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Propagation of *Euphorbia esula* for leafy spurge biocontrol agents[1]

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Abstract:

Efforts to screen and mass-rear insects and diseases for leafy spurge biocontrol agents have been hampered by low success in propagation and slow growth of leafy spurge in the greenhouse. The optimum greenhouse conditions for leafy spurge growth were determined. Leafy spurge was propagated from stem tip cuttings, with the basal end treated with 0.2% NAA, and the plants misted with water for 10 days. Optimum conditions for growth were 27ºC air temperature, application of a complete fertilizer at 70 kg ha^{-1} weekly or 135 kg ha^{-1} biweekly 20 days after stem tip propagation, in a peat/perlite/vermiculite growth medium at pH 7 and a 16-hour photoperiod. Regrowth from roots of parent plants was improved when cuttings were taken from plants at least 60 days old, and plants grew nearly twice as rapidly when the medium was maintained at 30ºC compared to 22ºC. Refrigeration of stem tip cuttings or roots before planting did not affect survival or growth vigor. Only gibberellic acid of nine plant growth regulators evaluated increased growth, but plants were etiolated. Biotypes from Nebraska and South Dakota were shorter than five others from the United States or Austria but had similar root and shoot dry weight. The time required to propagate vigorous leafy spurge was reduced to 2 months compared to 6 months required prior to the study.

Nomenclature:

NAA, 1-naphthalene acetic acid, gibberellic acid, *ent*-3α,10,13-trihydroxy-20-norgibberell-1, 16-diene-7,19-dioc acid 19,10 lactone; leafy spurge (*Euphorbia esula* L.) #[2] EPHES.

[1] Received for publication June 28, 1991, and in revised from December 16, 1991. Published with approval of the Director, Agric. Exp. Stn., North Dakota State Univ. as J. Art. No. 1949.

[2] Letters following this # symbol are a WSSA-approved computer code from Composite List of Weeds, Revised 1989. Available from WSSA, 309 West Clark Street, Champaign, IL 61820.

Additional index words:

Temperature, water, photoperiod, pH, fertilizer, chilling, heating, biotypes, EPHES.

Introduction

Leafy spurge infests pasture and rangeland, causing economic losses of over $75 million annually in North Dakota and probably over $350 million annually in the United States (26). The plant reduces carrying capacity of rangeland to near zero because cattle will not graze in areas with a 10 to 20% leafy spurge cover (13, 18). Landowners in the United States spend more than $5 million annually for chemical leafy spurge control. Cultural control including cropping, mowing, grazing, and cultivation has had only limited success (2, 14, 16).

Leafy spurge is a good candidate for biological control because it is difficult to control with conventional methods. Several insects have been introduced into the United States as potential biocontrol agents (6, 9). Insects successfully established in the northern Great Plains for leafy spurge biocontrol include several flea beetles: *Aphthona flava* Guill., *A. cyparissae* (Koch), *A. czwalinae* (Weise), and *A. nigriscutis* Foudias (Chrysomelidae); a long-horn beetle *Oberea erythrocephala* (Schrank.) (Cermambycidae); and a gall midge *Spurgea esulae* (Gagne) (Cecidomyidae) (6). More insect species are needed to control leafy spurge seed production, topgrowth, and roots during all leafy spurge growth stages if biocontrol is to be successful. However, screening, mass rearing, and field introduction of insects used as biocontrol agents has been hampered by the difficulty in propagating sufficient plant material in the greenhouse.

Plants propagated from seed or root cuttings often take up to 6 months to reach the maturity needed for experiments (12). Field cuttings often fail to grow more than 2 cm after 3 months in the greenhouse. Many researchers have evaluated the optimum growing conditions of poinsettia (*Euphorbia pulcherrima* Wild.), a related species, and the propagation methods described could be useful for leafy spurge propagation.

A large amount of plant material must be available continually to mass-rear biocontrol insects and for other laboratory research. The purpose of this research was to establish the optimum conditions for leafy spurge growth in greenhouse and controlled-environment chambers.

