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Freezing Resistance of Winter Annual and Biennial Legumes at Different Developmental Stages

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ABSTRACT

A requirement for developing cover crop systems is to find cover crop species and cultivars that are both suitable and adapted in such systems, including sufficient frost resistance, for the local climate. The objective of this study was to detect the freezing resistance of different winter annual and biennial legumes, and to clarify whether the freezing resistance is correlated to the developmental stages of the plants. The study included a growth chamber experiment with nine species-cultivars, including white clover (*Trifolium repens* L., cv. Milkanova) as a control plant, and three developmental stages. At different developmental stages the test plants were exposed to five different freezing temperatures. The best freezing resistance, independent of sowing time, was shown by hairy vetch (*Vicia villosa* Roth. cv.Welta). Crimson clover (*T. incarnatum* L. cv. Heusers Ostsaat) and yellow sweetclover [*Melilotus officinalis* (L.) Pall. cv. Nordgold Yellow] also showed good freezing resistance. Black medic (*Medicago lupulina* Gaertn. cv.Virgo Pajberg) showed medium freezing resistance, a little better than subclover (*T. subterraneum* L. cv. Denmark). Poorest freezing resistance was exhibited by barrel medic (*M. trunculata* Gaertn. cv. Parabinga) and snail medic [*M. scuttelata* (L.) Mill. cv. Kelson]. In hairy vetch, especially cv. AU EarlyCover, negative correlation between freezing resistance and age of the plants was detected. Generally, this was also true for crimson clover, subclover, barrel, and snail medic. In contrast, yellow sweetclover and white clover showed tendencies to positive correlation between freezing resistance and age of the plants. The results revealed the importance of developmental stage, at least in some species-cultivars, in the determination of freezing resistance.

EXPERIMENTS IN MANY COUNTRIES have shown that the use of cover crops has many potential benefits. Examples of such benefits are weed suppression (Moore et al., 1994), reduced damage from insect pests (Theunissen and den Ouden, 1982; Hofsvang, 1991; Brandsæter et al., 1998) and green manuring effects. These green manuring effects may be beneficial both spatially, regarding the main crop growing together with the cover crop (Grubinger and Minotti, 1990), and in time, regarding the subsequent crop (Brandsæter et al., 1998). These benefits may become very important in organic and integrated cropping systems.

Competition between the cover crop and the main crop is, however, a serious obstacle that has to be solved before cover crop systems can be included into the farmers practices. One approach to avoid or reduce the competition in such systems is to combine a main crop and a cover crop with a synchronized onset of maximum vegetative growth. The use of winter annual legumes sown in autumn is an example of such a cropping system (Ilnicki and Enache, 1992). Starting from seeds in late summer they grow vegetatively during autumn, become dormant in winter, and resume vegetative growth the following spring. Later in spring or early summer, the plants flower, senesce, and die. This makes it possible to transplant a main crop into, for example, a stand of subclover, without competition for nutrients and water (Ilnicki and Enache, 1992). Such a system may permit the legume to suppress weeds during the critical period of weed development early in the season and synchronizes maximum growth of the main crop with the senescence of the legume. Some other winter annual legume species, like hairy vetch and crimson clover, are too tall and have to be converted by mowing into an organic mulch before transplanting the crop (Brandsæter and Netland, 1999). Experiments of Abdul-Baki and Teasdale (1993) have shown that hairy vetch, if mowed, is very suitable as a cover crop.

Under Norwegian conditions, with cold winter climate, freezing resistance is an important character. Experiments by Brandsæter et al. (2000), showed differences in freezing resistance between winter annual legume species-cultivars.

Under field conditions, sowing date will strongly in-

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fluence the developmental stage reached by the winter annuals in autumn. Sowing date, however, will also influence the assimilate production before winter. Regarding freezing resistance, both the choice of species-cultivar and sowing date, and the interaction between these elements are of crucial importance. Brandsæter et al. (2000) showed that developmental stage may play a decisive role in optimizing freezing resistance of winter annual legumes. That study included two subclover cultivars and information is needed concerning freeze resistance of other winter annual legume species-cultivars. Interrelations between growth, development, and freezing resistance are described by Levitt (1980).

