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Source: Weed Technology, 25(3) : 454-458

Published By: Weed Science Society of America

URL: https://doi.org/10.1614/WT-D-09-00073.1

Weed Technology 2011 25:454–458

Possible Weed Establishment Control by Applying Cryogens to Fields Before Snowfalls

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Cryogens are defined as substances that produce low temperatures. In this study, cryogens refer to salts added to snow or ice to cool underlying soil, resulting in reduced weed establishment. In laboratory experiments, bags of ice mixed with cryogens were able to reach temperatures as low as −17 C. In soil-filled pots stored at 4 C, bags of cryogenic salts filled with ice chips reduced the soil temperatures to below 0 C and reduced the establishment of weeds significantly without salinity effects. The cryogen magnesium chloride-6-hydrate (MC) that was effective in pot experiments was tested in an oat field in 2008 and 2009. Plastic bags containing concentrated solutions of MC, perforated at the top, were placed on bare soil just before snowfall. Contact of snow with MC was expected to decrease the surface soil temperature enough to cause freezing injury to seeds in the soil. Although overall effects on weed establishment were small, the cryogenic effect did significantly reduce corn spurry establishment in 2008, and significantly reduced overall weed establishment in both years. These results show that weed management with cryogenic salts is possible in principle, but requires further technical improvements to be practical in the field.

Nomenclature: Corn spurry, *Spergula arvensis* var. *sativa* (Boenn.) Mert. & Koch.; oat, *Avena sativa* L.
Key words: Cryogen, freezing-point depression, thermal weed control.

Los criógenos se definen como substancias que producen bajas temperaturas. En este estudio, los criógenos se refieren a las sales añadidas a la nieve o hielo para enfriar el suelo subyacente, resultando esto en una reducción en el establecimiento de malezas. En experimentos de laboratorio, bolsas de hielo mezclado con criógenos alcanzaron temperaturas tan bajas como −17C. En maceteros llenos de tierra almacenados a 4C, bolsas de sales criógenas con hojuelas de hielo redujeron la temperatura del suelo a menos de 0C y se disminuyó el establecimiento de malezas significativamente, sin efectos de salinidad. El criógeno cloruro de magnesio hidratado (MC) que fue efectivo en experimentos de macetas fue probado en un campo de avena en 2008 y 2009. Bolsas de plástico conteniendo soluciones concentradas de MC, perforadas en la parte superior, fueron puestas en el suelo justo antes de una nevada. Se esperaba que el contacto de la nieve con MC disminuyera la temperatura de la superficie del suelo lo suficiente para causar daño por congelación a las semillas en el suelo. Aunque generalmente los efectos en el establecimiento de las malezas fueron pocos, el efecto criogénico redujo significativamente el establecimiento de Sp*ergula arvensis* en 2008, y disminuyó significativamente el establecimiento en general de las malezas en ambos años. Estos resultados demuestran que en principio el manejo de malezas con sales criogénicas es posible, pero se requieren mejoras tecnológicas adicionales para que sea práctica su aplicación en el campo.

Weed control is one of the most important components of any crop production system. Without chemical or biological herbicides, labor-intensive manual weeding is often unavoidable (Melander et al. 2005). With recent interest in organic agriculture, and also in the reduction of pesticide use to prevent environmental contamination, herbicide-free systems require investigation.

Thermal weed control methods have been tested in the 20th century, primarily on hard surfaces in urban areas or on railway tracks. Thermal methods can be categorized as direct heating (e.g., flaming, steaming), indirect heating (microwaves, ultraviolet light), or freezing (Rask and Kristofferson 2007). Weed control by freezing consumes more energy than flame weeding (Thomas 1993), and its effectiveness depends on the method chosen. Fergedal (1993) and Larsson (1993) studied weed control by flash-freezing emerged plants with carbon dioxide snow (CO_2: −78 C) or liquid nitrogen (LN_2: −196 C) and concluded that, while LN_2 was more effective than CO_2, neither was as effective as flaming. Weeds possibly survived after flash freezing because meristems or young leaves were protected by outer tissues (Rask and Kristofferson 2007). No further development of this approach has taken place. However, if imbibed cells freeze slowly and then thaw slowly, cells that have low freezing tolerance may be injured and lose the ability to germinate (Sakai and Larcher 1987).