Materials and methods

General

Leafy spurge plants initially were propagated from one accession (84ND001)[3] that was obtained from a natural infestation near Fargo, ND. Stem tips about 65 to 75 mm long were cut from parent clones during the vegetative growth stage. Stems of the parent plants were then cut back to the soil surface and plants regrew from roots. All but the upper three to four leaves were removed from the cuttings and the basal end was dipped in a commercial mixture of 0.2% naphthalene acetic acid (NAA)[4]. Cuttings treated with NAA established root systems much faster than nontreated cuttings in preliminary experiments and resulted in a two- and four-fold increase in stem and root growth, respectively, at harvest (data not shown). Each cutting was planted to a 40-mm depth in a 4-cm-diameter by 20-cm-long conical pot[5] which contained a potting medium of peat, perlite, and vermiculite[6]. The plants were misted for 10 days in an enclosed system that was cycled on and off by responding to water evaporation from a wire mesh simulated leaf[7]. As the water evaporated and the weight on the simulated leaf was no longer heavy enough to offset a counterbalance, an electrical switch was activated and the mister came on until the weight of the water on the simulated leaf again offset the counterbalance.

Preliminary experiments found misting was essential for the stem cuttings to begin growth but too much moisture resulted in rot. Thus, it was important to regulate the misting system (3). The growth media surface should be moist but not saturated. Plants were removed from the mist chamber, allowed to grow 10 more days, and 20-day-old cuttings were selected for uniformity for experiments.

Plants were harvested 36 days after treatment for each experiment, and stem height, diameter, and dry weight were determined for most experiments. This time interval was chosen because plants were then 56 days old, vegetative growth had declined, and flowering had started. An electronic caliper was used to measure stem diameter about 20 mm above the stem cut. Roots were washed to remove soil, and root diameter and dry weight were determined. Diameter of the largest root was measured about 5 mm below the callus tissue.

Initially, plants were grown in the greenhouse at 24 to 27ºC with supplemental light (400 $\mu E\ m^{-2}\ s^{-1}$ photosynthetic photon flux density) when necessary for a 16-hour light and 8-hour dark photoperiod. Each experiment was repeated once or twice and had similar variance, so the combined data are presented. Experiments were in a randomized complete block design and had 14 plants per treatment and three replications. The data were

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[3] Registry of leafy spurge accessions maintained by David G. Davis, USDA Biosciences Res. Lab., Fargo, ND 58105. Identification sequence is year collected, U.S. postal code for state, province, or country, and order of registration.

[4] Rootone. Pratt-Gabriel Div., Miller Chem. and Fertilizer Corp., Hanover, PA 17331.

[5] Cone-tainer Nursery, Canby, OR 97013

[6] Sunshine Mix No. 1, Patented formulation with wetting agents. Fisons Western Corp., Downers Grove, IL 60515

[7] Mist-A-Matic. E. C. Geiger, Inc., Harleysville, PA 19438

were subjected to analysis of variance and means were separated using a protected LSD test at P = 0.05.

Temperature, water, day length, and growth medium

Leafy spurge growth at 20, 23, 27, and 30ºC was determined in separate greenhouses. The plants were propagated as previously described and were fertilized once using a 23:19: 17 (N,P,K) water-soluble fertilizer at a rate of 110 kg N ha^{-1} (4.5 µg g^{-1} growth media) in 10 ml water when 20 days old. Plants were surface watered as needed for acceptable growth. However, it was observed that plants had to be watered thoroughly so soil at the top of the conical pot was wetted two to three times per watering. This allowed for thorough wetting of the growth medium throughout the length of the conical pot, otherwise dry zones developed in the middle of the pot between waterings. There were 24 plants per treatment rather than 14 for this experiment only, and greenhouses were replicated. Plants were grown at 27ºC in all subsequent experiments.

The effect of water quality on leafy spurge growth was determined using distilled and tapwater. Plants were propagated and fertilized as previously described and watered either with distilled or Fargo, ND municipal water when removed from the misting chamber (10 days old). The municipal water was chlorinated, had a pH of 8.9, with a Ca^{2+}, Mg^{2+}, Na^{2+}, Cl^{-} and HCO_3^{-} content of 31, 25, 27, 17, and 98 mg L^{-1}, respectively. Water quality had no effect on growth, so plants were surface watered using municipal water for all subsequent experiments.