Artificial freezing tests have been found to give an excellent agreement with winter survival in the field (Levitt, 1980). However, the freezing test only measures the freezing resistance and ignores other environmental factors in the field which affect the plants ability to survive the winter. The need for an absolute measure of freezing resistance is most simply met by determining the freeze killing point T_{k50}, the temperature required to kill 50% of the plants (Levitt, 1980). Additionally, regrowth ability after freezing is an important property of cover crops under northern conditions.

The studies by Brandsæter and Netland (1999) and Brandsæter et al. (2000) showed the following overall ranking of species, on the basis of freezing resistance: Hairy vetch (best), yellow sweetclover, crimson clover, black medic, subclover, barrel medic, and snail medic (poorest). The objective of this study was to clarify the effect of developmental stage of these species on freezing resistance. Knowledge about how developmental stages influence the freezing resistance is important for deciding the optimal time for sowing these species under field condition. As far as the authors know, no data on how developmental stages of these legumes influence on freezing resistance, have been published earlier.

MATERIALS AND METHODS

Experimental Design, Plant Material and Treatments

The experiment was carried out in growth- and freezing-chambers in accordance with a factorial, randomized complete block design with three replications. The following factors were included: (i) nine legume species-cultivars (Fig. 1); (ii) three sowing times to get three developmental stages; (iii) five different freezing temperatures (for freezing-test details, see Table 1). Milkanova white clover was used as a reference species-cultivar because it is a common legume in Norway. The three replications were sown at different times in 1999, starting 15 Mar., 26 July, and 8 Nov., respectively. Each treatment combination had two parallel pots.

About 25 seeds of each species-cultivar were sown in 10-cm or 12-cm diam. pots filled with limed peat [L.O.G. 'Gartnerjord', Mixture: 840 g kg^{-1} sphagnum peat, 100 g kg^{-1} fine sand, 60 g kg^{-1} clay, 5.5 kg dolomite lime m^{-3}, 1.2 kg fertilizer (NPK 15-4-12), 0.2 kg F.T.E. no. 36 (micro nutrients), pH 5.5–6.5, and density: 270 kg dm/m^{-3} (applied volume)]. The 10-cm pots were used for the latest sowing time.

The seeds were inoculated with compatible *Rhizobium* strains before sowing. After 1 wk, the seedlings were thinned to five plants per pot and the pots were placed randomly on carts in the growth chamber. To compensate for variations in light levels at different spatial positions the carts were rotated twice a week in the chamber.

The earliest sown pots, which received 5 wk long day (LD), were fertilized twice during the cultivation period, with 100 ml pot^{-1} nutrient solution (Hydro Superba brun NKP-fertilizer with micronutrients, Conductivity 1.5; Norsk Hydro, Oslo). The intermediately sown pots were fertilized once with 100 ml pot^{-1} nutrient solution. The latest sown pots, standing 1 wk at LD, were not fertilized.

The pots with hairy vetches had iron railing (wire support) to hold the plants upright. The treatment program for the experiment, including LD and SD conditions, hardening and freezing, is presented in Table 1.

Photon flux density during the period of LD and SD was 209 μmol s^{-1} m^{-2}. During the hardening period, the light level was reduced to 150 μmol s^{-1} m^{-2}. The light was supplied by high intensity lamps (KLORARC Daylight, High Intensity Discharge lamp/MB 1 D250/T/H, 250W, Base E 46, GE Lighting). The relative humidity (RH) in the chambers was about 65% throughout the cultivation period. Each replication in the experiment included two extra pots for each combination of species-cultivar and sowing time, on which visible flowers, primary shoot length and number of secondary shoots were recorded before destructive harvesting of the biomass. The harvested biomass was oven-dried for 72 h and then weighed. This assessment was carried out before the rest of the pots were exposed to the freezing treatments.

