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**Loblolly Pine (*Pinus taeda* L.): Stage-Specific Elemental
Analyses of Zygotic Embryo and
Female Gametophyte Tissue**

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Loblolly pine (*Pinus taeda* L.): Stage-specific elemental analyses of zygotic embryo and female gametophyte tissue

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Abstract

Stage-specific analyses for key elements (metals) for full-term seed and across seed development were completed for zygotic embryo and female gametophyte tissues of loblolly pine (*Pinus taeda* L.). The purpose of this research was to determine the natural range of elemental composition for embryo and female gametophyte during seed development to facilitate formulation of tissue culture growth medium and to provide targets for somatic embryos.

Tissue was analyzed in replicate for major and minor elements by the use of Inductively Coupled Plasma Emission Spectroscopy (ICPES). Variation in elemental composition among five full-term seed sources was minimal providing elemental composition targets for somatic embryos.

Seed tissues from two open-pollinated families, grown in different locations, were analyzed across the sequence of development. Significant changes in elemental composition occurred over time. Phosphorus, potassium, magnesium, calcium, and zinc contents in the female gametophyte were highest during early seed development while sulfur and iron were relatively constant. The lowest copper levels were detected during early female gametophyte development. Zygotic embryos attained maximum potassium, calcium, manganese, and zinc content during early embryo development. Embryo phosphorus, magnesium, and iron generally increased across embryo development. Both seed collections showed minimal values of embryo iron content at stages 4-7 followed by gradual increases until embryos stopped growth. Sulfur and copper were fairly constant; lowest copper occurred during early embryo stages. Patterns for sodium and boron were not consistent in the two half-sib families analyzed for either female gametophyte or embryo tissues. One family showed peaks in sodium and boron during early embryo development. The second family showed similar levels for sodium and boron through embryo development. These data provide stage-specific targets for somatic embryos and suggest element media composition changes for each step in the somatic embryogenesis protocol.

Keywords: loblolly pine, *Pinus taeda*, embryo development, metal analysis, megagametophyte, female gametophyte

Introduction

Loblolly pine (*Pinus taeda* L.) is an important fiber source for the North and South American pulp and paper industry. Loblolly pine (LP) is dominant on 11.7 million ha. and comprises over half of the standing pine volume in southern U.S. forests due to its fast growth, broad natural range, response to cultural practices, resistance to disease and ice damage, and genetic variability for breeding [1]. Over 1-1.5 billion seedlings of LP are planted annually [2].

In pine seed the zygotic embryo grows and develops within the female gametophyte (FG). Somatic embryos, however, are cultivated in the absence of this tissue, and the culture medium must provide nutrients and developmental signals. The more closely the culture medium resembles the environment of the FG, the more likely the development of vigorous somatic embryos. Nutritional, osmotic, hormonal, and gaseous environments surrounding an embryo control embryo growth. Optimization of these environments is critical for the growth and development of high-quality, vigorous somatic embryos. Most often media optimization is accomplished through a process of empirical trial and error. An alternative approach is to use analyses of zygotic tissues or growth environment to provide targets for the somatic tissues. In following this approach to improve elemental composition of tissue culture media for LP, metal content of embryo and FG tissue through the sequence of seed development was needed.

West and Lott [3,4] determined elemental concentrations in embryo and FG tissues for mature seeds from eleven *Pinus* species, not including LP. Their work further quantified elements in various seed regions. Reid et al. [5,6] quantified six elements in various mature seed parts of white spruce. Litvay et al. [7] determined metal content in Douglas-fir immature seeds prior to fertilization and for full-term embryo and FG tissue. These studies did not included data for LP, either by tissue type or developmental stage.

The objectives of this research were (1) to determine if elemental analyses for full-term seed of LP are similar for seed grown in different locations, and (2) to determine elemental profiles across the developmental sequence for embryo and FG from two sets of seed grown in different sites. If embryo and FG tissues from different sites contain similar concentrations of individual elements, then the averages of element contents could provide targets for somatic embryos. To accurately define elemental composition across growth, analyses were completed for staged embryo and FG tissue sorted by the embryo stage found in the gametophyte at the time of collection. If the patterns of elemental content change by zygotic embryo stage are similar for seed from different trees, stage-specific elemental content targets can be developed for somatic embryos. If FG elemental contents show similar patterns of change through seed development, then these changes may provide direct clues to formulate improvements in stage-specific medium elemental content.

Materials and Methods

Plant Material.

Full-term Seed. Full-term seed from five open-pollinated families from four locations and seed orchards were collected for metal analysis of embryo and FG. Dry seed normally requires an overnight soaking period to facilitate separation of seed tissues. To simplify seed dissection and avoid potential elemental loss due to seed soaking and imbibition, cones were requested at the end of seed development prior to the drying. Seed collections of 50-100 mg dry weight were obtained as follows. (1) BC-1 (S4PT6) Boise Cascade 1995 seed produced in a seed orchard near Lake Charles, Louisiana. (2) UC5-1036, Union Camp 1995 seed produced in a seed orchard near Bellville, GA. (3) UC10-1003, Union Camp 1995 seed produced in a seed orchard near Rincon, GA. (4) UC10-14, Union Camp 1995 seed produced in a seed orchard near Rincon, GA. (5) 7-56, Westvaco 1995 seed produced in a seed orchard

near Summerville, SC. Cones were opened and seeds isolated. Seeds were cracked using a hemostat, pried open with the aid of fine-tipped tweezers, and the integument and nucellus tissue removed from the ovule. FG and embryos were separated and collected tissues were dried overnight at 70°C and stored in a freezer prior to analysis.

Immature Seed. During 1994, LP cones were collected weekly throughout the sequence of embryo development from two open-pollinated mother trees. Union Camp tree UC5-1036 was located in a seed orchard near Bellville, GA. Tree BC-1 (S4PT6) was located in a Boise Cascade breeding orchard near Lake Charles, LA. Cones were shipped on ice to IPST and received within 24-48 hours of collection. Cones were opened and seeds dissected as above. The FG was slit, pried open, and the dominant embryo or mass of embryos removed. Individual embryos were quickly observed through a dissecting microscope, evaluated for stage of development (Figure 1, [8]), sorted by stage and tissue type, and placed in vials partially immersed in liquid nitrogen. Stage 9 embryos were also categorized by the week they were collected: 9.1 (stage 9, week 1), 9.2 (stage 9, week 2), etc. Twenty similar-staged embryos or FG were collected per vial, frozen, and stored at -70°C. Collections for each tree and tissue type spanned from stage 1, collected 1-2 weeks after estimated fertilization, to stage 9.10, shortly before cone harvest.

Fresh and Dry Weight Determinations for Embryo and Female Gametophyte. During 1994 three cones of BC1 and UC5-1036 were used each week to isolate 10-20 staged embryos and FG per cone for fresh and dry weight determinations.

Metal Analysis by Inductively Coupled Plasma Emission Spectroscopy (ICPES).

Full-term Seed. Approximately 50 mg of pre-dried sample was weighed into a labeled, graduated, screw-capped polyethylene centrifuge tube. The weight of the sample was recorded to the nearest 0.1 mg. Five mL of high-purity concentrated nitric acid (EM Science TracePur Plus Instrumental Grade) were added to each tube. The tube was capped and allowed to stand at room temperature for six

hours in a fume hood. The tube was uncapped and 2.0 mL of high-purity 30% hydrogen peroxide (J.T. Baker Ultrex Ultrapure Reagent Grade) was added. The tube was capped, inverted twice to mix the contents, and vented to release any evolved oxygen. The cap was loosely screwed on the tube to prevent pressure buildup from evolved oxygen. Each sample was allowed to digest at room temperature for 24 hours in a fume hood. At the end of the digestion period, ultrapure reagent-grade deionized water (ASTM Type I water) was added to each tube to bring the total solution volume to 10.0 mL. Prior to analysis, each sample was filtered through a 0.45- μ m membrane syringe filter. Analysis of the sample digests was conducted on a Perkin Elmer Optima 3000 DV ICP Emission Spectrometer. This instrument, equipped with an autosampler and integral computer workstation, was configured to detect elements simultaneously in less than 5 mL of sample solution. ICP analysis is based on the detection of characteristic ultraviolet and visible light emissions from metallic elements subjected to a high-temperature argon plasma torch. To improve instrument performance, an Yttrium internal standard was added to each sample, standard, and blank to compensate for small variations in sample flow rate, sample viscosity, and acid concentration, as well as to assist in the identification of potential interferences. Quantification of metallic analytes in samples is based on measuring specific wavelength intensities for each element and comparing these results to multipoint calibration standards analyzed in the same manner.