The effect of day length on leafy spurge growth was determined. Plants were propagated and fertilized as previously described. The photoperiod ranged from 10 to 20 hours. The experiment was conducted from November 1 to March 21 when the natural day length in Fargo, ND, was 10 hours or less. Plants were grown on separate tables that were separated by 0.23-mm black plastic tarps. The tarps were opened during the workday to allow air circulation but closed in the late afternoon to regulate day length. Plants were rotated among tables weekly during the experiment, and the photoperiod of the lights above each table was adjusted accordingly.

Effect of growth medium on leafy spurge growth was determined by comparing a commercial potting soil mix[8] containing a mixture of loam soil, sphagnum peat moss, perlite, and sand (91:5:2:1); a peat, perlite, and vermiculite mixture[6;] sand alone; and peat moss alone. All plants were fertilized weekly with 22 kg N ha^{-1} (100 µg g^{-1} growth medium) rather than 110 kg N ha^{-1} once because of rapid nutrient movement through the sand. The plants in sand were kept in the mist chamber during the entire experiment because the sand dried rapidly.

Effect of growth medium pH on leafy spurge growth was determined. Plants were propagated in the peat, perlite, and vermiculite medium[6] as previously described except the pH was adjusted to 5, 6, 7, 8, or 9 using aluminum sulfate [$A1_2(SO_4)_3$] or hydrated lime ($CaCO_3$).

[8] All Purpose Potting Soil. Hyponex Corp., 1411 Scottslawn Rd., Marysville, OH 43041

Fertilizer

The optimum rate and ratio of N,P,K was determined by varying either laboratory mixed formulations using water soluble 46-0-0, 0-44-0, and 0-0-60 N,P,K[9] or different commercially available water-soluble fertilizers. The N rate varied from 110 kg/ha^{-1} (456 µg g^{-1} growth medium) to 330 kg/ha^{-1} (1368 µg g^{-1} growth medium) in the first experiment and was held constant at 110 kg/ha^{-1} in the second experiment with commercially formulated fertilizers. Mo was also applied alone and with the fertilizer mixtures at various ratios from 1 to 40 µg g^{-1} only in the first experiment. Plants were propagated as previously described at 27ºC with a 16-hour photoperiod.

The optimum fertilizer rate was determined using water-soluble commercial N,P,K mixtures of 23-19-17, 15-30-15, and 36-6-6[9]. Each fertilizer was applied at 0, 110, 220, 440, 880, or 1760 kg N ha^{-1}. The optimum rate was further defined in a second experiment when the fertilizers were applied at various rates from 110 to 360 kg N ha^{-1}. The optimum frequency of fertilization was determined by applying a water-soluble fertilizer at 35 or 70 kg N ha^{-1} weekly; 100, 135, or 170 kg N ha^{-1} biweekly; or 200, 235, or 270 kg N ha^{-1} monthly. Plants were harvested 60 days after the first fertilizer application rather than 36 days as in other experiments.

Propagation techniques

Various greenhouse techniques and timing of treatments were evaluated to further increase the growth rate of leafy spurge. A water-soluble commercial N,P,K mixture of 15-30-15 was applied at 275 kg N ha^{-1} to 20-, 30-, 40-, and 50-day-old plants. The plants were harvested as previously described when 80 days old. Thus, the 20- and 50-day-old plants were harvested 60 or 30 days after fertilization, respectively.

Plants grown for several months in the greenhouse tended to become less vigorous than plants propagated from the field. Thus, the plants were "batted" daily; i.e. sprayed with water using a high-pressure garden nozzle, bending the plants nearly horizontal. Cuttings propagated from the batted plants were compared to nonbatted plants for both growth and vigor.

The age of plants required before cuttings could be taken without adversely affecting parent plant regrowth from the roots was determined. Plants were propagated as previously described and fertilized using the commercial mixture of 15-30-15 (N,P,K) at 270 kg N ha^{-1} when 20 days old. The plants were grown at 27ºC with a 16-hour photo period, stems were cut at the soil level 30, 45, 60, 75, or 90 days after treatment, and allowed to regrow from the roots. The regrowth was harvested 36 days after the first cutting.