Freezing Test

Controlled freezing of the plant material was conducted in freezing chambers, according to the method described by Larsen (1978). To assure slow initial freezing, the pots were placed in trays in which the bottom was covered with a 2-cm-thick styrofoam plate. The temperature was decreased by 1°C h^{-1} to −2.5°C and kept there for 24 h, before it was decreased further by 1°C h^{-1} to read temperatures of −4, 6, −8, and −10°C in the four freezing chambers, respectively. These temperatures were maintained for 36 h. In a separate control chamber the temperature was kept at 0°C for 60 h.

The thawing procedure for all units started with an increase in temperature at the rate of 1°C h^{-1} until the temperature reached 4°C, and was maintained for 24 h. The thawed plants were taken out of the chambers and placed in a greenhouse for 3 wk before freezing resistance was assessed. The parameters assessed in the experiment were as follows: biomass of plant material (dry weight of plant material expressed as percent of the control = 0°C) and T_{k50} (freezing temperature required to kill 50% of the plants). For the biomass assessments, dead and living tissues were identified by separating withered and green plant parts.

Statistical Analyses

Analysis of variance of the recorded results was carried out. The assumptions of normality and variance underlying the statistical analysis were tested for all response variables. A testing procedure as a part of the SAS/LAB software (SAS Institute Inc., 1992) for detecting outliers was performed. The results of the experiment were analyzed as a split-plot design, in which the freezing chamber is considered as "main-plots" and the species-cultivars and sowing times as "sub-plots." The biomass at 0°C in the freezing program was used as a reference of 100% in the statistical analyses. Level of significance was $P \leq 0.05$. Least square differences (LSD multiple-stage test) were used for comparing treatments (SAS Institute Inc., 1988).