The instrument was calibrated daily with three multicomponent standards and a blank. A series of verification standards, interference check solutions, and blanks were analyzed and evaluated before any samples were analyzed. At a frequency of every ten samples, a calibration verification standard and blank were analyzed. Acceptance criteria for each standard, blank, and sample measurement were used to accept or reject results.

Immature Seed. FG tissues and embryo tissues from stages 9.1 to 9.10 (full term) were analyzed for metals as described above. Due to the small amounts of embryo tissue available from stages 1-8, tissue sets were occasionally combined from two adjacent stages. In addition, the analysis method was modified as follows. An aliquot of predried sample was weighed into a new, labeled, graduated, screw-

cap polyethylene centrifuge tube. The weight of the sample was recorded to the nearest 0.1 mg. Weights ranged from a low of 0.5 mg for a mass of early stage embryos to 79.4 mg for later stage tissue. 0.5 mL of high-purity concentrated nitric acid was added to each tube. Capped tubes were incubated at room temperature for 18 hours in a fume hood. The tube was uncapped and 1.0 mL each of high-purity reagent water and 30% hydrogen peroxide were added. Caps were loosely screwed on the tube to prevent pressure buildup from evolved oxygen. Each sample digested at room temperature for 24 hours in a fume hood. The tube was uncapped and 1.0 mL of high-purity concentrated hydrochloric acid (J. T. Baker Instra-Analyzed Reagent Grade) was added. Each sample was allowed to digest an additional 24 hours. At the end of the digestion period, ultrapure reagent-grade deionized water (ASTM Type I water) was added to each tube to bring the total solution volume to 10.0 mL. Prior to analysis, each sample was filtered through a 0.45- μ m membrane syringe filter. Due to the extremely small weights of early stage embryos, a few collections only contained enough material even when combined with an adjacent stage for one analysis. When only one analysis was performed for a stage, the error bar is missing from the graphed data point.

Operation of the Perkin Elmer Optima 3000 DV ICP Emission Spectrometer was described above. However, due to the low amounts of tissue available for analysis, the standard operating mode was modified. To yield the lowest possible detection limits, the instrument was operated in the “axial” view mode to increase the path length of the spectroscopic measurement. In the “axial” mode, the spectrometer views down the length of the plasma, rather than across the width of the plasma as in the “radial” viewing mode. These viewing configurations are controlled within the instrument’s hardware and software environment [9,10].

Results

Fresh and Dry Weight Determinations for Embryo and Female Gametophyte. Figure 2 shows the patterns of fresh and dry weight accumulation for staged embryo and FG tissue. Embryo fresh and dry weights showed a typical sigmoidal pattern of weight accumulation with the greatest mass accumulation during stage 8 and the first two weeks of stage 9.

Metal Analysis by Inductively Coupled Plasma Emission Spectroscopy - Full-term Seed. The elemental analyses for zygotic embryos were very similar for the five seed sources tested. Summaries of the averages per seed source and for all replications are shown for zygotic embryos in Table 1. Analyses for FG tissue are also similar for the five seed sources and are shown in Table 2. Standard errors for zygotic embryo elemental variation between all of the replicates for Mn, Fe, Cu, Zn, P, S, Mg, and K are less than 4% of the mean values (Table 1). Nickel, B, Na, and Ca show greater variation with standard errors ranging from 5-16% of the mean (Table 1). Standard errors for FG elemental variation between all replicates for Fe, Ni, Cu, Zn, P, S, Mg, and K are less than 4% of the mean values (Table 2). Manganese, B, Na, and Ca show greater variation with standard errors ranging from 5-8%.

Individual replicate analyses for zygotic embryos are shown for two sites in Tables 3 and 4. Individual replicate analyses for FG for the same two sites are shown in Tables 5 and 6. Elemental concentrations detected for Co, Ni, Mo, and Na were sometimes below the accurate detection limits of the instrumentation equipment, and values for these replicates are shown as “<”.

Elemental concentrations of zygotic embryo and FG tissues were often different. Ratios of elemental compositions, on a dry weight basis, are shown in Table 7. Embryos contained low contents of Mn, B, and S, suggesting that these elements are selectively excluded from the embryo. Similar contents of Ca, Ni, Zn, and Cu were found in embryo and FG tissue, suggesting that these are taken into the

embryo by diffusion. Greater concentrations of P, K, Mg, Na, and Fe were found in the embryo compared to FG tissue. This finding suggests that the embryo actively takes up these elements.

Overall, the similarity in analyses of zygotic embryo tissues suggests that the mean elemental compositions provide reasonable targets for the elemental composition of somatic embryos.

Metal Analysis by Inductively Coupled Plasma Emission Spectroscopy - Immature Seed. Elemental analyses throughout the sequence of development for FG and embryo tissues from two half-sib families are shown in Figures 3-11. The FG tissue feeds the embryo during development and during germination. Elemental contents of the FG change over time (Figures 3, 5, 7, 9, 11A). Phosphorus, K, Mg, Ca, B, Na, and Zn contents in the FG are highest during early seed development. Sulfur, Fe, and Cu contents are relatively constant during FG growth. Element concentration was plotted vs. date of tissue collection or stage of embryo development. FG elemental concentrations correlated more closely with the date of tissue collection than to the stage of embryo contained within.

Zygotic embryo elemental contents also change over time (Figures 4, 6, 8, 10, 11B). Manganese showed a peak at stages 1-2. Peaks in B and Na occurred at stage 3-4. Since embryos were collected in sodium borosilicate glass vials during 1994, it is possible that some container contamination may be present. All samples were treated similarly, yet distinct peaks in B and Na are clearly present. Embryo collections for later years have used plastic cryostorage vials. Peaks in P, K, Ca, and Zn, occurred at stage 5-6. Iron showed an initial peak at stage 5-6 followed by a dip and then increased until embryos stopped growth. Magnesium rose slightly throughout embryo development. Sulfur and Cu were fairly constant through embryo development. In evaluating these mineral changes it is important to consider that early embryo stages 1-4 contain decreasing masses of suspensor tissue. Analyses of stage 1 embryo would consist mostly of suspensor tissue.

Discussion

To represent variation in mineral content five lots of full-term seed were chosen from four breeding orchards located in different LP growing regions. LP mineral contents were found to differ between FG and embryo tissues for most minerals quantified. The ratios of minerals for embryo vs. FG tissues are shown in Table 7. Phosphorus, Mg, K, Na, Fe, and Cu showed greater dry weight content in embryo than FG. Sulfur, Mn, and B were higher in FG than embryo tissue. Calcium, Ni, and Zn were similar in both tissues. Table 8 shows a comparison of mineral contents in conifer seed tissues by prior researchers. Eleven species of *Pinus* not including LP [3], Douglas-fir [7], and white spruce [5] full-term seed analyses are compared to our data on LP. Element contents for LP FG fall within the range of observations for eleven *Pinus* species for all elements but iron. LP FG contained more than twice as much iron when compared to the highest iron-containing *Pinus* species studied. For embryo tissue, Mn, S, and Ca were above the range reported for eleven *Pinus* species [3]. Analyses for Douglas-fir and white spruce also were similar to LP and the range of measured metal contents for *Pinus*.