Often plant cuttings must be shipped to other research labs or kept in short-term storage until ready for use. The effect of chilling prior to planting stem tip cuttings and on regrowth from roots of parent plants after topgrowth removal was determined. Stem tips used for propagation were wrapped with wet paper towels, placed in plastic bags, and

[9] Peters Fertilizer Products. W. R. Grace and Co., P.O. Box 789, Fogelsville, PA 18051. Sources of N:P:K nutrients were a mixture of NH_4^+, NO_3, and $(NH_2)_2$ (1:1:2.5); P_2O_5, and K_2O, respectively.

chilled at 4ºC for periods from 0 to 96 hours before planting. The number of plants successfully rooted and subsequent growth were determined. For the root experiment, top-growth was completely removed from 120-day-old plants while roots remained in the pots and growth media and were chilled to 2ºC for 48 hours, or 1, 2, 4, or 8 weeks. The plants were then moved to the greenhouse and plant survival and regrowth were evaluated 56 days after removal from the cold.

Effect of growth medium temperature during misting on establishment and subsequent growth was determined. The conical pots were heated while the cuttings were in the mist chamber to evaluate the effect of growth medium temperature on root growth. A commercial heating tape[10] was placed between rows of pots in the mist chamber so that one side near the middle of the pot was in contact with the heating element. Heated and control growth media were maintained at 30 and 22ºC, respectively. Plants were removed from the mist chamber, the heat tape was removed, and plants were harvested when 20 days old or were fertilized with a commercial mixture of 15-30-15 (N,P,K) at 275 kg N ha^{-1} and harvested 56 days after planting.

The effect of nine plant growth regulators (PGR)[11] applied as a soil drench at 1, 10, 100, and 1000µg g^{-1} soil on leafy spurge growth was evaluated. Plants were propagated as previously described and were fertilized with a commercial mixture of 15-30-15 (N,P,K) at 110 kg N ha^{-1} 3 days before the PGR was applied. Plants were harvested 36 days after PGR application.

Growth regulators evaluated included BA (6-benzylamino purine), chlormequat chloride [2-(chloroethyl)trimethylammonium chloride], ethephon [2-(chloroethyl)phosphonic acid], flurprimidol {α-(1-methylethyl)-α-[4-(trifluoromethoxy)phenyl]-5-primidine-methanol}, gibberellic acid (GA_3)[9,11] IAA (indole-3-acetic acid), kinetin [6-furfuryl-amino purine], MH (1,2-dihydro-3,6-pyridazinedione), and NAA.

Biotype response

Seven leafy spurge biotypes were propagated from cuttings and grown with the predetermined optimum greenhouse and growth techniques. The biotypes included 78AS001[3] from Austria; 79MB001 from Manitoba, Canada; and 80MT002, 79NE003, 84ND001, 80SD001, and 80WY001 from Montana, Nebraska, North Dakota, South Dakota, and Wyoming, respectively. Plants were propagated as previously described, grown at 27ºC with a 16-hour photoperiod in the peat, perlite, and vermiculite growth medium[6] at pH 7. The plants were fertilized with a commercial mixture of 15-30-15 (N,P,K) at 270 kg N ha^{-1} when 20 days old, watered as needed, and harvested when 56 days old.

[10] Easy-heat Electric Pipe Heating Cable, U.S. 20 East, New Carlisle, IN 46552.

[11] Abbreviations: PGR, plant growth regulator; GA, gibberellic acid.

Results and discussion

Temperature, water, day length, and medium

The optimum temperature for leafy spurge shoot and root growth in the greenhouse was 27ºC (Table 1). Plants tended to grow taller at 30ºC but the amount of root growth was less than at 27ºC. Temperature did not affect either stem or root diameter. Roots grew the length of the container in this and all subsequent experiments, so root length measurement was not useful in determining optimum growing conditions.

These results are similar to those with poinsettia, which produced more topgrowth and matured faster at warm (21 to 26ºC) than cool (14 to 18ºC) temperatures but often produced inferior flowers (1, 10, 24). Since flower production is not important with the insects currently being propagated for leafy spurge control, the temperature that rapidly produced the most stem and root biomass for insect feeding was considered optimum.

Poinsettia production problems occasionally have been traced to water quality (10). Also, poinsettia plants maintained in media with 35 to 75% moisture-holding capacity often develop root rot (4, 10). Leafy spurge growth, vigor, and color were not influenced by water source (Table 1). No root disease problems were encountered during these experiments. Conservation of water resources is becoming increasingly important and automated watering systems that have been developed for poinsettia (25) could be adapted to leafy spurge production.