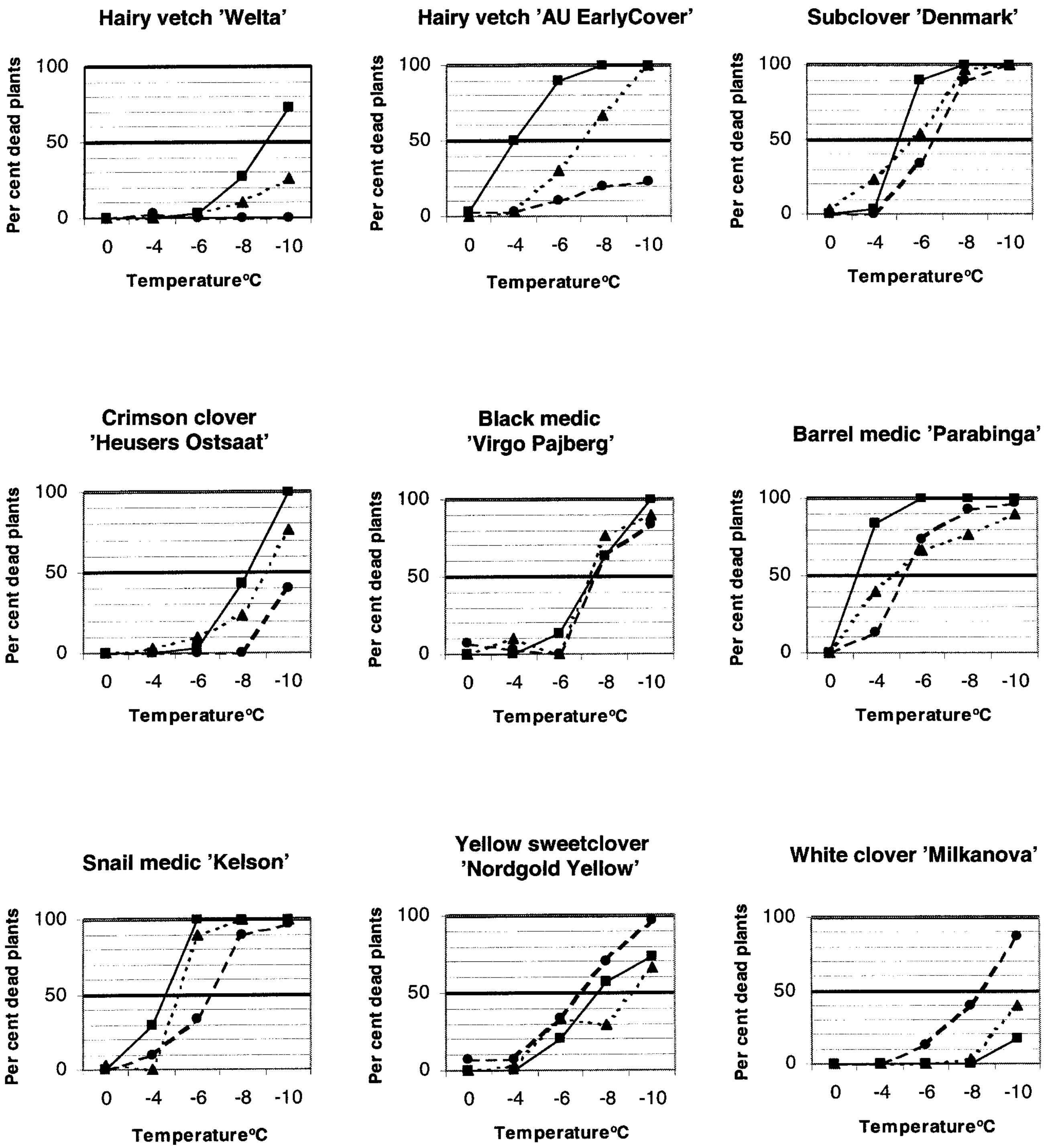

Fig. 1. Freezing resistance of species-cultivars at early (—■—), intermediate (...▲...), and late (—●—)sowing, in terms of percent dead plants following freezing treatments (0, −4, −6, −8, and −10°C).

T_{k50} values were estimated by simple linear regression with a quadratic model (SAS Institute Inc., 1992).

RESULTS

Development of Plants Prior to Freezing

Developmental characteristics at the end of the chosen growth periods are presented in Table 2. Some species-cultivars from early and intermediate sowing times had started flowering prior to freezing. AU EarlyCover hairy vetch and yellow sweetclover were blooming at both sowing times, and barrel medic, snail medic, and white clover were flowering when sown early.

Resistance to Low Temperatures

Freezing resistance evaluation in terms of percent dead plants (Fig. 1), indicated differences among species-cultivars and sowing times, and an interaction be-

Table 1. Growing periods of respectively 5, 3, and 1 wk long day, followed by 2 wk short day. Hardening conditions consisted of 2 wk short day at low temperature. Freezing procedure included 5 treatments before 3 wk recovery in greenhouse.

Period	Duration	Day length (PAR†)	Temperature
	wk	h	°C
Longday	Early sowing: 5 Intermediate sowing: 3 Late sowing: 1	20	18
Shortday	2	10	Day: 15 Night: 10
Hardening	2	10	2
Freezing	0.7 (total)	0 (darkness)	Chamber 1: 0 Chamber 2: −4 Chamber 3: −6 Chamber 4: −8 Chamber 5: −10
Recovery/Revival	3	18	Day: 18 Night: 12

† Photosynthetic active radiation.

tween these two factors. The analysis of T_{k50}, the parameter used for testing the freezing resistance (Table 3), revealed significant differences among species-cultivars and sowing times. The interaction between the two factors was not significant, but a tendency was detected ($P_{species \times sowing\ time} = 0.1$). However, although the interaction was weak, probably because of lack of degrees of freedom in the error term (DF = 8), the analyses were also carried out separately for each sowing time. Only results from each sowing time separately, are presented below. The effect of species-cultivars on T_{k50} was significant for all sowing times.

Some of the species-cultivars showed very good resistance to low temperature, even when frozen to −10°C (Fig. 1, Table 3). Consequently, for some combinations of species-cultivars and sowing time, T_{k50} values could not be estimated. These combinations, marked as < −10°C in Table 3, were excluded from the statistical analysis.

