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Title: FROST RESISTANCE OF PEA GENOTYPES (*PISUM SATIVUM* L.) GROWN IN THE FIELD ON GROUND LEVEL AND RAISED BEDS, AND UNDER CONTROLLED LABORATORY CONDITIONS

Abstract approved: Dr. J. R. Baggett

The effect of raised bed culture on winter survival of ten pea genotypes (*Pisum sativum* L.), differing in inherent cold hardiness and growth habit, was studied under two climatic conditions: a moist semi-maritime winter (Corvallis, Oregon) and a cold continental winter (Weston, Oregon). An assessment of winter injury was based on three indices: percent survival, plant weight, and a visually assigned damage rating. Varying percentages of seven genotypes survived at Corvallis and of six at Weston.

Planting on raised beds at Corvallis significantly increased the mean percent survival and plant weight and decreased the mean damage rating. Of two Corvallis planting dates, September 18 and October 10, 1975, the latter had a significantly higher mean percent survival and a lower mean damage rating.

At Weston, the mean percent survival was higher on ground level plantings. Plant weight was not affected by planting method. At this location there was a significant interaction between planting method and genotype. Of the six surviving genotypes only the two least hardy had significantly higher percent survival on ground level plantings.

Controlled freezing tests between -2 and -10° C were conducted on four genotypes, AW, WH2, INT1, and S4, to determine their relative levels of inherent cold hardiness and cold acclimating ability. An electrolyte leaching method of determining the extent of freezing injury ranked the genotypes as follows, from most hardy to least: AW = WH2 > INT1 > S4. A visual estimation of damage ranked the genotypes as follows: WH2 > AW > INT1 > S4. In comparison, percent survival in the field studies ranked the four genotypes: AW > WH2 > INT1 > S4. Genotypes with a compact growth habit, AW and WH2, acclimated $\geq$ 4° C, whereas those with an upright growth habit, INT1 and S4, acclimated 1-2° C.

Frost Resistance of Pea Genotypes (*Pisum sativum* L.) Grown in the Field on Ground Level and Raised Beds, and Under Controlled Laboratory Conditions

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Michael Ryan Becwar

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Head of Department of Horticulture

Dean of Graduate School

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

FROST RESISTANCE OF PEA GENOTYPES (PISUM SATIVUM L.) GROWN IN THE FIELD ON GROUND LEVEL AND RAISED BEDS, AND UNDER CONTROLLED LABORATORY CONDITIONS

INTRODUCTION

Commercially available pea cultivars (Pisum sativum L.) with seed desirable for culinary use are normally spring planted. Although such cultivars tend to grow well in cool temperatures, they lack the inherent cold hardiness needed for winter survival (11). A horticultural pea capable of being fall planted and surviving moderate winter conditions would be of economic importance.

Such a winter hardy cultivar would offer several advantages over normal spring planted types: 1) it would make use of a growing season when insects and disease organisms are less of a problem, 2) fall tillage and planting conditions are generally more favorable than in the early spring, 3) fall planting utilizes moisture more efficiently in areas of limited precipitation, and 4) the early harvest date would facilitate double cropping of the land.

At present, winter hardy lines are not desirable for culinary use. They cross readily with horticultural types resulting in lines with intermediate levels of hardiness (12). It is difficult to select winter hardy types and maintain desirable culinary seed characteristics. The compact autumn growth habit of overwintering genotypes appears to be associated with undesirable seed types. Overwintering trials of previous years at Oregon State University indicate that breeding lines with a compact autumn growth habit tend to have seeds resembling winter hardy types. In breeding programs a compromise between winter hardy traits and culinary quality has to be made. Therefore, intermediate types may have lower than ideal survival rates; and cultural practices that improve survival are important.

The primary purpose of this study was to evaluate the winter survival of ten pea genotypes, ranging from very hardy to non-hardy, as affected by: 1) ground level and raised bed planting methods, 2) early and late planting dates, and 3) mild and cold winter locations.

A secondary purpose was to determine the frost killing temperature of four selected genotypes through controlled laboratory freezing tests, and compare the inherent cold hardiness levels with observed field survival rates.

REVIEW OF LITERATURE

Cold Hardiness of Plants in General

The term cold hardiness describes the relative tolerance of a given plant to temperatures below 0° C. This is not the same as winter hardiness or "field hardiness" which refers to the plant's ability to survive all the stresses of the winter environment without injury (9).

Certain plants can increase their resistance to frost by undergoing the physiological process of hardening. One theory explains the difference between hardy and non-hardy plants to be mainly due to permeability differences in cytoplasmic membranes (1). When hardy plants are exposed to low temperatures (above 0° C), their cytoplasmic membrane becomes more permeable to water movement. Thus, when they are subjected to below 0° C temperatures, intracellular water can move more freely to extracellular freezing sites. Ice formation in this area is less likely to cause lethal damage than intracellular ice. This permeability effect is not observed in non-hardy plants (1).

There are other proposed theories to account for cold hardiness in plants. All are concerned with water removal from the cell during freezing and its effect on cellular constituents. Three such theories are: the sulfhydryl hypothesis of Levitt (9), the vital water exotherm hypothesis of Weiser (23), and the second supercooling point hypothesis of Tumanov and Kraszvtsey (20).

There is a wide range in the degree of cold hardiness in herbaceous crop plants grown in the temperate regions of the world. Certain cultivars of cabbage and winter wheat can withstand -25° C (2). In peas, the cultivar 'Austrian Winter' can survive -13° C (7), while certain horticultural types are hardy to -7° C (22).

The literature on cold hardiness and freezing injury of plants is voluminous and includes a number of recent reviews (2, 3, 8, 9, 14, 16).

Methods of Evaluating Hardiness

Field testing is a method of evaluating plants for relative levels of winter hardiness. Plants are grown and exposed to natural freezing conditions in the field. The degree of injury to the plants may be determined by bringing samples into a greenhouse at intervals during the winter or by examining the survivors in the spring. The most common criterion of injury for such studies is percent survival (3). A complicating feature of assessing relative levels of hardiness under field conditions is that a "test winter," in which conditions are right to give differential injury, may not occur.

Controlled laboratory freezing tests are also used to determine relative levels of plant hardiness. Plants are grown in the field or in controlled temperature growth chambers. Whole plants or excised plant parts are then subjected to test temperatures below 0° C. Since temperatures can be controlled it is easier to achieve differential injury for evaluating relative levels of plant hardiness. Common methods for determining the extent of freezing injury resulting from such tests are: a visual estimation of damage to foliage, electrolyte conductivity tests (4), triphenyl tetrazolium chloride (TTC) reduction tests (17), multiple freezing point curves (13), and electrical impedance (10).

The electrolyte conductivity test is based on the fact that when plant cells are injured by freezing, cellular electrolytes "leach" out through damaged cell walls. The amount of leaching is determined by measuring the conductivity of a solution (leachate) containing the frozen tissue sample (15, 18). In the laboratory work of this study, electrical conductivity tests and visual estimates of damage to foliage were used to quantify the extent of freezing injury.

Hardiness in Peas

There is a wide range in cold hardiness between cultivars of peas (*P. sativum*). Commercial cultivars with horticulturally desirable traits are the least resistant to frost and need to be spring planted. Wade (22) found certain cultivars able to tolerate -7° C (19° F) field temperatures. Such types tend to maintain vigorous upright growth in the fall if planted to overwinter. In contrast, the more winter hardy types form a compact rosette with creeping branches, small leaves, and shorter internodes under such growing conditions. The field pea, P. sativum var. arvense is an example. It is hardy to -13° C (11) and forms the compact growth habit associated with overwintering ability. 'Austrian Winter' is a typical cultivar and the most common in the United States. It has purple flowers in contrast to the white flowers of horticultural types. The seeds, which are not desirable for culinary use, are small, smooth, and dark colored at maturity.

The earliest report of an attempt to cross the culinary P. sativum type with the winter hardy P. sativum var. arvense was by Wellensiek in 1925 (24). The resulting F_2 plants overwintered.

More recently there have been several reported attempts to cross culinary types with winter hardy field peas and select horticulturally desirable types capable of overwintering. Holland and Frost (6) have attempted to develop an early maturing, winter hardy, wrinkle-seeded pea variety for the English climate. Markarian and Andersen (11) have evaluated the segregates of such a cross for their ability to form the compact rosette of tillers essential for winter survival. They reported that hardiness is inherited independently of other phenotypic characteristics. Thus, the possibility exists for the winter hardy character to be transferred to horticulturally desirable types.

