorn (Estados Unidos)</p> | <p>A vineyard information collection and management system is provided. The system allows a variety of information at one or more vineyards to be collected in real time, processed at a central server, and provided to users over the internet using customizable, easy-to-use forms and pages accessible using a conventional web browser. The system includes planning, analysis, and management tools that assist in making purchasing, operations, and management decisions at one or more vineyards The invention includes a plurality of software modules that provide useful, real-time information about vineyards.</p>   |
| <p><b><u>US8083481B2</u></b><br/>Method for siting and operating a crop protection wind machine array.</p> <p><b>Pub. Date</b><br/>2011-12-27</p> <p><b>Applicant(s)</b><br/>HILL DARYL G<br/>(Estados Unidos)</p>  | <p>A system for arranging and operating an array of wind machines to protect crops from damaging weather conditions, such as freezing frost, rain and heat. The method includes a wind machine positioned to force air across the crop. The wind machine is preferably a propeller/tower configuration. The operational method of the wind machine array includes the steps of sensing ambient meteorological and the hardiness of the crop to withstand a particular adverse weather condition and operating the wind machines in response to these factors. Multiples of wind machines are employed in the preferred embodiment of the method, the siting of the wind machines preferably based upon topographic and historical meteorological conditions. The operation of the wind machines can be automatically and remotely operated with the aid of satellite communications including internet links.</p>                                     |

## ANEXO 6: PUBLICACIONES Y PATENTES REFERIDAS A EQUIPOS CON USO DE AGUA

### Publicaciones

| Título          | Microclimate modification in pear plantation by using cooling irrigation            |
|-----------------|---|
| Autor(es)       | Lakatos, L., Dussi, M.C., Gonda, I., Soltasz, M., Nyaki, J., Szaba, Z., Zhongfu, S. |
| Año publicación | 2011  |
| Fuente          | Acta Horticulturae  |

Irrigation in some countries is a horticultural practice mainly used only to supply water. At the same time the use of microsprinklers have a powerful influence on the changes of temperature in orchards. When the air temperature is high (20°C or higher) evaporative cooling from irrigation significantly decreases the plant surface temperature and air temperature. The cooling effect is stronger when the air is dryer. By using cooling irrigation regularly, canopy temperature can be decreased so that the beginning of bloom can be delayed. Also if the bloom is early and frost probability is high, serious damages can happen in orchards. The beneficial effect of cooling irrigation is the temperature reduction and frost protection. In March 2010, one month before expected bloom, an irrigation system was established to produce anti-frost treatment and regulate the micro-climate of a 'Bosc' pear orchard which belongs to the University of Debrecen (Hungary). The objective of sprinklers was to cool the air by increasing water evaporation and relative humidity. The position of the microsprinklers was planned in three levels (around the tree trunks, a few cm near to the soil surface, in the crown region and above the crown, 0.5m higher). The results showed that the water sprayed in the orchard by micro-jets influenced the temperature decisively. At higher temperatures (20°C), temperature reduction may attain 5-7°C. Low relative humidity of the air may increase the relative effect. When water was applied at intervals of 15min ten times per day from 8:00 to 18:00, the air, flower and bud surface temperatures could be kept low. When the temperature was higher than 10°C, irrigation was used at night in similar 15min intervals, from 18:00 to 6:00. The beginning of bloom could be delayed for more than ten days. 'Bosc' pear blooming dynamics were characterized by a logistic curve in the treated as well as in the control plot. In the treated plot, the curve was steeper than in the control in spite of the equal temperatures measured in the plots. Under Hungarian climatic conditions, the method was successfully used to delay bloom dates. The main result was the diminution of the frost damage in the spring that assured pear yields.

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| <b>Título</b>          | <b>Tree-temperature monitoring for frost protection of orchards in semi-arid regions using sprinkler irrigation</b> |
| <b>Autor(es)</b>       | Ghaemi, A.A., Rafiee, M.R., Sepaskhah, A.R.   |
| <b>Año publicación</b> | 2009  |
| <b>Fuente</b>          | Agricultural Sciences in China  |

Automated over-tree sprinkler irrigation systems were developed and tested in two orchards located in two separate locations in southern Iran (a 0.17ha peach orchard and a 0.24ha orange orchard) to protect peach blossoms and orange trees leaves and fruits from low temperature damage. The experiment used a system that monitored the trees and air temperatures using two thermistors. The water application rate by the irrigation system was determined by an energy balance as implemented by the software FROSTPRO. In the peach orchard, the system was tested during three frost events during the spring of 2003 and three other events during the spring of 2004. The system successfully kept peach flowers above the critical temperature, i.e.,  $-4.0^{\circ}\text{C}$  in spring 2004 (control block  $-4.12^{\circ}\text{C}$ , and sprinkled block  $+0.5^{\circ}\text{C}$ ) during all events. Similar results were obtained in the orange orchard during three frost events in the winter of 2004, during which the tree temperatures were at least  $2.5^{\circ}\text{C}$  above the critical temperature. Results from field tests show that the system can effectively protect the peach blossoms from damage. Determination done after the frost events showed a 12% blossom kill in the sprinkled blocks while in the unsprinkled control block a 41.5% blossom kill. Calculations indicated that when using variable application rates, the amount of water used can be reduced by 54.3%. Spatial distribution of minimum temperatures during the three frosts was also studied in Jahrom, Iran. Results showed a significant temperature control in the experimental block, especially in the central part of the orchard, but the block margins (about 3.6% of the total area) were at the risk of low temperature due to the wind drift effects.