Many plant seeds can survive freezing temperatures under dry conditions, and this trait has been used for cryopreservation of plant genetic resources (Engelmann 2004). If seed tissue containing high amounts of cellular free water freezes, the cells are severely damaged by crystallization of intracellular water into ice (Mazur 1984). When plant tissues freeze slowly to a lower temperature, extracellular freezing occurs in dense tissues such as embryos or meristems. Ice crystals grow extracellularly and cells deform mildly. Cells die only if the deformation is strong enough to destroy the plasma membrane or other organelles (''freezing injury''; Fujikawa and Miura 1986; Jitsuyama et al. 1997). Complete weed control may be impossible with cold-thermal control because some weed seeds may have a high freezing tolerance (Li et al. 2008). Freezing below 0 C under sufficient water could be useful to reduce weed establishment in the following spring.

DOI: 10.1614/WT-D-09-00073.1
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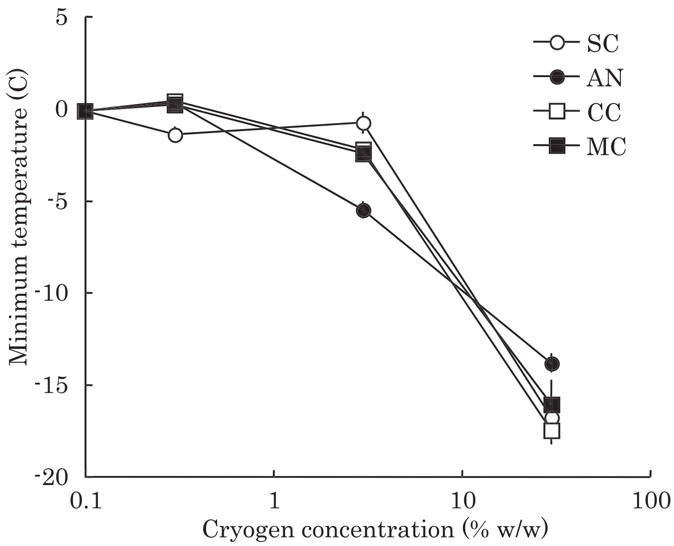

Figure 1. Schemes of experimental methods for cryogenic applications. These schemes represent the measurement of final minimum temperature in (A) ice–cryogen mixtures and cryogenic treatments for (B) pot and (C) field experiments.

Cryogenic chemicals that induce freezing-point depression are used to preserve food and medicine and to melt snow at temperatures below 0 C. The combination of sufficient amounts of cryogens and ice decreases the solidification point and also reduces temperature. If these types of cryogens are applied to snow-covered ground at about 0 C, the temperature drops below 0 C.

The study objective was to measure the temperature depression achievable with cryogens and to test the cryogens' effects on weed emergence under laboratory and field conditions.

Materials and Methods

Experimental Soil Material Containing Weed Seeds. In April 2007, soil for this experiment was collected from a rotational crop field at the Experiment Farm, Field Science Center for Northern Biosphere, Hokkaido University (43°30′N, 141°20′E). The soil was a brown lowland soil (Typic Udifluvent) and was known to contain seeds of various weeds, such as orchardgrass (*Dactylis glomerata* L.), cogongrass [*Imperata cylindrica* (L.) Beauv.], common lambsquarters (*Chenopodium album* L.), corn spurry, bog stitchwort [*Stellaria alsine* Grimm. var. *undulate* (Thunb.) Ohwi], Asian copperleaf (*Acalypha australis* L.), black nightshade (*Solanum nigrum* L.), dandelion (*Taraxacum officinale* G.H. Weber ex Wiggers), and white clover (*Trifolium repens* L.). This field is typically covered with common lambsquarters and monocotyledons when left fallow.