In 1968 Markarian *et al.* (12) released advanced generation breeding lines with levels of hardiness approaching and equaling that of the hardy parent. Three of these lines, which are similar to 'Austrian Winter' in most respects, are included in the present study.

It is generally known that decreased survival rates occur in low areas of field plots. No studies have been done to specifically quantify the interaction of improved drainage, genotype, and winter climatic conditions on winter hardiness and survival of peas. The field work of this study was designed to provide such information.

MATERIALS AND METHODS

Plant Materials

The genotypes included in the experiment (Table 1) represent a range of winter hardiness within *P. sativum*. The most winter hardy genotype is 'Austrian Winter'; a commercially available field pea. The three winter hardy lines are Michigan State University advanced generation breeding lines derived from 'Austrian Winter' x 'Early Perfection' (12). Two of these lines (coded WH2 and WH3) were crossed with Oregon State University (OSU) breeding lines, resulting in progeny lines B420 and B417 respectively. These lines were included in the study as intermediates in terms of fall growth habit and level of winter hardiness. The remaining lines, S433, S194-3, M183, and 'Alaska', are horticultural types with little or no winter hardiness. S433 is of the commercial freezing type and has been released by Oregon State University (OSU) for breeding purposes. S194-3 has been released as the home garden cultivar 'Corvallis'. M183 is an OSU breeding line of the commercial canning type. The OSU lines are wrinkle-seeded types resistant to pea enation mosaic virus. 'Alaska' is an early, smooth seeded commercial cultivar.

In the remainder of the text the code listed in Table 1 will be used to refer to genotypes.

Table 1. A coded description of genotypes included in the study.

Code	Hardiness class	Identification	Source	Autumn growth habit
AW	very winter hardy[a]	'Austrian Winter' (field pea)	commercial	compact, numerous tillers forming creeping rosette, short internodes
WH1	winter hardy	Pedigree: 'Austrian Winter' x 'Early Perfection'	Michigan State University breeding line	"
WH2	"	"	"	"
WH3	"	"	"	"
INT1	intermediate	OSU B420	OSU breeding line	semi-upright, moderately long internodes
INT2	"	OSU B417	"	"
S1	little winter hardiness	S433 (freezing type)	"	"
S2	"	S194-3 (home garden type)	"	"
S3	"	M183 (canning type)	"	"
S4	"	'Alaska' (canning type)	commercial	upright, one main stem, vigorous, long internodes

[a]Markarian and Andersen (10) reported 'Austrian Winter' hardy to -13°C.

Location

Field plots were planted at the Oregon State University Vegetable Research Farm at Corvallis, Oregon, on Chehalis silt loam and at Weston, Oregon, on Athena silt loam. The latter site was located 15 miles northeast of the Columbia Basin Research Station at Pendleton, Oregon. The two locations subjected the plants to different types of winter stress. Corvallis, located in the Willamette Valley of Western Oregon, has mild winter temperatures and a moist rainy semi-maritime climate. Weston is located in the foothills of the Blue Mountains of Northeastern Oregon. It has a more continental climate with colder temperatures and appreciable snowfall (see Table 2 for specific temperature and precipitation data).

Two plantings were made at Corvallis: September 18 and October 10, 1975. The field plot design was a randomized block, with four replications of a factorial arrangement of ten genotypes and two planting methods in each of the two dates.

One planting was made at Weston on September 23, 1975 as a randomized block, design with four replications of a factorial arrangement of ten genotypes and two planting methods.

Plot Establishment

Treflan herbicide (0.3 kg/h) was broadcast and worked into the soil prior to application of fertilizer (730 kg/h of 8N-10.3 P-6.6 K)

The raised beds (Figure 1), prepared with an implement designed[1] specifically for the purpose, were initially 46 cm (18 inches) wide at the base and 23 cm (9 inches) high at the center (Figure 2). A hand pulled garden roller was used to compact the soil in the beds to form a firm surface for planting. The final bed was 47 cm (18 inches) wide at the base and 15 cm (6 inches) high at the center.

Prior to planting all seeds were treated with 'Captan' fungicide and those planted at Weston were also treated with 'Dieldrin', an insecticide to protect against soil insects.

Two hundred seeds were planted in each 9.1 m (30 ft) plot. This resulted in a distribution of approximately 22 seeds/meter (7 seeds/ft). All plots were planted with a hand operated belt seeder. Stand counts were obtained as soon as germination was complete in the fall.

Temperature Data

At Corvallis, daily air and soil temperature minimums were obtained from the U. S. Weather Bureau site at the Hyslop Research Farm located four miles north of the plots. On January 24, 1976 a thermograph was placed in the plots to give a more accurate localized reading of air temperatures at 5.1 cm (2 inches) above the soil level. All temperature information for the Weston plots was obtained from

[1] The raised bed implement was designed and built by Terry Witham at the OSU Vegetable Research Farm.

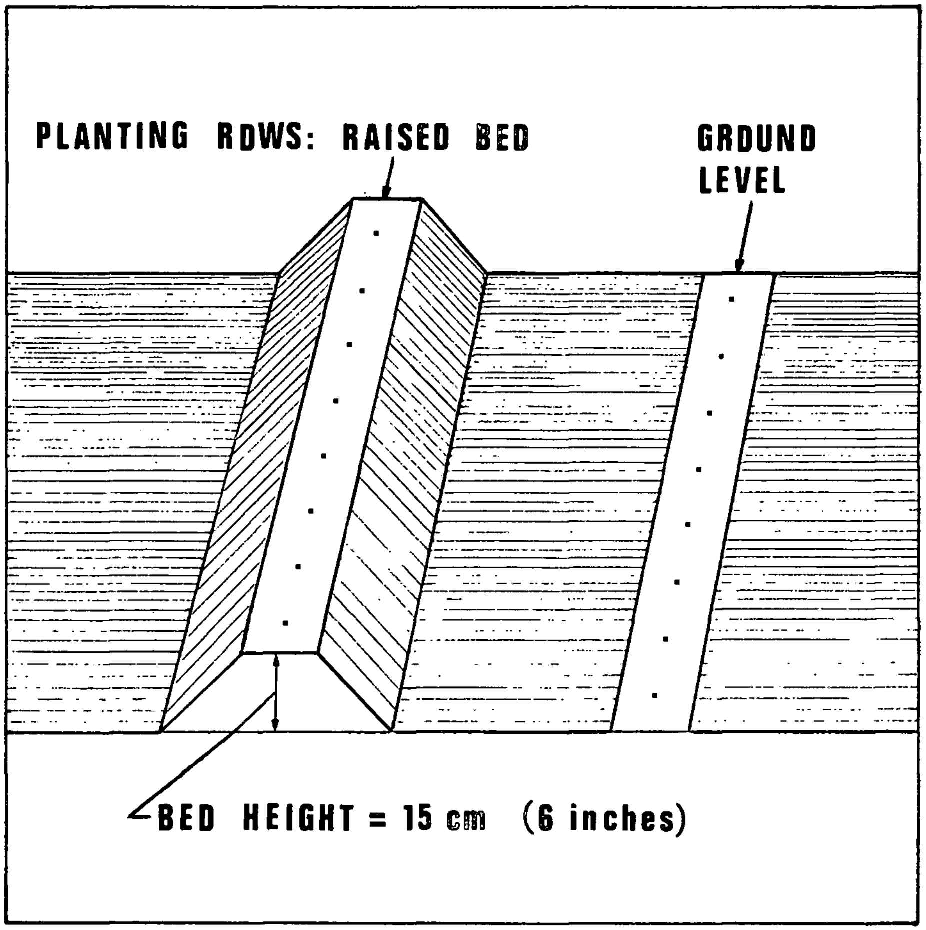

Figure 1. Raised bed and ground level planting rows.

Figure 2. Preparation of raised beds at Weston, Oregon.

the U. S. Weather Bureau site at the Pendleton Experiment Station.