A 16- to 20-hour photoperiod was optimum for increased leafy spurge height and root and shoot diameter (Table 1). The numerically highest shoot and root biomass was produced when the plants were exposed to 18- to 20-hour photoperiods. However, plants exposed to such long photoperiods became etiolated. Thus, the optimum day length for healthy growth was 16 hours.

Leafy spurge plants grew best in a commercial potting soil[8] or a peat, perlite, and vermiculite mixture[6] (Table 1). This was similar to poinsettia which grew best in a peat:perlite mixture (8). Leafy spurge grew slowly in sand alone, and no plants grew in peat moss alone, probably because of the low pH.

A medium pH of 7 was optimal for leafy spurge shoot and root growth (Table 1). Growth decreased dramatically at or below pH 6 and at pH 9. Thus, leafy spurge has a narrower pH range for good growth than poinsettia, which grew equally well in soil with pH levels between 4.4 and 8.0 (5).

Table 1. Effect of air temperature, water source, day length, and growth medium on leafy spurge growth 56 days after propagation from stem cuttings in the greenhouse.

Treatment	Shoot			Root	
	Height	Diameter	Dry weight	Diameter	Dry weight
	mm		mg	mm	mg
Temperature (C)					
20	90	12	160	1.0	240
23	90	1.2	150	0.9	200
27	100	1.3	210	0.9	230
30	110	1.3	205	0.9	190
LSD (0.10)	10	NS	22	NS	22
Regression[a]	L*	L*	C*	NS	C*
Water source:					
Municipal	130	1.4	290	0.9	220
Distilled	130	1.3	230	0.8	200
LSD (0.05)	NS	0.05	NS	NS	NS
Photoperiod (hour):					
10	160	1.6	270	1.0	190
12	150	1.7	300	1.0	230
14	160	1.7	350	1.0	250
16	180	1.8	420	1.1	290
18	170	1.8	440	1.0	300
20	180	1.8	480	1.1	320
LSD (0.05)	10	0.07	32	0.07	36
Regression[a]	NS	NS	L**	NS	L**
Growth medium:					
Peat moss	0	0	0	0	0
Peat/perlite/ vermiculite[b]	140	1.5	290	0.8	220
Loam/soil/peat perlite sand[c]	150	1.5	350	0.8	240
Sand	80	1.0	130	0.6	120
LSD (0.05)	11	0.1	30	0.1	22
pH of medium:					
5	25	0.2	35	0.2	20
6	30	0.4	40	0.2	20
7	140	1.5	265	0.7	150
8	95	1.2	130	0.5	110
9	40	0.7	55	0.4	60
LSD (0.05)	25	0.3	45	0.15	30
Regression	C**	C**	C**	C**	C**

[a]Best fit regression model L = linear, Q = quadratic, or C = cubic equation and significance at the P = 0.05 (*) or P = 0.01 (**) level.

[b]Sunshine Mix No. 1, patented formulation. Fisons Western Corp., Downers Grove, IL 60515.

[c](91:5:2:1), All Purpose Potting Soil. Hyponex Corp., 1411 Scottslawn Rd., Marysville, OH 43041.

Fertilizer

In general, leafy spurge grew similarly regardless of the N,P,K ratio (Table 2). High relative ratios of K tended to reduce plant height but there was no consistent effect on

stem diameter, foliage production, or root growth. Various ratios of N,P,K applied as commercial water-soluble fertilizers had little effect on leafy spurge growth at equal nitrogen concentrations. This is similar to poinsettia, which required a relatively high amount of nitrogen but both P and K were less important (10, 17, 23).

Poinsettia growth is sensitive to Mo and becomes chlorotic and/or slow growing when additional Mo is not added with the fertilizer (7, 10, 11). However, Mo did not affect leafy spurge even when applied up to 40 μg g^{-1}, which was three times the recommended rate for poinsettia (data not shown).

In the initial fertilizer rate experiment, leafy spurge grew best when a water-soluble fertilizer was applied at rates between110 and 440 kg N ha^{-1} (Table 3). Growth decreased when the nitrogen rate was increased to 880 kg N ha^{-1} or more, and the plants died at 1760 kg N ha^{-1}. The optimum fertilizer rate was further defined to be within a range of 165 to 330 kg N ha^{-1}.