Measurement of Final Minimum Temperature in Ice–Cryogen Mixtures. The cryogens sodium chloride (SC), ammonium nitrate (AN), calcium chloride-2-hydrate (CC),[1] and magnesium chloride-6-hydrate (MC)[2] were used. Artificial ice chips (100 g) were put into plastic bags[3] with a thermosensor connected to a recorder,[4] and 0.3, 3, or 30 g of one of the four cryogens were added (Figure 1A). For the control treatment, ice chips were put into a plastic bag without cryogens. The bags were kept in a household refrigerator at 4 C for 12 h and the temperature was recorded every 3 min. Each test was repeated four times.

Measurement of Freezing Tolerance of Weeds. Dry surface soil (depth 5 cm) sampled from the field was filtered through a 5-mm metal mesh, and 200 g of the fine-particle soil was placed in plastic trays (14 by 9.5 cm, depth 2 cm) with 50 ml distilled water. The soil contained natural weed seed populations (at least 10 seeds) that were shown to germinate within 1 wk under normal conditions in a separate experiment. Soils were kept at 25 C (control), in a domestic refrigerator (4 and −20 C) or in an experimental freezer (−5 and −80 C). After 48 h of these treatments, the soils were equilibrated at 25 C before the germination test. In addition to these cold treatments, soil was exposed to liquid nitrogen (LN_2; −196 C) to characterize weed seed survival under severe freezing conditions.

After these cold treatments, the soil was transferred to pots (diameter 6 cm, soil depth 5.5 cm) and incubated at 25 (± 1) C at 100 μmol m^{-2} s^{-1}, 16-h photoperiod in a growth chamber; 30 ml tap water was added once per week. After 3 mo, all emerged plants were counted. The experiment was conducted as a completely randomized design with six replications.

Tests of cryogens were done in the same way, but instead of the chilling treatment, the soil was kept at 4 C and plastic bags containing 30% (wt/wt) of ice–cryogen mixture were placed on top of the soil sample for 48 h (Figure 1B).

Field Experiment. Field experiments were conducted at the Experimental Farm of the Field Science Center for Northern Biosphere, Hokkaido University, Sapporo, Japan (43°04′N, a brown lowland soil, Typic Udifluvent) for 2 yr. Potatoes (*Solanum tuberosum* L.) were grown the year prior to each treatment. Rotary tilling was done to a depth of 10 cm each year on December 12, prior to heavy snowfalls. Plastic bags[5] were arranged on two experimental plots (1.6 by 1.6 m). Bags contained 2.2 L of agar (7 g L^{-1}) with or without 220 g L^{-1} MC. In each plot, nine plastic bags that had several holes (diameter < 1 mm) on their upper surface for contact between the cryogen and snow were placed on the ground and were covered with thin nonwoven fabric[6] (Figure 1C). Thermo-sensors were placed on the soil surface. The deepest snow cover was 86 cm and 79 cm recorded on February 2, 2008, and February 24, 2009, respectively. The lowest air temperatures (−18.4 and −16.9 C) were measured on 19 February 2008 and 23 February 2009. At that time, the lowest temperature recorded on the soil surface was about 0 C. In April 2008 and 2009, the snow had melted completely and the plastic bags and sensors were removed. In the beginning of May in both years, oats (200 kg ha^{-1}) were sown in rows 25 cm apart, with no pesticides or fertilizers applied. Growing conditions were mild during May and June of both years (Table 1). When oats reached the heading stage in early July, weed and crop densities were determined using quadrats (30 by 30 cm) with 10 subsamples per plot. Quantified weeds were common lambsquarters and corn spurry, which contributed 70 and 15% of the total weed dry weight, respectively. The remaining weeds were not individually assessed.