Table 2 gives the temperature data for the period November 1, 1975 to March 31, 1976 at the two locations. At Corvallis, the minimum air temperature for this period was -6° C (22° F) and occurred on February 5 and 6, 1976. The minimum soil temperature at this location was 0° C at a depth of 5. 1 cm (2 inches). This occurred on February 6, 8, and 9, 1976. At Weston, the minimum air temperature of -13° C (8° F) occurred on February 5, 6, and 7, 1976. The soil temperature reached a minimum of 0° C at a depth of 10. 2 cm (4 inches) for six days immediately after this period of low air temperatures.

Assessment of Winter Injury

During the first week of April, when the danger of freezing damage had passed, an assessment of winter injury was made based on the following criteria: 1) a damage rating (Table 3) was assigned to each plot at Corvallis based on the estimated percent foliage damage of the typical surviving plants, 2) the percent survival was determined by counting the number of surviving plants in each plot, and 3) the aboveground portion of the plants in the center 6 m (20 ft) of each plot was collected and weighed. Individual plant weight was calculated as: the net weight of all plants in the center 6 m of each plot divided by the number of plants.

Table 2. Temperature and precipitation data[a] for plots at Corvallis and Weston, Oregon, from Nov. 1, 1975 to March 31, 1976.

		Number of days at or below:				Minimum temperature		Average Minimum temperature		Precipitation		Snow (maximum depth)	
Location	Month	0°C (32°F)	-3°C (27°F)	-6°C (21°F)	-9°C (16°F)	(°C)	(°F)	(°C)	(°F)	(cm)	(inches)	(cm)	(inches)
Corvallis	Nov.	9	0	0	0	-2	28	+2	36	14.0	5.5	T(trace)	
	Dec.	11	7	0	0	-4	25	+2	36	16.5	6.5	T	
	Jan.	11	4	0	0	-4	25	+2	36	16.8	6.6	0	
	Feb.	14	8	0	0	-6	22	+1	33	17.0	6.7	0	
	March	12	4	0	0	-5	24	+1	33	11.4	4.5	0	
Weston													
	Nov.	20	12	5	2	-11	13	-1	30	3.8	1.5	12.7	5.0
	Dec.	21	13	2	1	-11	13	-1	30	8.6	3.4	10.2	4.0
	Jan.	22	12	2	0	-8	17	-1	30	5.3	2.1	T	
	Feb.	19	10	5	4	-13	8	-2	28	2.5	1.0	2.5	1.0
	March	14	10	3	0	-9	16	-1	30	4.3	1.7	0	

[a] All information was obtained from the U. S. Weather Bureau, Monthly Climatological Data Records for Corvallis and Pendleton, Oregon except Jan., Feb., and March temperature data at Corvallis. This data was recorded by a thermograph located in the plots.

Table 3. Winter damage rating system based on the estimated percent foliage damaged.

Rating	% Damage
0	0 (no injury)
1	0 - 12½
2	12½ - 25
3	25 - 37½
4	37½ - 50
5	50 - 62½
6	62½ - 75
7	75 - 87½
8	87½ - 100
9	100 (total injury)

Controlled Freezing Tests

Four genotypes representing a range of growth habits and inherent hardiness were included in the laboratory study. They were: AW, WH2, INT1, and S4. Table 1 describes the autumn growth habit and relative hardiness of each.

Six seeds of each genotype were planted in twenty 10 cm plastic pots containing a mixture of eight parts sandy loam soil to one part peat with 1.4 g of 8N-10.3 P - 6.6 K fertilizer added per 1 kg of soil mixture. Unhardened plants were grown in 21° C day/15° C night (±3° C) greenhouse conditions for 14 days. Hardened plants were grown in the same conditions as above for the first 11 days followed by ten additional days in a growth chamber at 10° C day/4° C night (±2° C), under $12\frac{1}{2}$ hour daylength conditions.

To insure uniformity in temperatures between unhardened and hardened plants, pots of each were held at 2° C for 12 hours (overnight) prior to subjecting the plants to a freezing test temperature. Individual freezing tests were conducted at each of the following temperatures: -2, -4, -6, -8, and -10° C in a walk-in freezing chamber. Temperatures were raised and lowered at a rate of 4° C/hour. Plants were held at the desired test temperature for three hours. Temperatures within the chamber were monitored with copper-constantan thermocouples and a Leeds and Northrup multipoint potentiometric recorder. Variation in temperature between test sites was less than 1° C. The on/off cycling of the refrigeration unit caused a continuous temperature fluctuation of 3° C.

After a freezing run, temperatures were warmed to 2° C and held constant for three hours. At this time two lower leaves were removed from three plants in each pot and immersed in individual test tubes containing 20 ml of distilled water. Test tubes containing the excised leaves were then held for 12 hours at ambient temperature and shaken for one hour. The conductance of three sample solutions of each genotype were measured with a "Solu-Bridge." The leachate and leaves were then boiled in an autoclave at 115° C for 15 minutes. Distilled water was added to each test tube to return the volume to 20 ml. The conductance was again measured.

Percent leaching was expressed as conductance of leachate after the test freeze × 100, divided by conductance of leachate after killing all cells by boiling in water (18). A mean percent leaching value was calculated from the three sample values. The temperature at which 50% leaching occurred was used in freezing studies of herbaceous tissue (15, 18) to indicate the temperature that caused lethal damage. Since 50% leaching does not always occur at a particular test temperature, the point at which it occurred was extrapolated from the percent leaching values of the two adjacent test temperatures.

After each freezing test, potted plants were returned to greenhouse conditions. A visual rating of percent foliage damage was assigned four days later according to the following scale:

damage rating	% foliage damage
0	0 (no injury)
1	<25
2	25 - 50
3	50 - 75
4	> 75
5	100 (total injury)

RESULTS

Field Survival Studies

Three genotypes, S1, S2, and S4, did not survive under any treatment at either location. The seven remaining genotypes were included in separate analyses of variance for the Corvallis and Weston data.

Corvallis

Percent survival, plant weight, and damage rating data were individually analyzed as a split-plot design with four replications, dates (two) as the whole plot, and a factorial set of treatments (seven genotypes and two planting methods) within each block.

Computer analysis indicated the following correlation coefficients (r) between the variables:

	r
percent survival and plant weight	+0.66
percent survival and damage rating	-0.93
plant weight and damage rating	-0.68

This shows that the indices percent survival and damage rating were in close agreement for indicating differences between treatments. However, a significant difference as indicated by percent survival or damage rating was not as likely to imply a difference in plant weight.

The mean percent survival and mean plant weight were significantly higher for plots grown on raised beds (Table 4). Figure 3A ranks the Corvallis genotypes from lowest to highest percent survival and shows the effect of planting method on each. Figure 4A ranks the Corvallis genotypes from lowest to highest plant weight and shows the effect of planting method on each. The mean damage rating was lower for plots grown on raised beds (Table 4).

Table 5 indicates that differences existed between certain genotypes. Genotype AW had higher percent survival and plant weight than all other genotypes and a lower damage rating. WH3 was different from all other genotypes as measured by percent survival and damage rating, but not plant weight. The two intermediate lines, INT1 and INT2, were different as measured by percent survival and damage rating, but not plant weight. The only horticultural type to survive, S3, was different from all other genotypes in terms of all three damage indices.

There was no interaction between planting method and genotype. Percent survival was consistently higher on raised bed plantings for all genotypes that survived at Corvallis (Figure 3A).

Planting date significantly affected the mean percent survival and mean damage rating, but not mean plant weight (Table 4). The mean percent survival was higher for the later planting date. The mean damage rating was lower for the later planting date.

Table 4. Mean effect of planting date (Sept. 18, 1975 and Oct. 10, 1975) and planting method on percent survival, plant weight, and damage rating at Corvallis, Oregon.