Frequency of fertilizer application was less important than rate. In general, leafy spurge growth was similar when the plants received at least 70 kg N ha^{-1} weekly or 135 kg N ha^{-1} biweekly but tended to decline when fertilizer was applied monthly (Table 3). Poinsettia should be fertilized weekly with a water-soluble fertilizer to avoid cyclic nutrient concentration (10). A water-soluble fertilizer applied weekly with irrigation in this study was convenient and resulted in healthy and vigorous growth.

Table 2. Effect of various N,P,K rates and ratios and water-soluble fertilizer rates on leafy spurge growth when applied 20 days after planting stem cuttings.

	Shoot[a]			Root[a]	
Nutrient	High	Diameter	Dry weight	Diameter	Dry weight
	——— mm ———		mg	mm	mg
N,P,K ratio[b]:					
2-2-1	170	2.0	570	1.2	370
2-1-2	150	1.9	490	1.1	370
4-2-1	170	2.0	570	1.2	360
4-1-2	160	2.0	540	1.1	350
6-2-1	170	1.9	540	1.2	370
6-1-2	170	1.9	570	1.2	400
4-4-2	180	2.0	600	1.2	390
4-2-4	160	2.0	520	1.2	340
6-6-3	190	2.1	620	1.2	360
6-3-6	170	1.9	560	1.2	340
LSD (0.05)	22	NS	NS	NS	NS
N,P,K ratio[c]:					
15-30-15	200	1.9	590	1.1	340
18-24-16	200	1.9	570	1.1	370
19-24-18	190	1.8	540	1.1	360
20-20-20	210	1.9	560	1.1	350
23-19-17	190	1.8	530	1.1	350
30-10-10	200	1.8	530	1.1	320
36-6-6	190	1.7	505	1.0	310
LSD (0.05)	NS	NS	NS	NS	32

[a]Plants were harvested 36 days after application (56 days after planting).
[b]1 = 56 kg ha^{-1} or 228 μg g^{-1} for each nutrient.
[c]N fertilization rate was kept constant for each treatment at 110 kg ha^{-1} or 456 μg g^{-1} growth medium.

Propagation techniques

Leafy spurge grew best when fertilized 10 days after removal from the mist chamber (20-day-old cutting) (Table 4). Shoot height, dry weight, and root dry weight declined by at least 27, 46, and 54%, respectively, when fertilization was delayed by 10 days or more. Plants fertilized when less than 20 days old were stunted or died (data not shown).

Table 3. Effect of nitrogen rate and application frequency on leafy spurge growth when applied 20 days after planting stem cuttings.

	Shoot			Root	
Nutrient parameter	Height	Diameter	Dry weight	Diameter	Dry weight
	——— mm ———		mg	mm	mg
Nitrogen rate (kg ha^{-1})[a] (Experiment 1):					
0	80	1.3	170	0.7	180
110	170	1.8	450	1.0	310
220	190	1.9	520	1.0	310
440	160	1.7	440	0.9	220
880	70	0.7	170	0.3	80
1760	0	0	0	0	0
LSD (0.05)	20	0.2	71	0.1	38
Regression[b]	C**	C**	C**	C**	C**
Nitrogen rate (kg ha^{-1})[a] (Experiment 2):					
110	120	1.5	300	0.9	270
165	150	1.7	480	0.9	340
220	160	1.7	480	1.0	340
250	180	1.8	570	1.0	350
275	170	1.8	580	1.2	400
300	170	1.8	530	1.0	340
330	170	1.8	540	1.0	360
360	140	1.7	380	0.9	240
LSD (0.05	31	0.2	147	0.1	70
Regression[b]	L*	Q**	Q**	Q**	NS
Application frequency[c] (kg N ha^{-1})[d]:					
35 weekly (8)	340	2.6	1720	1.9	1030
70 weekly (8)	430	3.2	2720	2.2	1640
100 biweekly (4)	400	2.9	2380	2.1	1380
135 biweekly (4)	430	3.3	2790	2.0	1520
170 biweekly (4)	410	3.3	2920	2.3	1640
200 monthly (2	400	2.8	2100	2.0	1360
235 monthly (2)	380	2.9	2320	2.1	1490
270 monthly (2)	350	2.9	2420	2.1	1470
LSD (0.05)	42	0.2	330	NS	200

[a]Water-soluble 23-19-17 (N,P,K) formulation, and plants were harvested 36 days after application (56 days after planting).