Sown early, white clover exhibited very good freezing resistance and accordingly was excluded from the analysis. Of the remaining species, yellow sweetclover and Welta hairy vetch obtained the lowest T_{k50} values. Crimson clover and black medic exhibited T_{k50} values statistically similar to yellow sweetclover and Welta hairy vetch and also to subclover. The T_{k50} temperatures for other species-cultivars were higher than for all previously mentioned species except subclover.

For the intermediate sowing time, Welta hairy vetch, yellow sweetclover, and white clover showed very good resistance, and T_{k50} could not be estimated. When excluding these species, crimson clover, followed by black medic and AU EarlyCover hairy vetch obtained the lowest T_{k50} values. However, crimson clover was the only one that was significantly more resistant than the three weakest species-cultivars which are subclover, snail medic, and barrel medic.

Following late sowing, T_{k50} values were not obtained

Table 2. Developmental characteristics of legume species-cultivars prior to the freezing treatment: Percent flowering plants, biomass dry weight, primary shoot length, and secondary shoot number.

Sowing time	Species-cultivar	Flowering	Biomass	Shoot length	Sec. shoots (number) basal/axial/terminal
		%	g (DW)pot^{-1}	cm	
Early	Hairy vetch ‘Welta’	0	6.23	59.4	5/18/0
	Hairy vetch ‘AU EarlyCover’	90	4.7	77.6	3/13/0
	Subclover ‘Denmark’	0	6.2	2.7	9/0/0
	Crimson cl. ‘Heusers Ostsaat’	0	6.67	4.3	8/0/0
	Black medic ‘Virgo Pajberg’	0	4.04	7.7	6/2/3
	Barrel medic ‘Parabinga’	100	6.47	35.1	2/8/2
	Snail medic ‘Kelson’	20	9.44	41.1	2/7/3
	Yellow sweetcl. ‘Nordgold Yellow’	86.7	5.63	39.8	1/9/3
	White clover ‘Milkanova’	16.7	5.9	2.4	7/0/0
Intermediate	Hairy vetch ‘Welta’	0	3.55	44.4	3/15/0
	Hairy vetch ‘AU EarlyCover’	31.7	2.7	56.6	2/12/0
	Subclover ‘Denmark’	0	3.59	1.5	7/0/0
	Crimson cl. ‘Heusers Ostsaat’	0	3.55	2.0	6/0/0
	Black medic ‘Virgo Pajberg’	0	1.17	1.9	6/0/0
	Barrel medic ‘Parabinga’	0	3.93	22.1	2/5/3
	Snail medic ‘Kelson’	0	3.81	14.9	0/4/3
	Yellow sweetcl. ‘Nordgold Yellow’	20	2.54	17.5	2/4/3
	White clover ‘Milkanova’	0	1.69	1.0	6/0/0
Late	Hairy vetch ‘Welta’	0	0.58	22.0	3/8/0
	Hairy vetch ‘AU EarlyCover’	0	0.49	24.6	2/8/0
	Subclover ‘Denmark’	0	0.45	1.2	6/0/0
	Crimson cl. ‘Heusers Ostsaat’	0	0.43	1.0	5/0/0
	Black medic ‘Virgo Pajberg’	0	0.16	0.8	4/0/0
	Barrel medic ‘Parabinga’	0	0.43	2.2	4/0/0
	Snail medic ‘Kelson’	0	0.76	2.6	0/1/3
	Yellow sweetcl. ‘Nordgold Yellow’	0	0.21	3.2	0/0/3
	White clover ‘Milkanova’	0	0.14	0.5	3/0/0

Table 3. Estimated T_{k50} values (°C) for legume species-cultivars following early, intermediate, and late sowing. Figures in the same column followed by different letters are significantly different at $P = 0.05$.