	Percent survival			Plant weight (g)			Damage rating		
	Planting method			Planting method			Planting method		
Planting date	Ground level[C]	Raised bed[C]	Planting date means[B]	Ground level[F]	Raised bed[F]	Planting date means[E]	Ground level[I]	Raised bed[I]	Planting date means[H]
Early	16.3	33.5	24.9	31.5	38.8	35.1	7.5	6.4	7.0
Late	50.3	57.7	54.0	25.1	32.5	28.8	5.2	3.5	4.4
Planting method means	53.3[A]	45.6[A]		28.3[D]	35.6[D]		6.4[G]	4.9[G]	

	Percent survival	Plant weight (g)	Damage rating
Planting method means	[A] 5% LSD = 4.0	[D] 5% LSD = 3.4	[G] 5% LSD = 0.4
Planting date means	[B] 5% LSD = 9.2	[E] NSD	[H] 5% LSD = 0.5
Planting date x method means	[C] 5% LSD = 4.8	[F] 5% LSD = 6.4	[I] 5% LSD = 0.5

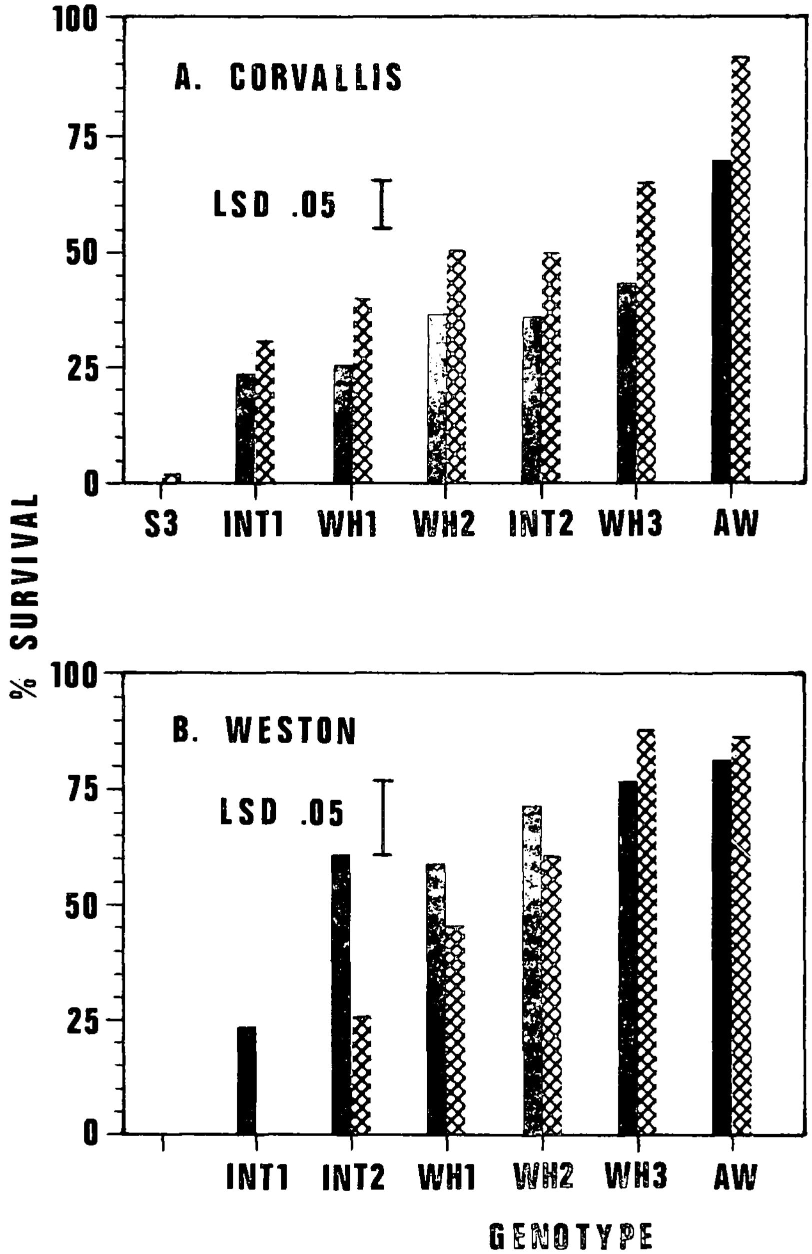

Figure 3. Effect of planting method (■ = ground level; ⊠ = raised bed) on percent survival of seven genotypes at: A) Corvallis, and B) Weston.

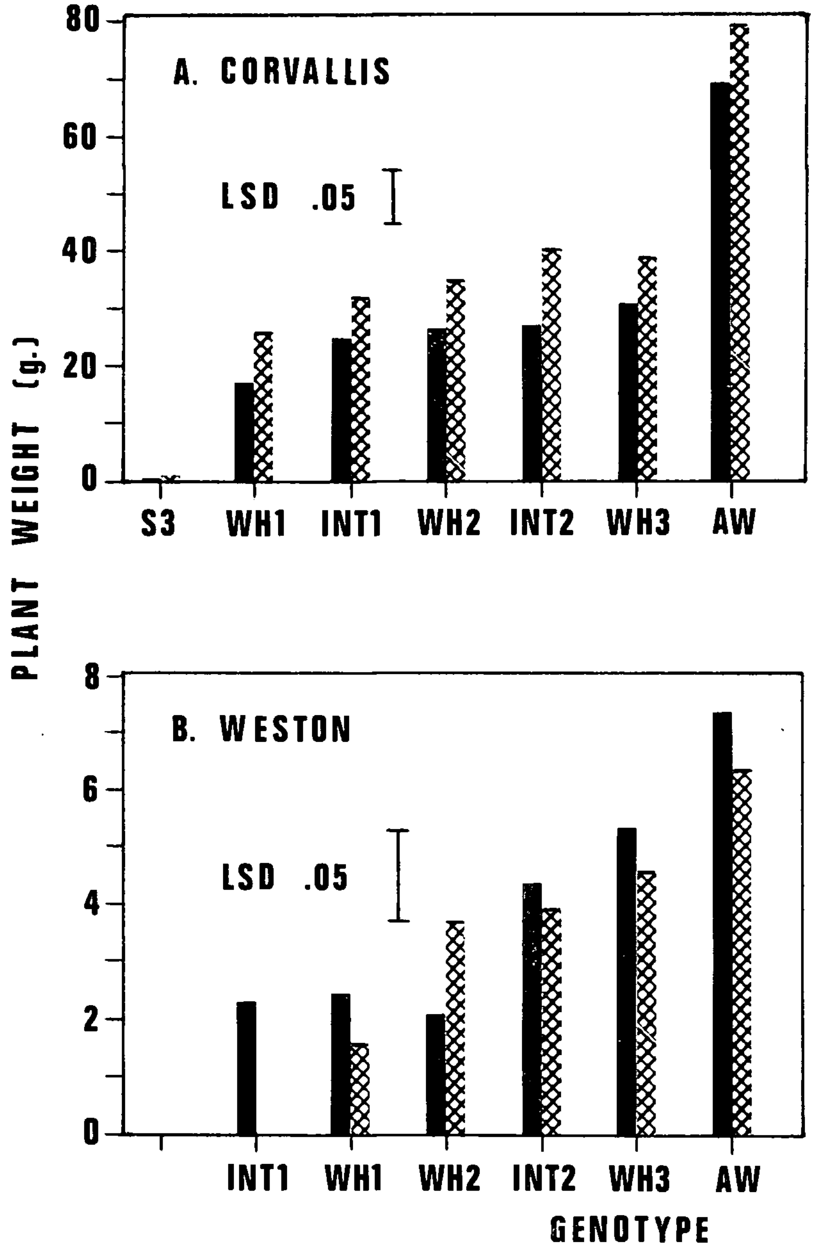

Figure 4. Effect of planting method (■ = ground level; ✕✕ = raised bed) on plant weight (g) of seven genotypes at: A) Corvallis, and B) Weston.

Table 5. Effect of planting method on percent survival, plant weight, and damage rating of seven genotypes at Corvallis, Oregon.