[b]Best fit regression model L = linear, Q = quadratic, or C = cubic equation and significance at the P = 0.05 (*) or P = 0.01 (**) level.

[c]Number in ( ) is total number of applications.

[d]Water-soluble 23-19-17 (N,P,K) formulation, and plants were harvested 60 days after first application (80 days after planting).

Batting leafy spurge plants with high water pressure did not increase shoot diameter, dry weight, or height compared to nonbatted plants (data not shown). However, this practice appeared to reduce insect problems and kept leaf litter from accumulating between the plants.Occasionally the parent leafy spurge plant would fail to regrow after a stem tip cutting and remaining topgrowth was removed for propagation, which resulted in no net population gain. Leafy spurge shoot regrowth and root diameter and dry weight increased as the age of the parent plant at harvest increased (Table 4). In general, parent plants less than 60 days old either failed to regrow (<90% survival) or the regrowth was often less than the initial growth from stem tip cuttings.

Previous research has shown leafy spurge growth from roots is stimulated by chilling at 0 to 6ºC (12, 20). Roots from presenescent plants required chilling for 8 days for maximum regrowth (20), while roots gathered from postsenescent plants required chilling for up to 42 days (12). There was no discernible effect of chilling roots or stem tip cuttings on subsequent leafy spurge shoot and root dry weight (Table 4). However, these results show that stem cuttings can be refrigerated for several days before planting without a decrease in survival or growth vigor.

Growth of heated and nonheated cuttings was similar when 20 days old (10 days in mist chamber plus 10 days growth), but plant stem height and dry weight and root dry weight were three- to five-fold greater in 56-day-old plants that had been heated for the first 10 days compared to unheated plants (Table 4). Fertilizer applied to unheated cuttings did not overcome the increased growth of cuttings which had the growth medium heated while in the mister, and heat plus fertilizer produced the largest plants. Poinsettia plants grown on heated benches were shorter, had more prominent axillary shoots, and developed anthocyanin sooner than unheated controls (15). However, heat treatment resulted in an increase in leafy spurge height and shoot dry weight but no change in bract color was observed. It was important that the heat tape touched only one side of the pot; if placed on both sides, the plant did not root, presumably because the growth medium was too warm (>30ºC).

Of the nine PGRs evaluated, only GA increased leafy spurge growth (data not shown). GA increased plant height up to 250% of the untreated control, but the plants were etiolated and there was no corresponding increase in stem dry weight. Root diameter and dry weight decreased in GA-treated plants. GA also increased shoot height of caper spurge (*Euphorbia lathyris* L.) (21). Ethephon, flurprimidol, and IAA decreased growth, and BA and NAA at 1000 $\mu g\ g^{-1}$ were toxic to plants (data not shown). MH decreased shoot length slightly but chlormequat chloride did not affect growth. Most PGRs used in the poinsettia industry are applied to control height and/or branching (10, 19, 22, 27) but usually decrease overall plant dry weight, which is opposite the goal of the present research.

Table 4. Effect of fertilization date on stem cutting growth, age of parent plant when top growth was removed on regrowth from roots, and temperature on stem growth from roots and cuttings.