In our study, the five seed lots from different mother trees and locations contained similar mineral contents (Tables 1 and 2). For most elements the standard error was less than 5% of the mean. For Ca, B, and Ni the variation was as high as 16%. These similarities in mineral content for full-term seed prompted us to measure mineral content across seed development. Seeds from two of these mother trees were collected weekly across the sequence of development and analyzed in triplicate for mineral content. Patterns of mineral content across embryo and FG development are similar for both trees (Figures 1-10). It is interesting to note that when relationships were presented graphically, embryo analyses were portrayed well by plotting mineral content by embryo stage, even when seeds at the same stage were collected on different dates. For FG, plotting mineral content by date of collection best represented the pattern of change. This observation suggests that mineral accumulation in embryos is driven by physiological stage, while accumulation in FG tissue progresses with time regardless of the physiological

age of the embryo contained within. Full-term whole seed of Norway spruce collected from 24 locations throughout Poland showed greater variation in mineral content (Table 8, [11]).

In this study most of the minerals quantified showed the highest levels during early embryo and FG development. Few studies are available where inorganic ions were measured during embryo development. The most abundant information is for full-term seed. Probably the most significant barrier to obtaining information on nutritional components during early and mid embryo development is the small amount of embryo tissue present. Hundreds of embryos requiring laborious dissection may be required for a single non-replicated analysis. For the few systems where inorganic ions have been studied in embryo or endosperm tissue, the general pattern is of decreasing metal content as embryos develop. Maes et al. [12] measured free inorganic ions in wheat ovule extracts. Potassium, Mg, Ca, Na, chloride, ammonium, phosphate, nitrate, and sulfate were measured on a fresh weight basis from 0 to 11 days post anthesis (DPA). All inorganic ions quantified increased to a peak about 1 DPA and decreased to the lowest levels at about 3 DPA. Thereafter, levels rose slightly and remained relatively constant. Carman et al. [13] measured total minerals in wheat kernel supernatants and in wheat seed parts for twelve metals. Mineral content was measured at 3-18 DPA. Most minerals rose slightly over time or remained constant. Since Carman et al. [13] began collecting tissue at 3 or 6 DPA, they may have missed the mineral peaks and started sampling at the low point. Chloride, sulfate, nitrate, and phosphate were measured in developing coffee seed [14]. *Coffea canephora* and *C. arabica* whole grains were analyzed at four dates following flowering. The four anions quantified generally declined as seed development progressed. In *Phaseolus vulgaris* inorganic ion measurements of three macroelements present in liquid endosperm around heart or late cotyledon-stage embryos showed higher levels in the heart stage [15].

Assigning physiological age to embryos and surrounding tissue by visually staging the dominant embryo allows improved comparisons of tissue from tree to tree and year to year. Most studies concerned with nutritional components in developing embryo and surrounding tissues monitor tissue by time after flowering, time after fertilization, or date of collection. Plant growth is controlled by genetics and

physiological time such as the accumulation of degree-days above a minimum temperature, rather than Julian day. Since genetics and climate vary, the timing of embryo development also varies by tree and year. In the fall loblolly pine mother trees may vary as much as 50 days in cone ripening [16]. In our tissue collections spanning several years, stage one embryos were often isolated from immature seed for three consecutive weeks in late June and early July. In addition, mid-development collections often contained multiple stages in cones collected from a single tree on a single date. For example, cones of BC-1 collected on July 15, 1996 contained embryo stages 3-8. When embryo mineral content was plotted by date, mixtures of embryo stages produced variable patterns of change through time. But when mineral contents were plotted by embryo stage, clear repeatable patterns of change by stage emerged allowing comparison of results between trees and years.

The ion and metal analyses performed in this work represent total metals by acid digestion including free and bound forms. Of greater interest are the free metal contents, those minerals that are immediately available for uptake and not bound. Thus, an improvement would be to determine water-soluble free nutrients. This may be especially important if free macroelements contribute significantly to the osmotic environment during embryo development.

A second improvement would be to develop specific information for particular areas of the seed that are important to embryo development such as the archegonium during early stages or the corrosion cavity at mid to late embryo development. West and Lott [4] used energy dispersive x-ray analysis to compare female gametophyte and various regions of the embryo for peak-to-background ratios for P, Mg, K, and Fe in globoid crystals ($\geq 0.33 \mu\text{m}$) or electron-dense particles ($\leq 0.33 \mu\text{m}$). Regions of the embryo or female gametophyte did not differ significantly. Little work has been done to study transfer of nutrients from the FG to the developing conifer embryo. Currently, water-soluble materials are thought to move from the FG through a moist film surrounding the embryo and then move into the embryo. An alternate route is to diffuse into and through the suspensor. Regardless of the mechanism, the concentration of nutrients within the water layer or film would be of great interest in stimulating and

improving tissue culture media oriented to a specific growth stage(s). Timmis [17] suggested biochemical analysis of the liquid or water film present between the FG and embryo during early to mid-development. Grob et al. [18] speculated that the embryo receives differing nutritional components depending on the layer or region of the FG that the embryo is in contact with, early-stage embryos being in contact with the inner FG and late-stage embryos contacting the outer FG. Litvay et al. [7] attempted to analyze mineral components in the archegonium, erosion zone, and FG in unfertilized Douglas-fir seed. They compared these preliminary nonreplicated values to embryo and FG tissue from mature seed. Elemental contents ($\mu\text{g/g}$ oven-dried tissue) for K, Ca, Na, and Zn in the prefertilization tissues were 4-10 times higher than the mature tissues. Boron in the erosion zone was more than 7 times greater than levels found in other prefertilization or mature seed tissues. Magnesium, Mn, and Cu did not differ greatly between tissue types. While these data were only preliminary they are remarkably similar to the replicated trends we see through time in the FG tissue for LP.

For SE to be successful commercially it must work with many genotypes of diverse genetic backgrounds. Both embryo quantity and quality need to be maximized at each stage of embryo development. SE in conifers usually occurs in a sequence of protocol steps such as initiation, multiplication (maintenance), development and maturation, and germination. Specific media are researched and developed for each step. These media normally differ in mineral, hormonal, organic components, and osmoticant content. In loblolly pine, initiation and multiplication media support growth of somatic embryos at stages 1-2. Development and maturation media grow more advanced stages, usually 3-9.1, as well as some additional multiplication of stages 1-2. One of the objectives of this research was to develop stage-specific mineral targets for somatic embryos. We met this objective by showing repeatable patterns of mineral change through the sequence of embryo growth. The actual stage-specific values from zygotic embryos provide targets for the somatic embryo. Further, if we consider the female gametophyte tissue to be the “medium” for the zygotic embryo, then changes in the FG mineral content through time could be simulated in the tissue culture environment. The data obtained in this

study provide abundant suggestions for the mineral content modification of initiation, maintenance, development, and germination media.

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Table 1. Summary of averages of replicated elemental analysis ($\mu\text{g/g}$ dry wt.) of zygotic embryo tissues collected from loblolly pine seeds grown on different mother trees and in different locations.

Tree	Location	Reps	Mn	Fe	Co	Ni	Cu	Zn	B	P	S	Mo	Na	Mg	K	Ca
S4PT6	Lake Charles, LA	5	89.4	258		3.4	28.7	146	6.0	17053	2747			7671	12678	173
UC5-1036	Bellville, GA	4	90.6	279		1.0	24.6	133	8.7	17432	2619			8122	13092	275
7-56	Summerville, SC	3	66.4	221		1.0	22.7	113	1.2	15440	2242		7.1	7352	11552	140
UC10-14	Rincon, GA	2	76.7	181			31.0	113	2.2	14883	2312		6.6	7283	11219	174
UC10-1003	Rincon, GA	1	82.1	215	<0.40	<0.59	30.0	147	<.36	16421	2410	<0.52	<3.23	7618	11833	146
Mean			83.0	243		2.1	26.8	132	6.2	16500	2531		6.6	7672	12312	192
Std Error			3.0	9.7		0.3	0.8	4.3	1.0	282	57		0.3	99	213	28
Std			0.04	0.04		0.16	0.03	0.03	0.16	0.02	0.02		0.05	0.01	0.02	0.15
Er/Mean																

Table 2. Summary of averages of replicated elemental analysis ($\mu\text{g/g}$ dry wt.) of female gametophyte tissues collected from loblolly pine seeds grown on different mother trees and in different locations.