Species-cultivars	Early sowing T_{k50}	Intermediate sowing T_{k50}	Late sowing T_{k50}
Hairy vetch 'Welta'	−9.11a	<−10	<−10
Hairy vetch 'AU EarlyCover'	−3.32c	−7.09ab	<−10
Subclover 'Denmark'	−5.35bc	−5.34b	−6.77b
Crimson clover 'Heusers Ostsaat'	−8.19ab	−8.95a	<−10
Black medic 'Virgo Pajberg'	−7.6ab	−7.63ab	−8.3a
Barrel medic 'Parabinga'	−4.12c	−5.33b	−4.98c
Snail medic 'Kelson'	−2.67c	−5.34b	−6.43b
Yellow sweetclover 'Nordgold Yellow'	−9.14a	<−10	−7.12ab
White clover 'Milkanova'	<−10†	<−10	−8.46a

† Some of the species-cultivars showed very good low temperature resistance, even when frozen to −10°C. Consequently, T_{k50} values could not be estimated for some combinations of species-cultivars and sowing times. These combinations, which are not followed by letters, were not included in the statistical analysis.

for the two hairy vetch cultivars and crimson clover. Of the remaining species-cultivars, white clover, black medic, and yellow sweetclover showed the lowest T_{k50} values. Yellow sweetclover was, however, not significantly different from subclover and snail medic. Barrel medic had significantly higher T_{k50} value than all other species-cultivars.

Regrowth Ability after Freezing

The effect of the main factors species-cultivars and sowing time on regrowth ability, relative to regrowth biomass of control treatment, were statistically significant. Additionally, the interaction between species-cultivars and sowing time was significant. Because of this interaction, only results from analyses with separated sowing times, are presented below. For all sowing times, analyzed separately, significant differences among species-cultivars were detected.

Relative biomass regrowth was lower following early sowing than from both intermediate and late sowing (Table 4). Species-cultivars divided into two distinct groups when comparing relative regrowth within the early sowing treatment. Subclover, snail medic, barrel medic, and AU EarlyCover hairy vetch, produced less relative biomass than other species-cultivars.

At the intermediate sowing time, relative biomass regrowth of Welta hairy vetch was greater than all other species-cultivars except that of yellow sweetclover. Yellow sweetclover did not differ from crimson clover and white clover. Black medic had less relative biomass, but not significantly different from that of crimson clover and white clover. AU EarlyCover hairy vetch did not differ significantly from black medic. The four species, Barrel medic, snail medic, subclover, and AU EarlyCover hairy vetch exhibited the lowest relative biomass regrowth.

When sown late, Welta hairy vetch exhibited the highest relative biomass regrowth, but did not differ signifi-

Table 4. Biomass of species-cultivars from early, intermediate, and late sowing, three weeks after freezing to −4, −6, −8, −10°C, expressed as percent of control. No frozen controls (0°C) are set to 100%. Different letters indicate significant differences between species-cultivars within each sowing time. Figures in brackets give the dry weight pot^{-1} of the control.