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|-----------------|---|
| Título          | A comparison of traditional and novel methods of antifrost irrigation. Experiences in Trentino and in Emilia, Italy |
| Autor(es)       | Pratizzoli, W., Zinoni, F., Eccel, E.   |
| Año publicación | 2008  |
| Fuente          | Italian Journal of Agrometeorology  |

This work presents the results of several experimental trials on Anti-frost irrigation methods alternative to the present standard protection on fruit crops in Trentino; the latter is carried out by high-rate overcanopy irrigation. The comparison among different types of micro-sprinklers, managed both in continuous and in alternate operation, yielded suggestions on the importance of the drop size in the efficiency of protection, as well as on the possibility of applying an alternative functioning. Field trials confirmed the chance of saving up to 75% of the water volume by implementing an overcanopy jet “localization” technology. The feasibility of sprinkler timing opens the way to dynamic protection techniques, with watering volumes proportional to the extent of the event to face. The comparisons performed between overcanopy and undercanopy plants corroborate the use of undercanopy techniques for protecting fruit crops from minor frosts, but, at the same time, they highlight their limitations in contrasting the temperature fall in alpine areas for intense and persistent episodes; nevertheless, such events represent a small amount of the spring frosts recorded in Trentino.



| Título  |              |
|---|--------------|
| Spring cold injury to winegrapes and protection strategies and methods  |              |
| Autor(es)   | Poling, E.B. |
| Año publicación   | 2008         |
| Fuente  | HortScience  |
| <p>Active frost protection methods may be expensive, but a correctly selected and operated system can provide more consistent crops and improved cash flow in years of potentially damaging cold events at postbudbreak stages of grape development. The selection of an active frost protection system depends on a number of factors, including the prevailing climatic conditions that occur during the spring season at the vineyard location, the costs associated with different frost control systems as well as considerations related to the reliability and relative simplicity of operating the equipment associated with each method. In winegrape production areas in North Carolina, hoar (white) frosts are the predominant cold threat in the postbudbreak period. A wind machine can be a very cost-effective investment on sites that are prone to a damaging frost event in 1 of 5 years or with a higher frequency of occurrence. However, wind machines have less overall usefulness in growing areas where there is also potential for black frosts (a more damaging radiational event than a hoar frost) and frosts/freezes (events with subfreezing temperatures and winds in the range of 2.2 to 4.5m·s<sup>-1</sup>). For black frost events, a well-designed over-vine sprinkling system can provide nearly 6°C protection; sprinkling is also an appropriate management option for frost/freeze events. Windborne freezes are far less common at the postbudbreak stages but represent the most damaging type of cold event that can occur in eastern and midwestern winegrape regions in the United States. Methods that reliably delay budbreak (e.g., dormant oils, evaporative cooling with targeted sprinklers) may represent the best near-term opportunity for growers to decrease or avoid vine injury from freeze events such as the early April Easter freeze of 2007 that devastated grape vineyards through much of the midwest and southeastern United States.</p> |              |

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|-----------------|---|
| Título          | Dynamics of heat exchange in strawberries in an open field at night-frost conditions  |
| Autor(es)       | Pennar, M., Palge, V.   |
| Año publicación | 2012  |
| Fuente          | Actual Tasks on Agricultural Engineering: Proceedings of the 40. International Symposium on Agricultural Engineering, Opatija, Croatia, 21-24 February 2012<br>Zagreb: University of Zagreb Faculty of Agriculture, 2012, 577-585 |

Protecting strawberry plants against low temperatures must be considered in plantation equipment planning. Typically, the plants must be protected at “radiation” frost conditions when the skies are clear. This can occur in spring or fall. In this paper, we model the dynamics of heat exchange of strawberries at night frost conditions in an open field, and create a control algorithm for frost protection sprinkler systems. The algorithm takes account of air temperature, humidity, and both the convective and radiation heat loss intensity. The algorithm increases the degree to which plants are protected against frost and reduces the amount of water needed.

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|------------------------|---|
| <b>Título</b>          | <b>Possibility for modification of microclimate in orchards by using evaporative cooling irrigation</b> |
| <b>Autor(es)</b>       | Lakatos, L., Zyromski, A., Biniak-Pierog, M.  |
| <b>Año publicación</b> | 2012  |
| <b>Fuente</b>          | Journal of Water and Land Development (16) Warsaw: Versita, 2012, 29-34                                 |

Micro irrigation is a horticultural practice mainly used to supply water to the orchard. Nevertheless the micro sprayed irrigation has a powerful influence on fruit microclimatic parameters as temperature and air humidity. By the application we can improve the fruit quality parameters as anthocianine, C-vitamin, sugar content of the fruits. When the air's temperature is high (about 20°C or higher) the evaporative cooling irrigation significantly decreases the plants' surface temperature and air temperature. The cooling effect is stronger when the air is dryer. The beneficial effect of cooling irrigation is the temperature reduction and frost protection. In March 2010, one month earlier than the expected blooming an irrigation system was established to produce anti-frost treatment and regulate the micro-climate of a apricot, peach, plum, apple and pear orchard which belongs to the University of Debrecen (Hungary). The objective of sprinklers was to cool the air by increasing water evaporation and relative humidity. The results showed that the water sprayed in the orchard by micro-jet influenced decisively the temperature of the plantation. At higher temperatures (around 20°C), the drop of temperature may attain 5-7°C. A low relative humidity of the air may increase the relative effect. When water was applied at 15 minutes intervals for ten times a day, the temperature could be kept low also in the buds. The beginning of bloom could be delayed for 8-14 day at different fruit species. Blooming dynamics was characterized by a logistic curve in the treated as well as in the control plot. In the treated plot, the curve was steeper than in the control one, in spite of equal temperatures measured in the plots. Under Hungarian climatic conditions, the method was successfully used to delay blooming dates. The main result was the diminution of the frost damage in the spring that assured fruit yields.

| Título          | Current status of microsprinkler irrigation in the United States   |
|-----------------|--|
| Autor(es)       | Boman, B., Sanden, B., Peters, T., Parsons, L.   |
| Año publicación | 2012   |
| Fuente          | Applied Engineering in Agriculture 28 (3) St. Joseph: American Society of Agricultural and Biological Engineers, 2012, 359-366 |