Tree	Location	Reps	Mn	Fe	Co	Ni	Cu	Zn	B	P	S	Mo	Na	Mg	K	Ca
S4PT6	Lake Charles, LA	5	243	74		3	20	127	28	12415	5867			5019	9410	203
UC5-1036	Bellville, GA	4	233	82			22	143	21	13525	5547			5453	9744	344
7-56	Summerville, SC	3	121	53			16	126	17	10804	4633		4	4621	7843	270
UC10-14	Rincon, GA	2	251	79			18	155	19	11981	5430		4	5435	9102	150
UC10-1003	Rincon, GA	1	287	66	<0.35	<0.55	21	193	12	12348	5465	<0.45	<2.83	5129	9460	311
Mean			221	72		2.6	20	139	22	12327	5450		3.9	5118	9148	254
Std Error			14.8	3.1		0.1	0.6	4.9	1.5	256	120		0.2	88	199	21
Std Er/Mean			0.07	0.04		0.03	0.03	0.04	0.07	0.02	0.02		0.05	0.02	0.02	0.08

Table 3. Elemental analysis ($\mu\text{g/g}$ dry wt.) for zygotic embryo tissues of S4PT6 (Lake Charles, LA, Site) from full-term seed collected prior to drying in 1995.

Tree	Mn	Fe	Co	Ni	Cu	Zn	B	P	S	Mo	Na	Mg	K	Ca
S4PT6	89.3	283.4	<0.43	3.5	29.2	154.7	7.7	17302	2715	<0.55	<3.44	7740	12675	171.9
S4PT6	87.9	249.8	<0.43	4.1	28.9	139.5	6.9	16806	2711	<0.55	<3.45	7617	12708	200.7
S4PT6	89.1	271.3	<0.40	2.3	30.4	160.6	4.2	17543	2824	<0.52	<3.24	7924	13009	137.0
S4PT6	97.8	266.3	<0.40	3.3	28.4	147.0	3.1	17525	2793	<0.51	<3.22	7823	13050	180.2
S4PT6	83.2	218.3	<0.42	3.9	26.7	129.5	8.1	16091	2692	<0.54	<3.40	7253	11948	175.6
Mean	89.4	257.8		3.4	28.7	146.3	6.0	17053	2747			7671	12678	173.1
Std Error	2.4	11.3		0.3	0.6	5.5	1.0	274.9	25.8			116.1	197.8	10.3

Table 4. Elemental analysis ($\mu\text{g/g}$ dry wt.) for zygotic embryo tissues of Union Camp 5-1036 (Belville, GA, Site) from full-term seed collected prior to drying in 1995.

Tree	Mn	Fe	Co	Ni	Cu	Zn	B	P	S	Mo	Na	Mg	K	Ca
UC5-1036	83.2	267.3	<0.42	1.4	24.6	129.4	8.0	16961	2539	<0.54	<3.35	7958	12791	150.6
UC5-1036	97.6	291.6	<0.42	0.9	24.6	126.6	3.1	17630	2652	<0.54	<3.39	8158	13196	188.9
UC5-1036	100.6	292.7	<0.38	0.7	25.2	143.7	14.3	17700	2595	<0.48	5.1	8273	13120	574.1
UC5-1036	81.2	265.5	<0.42	1.1	24.0	130.5	9.3	17435	2688	<0.54	<3.37	8098	13259	185.1
Mean	90.6	279.3		1.0	24.6	132.5	8.7	17432	2619			8122	13092	274.7
Std Er	4.9	7.4		0.2	0.2	3.8	2.3	167	33			66	104	100.2

Table 5. Elemental analysis ($\mu\text{g/g}$ dry wt.) for female gametophyte tissue of S4PT6 from full-term seed collected prior to drying in 1995.

Tree	Mn	Fe	Co	Ni	Cu	Zn	B	P	S	Mo	Na	Mg	K	Ca
S4PT6	237.2	78.5	<0.37	2.2	21.2	129.2	28.3	12624	5774	<0.48	<3.00	4934	9503	240.8
S4PT6	264.1	70.5	<0.38	2.8	19.5	131.8	23.3	12291	5883	<0.49	<3.06	4785	9343	198.6
S4PT6	248.2	79.8	<0.38	2.3	19.8	129.4	31.2	12619	5775	<0.49	5.4	5128	9530	208.0
S4PT6	257.3	77.3	<0.41	2.7	21.2	130.5	28.0	12636	5977	<0.52	<3.26	5199	9943	111.7
S4PT6	205.8	65.6	<0.40	3.1	18.6	114.7	28.1	11907	5925	<0.51	<3.22	5050	8732	257.9
Mean	242.5	74.4		2.6	20.1	127.1	27.8	12415	5867			5019	9410	203.4
Std Er	10.2	2.7		0.2	0.5	3.1	1.3	143	41			73	197	25.3

Table 6. Elemental analysis ($\mu\text{g/g}$ dry wt.) for female gametophyte tissues of Union Camp 5-1036 from full-term seed collected prior to drying in 1995.

Tree	Mn	Fe	Co	Ni	Cu	Zn	B	P	S	Mo	Na	Mg	K	Ca
UC5-1036	231.8	72.9	<0.40	<0.60	22.2	141.5	16.1	13448	5606	<0.52	<3.25	5455	9761	294.5
UC5-1036	241.5	88.4	<0.39	<0.58	22.2	151.9	20.8	13944	5600	<0.50	<3.14	5600	10024	402.2
UC5-1036	248.5	86.1	<0.39	<0.57	22.3	138.2	22.7	13218	5537	<0.50	<3.11	5278	9449	292.0
UC5-1036	211.7	79.2	<0.41	<0.60	21.2	141.2	22.9	13493	5444	<0.52	3.8	5477	9743	386.5
Mean	233.3	81.7			21.9	143.2	20.6	13525	5547			5453	9744	343.8
Std Er	8.0	3.5			0.3	3.0	1.6	152	38			66	118	29.4

Table 7. Ratio of average elemental concentrations ($\mu\text{g/g}$ dry wt.) in dried zygotic embryo vs. female gametophyte tissues.

Ratio	Mn	Fe	Ni	Cu	Zn	B	P	S	Na	Mg	K	Ca
Zyg/FG	0.38	3.37	0.80	1.37	0.95	0.29	1.34	0.46	1.72	1.50	1.35	0.75

Table 8. Comparison of metal analyses ($\mu\text{g/g}$ dry wt.) for full-term loblolly pine and other coniferous seeds.

Tissue Type & Species	Replication	Mn	Fe	Ni	Cu	Zn	B	P	S	Na	Mg	K	Ca	Reference
Embryo Tissue														
<i>Pinus taeda</i>	5 Sites	221.0	72.0	2.6	20	139	22	12327	5450	3.9	5118	9148	254	
Pinus	11 species													[3]
Average		56.0	129.7			98		11607	2533		4585	10004	140	
Highest in Range		141.7	215.5			160		14800	3800		6147	13283	212	
Lowest in Range		12.2	43.5			50		7000	1500		2881	7939	74	
Douglas -fir	1	100.0	100.0		<50	<50	50	5700		100	2400	4000	250	[7]
White Spruce	3-4		242.0			149		10697			3708	7738	94	[5]
Female Gametophyte														
<i>Pinus taeda</i>	5 Sites	83.0	243.0	2.1	26.8	132	6.2	16500	2531	6.6	7672	12312	192	
Pinus	11 species													[3]
Average		228.8	73.5			147		11927	5507		5294	10342	274	
Highest in Range		608.1	106.8			231		15600	8900		7720	12769	435	
Lowest in Range		65.3	29.0			52		5200	1600		2225	7127	110	
Douglas -fir	1	250.0	75.0		<50	200	<50	2400		400	2800	10000	150	[7]
White Spruce	3-4		144.0			195		11630			4910	8236		[5]
Whole Seed														
<i>Picea abies</i>														
Average	24 sites							10549		62	4543	6615	310	[11]
Highest in Range								14420		79	5040	8370	430	
Lowest in Range								4410		43	3940	3700	230	

Figures

Figure 1. Photographs of loblolly pine zygotic embryos stages 1-9.2.