Sowing time	Species-cultivar	Relative biomass after freezing 0°C	−4°C	−6°C	−8°C	−10°C	LSD
Early	Hairy vetch 'Welta'	100 (11,1g)	77,7	49,1	34,8	13,5	a
	Hairy vetch 'AU EarlyCover'	100 (9,8g)	20,5	2,6	0	0	b
	Subclover 'Denmark'	100 (10,2g)	48,1	0,5	0	0	b
	Crimson clover 'Heusers Ostsaat'	100 (10,0g)	84,8	47,7	46,1	0	a
	Black medic 'Virgo Pajberg'	100 (7,5g)	78,4	49,5	18,5	2,2	a
	Barrel medic 'Parabinga'	100 (8,9g)	32,6	0	0	0	b
	Snail medic 'Kelson'	100 (17,3g)	35,5	0	0	0	b
	Yellow sweetcl. 'Nordgold Yellow'	100 (10,5g)	76,5	38,7	21,3	16,3	a
	White clover 'Milkanova'	100 (9,8g)	81,1	68,2	25,7	10,4	a
Intermediate	Hairy vetch 'Welta'	100 (8,0g)	102,6	83,6	73,8	50,5	a
	Hairy vetch 'AU EarlyCover'	100 (8,1g)	66,3	51,8	8,0	0	de
	Subclover 'Denmark'	100 (6,2g)	68,4	21,4	1,6	0	e
	Crimson clover 'Heusers Ostsaat'	100 (4,5g)	92,0	76,7	57,9	10,8	bc
	Black medic 'Virgo Pajberg'	100 (2,7g)	67,3	74,6	16,2	7,5	cd
	Barrel medic 'Parabinga'	100 (7,7g)	43,4	8,9	6,7	2,1	e
	Snail medic 'Kelson'	100 (9,2g)	60,8	14,4	3,1	0	e
	Yellow sweetcl. 'Nordgold Yellow'	100 (5,9g)	80,7	61,1	63,5	33,3	ab
	White clover 'Milkanova'	100 (4,2g)	87,7	81,6	29,9	18,6	bc
Late	Hairy vetch 'Welta'	100 (2,0g)	82,3	100,5	105,4	69,3	a
	Hairy vetch 'AU EarlyCover'	100 (1,9g)	101,7	91,4	71,5	35,5	a
	Subclover 'Denmark'	100 (1,3g)	88,2	62,5	3,9	0	bc
	Crimson clover 'Heusers Ostsaat'	100 (1,3g)	115,0	107,6	89,5	19,1	a
	Black medic 'Virgo Pajberg'	100 (0.9g)	57,0	71,4	8,4	1,1	bcd
	Barrel medic 'Parabinga'	100 (1,7g)	33,4	6,3	1,4	0,7	d
	Snail medic 'Kelson'	100 (2,5g)	57,9	31,3	2,8	1,3	cd
	Yellow sweetcl. 'Nordgold Yellow'	100 (1,2g)	112,8	71,2	17,4	1,0	b
	White clover 'Milkanova'	100 (1,0g)	82,9	53,3	18,1	0,5	bc

cantly from that of crimson clover and AU EarlyCover hairy vetch (Table 4). Yellow sweetclover, subclover, white clover, and black medic formed an intermediate group for relative biomass production although performance of snail medic was similar to several species in the group. Barrel medic had the lowest relative biomass but did not differ significantly from black medic or snail medic.

DISCUSSION

The ranking of the species-cultivars for freezing resistance was quite similar to that of the experiments by Brandsæter et al. (2000). This was true for both T_{k50} and regrowth ability. Regarding time of sowing, the general pattern was that as planting was delayed and the plants had not reached flowering, regrowth ability after freezing improved and T_{k50} values were lower. Connection between growth stage of winter annual legumes and freezing resistance or winter survival, were shown by Brandsæter and Netland (1999) and Brandsæter et al. (2000). However, the present experiment revealed some very distinct interactions between species-cultivars and sowing time.

Welta hairy vetch exhibited the best overall freezing resistance and regrowth ability although the cultivar did respond in a general pattern to sowing time. Since the incidence of flowering frequency in Welta hairy vetch was zero for all sowing dates, the results were seemingly not affected by a transition from vegetative to generative phase of development. However, flower initiation may have been a better parameter to use than flower formation to determine the effect of development phase on freezing resistance.

AU EarlyCover hairy vetch showed poorer results for freezing resistance and regrowth ability than Welta hairy vetch but responded even stronger to sowing time than Welta. The two earliest sowing dates gave plants with high T_{k50} and low regrowth ability. Plants in young developmental stages, however, gave promising results regarding freezing resistance and regrowth ability. Actually, when sown late AU EarlyCover hairy vetch was the second most freezing resistant species-cultivar. For this cultivar, the freezing resistance and regrowth ability were clearly negatively correlated with incidence of flowering prior to freezing. The early sowing resulted in loss of freezing resistance compared with intermediate sowing (medium freezing resistance) and late sowing (very good freezing resistance). In an earlier study, hairy vetch AU EarlyCover showed rather good freezing resistance, with a 95% survival when exposed to −9°C (Brandsæter et al. 2000). In the latter study, the LD period used corresponded to the intermediate sowing time of the present study. However, it is possible that a prolonged SD period after the LD period, used in that study, improved freezing resistance in AU EarlyCover hairy vetch (Brandsæter et al., 2000). In field experiments by Brandsæter and Netland (1999), Welta hairy vetch survived very well at all locations while AU EarlyCover was completely dead in the spring.