Table 1. Weather conditions at the field site during May and June.

Parameter	Year 2008	Year 2009
Air temperature (C)		
Average	14.0	15.1
Minimum	1.6	1.5
Maximum	25.3	29.2
Soil temperature at 5-cm depth (C)		
Average	14.7	15.1
Minimum	8.9	8.9
Maximum	23.5	23.1
Relative humidity (%)	77	73
Precipitation (mm)	88.8	91.2

Soil and Plant Element Analysis. To check for cryogen leakage from the plastic bags and uptake by plants, soil and plant dry matter were analyzed by using X-ray fluorescent spectrometry (XRF).[7] Soil samples and plant shoots from the field were dried at 80 C, milled, and filtered through a 1-mm metal mesh, and coin-shaped pellets (diameter 10 mm, thickness 1 mm) were made under 40 MPa compression by using a mechanical press.[8] The pellets underwent XRF at 30 kV to quantify 12 elements, including magnesium, chloride, and calcium, which were present in three of the four cryogens. Sulfur, chlorine, copper, and molybdenum were not detected in the soil and plant samples.

Statistical Analysis. Experiments were completely randomized designs. Treatment differences from controls were determined using Student's *t* tests. Statistical analysis was conducted using SPSS software.[9]

Results and Discussion

Minimum Temperature in Ice–Cryogen Mixtures. Within 30 min after adding a cryogen, the temperature in the ice-filled plastic bags reached a minimum that was maintained for at least 30 min. In all cases the minimum temperature in the bag was lower than the ambient temperature of 4 C (Figure 2). All cryogens caused significant rate-dependent declines in temperature. AN initially had a faster rate of temperature suppression to the 3% concentration, but then had the least suppression at the highest concentration. The remaining three cryogens behaved similarly, achieving comparatively little temperature suppression at 3%, but resulting in temperatures as low as −16 to −17.5 C at the 30% concentration.

The theoretical minimum temperatures that can be achieved with 23.1% (wt/wt) MC and 25% (wt/wt) SC mixed with ice are −33 and −21 C, respectively. We detected temperature drops to −16.1 and −16.8 C induced by 30% (wt/wt) MC and SC, respectively (Figure 2). The theoretical minimum temperature was probably not reached because of incomplete insulation of our experimental equipment. Nevertheless, the salts tested produced significant temperature drops under our experimental conditions.

Freezing Tolerance of Weeds and the Effects of Cryogens. The total emerged weeds population tended to decrease with decreasing temperature, but this effect was statistically significant only at temperatures −80 or −196 C (Figure 3A).

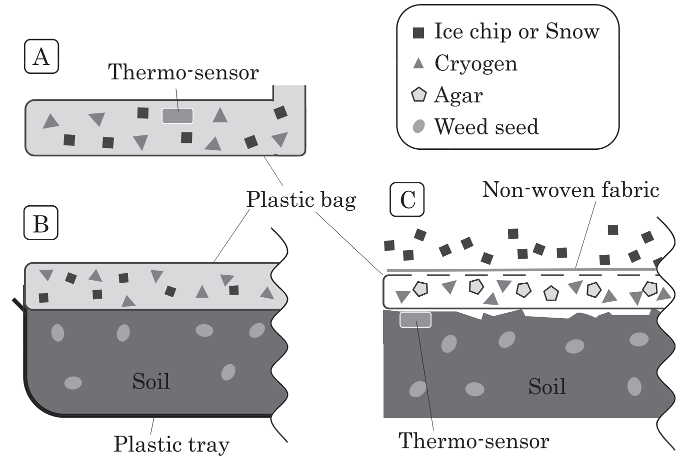

Figure 2. Final minimum temperature in mixtures of ice and various amounts of cryogenic salts. SC = sodium chloride; AN = ammonium nitrate; CC = calcium chloride-2-hydrate; MC = magnesium chloride-6-hydrate.