Figure 2. *Pinus taeda* embryo and female gametophyte tissue fresh and dry weights during seed development in 1994. (A) Embryo fresh and dry weights for S4PT6 seed. (B) Embryo fresh and dry weights for UC 5-1036 seed. (C) Female gametophyte fresh and dry weights for S4PT6 seed. (D) Female gametophyte fresh and dry weights for UC5-1036 seed.

Figure 3. Elemental analysis ($\mu\text{g/g}$ dry wt.) for *Pinus taeda* female gametophyte tissue of (Top) Union Camp 5- 1036 and (Bottom) S4PT6 seed collected in Summer 1994. Sulfate, magnesium, phosphorus, and potassium shown by date of collection of female gametophyte tissue.

Figure 4. Elemental analysis ($\mu\text{g/g}$ dry wt.) for *Pinus taeda* zygotic embryo tissue for (Top) Union Camp 5-1036 and (Bottom) S4PT6 seed collected in Summer 1994. Phosphorus, sulfate, magnesium, and potassium content are shown by embryo stage.

Figure 5. Elemental analysis ($\mu\text{g/g}$ dry wt.) for *Pinus taeda* female gametophyte tissue of (Top) Union Camp 5-1036 and (Bottom) S4PT6 seed collected in Summer 1994. Calcium content is shown by date of collection of female gametophyte tissue.

Figure 6. Elemental analysis ($\mu\text{g/g}$ dry wt.) for *Pinus taeda* zygotic embryo tissue of (Top) Union Camp 5-1036 and (Bottom) S4PT6 seed collected in Summer 1994. Calcium content is shown by embryo stage.

Figure 7. Elemental analysis ($\mu\text{g/g}$ dry wt.) for *Pinus taeda* female gametophyte tissue of (Top) Union Camp 5-1036 and (Bottom) S4PT6 seed collected in Summer 1994. Copper, iron, manganese, and zinc content are shown by date of collection of female gametophyte tissue.

Figure 8. Elemental analysis ($\mu\text{g/g}$ dry wt.) for *Pinus taeda* zygotic embryo tissue of (Top) Union Camp 5-1036 and (Bottom) S4PT6 seed collected in Summer 1994. Manganese, iron, copper, and zinc content are shown by embryo stage.

Figure 9. Elemental analysis ($\mu\text{g/g}$ dry wt.) for *Pinus taeda* female gametophyte tissue of (Top) Union Camp 5-1036 and (Bottom) S4PT6 seed collected in Summer 1994. Boron and sodium content are shown by date of collection of female gametophyte tissue.

Figure 10. Elemental analysis ($\mu\text{g/g}$ dry wt.) for *Pinus taeda* zygotic embryo tissue of (Top) Union Camp 5-1036 and (Bottom) S4PT6 seed collected in Summer 1994. Boron and sodium content are shown by embryo stage.

Figure 11. *Pinus taeda* elemental analysis ($\mu\text{g/g}$ dry wt.) (A) Female gametophyte tissue of S4PT6 seed collected in summer 1994. Nickel content is shown by date of collection. (B) Zygotic embryo tissue of S4PT6 seed collected in summer 1994. Nickel content is shown by embryo stage.

Figure 1.

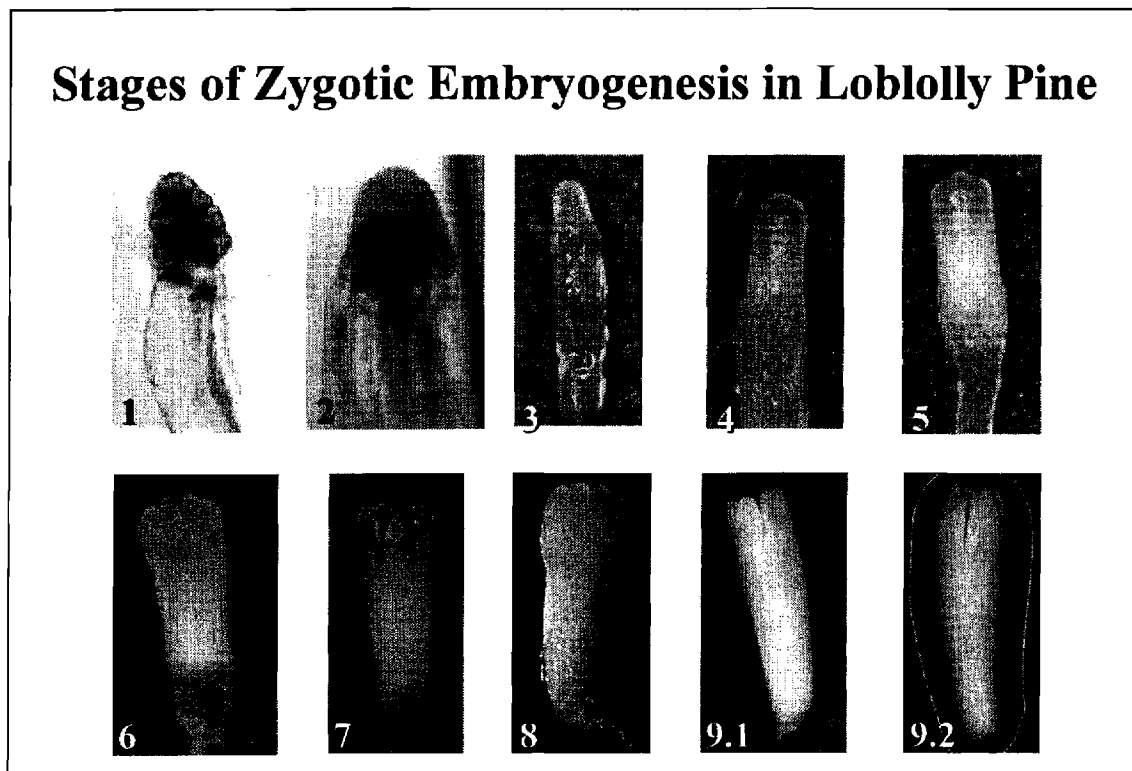


Figure 2.