Microsprinkler irrigation is often the preferred method of irrigation for tree and vine crops since it provides a greater degree of freeze protection than drip irrigation and provides water and energy savings over sprinkler and flood irrigation methods. With chemigation, microirrigation also provides an economical method of applying fertilizer and other agricultural chemicals on a timely basis. However, microsprinkler systems generally require more maintenance than drip or overhead systems, and they require a higher level of management expertise than sprinkler or flood irrigation methods. This article discusses the current status of design, operation, and maintenance of microsprinkler systems and the benefits and limitations of these systems for several agricultural crops. Types and characteristics of available microsprinklers are discussed in relation to appropriate application considering crops, soils, and management philosophy. Topics include uniformity, clogging, insect problems, wetting patterns, emitter maintenance, chemigation, system evaluation, management for both young and mature trees, crop response, and freeze protection.

| Título          | Low cost irrigation and frost protection with Centauro                                |
|-----------------|---|
| Autor(es)       | Toller, G., Corradini, S., Biasi, A.  |
| Año publicación | 2010  |
| Fuente          | Informatore Agrario 66 (5) Verona: Edizioni l'Informatore Agrario S.r.l., 2010, 60-62 |

Centauro is a system aimed at making best use of water, for which competition is constantly growing. It consists of sensors to measure the soil moisture. These are linked to a computer that will alert the grower when a level requiring irrigation is reached. The costs of the installation are discussed and it is suggested that with a little training non-specialists could construct the sensors, so as to keep costs down. At the heart of the system is an EZ10 modem for transmitting the sensor data to the computer. Other sensors could be used to monitor temperature and alert growers to the risk of frost. Trials are ongoing in orchards and vineyards.

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| Título          | Microsprinkler irrigation for frost protection of citrus in Florida |
| Autor(es)       | Parsons, L. R.  |
| Año publicación | 2015  |
| Fuente          | International Society for Horticultural Science (ISHS)              |

In 1979, it was not known if microsprinkler irrigation could provide any frost protection for citrus, and some assumed that this irrigation method would not. Tests during freezes between 1980 and 2010 showed that microsprinklers were effective in protecting the lower 0.9m of young citrus trees, thereby saving the bud union and allowing later regrowth. Microsprinklers also benefit mature trees. The amount of air temperature warming depends on several factors, including volume of water applied, dew point temperature, and wind speed. Microsprinkler irrigation is more effective for cold protection when higher volumes of water are applied. Irrigation rates of 18.7m<sup>3</sup> ha<sup>-1</sup> h<sup>-1</sup> have been recommended. At these rates, average air temperature warming at a 1.2m height is 0.6 to 1.4°C. At tree heights of more than 2.4m, warming is commonly less than 0.5°C. In windy, low humidity freezes, evaporative cooling can damage trees, particularly if the emitter is on the downwind side of the tree. Protection is better if the microsprinkler is placed on the upwind (north or northwest in Florida) side of the tree. Microsprinklers elevated to a height of 0.6 to 0.9m protect more of the tree than those at 0.2m. While it was initially thought that microsprinklers provided no frost protection to the fruit, recent research has shown that this irrigation can promote better production of juice and kilos of soluble solids than when no irrigation is used. Microsprinkler irrigation has become the most commonly used form of frost protection in Florida citrus.

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| <b>Título</b>   | <b>Efficient irrigation against frost uses less water</b> |
| <b>Autor(es)</b>  | Pratizzoli, W.  |
| <b>Año publicación</b>  | 2007  |
| <b>Fuente</b>   | Edizioni l'Informatore Agrario S.r.l.                     |
| <p>Over the past few years the technology of irrigation interventions to protect fruit crops from late spring frosts has been refined and the quantity of water required has diminished; whereas 5-6mm/h were required previously, modern systems now only use 3.5-4mm/h. The GEPRI project, which studies spring frosts in mountainous areas of Italy, evaluated the use of a new type of automatic microirrigator, namely the Naan Dan model Flipper, mounted above the plant [apples?] canopy at a height of 3m. Trials were held in Rupe di Mezzolombardo in Trento province in 2006. As there were no significant spring frosts that year, the trial was undertaken later in winter when the first frosts occurred on the nights of December 20 and 21. Results showed that it was possible with Flipper at the appropriate locations and rotations to obtain consistent water savings of around 75% compared to conventional systems. Specifically, Flipper used 1.14mm water/h when in intermittent mode (45 seconds on, 45 seconds off), giving the same frost protection as 4mm/h in the conventional system. Even when used continuously, Flipper only consumed 2.29mm water/h.</p> |   |

## Patentes

| PATENTE   | RESUMEN  |
|---|--|
| <p><b><u>US8919036B2</u></b><br/>Trellis system for irrigation and frost prevention</p> <p><b>Pub. Date</b><br/>2014-12-30</p> <p><b>Applicant(s)</b><br/>Edge Technology<br/>(Estados Unidos)</p>  | <p>A trellis mounted system for irrigation and frost prevention is described. The irrigation system comprises a trellis, a first conduit coupled to the trellis, and a second conduit coupled to the trellis. The first conduit delivers a heated water stream to a nozzle that provides a heated water spray to an area surrounding the trellis. The second conduit provides drip irrigation to a ground area surrounding the trellis. The heated water supplied by the trellis mounted irrigation system prevents the development of damaging frost on agricultural plants.</p>  |
| <p><b><u>WO2014068536A1</u></b><br/>Pulsating device with two preset pressure-responding normally-closed valves</p> <p><b>Pub. Date</b><br/>2014-05-08</p> <p><b>Applicant(s)</b><br/>Developed Research For Irrigation Products, Inc.<br/>(Estados Unidos)</p> | <p>A pulsating device with two preset pressure-responding normally-closed valves is disclosed. The first valve is used for accumulating fluid. The second valve is used for creating resistance so as to force the first valve to open widely. The second valve may be configured so it creates little to no resistance once opened. In some embodiments, the pulsating device converts a low controlled and/or continuous flow of fluid, such as water and/or air, to a high pulsating and/or intermittent flow. A pulsating device may operate, for example, one or more drip lines, pop ups, sprinklers, misters and/or other irrigation devices.</p> |