The responses to the chilling treatment at 4 C were compared with responses produced by exposure to plastic bags containing ice–cryogen mixtures (Figure 3A). Treatments in which the soil temperature was reduced (SC, CC, and MC) had significantly lower plant numbers ($P < 0.05$, Figure 3B). SC and MC were the most effective cryogens for killing weeds, reducing the seedling number by about 70% compared with the 4 C chilling treatment ($P < 0.01$). Analysis of soil and weed elements by using XRF showed no significant increase in levels of free magnesium and calcium in the cryogen treatments ($P < 0.05$, Student's *t* test, $n = 8$), indicating that no cryogen had leaked from the plastic bags.

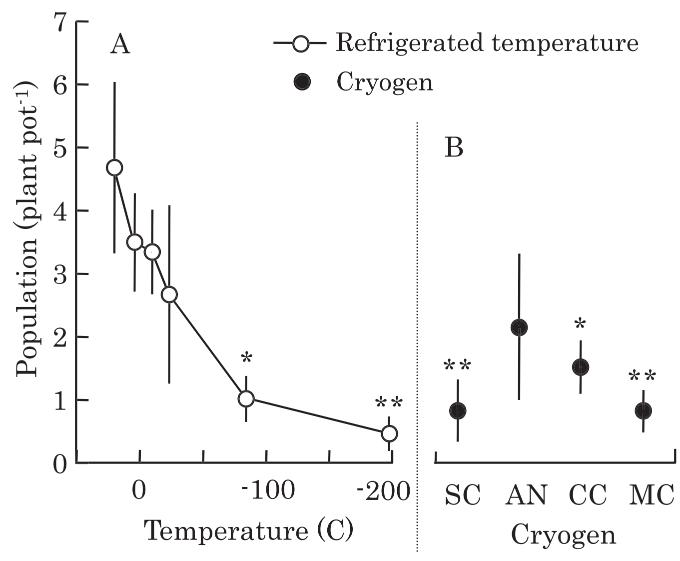

Figure 3. Effects of (A) freezing and (B) adding plastic bags containing cryogens on weed population density. Vertical bars represent SE calculated for the cumulative values (monocotyledons and dicotyledons). Values are significantly different from controls at (A) 25 C or (B) 4 C, at $*P < 0.05$ and $**P < 0.01$, respectively. Values were compared by using the Student's *t* test ($n = 6$). See Figure 1 for cryogen abbreviations.

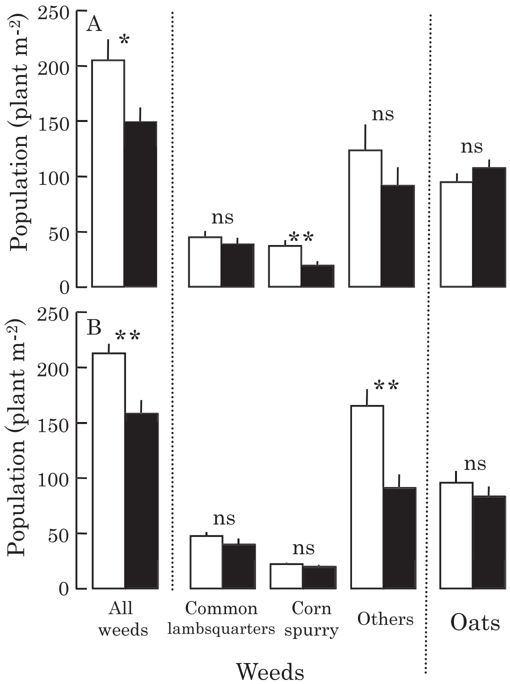

Figure 4. Effects of spreading plastic bags containing cryogens on weed and oat population densities in the field trial through 2 years (A: 2008, B: 2009). "All weeds" are the total weed populations emerged. Open and filled bars are weed populations in control and cryogen plots, respectively. Vertical bars are SE. Significant differences between control and cryogens existed at *$P < 0.05$ and **$P < 0.01$. Values were compared by using the Student's t test ($n = 10$).