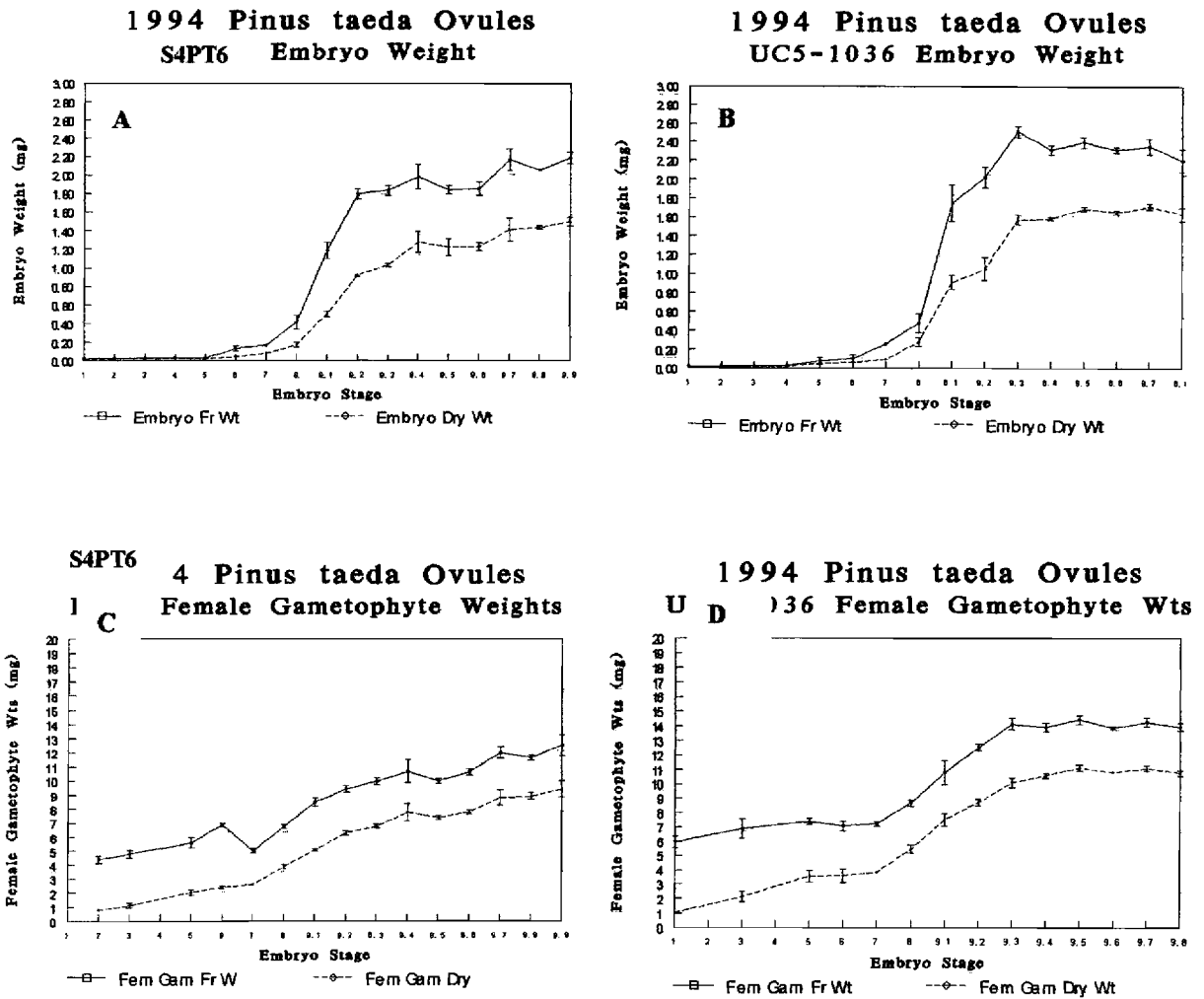
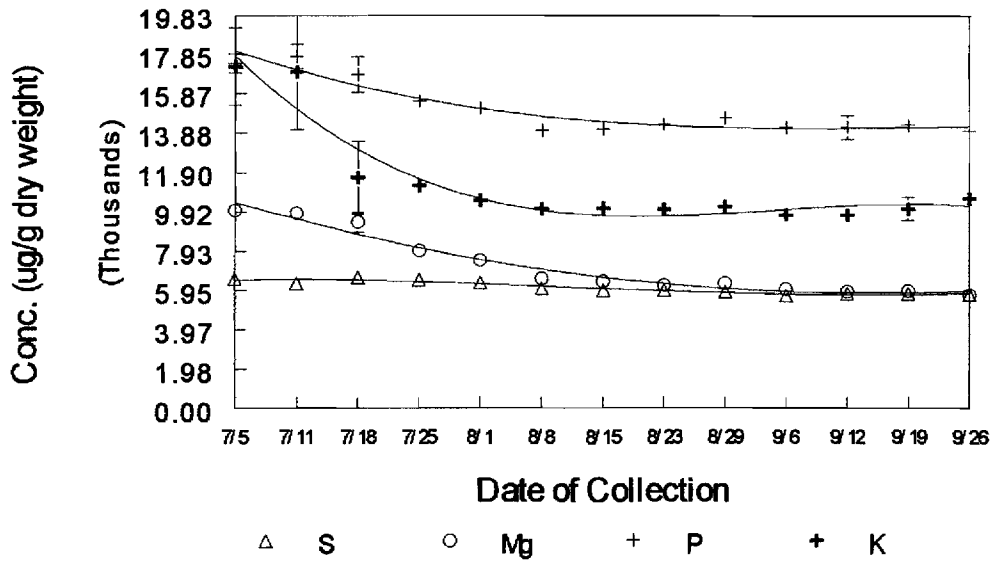


Figure 3.

Loblolly Pine Female Gametophyte UC5-1036 Macroelements, 1994



S4PT6 Macroelements, 1994

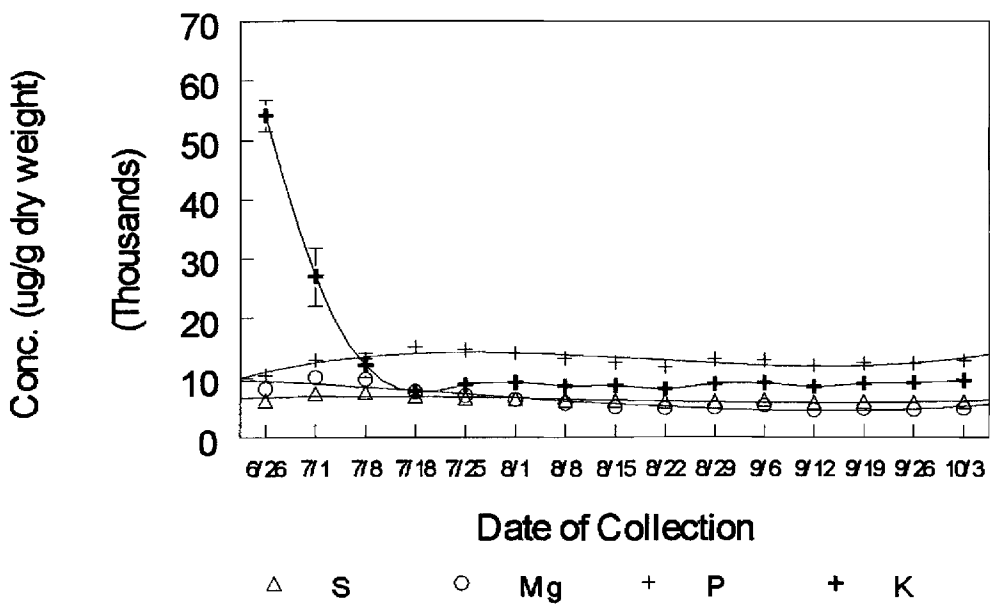
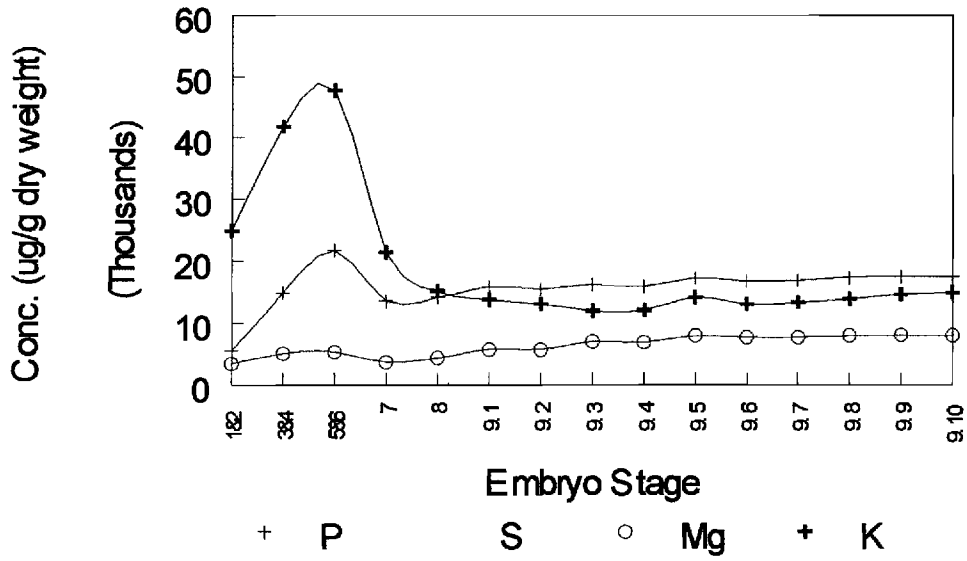


Figure 4.