## ANEXO 7: PUBLICACIONES Y PATENTES REFERIDAS A MÁQUINAS DE VIENTO

### Publicaciones

|                        |   |
|------------------------|---|
| <b>Título</b>          | An improved design on suction-exhaust duct for frost protection in tea fields               |
| <b>Autor(es)</b>       | Wu, W., Hu, Y., Zhang, H., Sun, H.  |
| <b>Año publicación</b> | 2014  |
| <b>Fuente</b>          | American Society of Agricultural and Biological Engineers Annual International Meeting 2014 |

The late spring frost is one of the frequent agrometeorological disasters for tea farms or orchards with large area of damage influence and sizable economic losses. Thermal inversion near ground often occurs with frost events, and air temperature increases with height. Based on the feature of thermal inversion, frost protection equipment is developed by convecting near-ground air with wind machines or helicopters. An upward-blowing wind machine was developed and introduced to many countries for frost protection. Although it is commercially available in the market, limited frost protection effects and obvious defects were investigated through previous simulation and field experiments. Some improvements are made with the combination of upward-blowing and conventional wind machines. The airflow drawn through the duct is reinforced on the top and used as driving force to convect the thermal inversion from the top, covering a large area when swaying. The new design makes good use of the advantages of both machines and made up for their defects. The computational fluid dynamics simulation shows that the new model could provide good protection by increasing the canopy temperature up to 3°C. In the meanwhile cold air would not be accumulated in the depressions and the dead zone of frost protection is avoided. The design makes low operation cost, ease of installation and maintenance possible.



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|------------------------|--|
| <b>Título</b>          | Effective experiment of elevated wind machine for frost protection with long blade in large apple orchards |
| <b>Autor(es)</b>       | Yin, X., Wang, Y., Ding, R., Fu, S., Zhang, F., Chen, Q.   |
| <b>Año publicación</b> | 2014   |
| <b>Fuente</b>          | Nongye Gongcheng Xuebao / Transactions of the Chinese Society of Agricultural Engineering                  |

In recent years, the crops have severely suffered from frost damage with the deterioration of the global climate. Conventional methods of frost protection, such as smoking, covering, and irrigation may be effective in many cases, but they are time-consuming, and some methods even easily caused environmental contamination. So study of scientific and high-effective artificial frost prevention equipment has become very necessary and an urgent need. Most wind machines for frost protection are still in the pilot stage at home and abroad, and their height and blade length, etc, are not for taller plants, such as apple trees, peach trees, and cherry trees. The Gansu meteorology department, using the principle of disturbing the air of mechanical power, invented the first domestic elevated wind machine for frost protection with a long blade, which proved the feasibility of frost protection by air in disturbing surface layer. The machine construction has the main components as follows: blade, pipe tower, fan base, underground base, engine and ladder. In order to study the effect of frost prevention and the extent of protection for an elevated wind machine, the instrumentation layout was as follows: at a distance of 53m from the wind machine, installing a 10m gradient observation tower of temperature within protected areas, and at a distance of 190m from the wind machine, also installing a 10m gradient observation tower of temperature outside protected areas as the effect of frost prevention control. Two gradient tower of temperature at 1, 2, 3, 4, 5, 8 and 10m from the ground installed a temperature sensor respectively, which observed vertical temperature distribution; also at 2m from the ground install a humidity sensor observed relative humidity. A projector to the ground of the wind wheel rotation axis of the wind machine for the center line, arranged the horizontal distribution of an observation point at intervals of 10m, a total of 10 observation points, and each observation point 1, 2 and 3m from the ground were erecting a portable anemometer for wind observation. In this paper, based on the contrast test observation data of the processes of a strong decrease in temperature from October 19 to 21, 2013, the frost protection effect of the first self-designed domestic elevated wind machine for frost protection with a long blade was evaluated. The main analysis results were as follows: 1) Under the temperature inversion condition, the upper and lower air disturbance caused by one elevated wind machine with a 6m diameter blade of 12kW power and 8.5m in height made the temperature inversion disappear and the temperature within 1-3m greatly increased. Relative humidity decreased rapidly, effectively preventing the generation of frost in the protected area. 2) The disturbances-affected area of strong wind was about 20m, and wind speeds at 3, 2 and 1m from the ground were 4, 2.1 and 1.6m/s respectively. The effective protection range of one wind machine was 20 to 100m, within level range of 20-100m, wind speed changes of 1-3m showed a reduced trend with fluctuations. The effective protection area of one wind machine was 1.73-3.07hm<sup>2</sup>. The result of the research provides a reference for scientific and high-effective artificial frost prevention and promotion of an elevated wind machine for frost protection with a long blade use.

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|------------------------|--|
| <b>Título</b>          | Apple orchard frost protection with wind machine operation |
| <b>Autor(es)</b>       | Ribeiro, A.C., De Melo-Abreu, J.P., Snyder, R.L.           |
| <b>Año publicación</b> | 2006   |
| <b>Fuente</b>          | Agricultural and Forest Meteorology                        |

Research has shown that wind machines are more effective under conditions of strong thermal inversions. Quantitative relationships between the level of protection and inversion strength, however, are not well known, and there are few reports on the effect of fan operation on energy balance. Whether the wind machines should be started before surface cooling causes turbulence damping and atmospheric stratification or if it is possible to delay starting until just before the critical temperature occurs remains a matter of discussion. Therefore, experiments were conducted on 11 spring frost nights during the 1999 and 2000 to assess the effectiveness of a fan operation on frost protection of an apple orchard under different microclimatic conditions. The 11 frost events were characterized by light winds (0.58-1.92m s<sup>-1</sup>) and clear skies for most of the night, resulting in an average accumulated radiative loss of 2.67 ± 0.38MJ m<sup>-2</sup>. The air temperature increased immediately after the wind machines were started and the temperature rise depended on inversion strength. For each 1°C increase in temperature inversion strength between 1.5 and 15m height, wind machine operation caused a 0.3°C increase of air temperature at a 1.5m height within the main area affected by the fan operation. Using multiple regression, the area protected was significantly related to the temperature increase and the inversion strength. Wind machine operation reduced flower damage by 60% in 1999 and 37% in 2000. Distribution of flower damage varied spatially, and it was related to wind drift.