Genotype	Percent survival: Planting method: Ground level[C]	Percent survival: Planting method: Raised bed[C]	Percent survival: Genotype means[B]	Plant weight (g): Planting method: Ground level[F]	Plant weight (g): Planting method: Raised bed[F]	Plant weight (g): Genotype means[E]	Damage rating: Planting method: Ground level[I]	Damage rating: Planting method: Raised bed[I]	Damage rating: Genotype means[H]
AW	67.8	89.9	78.9	69.9	78.7	74.3	1.9	0.4	1.1
WH1	25.4	38.2	32.2	16.7	26.0	21.4	8.1	6.3	7.2
WH2	36.1	47.7	41.9	26.8	33.4	30.1	7.3	5.3	6.3
WH3	43.1	63.0	53.1	30.9	37.9	34.4	5.0	3.5	4.3
INT1	23.8	29.9	26.8	25.9	31.9	28.9	7.6	5.9	6.7
INT2	36.2	48.6	42.4	27.0	40.1	33.6	5.9	4.6	5.3
S3	0.5	1.2	0.8	0.8	1.4	1.1	8.8	8.7	8.8
Planting method means	33.3[A]	45.6[A]		28.3[D]	35.6[D]		6.4[G]	4.9[G]	

	Percent survival	Plant weight (g)	Damage rating
Planting method means	[A] 5% LSD = 4.0	[D] 5% LSD = 3.4	[G] 5% LSD = 0.4
Genotype means	[B] 5% LSD = 7.4	[E] 5% LSD = 6.4	[H] 5% LSD = 0.7
Planting method x genotype means	[C] 5% LSD = 10.5	[F] 5% LSD = 9.0	[I] 5% LSD = 0.9

There was a significant interaction between planting method and date as measured by percent survival and damage rating. Figure 5A shows that for percent survival there was a different response to planting methods, in relation to each other, at different planting dates. However, in terms of plant weight, there was no interaction between planting method and planting date (Figure 5B).

The effect of planting date on the three survival indices of the seven genotypes is shown in Table 6. There was an interaction between planting date and genotype (Figure 6). There was a wider range in percent survival at the earlier planting date than at the later. Thus, the earlier planting date is more effective in differentiating relative levels of hardiness for selection purposes.

The analysis of variance also indicated a significant difference in mean percent survival and mean plant weight between replications, but no significant difference in mean damage rating between replications. The high variability in percent survival and plant weight could be due to herbicide damage of pea plants in specific areas of the field plots. The damage, noticed soon after emergence, reduced the size and vigor of plants in certain plots. Such damage was not readily noticed in the spring when visual damage ratings were assigned.

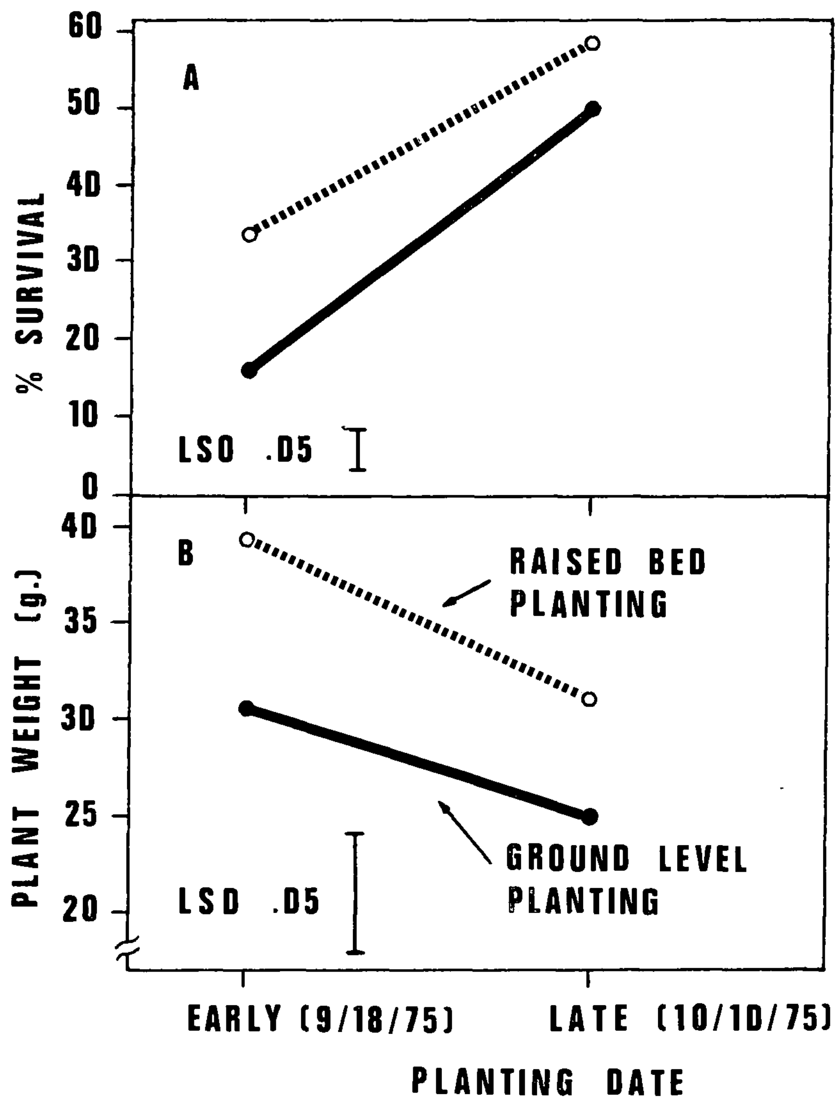

Figure 5. Effect of planting method (▮▮▮▮ = raised bed; ▬ = ground level) and planting date on: A) percent survival, and B) plant weight at Corvallis.

Table 6. Effect of planting date (Sept. 18, 1975 and Oct. 10, 1975) on percent survival, plant weight, and damage rating of seven genotypes at Corvallis, Oregon.

Genotype	Percent survival			Plant weight (g)			Damage rating		
	Planting date			Planting date			Planting date		
	Early[C]	Late[C]	Genotype[B] means	Early[F]	Late[F]	Genotype[E] means	Early[1]	Late[I]	Genotype[H] means
AW	70.7	87.1	78.9	87.6	60.9	74.3	1.9	0.4	1.2
WH1	11.9	52.5	32.2	20.2	22.5	21.4	8.1	6.3	7.2
WH2	31.8	51.9	41.9	39.8	20.4	30.1	7.2	5.4	6.3
WH3	34.1	72.0	53.1	42.3	26.5	34.4	6.5	2.0	4.3
INT1	8.8	44.9	26.8	27.7	30.1	28.9	8.1	5.4	6.7
INT2	16.8	68.0	42.4	28.3	38.8	33.6	7.9	2.6	5.3
S3	0.0	1.7	0.8	0.0	2.2	1.1	9.0	8.6	8.8
Planting date means	24.9[A]	54.0[A]		35.1[D]	28.8[D]		7.0[G]	4.4[G]	

	Percent survival	Plant weight (g)	Damage rating
Planting date means	[A] 5% LSD = 9.2	[D] NSD	[G] 5% LSD = 0.5
Genotype means	[B] 5% LSD = 7.4	[E] 5% LSD = 6.4	[H] 5% LSD = 0.7
Planting date x genotype means	[C] 5% LSD = 10.5	[F] 5% LSD = 9.0	[1] 5% LSD = 0.9