Loblolly Pine Embryo Analysis UC5-1036 Macroelements, 1994



S4PT6 Macroelements, 1994

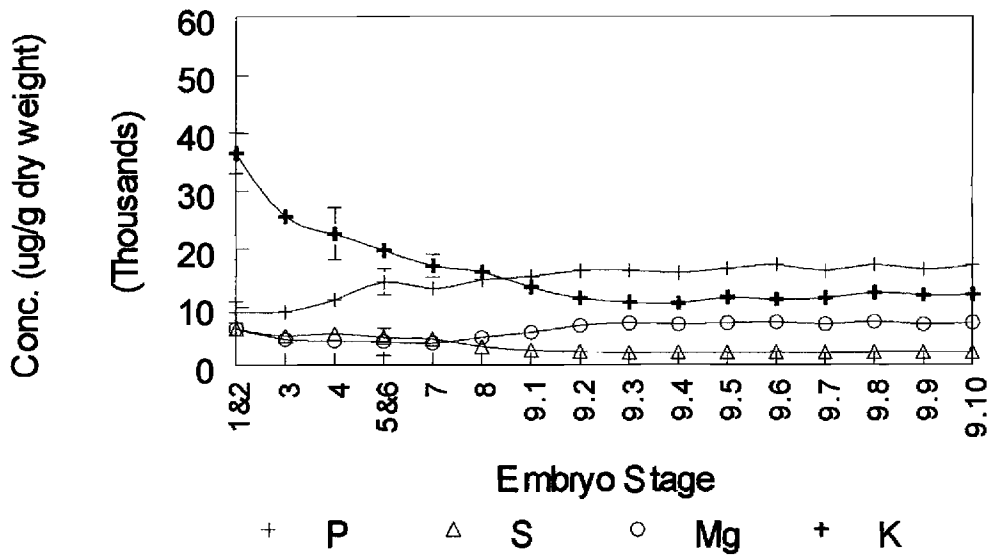
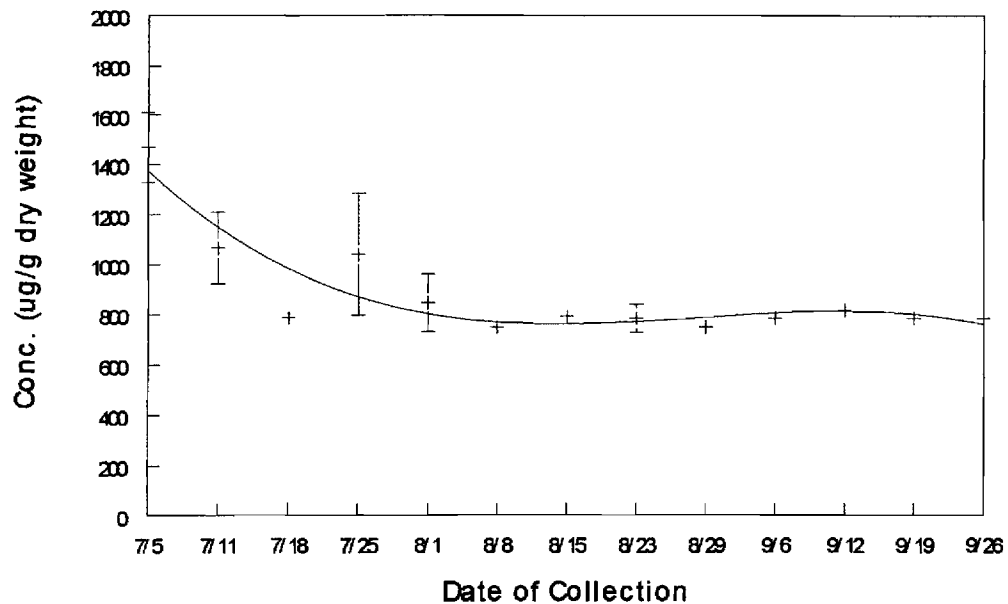


Figure 5.

Loblolly Pine Female Gametophyte UC5-1036 Calcium, 1994



S4PT6 Calcium, 1994

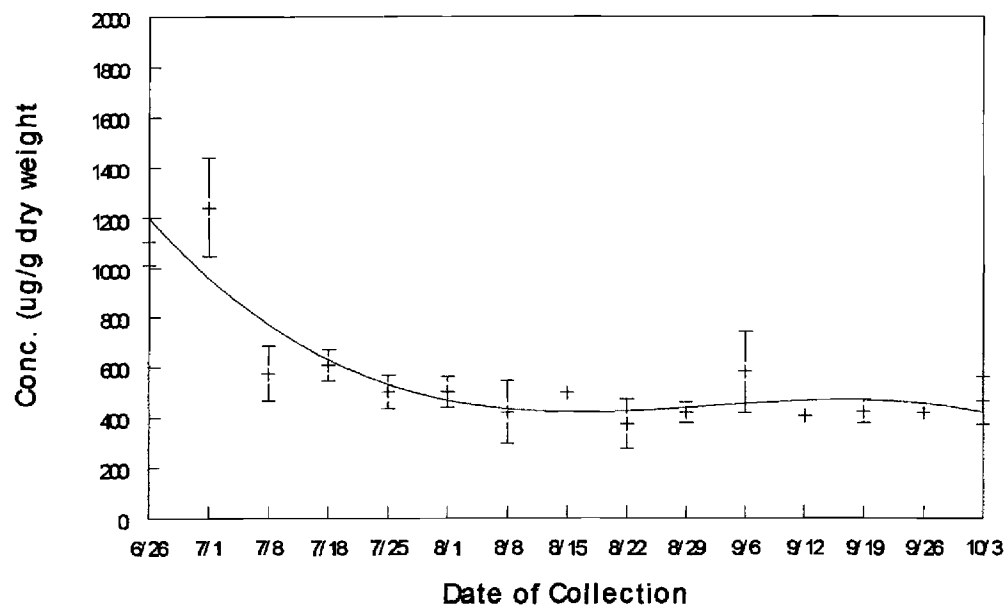
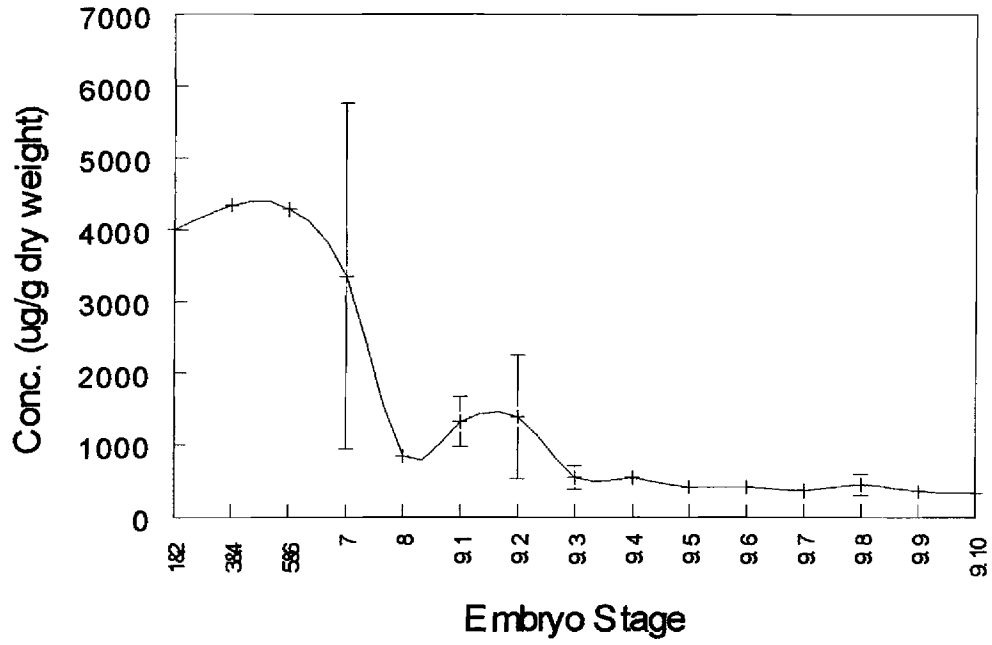


Figure 6.