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| <b>Título</b>          | Frost protection experiment in tea fields using an unmanned helicopters |
| <b>Autor(es)</b>       | Hu YongGuang, Liu ShengZhong, Shen JianWen                              |
| <b>Año publicación</b> | 2013  |
| <b>Fuente</b>          | Journal of Shenyang Agricultural University                             |

To develop the efficient equipment and technology of frost protection on a large scale for agricultural use, the experiment of mobile frost protection in tea fields was conducted with an unmanned helicopter for plant protection. Based on the analysis on frost protection principles through air disturbance of low-altitude flight, the impact of the unmanned helicopter hovering on the air flow distribution near ground was tested at different hovering heights. Then the field experiment of frost protection effects was conducted under different flight combinations of height, speed and flying interval. The preliminary results showed that the helicopter had the strongest disturbance to the ground below the outermost edge of the rotors within 5-10m of the hovering height, but the disturbance increment here reduced with the hovering height. The best frost protection effect was achieved with the height of 5m, flight speed of 6m.s-1 and the interval of 30min, and the temperature of the tea canopy was increased by 3.83°C. Therefore, current unmanned helicopters for plant protection have the expanding application potential for mobile frost protection by low-altitude flight above tea fields, which could enhance frost protection efficiency and be applied for large-scale operation.

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|------------------------|---|
| <b>Título</b>          | Vineyard frost protection with upward-blowing wind machines               |
| <b>Autor(es)</b>       | Battany, M. C.  |
| <b>Año publicación</b> | 2012  |
| <b>Fuente</b>          | Agricultural and Forest Meteorology 157 Oxford: Elsevier Ltd, 2012, 39-48 |

Upward-blowing wind machines have been commercialized for use in frost protection but little quantitative information exists regarding how their operation alters site temperatures. In particular, their performance relative to conventional wind machines has been debated. To address this need, experiments were conducted on 12 spring frost nights in 2010 and 2011 in a commercial winegrape vineyard where either two upward-blowing wind machines or a single conventional wind machine were operated. Comprehensive measurements of air temperature changes caused by wind machine operation were evaluated on multiple transects at heights of 1.1, 4, 7 and 10m. All 12 frost nights were characterized by low wind and clear sky conditions, with temperature inversion strengths commonly associated with beneficial wind machine use occurring on 9 of the 12 nights. The operation of the conventional wind machine produced consistently larger and more statistically significant increases in temperature, particularly at the 1.1m vine level, as compared to the operation of the upward-blowing wind machines which produced very minor increases in temperature at the 1.1m level under strong inversion conditions and either no change or decreases in temperature under weaker inversion conditions. Based on the summary relationships between temperature changes as a function of inversion strength, under conditions of an inversion gradient of 0.2°C m<sup>-1</sup> the conventional wind machine would be expected to raise target area temperatures by 1.6°C at the vine level, while the upward-blowing wind machines would have no net effect under the same inversion conditions. Smoke tracking of the air flow from the upward-blowing wind machines indicated that the air jet reached 25m height, and then tended to slowly settle back towards the ground. These results indicate relatively poor performance of this type of low-powered (6.3kW) upward-blowing wind machine compared to a conventional wind machine under the conditions of this study.

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|------------------------|---|
| <b>Título</b>          | Operation effects of wind machines for frost protection of tea trees on different time scales |
| <b>Autor(es)</b>       | Hu YongGuang, Zhu XiaoLan, Zhao MengLong, Snyder, R. L., Li PingPing                          |
| <b>Año publicación</b> | 2013  |
| <b>Fuente</b>          | Chinese Society for Agricultural Machinery  |

Frost protection effect experiments in tea fields were conducted according to wind machine operation timing on different time scales. The treatments of starting to use wind machines before tea sprouting, starting before frost occurrence and stopping after sunrise were set up respectively. And the impact of wind machine operation on the growth of tea trees, temperature rise in the canopy and protection coverage was measured. The results indicated that starting to use wind machines 20, 15 and 7 days before tea sprouting increased the length of young shoot, bud density and 100-bud weight by over 20% compared with the control test. Starting wind machines 2, 1.5 and 1 hour before frost occurrence led to better protection and enlarged the coverage radius by more than 60% compared with the control test. Delayed stopping of wind machines by 2, 1.5 h and 1 hour after sunrise reduced the temperature rise of the canopy by 45.76% in the first hour, and narrowed the reduction of the photosynthetic intensity of tea leaves. Therefore, to protect tea trees from late frost cold for better growth and yield, the appropriate application of wind machines should be more than 7 days prior to the tea budding, and the operation timing of starting and stopping for a certain frost event should be 1 hour before frost occurrence and 1 hour after sunrise, respectively.

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|------------------------|--|
| <b>Título</b>          | Selective inverted sink efficiency for spring frost protection in almond orchards northwest of Isfahan, Iran |
| <b>Autor(es)</b>       | Yazdanpanah, H., Stigter, C. J.  |
| <b>Año publicación</b> | 2011   |
| <b>Fuente</b>          | Theoretical and Applied Climatology 105 (1/2) Wien: Springer-Wien, 2011, 27-35                               |

A so-called selective inverted sink (SIS) was validated in frost protection of a 20ha almond orchard. Daily counts of flower buds were made for two branches of some selected almond trees in every plot to determine frost damage percentage. Temperatures increased due to the SIS system, but there was an average gradient of temperature decrease of about 0.4°C per 100m with distance from the SIS. The minimum air temperature increased from 0.5 to 2.8°C, with the highest increase closest to the SIS. The percent of frost-damaged flower buds of almond relative to the control plot with distance to the SIS system had its maximum gradient (8% per 100m) in 100-200m distance from the SIS, but this gradient decreased to a minimum (4% per 100m) in 500-700m distance from the SIS. The ANOVA and Duncan's multiple-range test of air temperature and frost damage data confirm that the significant influence zone of this local SIS was about 500m.