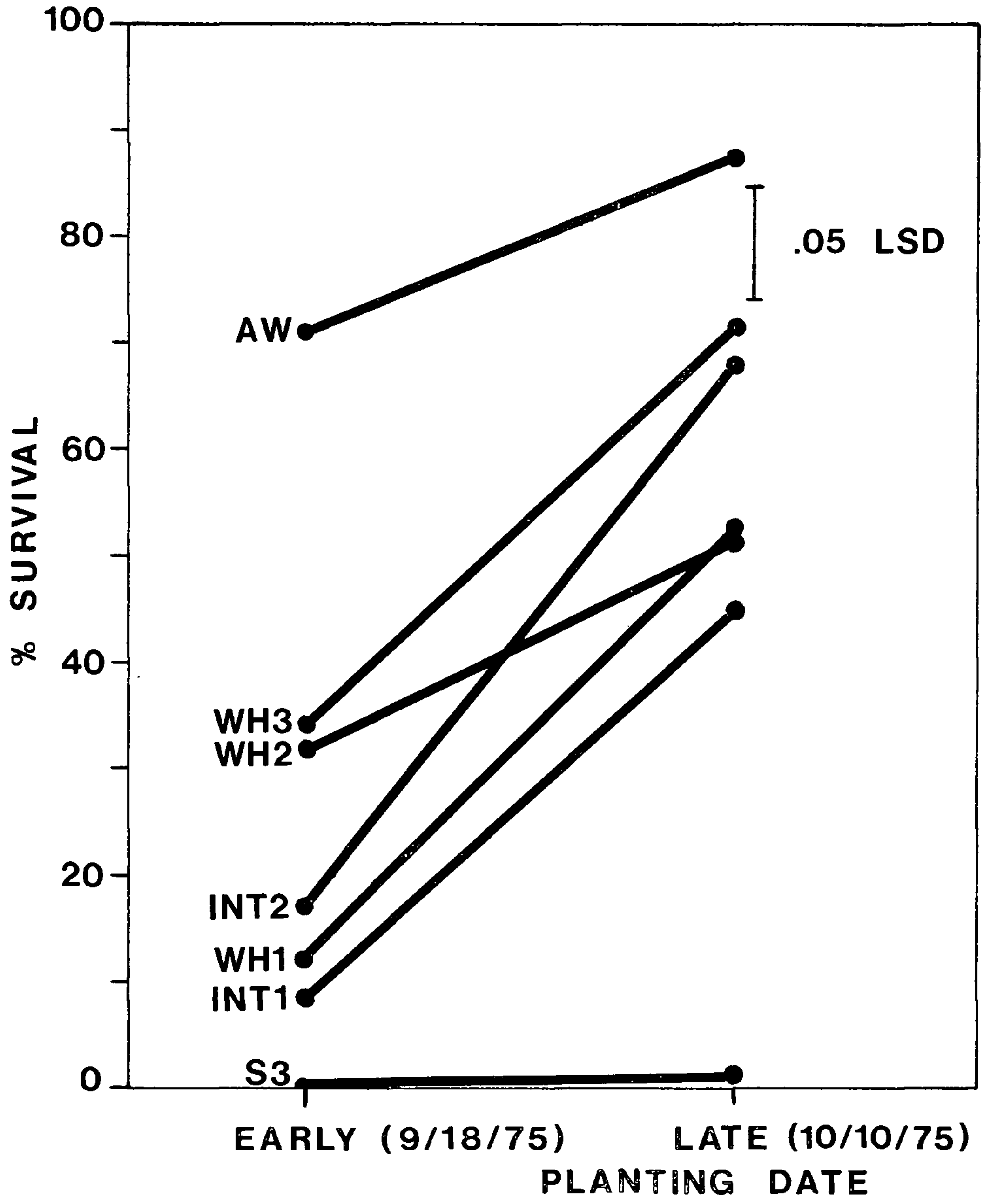

Figure 6. Effect of genotype and planting date on percent survival at Corvallis.

Weston

The surviving plants did not show damage that could readily be attributed to frost. Thus, the damage rating was not applicable.

Percent survival and plant weight data were individually analyzed as a randomized block design with four replications of a factorial arrangement of ten genotypes and two planting methods. The correlation coefficient (r) between the variables percent survival and plant weight was +0. 76.

The mean percent survival was higher for ground level plots than raised beds (Table 7). There was no significant difference in mean plant weight between the two planting methods (Table 7). There were differences in percent survival and plant weight between certain genotypes (Table 7). Genotypes AW and WH3 had higher percent survival than all others.

Figure 3B ranks the Weston genotypes from lowest to highest percent survival and shows how planting method affected each. The interaction was significant for percent survival. Ground level planting significantly increased survival rates for the two least hardy genotypes, INT1 and INT2 (Figure 3B). The remaining genotypes were not significantly affected by planting method.

The analysis of variance also indicated a significant difference in mean percent survival between replications but no significant difference in plant weight between replications.

Table 7. Effect of planting method on percent survival and plant weight of seven genotypes at Weston, Oregon.

Genotype	Percent survival			Plant weight (g)		
	Planting method			Planting method		
	Ground level[C]	Raised bed[C]	Genotype means[B]	Ground level[F]	Raised bed[F]	Genotype means[E]
AW	80.0	84.8	82.4	7.4	6.3	6.9
WH1	58.1	43.3	50.9	2.5	1.6	2.1
WH2	70.5	59.0	64.8	2.2	3.7	3.0
WH3	77.3	87.3	82.3	5.4	4.6	5.0
INT1	23.8	0.0	11.9	2.3	0.0	1.2
INT2	61.3	25.5	43.4	4.4	3.9	4.2
S3	0.0	0.0	0.0	0.0	0.0	0.0
Planting method means	53.0[A]	42.8[A]		3.5[D]	2.9[D]	

	Percent survival	Plant weight
Planting method means	[A] 5% LSD = 6.2	[D] NSD
Genotype means	[B] 5% LSD = 11.5	[E] 5% LSD = 1.1
Planting method x genotype means	[C] 5% LSD = 16.3	[F] 5% LSD = 1.6

Controlled Freezing Tests

Table 8 indicates the temperature at which 50% leaching occurred. According to the percent leaching technique of assessing injury, this represents the frost killing temperature.

Figure 7 shows the mean percent leaching plotted as a function of test temperature for the genotypes AW, WH2, INT1, and S4. Studies utilizing this technique (18, 19) indicate percent leaching will continue to increase as the temperature is decreased below the frost killing temperature. For all four genotypes a maximum percent leaching value was attained and a substantial decrease in percent leaching followed. This may be attributed to the fluctuation of temperature within the chamber resulting from the refrigeration unit cycling on and off. The range in temperature during such a cycle was approximately 3° C. Thus plants that were subjected to test temperatures just above the killing temperature were continually cycled through this critical temperature. This may have resulted in more severe damage and higher than normal percent leaching.

This method of assessing injury ranks the hardened genotypes as follows (from most hardy to least hardy): WH2 > AW > INT1 > S4. In field survival studies at Weston, Oregon (see Table 7), where the minimum temperature was -13° C, the genotypes were ranked as follows according to percent survival: AW = 82.4% > WH2 = 64.8% > INT1 = 11.9% > S4 = 0%. Thus, the percent leaching method showed

Table 8. Temperatures that resulted in 50% leaching and at least 50% foliage damage of four hardened (H) and unhardened (U) pea genotypes. Cold acclimation (CA) equals the temperature difference between H and U for each genotype.

	50% leaching temperature (^oC)			$\geq$ 50% foliage damage temperature (^oC)		
Genotype	H	U	CA	H	U	CA
AW	-8	-3	5	<-10	-4	> 6
WH2	<-10	-5	> 5	<-10	-6	> 4
INT1	-7	-5	2	-7	-5	2
S4	-4	-3	1	-5	-3	2