	Shoot			Root	
Technique	Height	Diameter	Dry weight	Diameter	Dry weight
	——— mm ———		mg	mm	mg
Age when fertilized[a] (days):					
20	230	2.2	1100	1.7	1210
30	190	2.0	700	1.5	680
40	190	2.2	790	1.3	610
50	170	2.0	570	0.9	320
LSD (0.10)	25	0.1	104	0.1	97
Regression[b]	L**	C**	L**	L**	Q**
Age before cutback[c] (days):					
30	100	1.0	150	0.7	130
45	110	1.0	210	0.8	180
60	160	1.5	430	1.1	330
75	190	1.6	550	1.8	670
90	200	1.7	420	2.1	1000
LSD (0.05)	18	0.5	82	0.2	84
Regression[b]	L**	L**	C*	C**	Q*
Roots chilled before planting[d]:					
0	230	1.8	590	1.9	1020
48 hours	240	1.9	680	1.6	870
1 week	240	1.8	650	1.7	1020
2 weeks	240	1.9	570	1.7	780
4 weeks	260	1.8	820	1.6	850
8 weeks	260	1.9	780	1.9	940
LSD (0.05)	NS	NS	NS	NS	180
Regression[b]	NS	NS	NS	NS	Q*
Stems chilled before planting:					
0 hours	110	1.6	220	0.7	220
24 hours	110o	1.7	320	0.8	240
48 hours	100	1.5	230	0.8	180
72 hours	110	1.6	280	0.9	220
96 hours	110	1.7	270	1.0	230
LSD (0 05)	NS	NS	NS	0.1	NS
Regression[b]	NS	NS	NS	L**	NS
Growth media heated in mist chamber[e] harvested 20 days after planting:					
22 C medium/no fert.	20	1.1	30	0.6	30
30 C medium/no fert.	20	1.2	40	0.5	30
LSD (0.05)	NS	NS	NS	NS	NS
Harvested 56 days after planting:					
22 C medium/no fert.	40	1.3	100	0.6	130
30 C medium/no fert.	190	2.2	660	1.1	430
22 C medium/fert.[f]	60	1.3	130	0.6	160
30 C medium/fert.[f]	230	2.5	1170	1.3	720
LSD (0.05)	18	0.1	80	0.1	55

[a]275 kg N ha^{-1} applied as part of fertilizer (15-30-15) at age indicated and all plants were harvested when 80 days old.
[b]Best fit regression model L = linear, Q = quadratic, or C = cubic equation and significance at the P = 0.05 (*) or P = 0.01 (**) level.
[c]Topgrowth removed for propagation from stem tip at age indicated and regrowth from roots of parent plants harvested after 36 days.
[d]Topgrowth removed from 120-day-old plants while roots remained in the pot and were cooled to 2ºC for time indicated, moved to a greenhouse bench, and regrowth harvested after 56 days.
[e]Growth medium heated for 10 days only while plants were in a mist chamber, then moved to a greenhouse bench and harvested 20 or 55 days after cuttings were planted.
[f]275 kg N ha^{-1} applied as part of fertilizer (15-30-15) when plants were 20 days old.

Biotype response

The Nebraska and South Dakota leafy spurge biotypes were shorter than the other five biotypes evaluated but had similar shoot dry weight and root growth (Table 5). All biotypes had at least a 90% regeneration rate. Previous research has shown that the Nebraska and South Dakota biotypes do not grow as tall as the standard Austrian biotype but this difference has not affected insect propagation[12].

Leafy spurge plants grew best in the greenhouse at 27ºC, fertilized once with a balanced fertilizer at approximately 250 kg N ha^{-1} when 20 days old, in a potting medium at pH 7 with a 16-hour photoperiod. Other propagation techniques such as heating the growth media while the cuttings were in the mist chamber and taking stem cuttings from 60-days or older plants increased the survival and growth of the cuttings. The time required for leafy spurge propagation from cuttings was reduced to 2 months compared to 6 months or longer for previous propagation methods. Leafy spurge plants can now be grown rapidly to provide plants for biocontrol agents as well as herbicide plus insect integrated research.

Table 5. Growth of leafy spurge biotypes with optimum[a] greenhouse conditions 56 days after cuttings were planted.

	Shoot			Root	
Biotype[b]	Height	Diameter	Dry weight	Diameter	Dry weight
	——— mm ———		mg	mm	mg
78AS001	300	2.2	1020	1.5	730
80MB00l	300	2.9	1320	1.3	820
80MT002	280	2.4	1280	1.3	940
79NE003	200	2.6	1230	1.4	730
84ND001	310	2.3	1160	1.5	740
80SD001	230	2.3	1310	1.3	690
80WY001	310	2.3	1110	1.5	760
LSD (0.05)	40	0.2	NS	NS	NS

[a]27ºC air temperature, 16-hour photoperiod, fertilized with 275 kg N ha^{-1} 15-30-15 (N,P,K) 20 days after stem cuttings planted in a peat, perlite, and vermiculite growth medium[6] at pH 7.

[b]Identification sequence is year collected, U.S. postal code for state, province, or country, and order of registration.

Acknowledgments

This research was funded in part by USDA-APHIS. Orval R. Swenson and Cheryl A. Mihelich provided technical assistance and helpful suggestions.

[12] Lym, R. G. Unpublished data

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