Although SC and MC did not decrease temperatures as low as in other studies (Fergedal 1993; Larsson 1993), these cryogens produced the same weed control as was achieved by freezing to below −20 C. Several seeds in the soil samples survived even after three repeated rapid freeze–thaw cycles in LN_2. This also may have been caused by some weed seeds having high freezing tolerance (Li et al. 2008). Therefore, general weed control may be impossible by using cold-thermal control alone.

Effect of Cryogens in the Field. Soil temperatures in experimental field plots were recorded by using thermosensors once an hour, including the minimum temperature in a day, from December 12 to March 31 for 2 yr. The minimum temperatures were −1.4 and −1.9 C in the control plots, and −4.0 and −5.3 C in the cryogen plots in 2008 and 2009, respectively.

No significant differences in oat plant population were detected between control and cryogen-treated plots sown after the cryogen treatment in both years (Figure 4). Total weed population density was suppressed significantly by treatment with cryogens in both years. The density of common lambsquarters (evaluated alone) and bog stitchwort, Asian copperleaf, black nightshade, dandelion, and white clover (evaluated collectively, "Others" in Figure 4), showed no significant response to cryogen treatment in 2008, while the corn spurry population was reduced significantly with cryogens (Figure 4A). In 2009, the density of weeds other than corn spurry or common lambsquarters was reduced significantly by the cryogen treatment (Figure 4B). XRF analysis of field soil produced no evidence of cryogen leakage ($P < 0.05$, Student's t test, $n = 4$).

We had hoped that enclosing cryogens in plastic bags might produce good results, but further improvements appear necessary. The plastic bags had holes in the upper side to allow contact between the cryogen and snow; these holes might have been too small for enough contact. Further attempts will be made using a special sheet design to improve the contact between snow and the cryogen. Despite low weed control, cryogen application in the field led to a small, but statistically significant, temperature drop. Cryogen-induced temperature drops had a significant impact on weed seed germination in the pot experiment; therefore, the method proposed here should continue to be improved and studied.

The effects of cryogen application in the field were smaller than expected, and whether new types of sheet-like cryogen containers that prevent cryogen leakage and promote direct contact between the cryogen and snow will yield better results remains to be seen. The application might be suitable for small, high-value organic crops. Although the study was based on data limited by experimental year and location, the possibility of effective weed management with cryogens was shown. Further study is needed to investigate if cryogenic weeding in agricultural fields before snow fall is useful or sufficient.

Sources of Materials

[1] Calcium chloride-2-hydrate, extra-fine grade agents, Kishida Chemical Co., Ltd., Osaka, Japan.

[2] Sunny Keeper, magnesium chloride-6-hydrate, Nio Kosan Co., Ltd., Kagawa, Japan.

[3] Plastic bag, 17 by 24 cm, H-4, Seisannipponsha Ltd., Tokyo, Japan.

[4] Thermosensor connected to a recorder, TR-71U, T&D Co., Nagano, Japan.

[5] Plastic bag, 40 by 56 cm, SL-4, Seisannipponsha Ltd., Tokyo, Japan.

[6] Nonwoven fabric, Pao-Pao90, MVK PLATEC Co., Ltd., Tokyo, Japan.

[7] X-ray fluorescent spectrometry, element analyzer JSX-3202M, JEOL Co., Tokyo, Japan.

[8] Mechanical press, B-012C, Imoto Co., Kyoto, Japan.

[9] Software SPSS ver. 14.0J for Windows, SPSS Japan, Tokyo, Japan.

Acknowledgments

This study was supported by grants from the Japan Society for the Promotion of Science (nr. 19658005).

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Received November 25, 2009, and approved March 2, 2011.