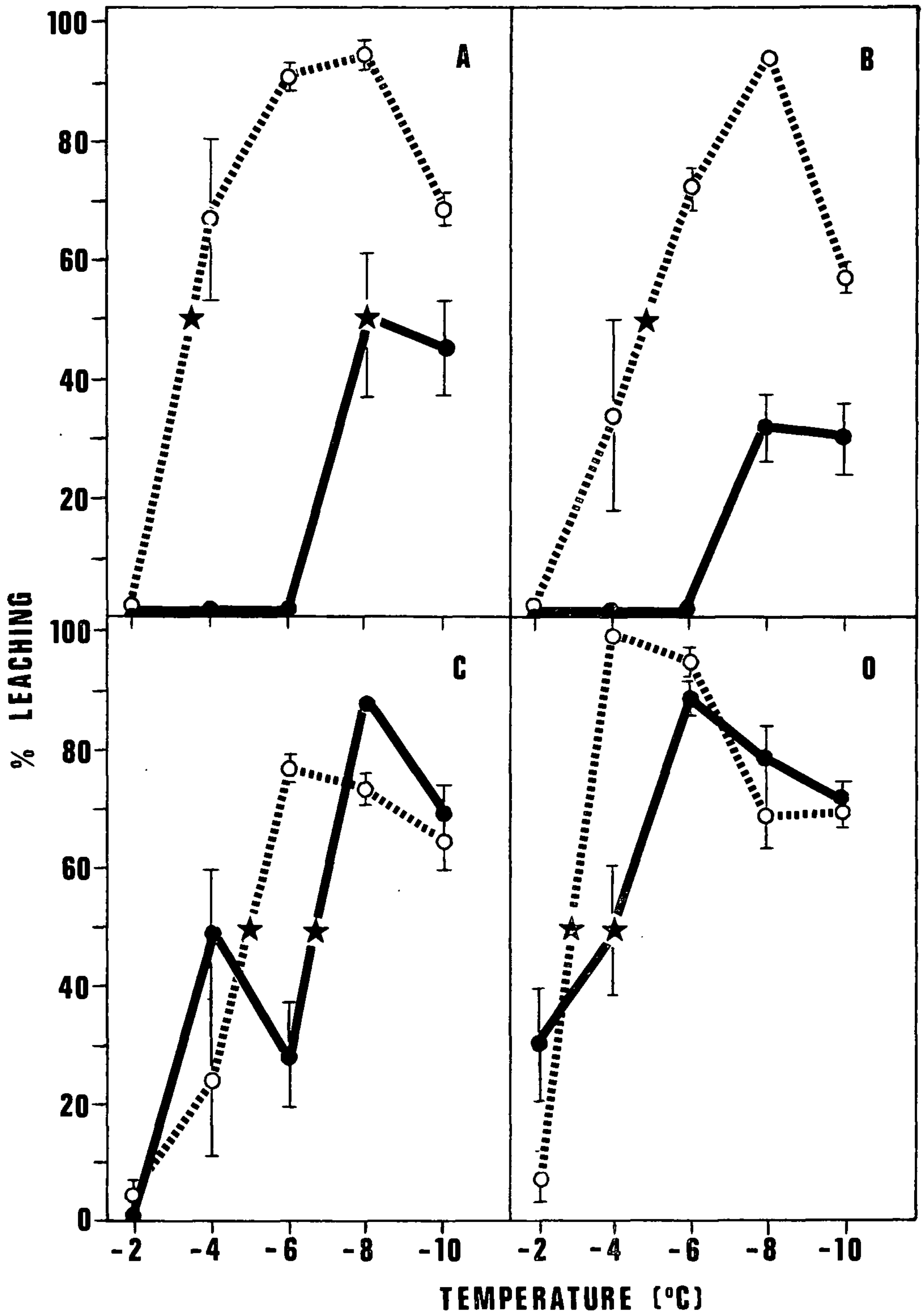

Figure 7. Percent leaching at five test temperatures, as a measure of freezing injury, of four unhardened (▪▪▪▪▪▪) and hardened (▬▬) genotypes. A) AW, B) WH2, C) INT1, and D) S4. The 50% leaching temperature (★) represents the frost killing temperature. Vertical bars represent the standard error of the mean.

WH2 to be hardier than AW, in disagreement with the results of field survival studies. This difference may be due to the large amount of variation between sample values that resulted in a high standard error of the mean, or could be caused by microclimate variation at test sites. It is suggested that a larger number of samples per test temperature should be used to give a more accurate mean percent leaching value.

Table 8 also gives the test temperature at which 50% foliage damage occurred according to a visual estimation. Preliminary freezing tests showed that when 50% or more of the foliage was damaged, little or no regrowth and recovery resulted. Figure 8 shows the frost damage index plotted as a function of test temperature for each hardened and unhardened genotype. This method ranked the genotypes in terms of hardiness as follows: AW = WH2 > INT1 > S4. Since both AW and WH2 survived the lowest test temperature, -10° C, this test did not determine which was the hardiest genotype. Field survival studies at both Corvallis and Weston ranked the genotypes as follows: AW > WH2 > INT1 > S4.

The ability of each genotype to cold acclimate (CA) was determined by calculating the difference between the hardened (H) and unhardened (U) killing temperatures (Table 8). According to both the percent leaching technique and a visual estimation of damage, genotypes with a compact growth habit, AW and WH2, acclimated more than upright genotypes, INT1 and S4 (Table 8).

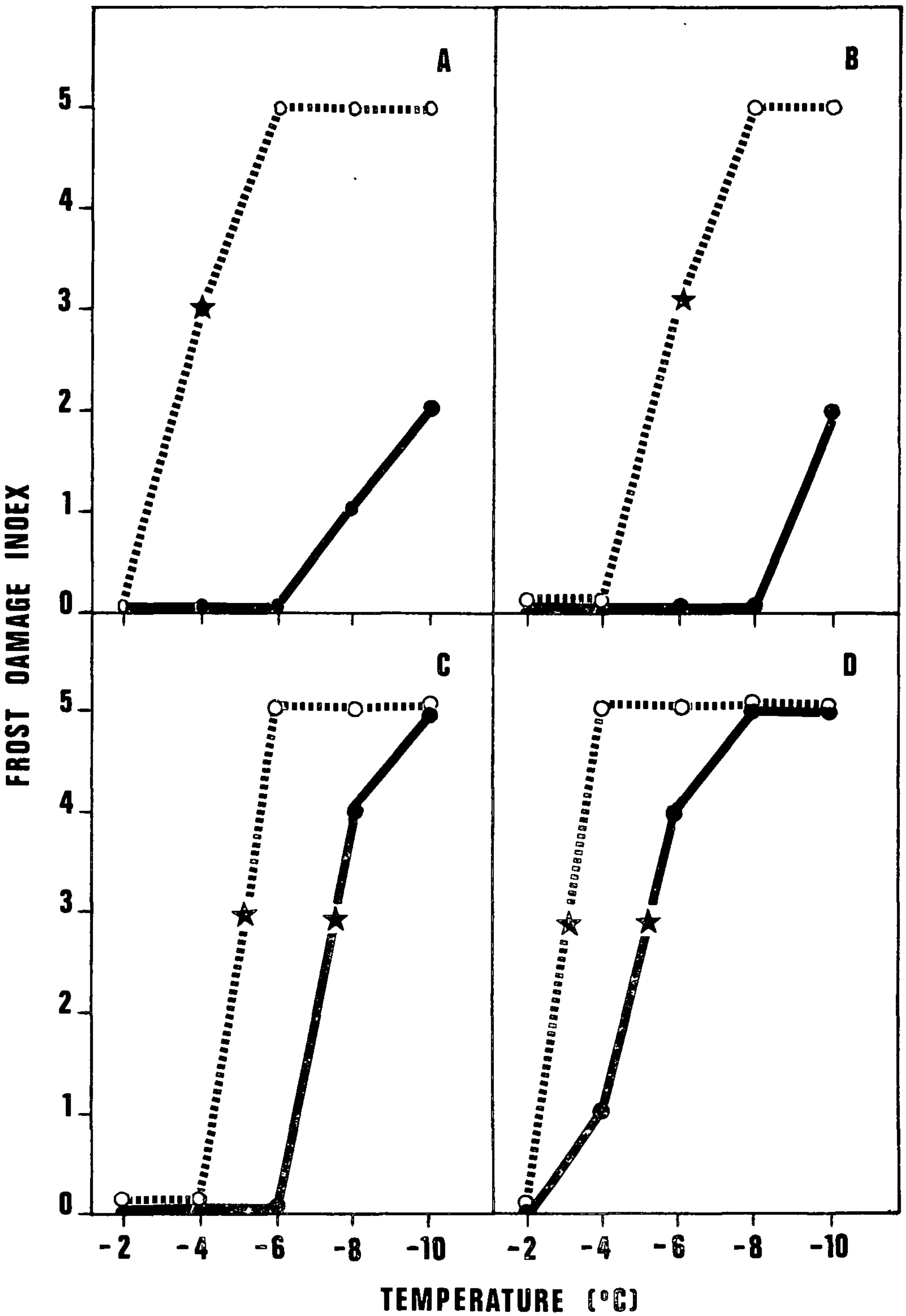

Figure 8. Frost damage index at five test temperatures of four unhardened (▪▪▪▪▪▪▪) and hardened (▬▬▬) genotypes: A) AW, B) WH2, C) INT1, and D) S4. The frost killing temperature (★) corresponds to a damage rating of 3.

DISCUSSION

Field Survival Studies

The immediate environment of the overwintering plant can greatly influence its resistance to winter stress. This study showed that winter survival of fall planted peas was improved by planting on raised beds at the Corvallis location. This was true for all genotypes that survived. Raised beds at this location improved drainage and allowed the crown region of the plant to be free from standing surface water (Figure 9). Temperature minimums that resulted in the freezing of such surface water were numerous.

At Corvallis the typical winter damage to genotypes that showed appreciable survival was confined to the crown area of the plant (Figure 10). Defoliation and decay of the exterior of the stem was common. A cross sectional cut in this area revealed living tissue in the interior of the stem. The distal end of damaged tillers remained uninjured during the period of winter stress and in the spring resumed growth, set flowers, and developed normally.

No attempts were made to isolate any fungal growth from the damaged crown area of the plants. Thus, it is not known whether the damage is due to direct freeze injury of the tissue or a secondary infection caused by microorganisms.