## Patentes

| PATENTE  | RESUMEN  |
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| <p><b><u>US20150024674A1</u></b><br/>Agricultural frost protection using induction fans</p> <p><b>Pub. Date</b><br/>2015-01-22</p> <p><b>Applicant(s)</b><br/>McGregor, Michael<br/>(Estados Unidos)</p>   | <p>A system for protecting an agricultural field from frost damage, comprising: one or more induced flow apparatuses, each comprising an induced flow fan that has a primary air flow inlet, a primary air flow outlet, a secondary flow inlet and a secondary flow outlet wherein said primary air flow induces a larger volume of secondary air flow; a primary air flow impeller, powered by a motor, and a duct arrayed to conduct the primary flow from the primary flow impeller to the induced flow fan. The induced flow apparatuses are disposed to induce the flow of warmer, upper-level, air into a lower level, displacing lower, cooler, air in an agricultural field.</p>   |
| <p><b><u>US8701341B2</u></b><br/>Control method and apparatus of wind machine for plant frost protection</p> <p><b>Pub. Date</b><br/>2014-04-22</p> <p><b>Applicant(s)</b><br/>Jiangsu University; Jiangsu Yinchunbiya Tea Reseach Institute Co., Ltd.<br/>(China)</p> | <p>This invention relates to control method and apparatus of wind machine for plant frost protection, and belongs to the domain of agro-meteorological disaster monitoring and control. Existing wind machines for plant frost protection only rely on critical damage temperature or wind speed as a condition to start. If there is no thermal inversion, operation of the wind machine will aggravate freezing injury to plants. If the strength of thermal inversion is weak, the effect will be minimal. The feature of this invention is that when temperature at the plant canopy is lower than the critical damage temperature of a certain plant and temperature difference between the top and bottom of the thermal inversion layers exceeds set threshold, wind machine will start automatically. Otherwise it will remain off. The control apparatus mainly consists of sensor 5 at the top of thermal inversion layer, sensor 7 at bottom of thermal inversion layer, and controller 4. This invention of the control of frost protection wind machine ensures rational and effective operation and avoids aggravated freezing risk due to misuse of energy and incorrect operation.</p> |
| <p><b><u>US7654035B2</u></b><br/>Device for the creation of containment barriers for cold air in atmospheric conditions corresponding to radiation frosts</p> <p><b>Pub. Date</b><br/>2010-02-02</p> <p><b>Applicant(s)</b><br/>Guarga Ferro, Rafael<br/>(Uruguay)</p> | <p>A method for generating a pneumatic barrier with a device that operates in a stratified atmosphere associated with the occurrence of radiation frost. This device takes cold and dense air found close to the ground by means of an axial fan with a vertical axle that propels the air upward to an elbow that forces the airflow to make a 90° turn. The airflow discharged from the device through the outlet section forms a jet with a horizontal axis. The jet's vertical cross-section, perpendicular to its axis, has a rectangular shape and sides that grow with the distance to the outlet. The momentum transported by the jet is able to create at a certain distance from the outlet a pneumatic barrier that fits the jet's section. This pneumatic barrier faces an air mass that is colder and denser than the air transported by the jet and prevents such colder and denser air from advancing toward the device.</p>  |

## ANEXO 8: PUBLICACIONES Y PATENTES REFERIDAS A CALEFACTORES Y/O QUEMADORES

### Publicaciones

|                        |   |
|------------------------|---|
| <b>Título</b>          | Electrically heated cables protect vines from frost damage at early flowering |
| <b>Autor(es)</b>       | Lamb, D.W.  |
| <b>Año publicación</b> | 2009  |
| <b>Fuente</b>          | Australian Journal of Grape and Wine Research                                 |

Background and Aims: Current methods of frost protection in vineyards involve fans, air heaters or sprinklers; each is limited by environmental constraints or available water. An alternative, all-electrical technique offers growers wider choice to match options with their vineyard operations. This study evaluates the ability of electrical heating cables, wrapped around the vine cordons, to protect inflorescences from frost damage. Methods and Results: Five heating cable treatments in six replicates were applied to a 2ha block of Sauvignon Blanc in the southern New England Region of Australia. Vines were subjected to a single -3°C frost event in November 2006 when at approximately 30% capfall. Non-heated vines suffered 41% (Control) and 46% (No heat) inflorescence loss. Those subjected to Low heat suffered a 28% loss, Medium-heated vines suffered a 16% loss and High-heated vines suffered a 13% loss. Qualitative scoring of the vines indicated that more than half of the Medium-/High-heated vines suffered no appreciate damage, whereas all non-heated vines suffered some form of potential crop loss or damage. Conclusion: Electrical heating cables of minimum 10W/m power rating were found to significantly reduce frost damage to inflorescences at 30% capfall. Significance of the study: At approximately 43kW/ha, electrical heating cables offers an alternative frost protection method for small vineyards.