Subclover showed generally weak freezing resistance and regrowth ability. Differences in freezing resistance and regrowth ability between sowing times were observed, but were less distinct than in the hairy vetches. Late sowing time gave lower T_{k50} than intermediate and early sowing and consequently the best and poorest regrowth. These results could indicate a transition to reproductive phase in plants from early sowing, even if the incidence of flowering was zero for all sowing dates. Field experiments by Brandsæter and Netland (1999) within subclovers showed generally poor winter survival.

In general, crimson clover showed good freezing resistance and regrowth ability. Only Welta hairy vetch had better freezing resistance. Although no visible flowering occurred at any sowing time, the response of crimson clover to sowing time followed the general pattern. Late sowing time gave the best freezing resistance compared with intermediate and early sowing times. Crimson clover (cultivar not specified) has shown poor winter survival in field experiments by Brandsæter and Netland (1999). However, we have some preliminary field data indicating that Heusers Ostsaat crimson clover is better fitted to survive winter conditions.

Barrel medic and snail medic generally showed the highest T_{k50} and the poorest regrowth ability of all species-cultivars at all sowing times. Also for these species the response of sowing time were similar to the previously mentioned winter annual species. However, it was more distinct in snail medic than in barrel medic. Freezing experiments by Brandsæter et al. (2000) also showed poor freezing resistance of these two species. Sown early, both reached the flowering stage before freezing. Intermediate and late sowing time improved the freezing resistance to some extent.

The last three species, black medic, white clover, and yellow sweetclover, had a less typical response to sowing time. Although no visible flowering occurred after any sowing time, black medic showed medium freezing resistance after all sowing times. Almost no differences in T_{k50} and regrowth ability between early, intermediate, and late sowing were detected. White clover and yellow sweetclover showed, in contrast to the other species-cultivars, poorest freezing resistance in terms of T_{k50} after the late sowing time. After intermediate and early sowing times, however, white clover exhibited excellent freezing resistance (T_{k50} below −10°C). Yellow sweetclover exhibited T_{k50} below −10°C after intermediate sowing time and somewhat higher after early sowing time. In accordance to the present results of yellow sweetclover, we have preliminary field data indicating that winter survival is considerably improved if this species is sown in early summer compared with late summer or autumn. The observed biomass of yellow sweetclover and white clover of the late sown plants was very low at all freezing temperatures. Brandsæter et al. (2000) showed that white clover Milkanova had lower relative biomass than winter annual legume species. Plants ability to harden depends on products of photosynthesis (Tronsmo, 1978). It seems reasonable to assume that late sown white clover and yellow sweetclover could not develop freezing resistance due to lack of assimilate products.

When comparing freezing chamber tests with field experiments, it is important to realize that freezing resistance is only one of many factors that determine field survival. When sown in the field in Norway, the sowing times corresponding to early, intermediate, and late are about mid July, early August and early September, respectively. The climatic conditions during the fall, however, will affect growth and development and therefore make the comparison between growth chamber and field experiments more complicated.

In conclusion, the best freezing resistance regardless of sowing time was shown by Welta hairy vetch. However, not all hairy vetch cultivars are similar since the present results show that the freezing resistance of AU EarlyCover highly depend on sowing time. Also, the tested cultivars of crimson clover, yellow sweetclover, and black medic generally showed good freezing resistance, while subclover, barrel medic, and snail medic showed poor freezing resistance. In contrast to, for example, the winter annual species, hairy vetch, freezing resistance of the biennial species, yellow sweetclover, decreased as planting was delayed. Species-cultivars that did not reach the flowering stage, did not differ much between sowing times in their freezing resistance. White clover and yellow sweetclover had poorest freezing resistance at late sowing time probably because of weak biomass production and lack of assimilate products. The data of the present study show that Welta hairy vetch, crimson clover, and yellow sweetclover are the most promising species for northern condition. Our observations may suggest that for optimizing winter survival, sowing time of hairy vetch and crimson clover should be between mid August and early September, while the sowing time of yellow sweetclover should be in spring or early summer.

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