Plants of various genotypes were lifted from the ground with

Figure 9. Improved drainage provided by raised beds. The plot in the center foreground (B) is planted on a ground level row. Plants in ground level rows are surrounded by frozen standing surface water (A). The plot in the right foreground (C) is planted on a raised bed and is free of standing surface water.

Figure 10. Typical winter damage at Corvallis confined to the crown area of genotype INT2.

their root systems intact and examined for damage. Figure 11 illustrates one such plant from Corvallis. The roots appeared healthy and undamaged. This fact is supported by soil temperature data. A minimum soil temperature of 0° C at a depth of 5.1 cm (2 inches) occurred only three days during the winter at Corvallis.

All genotypes had higher survival rates at the later planting date. This is in agreement with other studies that indicate an early planting to be better for selecting winter hardy types because it exposes any tendency to form lush non-hardy growth (5, 6, 11).

At Weston, genotypes having horticulturally desirable traits and low levels of inherent winter hardiness had higher survival rates when planted on ground level rows. Plants grown on raised beds at this location had two environmental disadvantages affecting survival compared to ground level plants: 1) the root system was exposed to lower temperatures and for longer periods of time, and 2) the above ground portion of the plant was less likely to be protected by snow cover from wind and low air temperatures.

Winter survival of peas in Michigan was better in drifted areas than in exposed (11). During the winter at Weston there were varying amounts of snow cover. Such may have been sufficient to protect certain plants from air temperatures that killed exposed plants. This would explain why little variation in the degree of winter injury was observed between plants.

Figure 11. Winter damage at Corvallis confined to above ground portion of genotype INT2. Roots appear healthy and undamaged.

At this location the typical damage was confined to the root system. Decay and blackening of the exterior of the tap root was the common symptom of numerous plants dug from the ground. Figure 11 shows that the damage to genotype INT2 is confined to the below ground portion of the plant. The subcircular notches in the leaf margins of the plant is pea leaf weevil damage and not related to winter injury.

Although mean percent survival rates of the genotypes were similar between locations, there was a large difference in individual plant weights (compare Figure 4A and 4B). Figures 11 and 12 show the same genotype, INT2, from Corvallis and Weston respectively. The Corvallis plant developed several tillers and considerably more foliage than the Weston plant. In Weston the severity of the late fall to winter temperatures did not allow for such development.

Controlled Freezing Tests

Both the percent leaching technique and the visual estimation of foliage damage could be useful techniques to utilize in a breeding program for screening winter hardy segregates. The latter involves less equipment and is less time consuming. It would be more conducive to screening large populations of plant material.

Both methods of determining freezing injury gave similar results. According to percent leaching, genotypes with a compact

Figure 12. Winter damage at Weston confined to the below ground portion of genotype INT2. The exterior of the roots appear blackened and decayed. The horizontal line represents the soil surface.

growth habit, AW and WH2, cold acclimated more ($\geq$5° C) than genotypes with an upright growth habit, INT1 and S4 (1-2° C). This difference is due to cold acclimation (a physiological response) and not growth habit alone since both compact and upright genotypes were killed at similar test temperatures (-3 to -5° C) in the unhardened state.

In controlled freezing studies, the percent leaching technique showed the compact and upright genotypes, AW ('Austrian Winter') and S4 ('Alaska') respectively, were killed at the same test temperature in the unhardened state. Thus, it appears that the compact growth habit in itself, without physiological hardening of the tissue, does not allow for survival at lower temperatures under laboratory conditions.

SUMMARY AND CONCLUSION

1) Since damage rating and percent survival were closely correlated (r=-0.93), it is suggested that a visual estimation of damage alone would suffice to distinguish relative levels of winter hardiness within pea genotypes. Such a method of determining differences is much less time consuming than determination of percent survival.

2) Planting on raised beds at Corvallis improved the percent survival of all surviving genotypes.

3) Planting on raised beds at Weston significantly decreased the percent survival of the least hardy surviving genotypes.

4) The later planting date improved survival rates of all surviving genotypes. The earlier planting date was conducive to selecting winter hardy types.

5) At Corvallis the typical winter damage was confined to the crown area of the plants. This type of damage did not occur in Weston. There, frost damage was confined to the roots.

6) Both methods used for differentiating levels of cold hardiness in controlled freezing studies gave similar results. A visual estimation was preferred over the electrolyte leaching technique because of the simplicity and rapidity of the method.

7) Genotypes that cold acclimated the most under laboratory conditions also had the highest winter field survival rates.

8) In the controlled freezing tests inherent cold hardiness was associated with the compact growth habit. Breeding lines having a compact growth habit tend to have undesirable culinary seed. Thus, it appears that cold hardiness in peas may not be inherited independently of other characteristics.

BIBLIOGRAPHY

1. Bleasdale, J. K. A. 1973. Plant physiology in relation to horticulture. Macmillan, London. 144 pp.

2. Burke, M., L. V. Gusta, H. A. Quamme, C. J. Weiser, and P. H. Li. 1976. Freezing and injury in plants. Ann. Rev. Plant Physiol. 27:507-527.

3. Dexter, S. T. 1956. The evaluation of crop plants for winter hardiness. Advances in Agronomy. 8:203-236.

4. ___________, W. E. Tottingham, and L. F. Graber. 1932. Investigations of the hardiness of plants by measurements of electrical conductivity. Plant Physiol. 7:63-78.

5. Dowker, B. D., 1969. Field methods of assessing winter hardiness in peas. Euphytica. 18:398-402.

6. Holland, H., and G. J. Frost. 1963. Annual Report, Nation Vegetable Research Station, Wellesbourne. 13:21-23.

7. Khitrinskii, V. F. 1960. Guided modification of the heredity of nonwintering pea plants to overwinter pea plants. Agrobiologia. 6:860-865.

8. Levitt, J. 1956. The hardiness of plants. Academic Press Inc., N. Y. 278 pp.

9. ________. 1972. Responses of plants to environmental stresses. Academic Press Inc., N. Y. 697 pp.

10. Luyet, B. J. 1931. Variation of the electrical resistance of plant tissue for alternating currents of different frequencies during death. J. Gen. Physiol. 15:283-287.

11. Markarian, D., and R. L. Andersen. 1966. The inheritance of winter hardiness in pisum. I. Euphytica. 15:102-110.

12. ____________, R. R. Harwood, and P. R. Rowe. 1968. The inheritance of winter hardiness in Pisum. II. Description and release of advanced generation breeding lines. Euphytica. 17:110-113.

13. McLeester, R. C., C. J. Weiser, and T. C. Hall. 1968. Multiple freezing points as a test for viability of plant stems in the determination of frost hardiness. Plant Physiol. 44: 37-44.

14. Olien, C. R. 1967. Freezing stresses and survival. Ann. Rev. Plant Physiol. 18:387-408.

15. Richardson, D. G., and C. J. Weiser. 1972. Methods for evaluating potato foliage frost resistance. Prospects for the potato in the Developing World. E. R. French (ed.), Centro Internacional de la Papa, Lima Peru. pp. 217-221.

16. Senate Report. 1960. Cold weather agriculture. 86th Congress, Document no. 91. U. S. Government Printing Office, Wash., D. C. 48 pp.

17. Steponkus, P. J., and F. O. Lanphear. 1967. Refinement of the triphenyl tetrazolium chloride method of determining cold injury. Plant Physiol. 42:1423-1426.

18. Sukumaran, N. P., and C. J. Weiser. 1972a. An excised leaflet test for evaluating potato frost tolerance. HortScience. 7:467-468.

19. ______________, and ____________. 1972b. Freezing injury in potato leaves. Plant Physiol. 50:564-567.

20. Tumanov, I. I., and O. A. Krasavtsev. 1966. Elucidation of the conditions required for vitrification of plant cells. Soviet Plant Physiol. 13:127-133.

21. Vasil'yev, I. M. 1956. Wintering of plants. Translated by American Institute of Biological Science, (1961), Washington, D. C. 300 pp.

22. Wade, B. L. 1941. Frost tolerance of strains of market garden peas. Proc. Amer. Soc. Hort. Sci. 38:530-534.

23. Weiser, C. J. 1970. Cold resistance and injury in woody plants. Science. 169:1269-1278.

24. Wellensiek, S. J. 1925. Genetic monograph on Pisum. Bibliographia Genetica. 2:343-476.