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|------------------------|--|
| <b>Título</b>          | Pulse jet orchard heater system development: Part II. System scaling and application |
| <b>Autor(es)</b>       | Alshami, A.S., Evans, R.G.   |
| <b>Año publicación</b> | 2009   |
| <b>Fuente</b>          | Transactions of the ASABE  |

This article reports the results of a six-year project in which an efficient and effective supplemental orchard field heater system was successfully developed. In addition to being low-cost and mobile, the criteria met by this system design included: (1) usage with or without wind machines or as stand-alone units, (2) reduced labor requirements, and (3) reduced air pollutants compared to conventional supplemental, fossil fuel combustion or wind machines chard heating systems. The resulting new generation heaters were portable, auto-regulating, horizontal pulse jet engines (combustors) with no moving parts. Scaling criteria were formulated and utilized for fabrication of various sizes of portable horizontal pulse jet engines optimized for stable operation. Scale-up parameters for multiple sizes of these combustor systems for agricultural heating and frost protection purposes were both theoretically and qualitatively described. Theoretical and empirical approaches resulted in similarity parameters that played an important role in the scale-up for field applications. The scaled and optimized heater prototypes were constructed and field tested. Experimental results confirmed the applicability of using both gaseous and liquid propane as fuel supplies. Detailed field testing showed that mixing of warm air by the high-velocity exhaust stream into cold air (0 to 4m height) was quite effective in reducing buoyant heat losses. Pulse jet heater prototypes were also tested under orchard conditions and found to circumvent the many disadvantages of conventional oil and liquid propane (LP) gas orchard heating systems, especially when used in combination with wind machines.

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|------------------------|---|
| <b>Título</b>          | Pulse jet orchard heater system development: Part I. Design, construction, and optimization |
| <b>Autor(es)</b>       | Alshami, A.S., Evans, R.G.  |
| <b>Año publicación</b> | 2009  |
| <b>Fuente</b>          | Transactions of the ASABE   |

The objective of this six-year project was to develop a more efficient and effective supplemental orchard field heater technology. In addition to being low-cost and mobile, these devices should be useable with or without wind machines, reduce labor requirements, and greatly reduce air pollutants compared to conventional orchard heating systems. The new generation orchard heaters that were developed in this process are portable, auto-regulating, horizontal pulse jet engines (combustors) with no moving parts. These devices utilize air ejector technology to reduce the temperature of the high-velocity combustion stream by thoroughly mixing it with the surrounding ambient (cold) air, producing a net effective temperature increase of only 2°C to 5°C above ambient. Buoyant forces of warm air are therefore small, and fewer heaters would be needed. The design achieved pressure oscillations that were free of combustion instabilities, and amplitudes were maximized for high exhaust jet velocities for deep hot jet penetration and mixing into the surrounding cold air. The overall size and weight of the heater system were minimized while maintaining optimum operation. Finally, anti-phase operation of paired heaters for noise level reduction from 130dB to near 75dB was achieved by implementing optimized silencing plenums at the inlet and exhaust of the paired heaters.

|                        |  |
|------------------------|--|
| <b>Título</b>          | Efficiency of air heating trailed machine for protection against spring frost in apple orchard                                     |
| <b>Autor(es)</b>       | Rabcewicz, J., Biakowski, P., Konopacki, P.  |
| <b>Año publicación</b> | 2012   |
| <b>Fuente</b>          | Zeszyty Naukowe Instytutu Sadownictwa i Kwiaciarnictwa im. Szczepana Pieniazka 20 Skierniewice: Research Institute of Horticulture |

The aim of research was evaluation of efficiency of air heating trailed machine for protection against spring frost in apple orchard with frost pocket. During the occurrence of night frost the machine was trailed along tree rows and the warmed air is distributed across the rows. The generated heat caused fluctuations of air temperature in the tree rows at the distance of 25m from the machine. At the distance of 15m, during the night frost -2°C and at the height of 1m the heat pulses of 1.7°C were observed. The mean and minimal air temperatures at the protected area were slightly higher (up to 0.5°C) than at non-protected area. The spring night frosts observed during evaluation period were not sufficiently severe to cause the frostbite of buds and blossom. The influence of use of air heating trailed machine on reduction of blossom frost damages was not proved. The air heating increased, however, the number of primordia and apple crop in the season 2011.

|                        |   |
|------------------------|---|
| <b>Título</b>          | Pistachio frost damage in Iran and new methods of frost protection  |
| <b>Autor(es)</b>       | Hokmabadi, H.   |
| <b>Año publicación</b> | 2010  |
| <b>Fuente</b>          | Options Méditerranéennes. Série A, Séminaires Méditerranéens (94) Montpellier: Centre International de Hautes Etudes Agronomiques Méditerranéennes, 2010, 71-78 |

Because of global warming as levels of CO<sub>2</sub> concentration rise, climate has changed nowadays. With climate change we can expect increased damage to orchards, such as increased winter and spring frost, summer droughts, storms, and increased damages from fungi and insect attacks. Particularly in winter we can expect a temperature increase, and the trees natural annual rhythm will gradually fall further out of step with the climate of the region. Higher winter temperatures will reduce the hardiness and lead to an earlier growth start. The tree mechanisms for avoiding such damage do not appear to be able to prevent this. First, trees can have problems with de-hardening in mild weather periods and frost damage in the subsequent cold periods. Particularly unfortunate are fluctuations between above-and below-freezing temperatures (thaw-freeze cycles) for trees that are adapted to a continental climate. In the south and west parts of Iran, there has been a wide-spread and increasing problem with such damage over several years. In average 50% of pistachio was damaged by spring frost in the last five years. New methods of frost protection were applied in the last few years for controlling frost damage in pistachio orchards in Kerman province. One of these methods was the design and manufacturing of a SIS (selective inverted sink) system and testing it in pistachio orchards. Results showed that this system can protect orchards and can increase temperature by 2°C. The other system was the fogger machine. This system was also designed and manufactured and tested in pistachio orchards. The results of this system showed 11.5% increase in relative humidity and 1.9°C increase in temperature of orchards. The new methods of frost control in orchards in Iran are explained in this paper and the importance of frost damage in horticultural plants in Iran is discussed.

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|------------------------|---|
| <b>Título</b>          | Frostguard: a new technique of frost control in small orchards? |
| <b>Autor(es)</b>       | Deckers, T., Schoofs, H.  |
| <b>Año publicación</b> | 2006  |
| <b>Fuente</b>          | Fruitteelt-nieuws   |

A small machine was developed in Belgium, based on a propane gas cylinder with a ventilator. One machine could protect 0.3-0.5ha against frost. Field test were carried out during April 21-22, 2005, in an orchard (2ha) with Jonagold apples. Four machines were used. The temperature in the unheated control plot dropped to -1.2°C, while temperatures in heated plots varied between 0.6 and 1.4°C at distance of 30m from the machine, and between 2.1 and 3.2°C at 10m distance. Another study was carried out in strawberry cv. Elsanta (2 ha) where the temperature in the unheated control plot dropped to -2.7°C. Heated plots showed temperatures of at least 2°C, thus indicating less circulation problems of the warm air (compared to the orchard).

## Patentes

| PATENTE   | RESUMEN  |
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| <p><b><u>WO2014200367A1</u></b><br/>Heat distribution apparatus and method</p> <p><b>Pub. Date</b><br/>2014-12-18</p> <p><b>Applicant(s)</b><br/>Heat Ranger Limited<br/>(Nueva Zelanda)</p>  | <p>In one aspect the invention provides a heating apparatus adapted to modify local climatic conditions which includes a base arranged to support the heating apparatus on a support surface. An inlet stage is associated with the base and a mixer stage connected on top of the inlet stage. At least one outlet nozzle is connected to the mixer stage, with the outlet nozzle or nozzles being adapted to rotate relative to the base. At least one heating element is located inside the mixer stage, and an impeller is arranged to drive air through the inlet stage into the mixer stage and out at least one outlet nozzle, where the mixer stage defines an air flow path which elevates air drawn through the mixer stage.</p> |
| <p><b><u>EP1834520B1</u></b><br/>Method for avoiding frost damage in crops and/or for improving the fructification at low temperatures and device applied with such a method</p> <p><b>Pub. Date</b><br/>2013-06-05</p> <p><b>Applicant(s)</b><br/>Agrofrost NV<br/>(Bélgica)</p> | <p>Method for avoiding frost damage in crops (35) and/or for improving the fructification at low temperatures, characterized in that it consists in periodically heating and cooling the air on the site of the crops (35) between a minimum and a maximum temperature with a minimal temperature variation of 0.2°C.</p>  |
| <p><b><u>WO2009103488A1</u></b><br/>Device and method for the frost protection of grape vines</p> <p><b>Pub. Date</b><br/>2009-08-27</p> <p><b>Applicant(s)</b><br/>Integrated Solutions GMBH<br/>(Suiza)</p>   | <p>The invention relates to a method and a device for the frost protection of grape vines, wherein a gas, particularly air, is heated at least at one station (26) by means of compression, is conducted to the grape vines via a distribution system (28, 30, 32, 34), and is discharged from the distribution system through outlet openings. The distribution system may particularly comprise flexible hoses (30) having capillary openings, which uniformly deliver the heated air across nearly the entire hose surface.</p>   |

## ANEXO 9: PUBLICACIONES REFERIDAS A CUBIERTAS PROTECTORAS

| Título   | Study on using shade net for protection of frost damage for longan             |
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| Autor(es)  | Huang JiangLiu, Zhu JianHua, Luo MuLin, Zhu AnDing, Li GuiFen                  |
| Año publicación  | 2008   |
| Fuente   | South China Fruits (4) Chongqing: Citrus Research Institute, CAAS, 2008, 40-42 |
| <p>Fifteen years old trees of longan cv. Shixia were covered with shade net to protect from frost damage in mid-December 2006 when the lowest temperature reached -4.5°C. Covering the whole of the tree crown protected the trees from frost damage whereas covering only the top of the crown did not give adequate protection. Control trees suffered 87.7% damage.</p> |  |

| Título  | Evaluation of containers and plastic covering for coffee seedlings, for protection against low temperatures |
|---|---|
| Autor(es)   | Morais, H., Carneiro Filho, F., Caramori, P. H., Mariot, E. J., Ribeiro, A. M. de A.                        |
| Año publicación   | 2004  |
| Fuente  | Acta Scientiarum - Agronomy 26 (4) Maringa: Universidade Estadual de Maringa, 2004, 401-406                 |
| <p>The production of coffee (<i>Coffea arabica</i>) seedlings in the south and southeast regions of Brazil is constrained by eventual frosts. The protection of coffee seedlings, which are grown by the utilization of containers and plastic coverings, was evaluated in Londrina, State of Parana, during the winter of 2000. The utilized plastic coverings were composed of single and double layers of black and white polyethylene. The seedlings produced in both materials were also evaluated in cold chamber, to verify the vulnerability of root system. The temperatures of leaves and root system were monitored by thermocouples connected to a data logger. The results of this experiment showed that plastic coverings of double layers were efficient to protect the seedlings against low night temperature of leaves. The seedlings produced in containers showed lower temperatures in the roots, which suggests more susceptibility to frost damages. Therefore, a special caution must be taken with seedlings grown in containers during the winter, to avoid frost damages.</p> |   |

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| Título          | Covering of peach ( <i>Prunus persica</i> ) flowers for early spring frost protection                                 |
| Autor(es)       | Drogoudi, P., Tsipouridis, C., Thomidis, T., Terzis, T.   |
| Año publicación | 2006  |
| Fuente          | New Zealand Journal of Crop and Horticultural Science 34 (1)<br>Wellington: Royal Society of New Zealand, 2006, 51-53 |

The aim of this study was to investigate the covering of peach (*Prunus persica*) flowers by single and double layered polyethylene foil bag, foam (starch-based foam), thin vermiculite, perlite, or sawdust as methods of protection from early spring frost. Compared with uncovered flowers, the results showed that using a double bag or foam significantly reduced the percentage of damaged flowers caused by low temperatures. In contrast, no significant differences in damaged flowers were found between flowers covered with thin vermiculite, perlite, a single bag, or sawdust compared with those uncovered (control). The existence of the ice nucleation bacterium *Pseudomonas syringae* pv. *syringae* in the peach flowers of four cultivars was also investigated. It was not found in any peach flowers of 'Arm King', 'Silver King', 'May Grand', or 'Andross' examined by ELISA.







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