Loblolly Pine Embryo Analysis UC5-1036 Calcium, 1994



S4PT6 Calcium, 1994

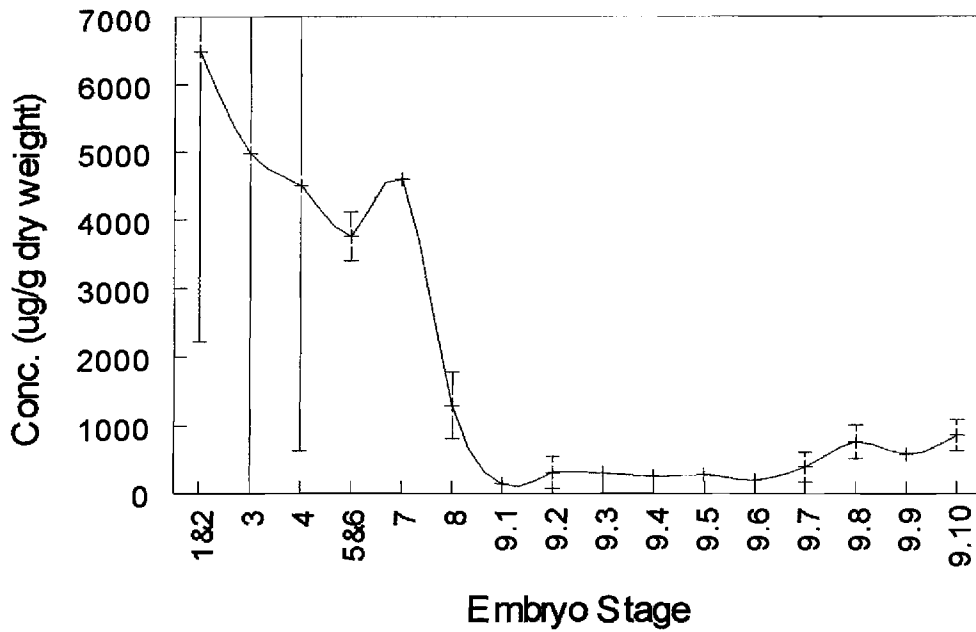
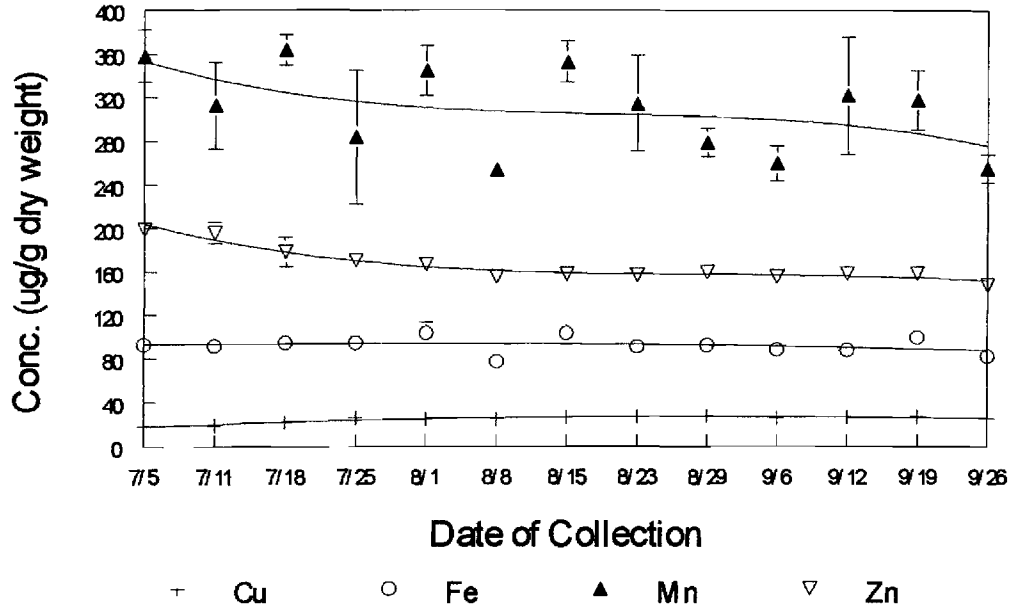


Figure 7.

Loblolly Pine Female Gametophyte UC5-1036 Microelements, 1994



S4PT6 Microelements, 1994

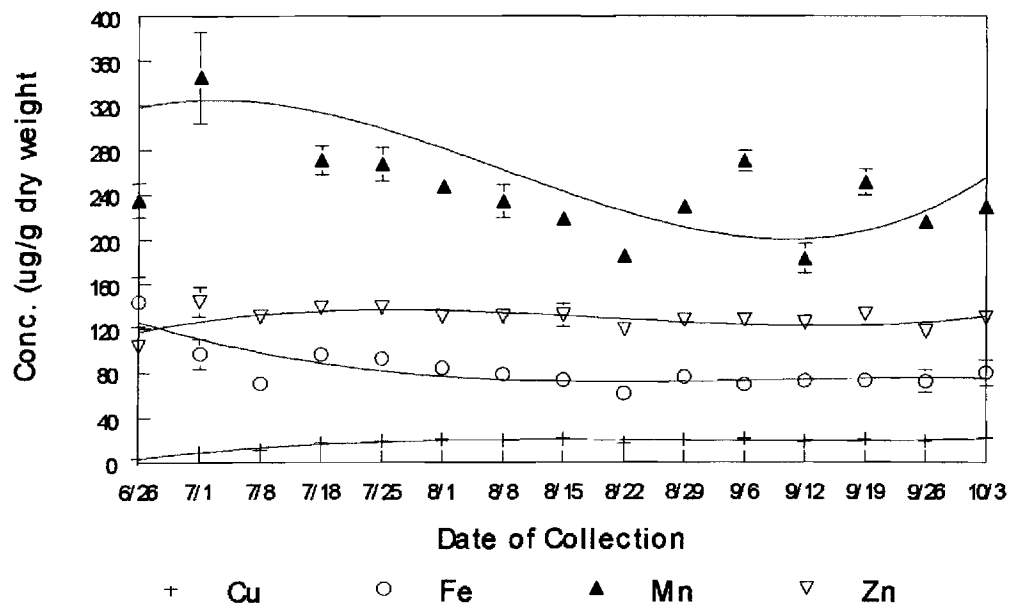
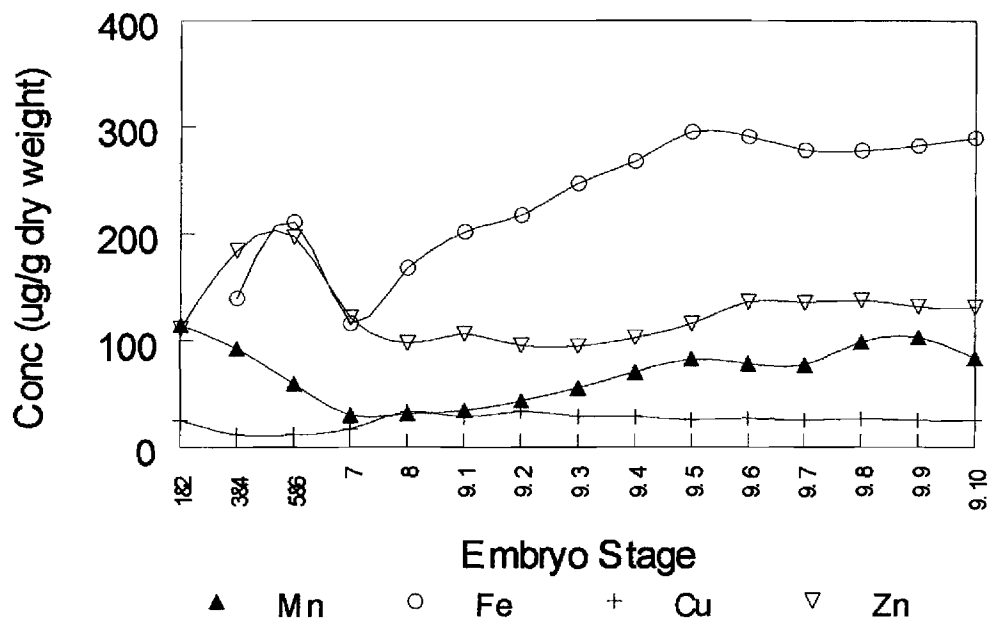


Figure 8.

Loblolly Pine Embryo Analysis

UC5-1036 Microelements, 1994



S4PT6 Microelements, 1994

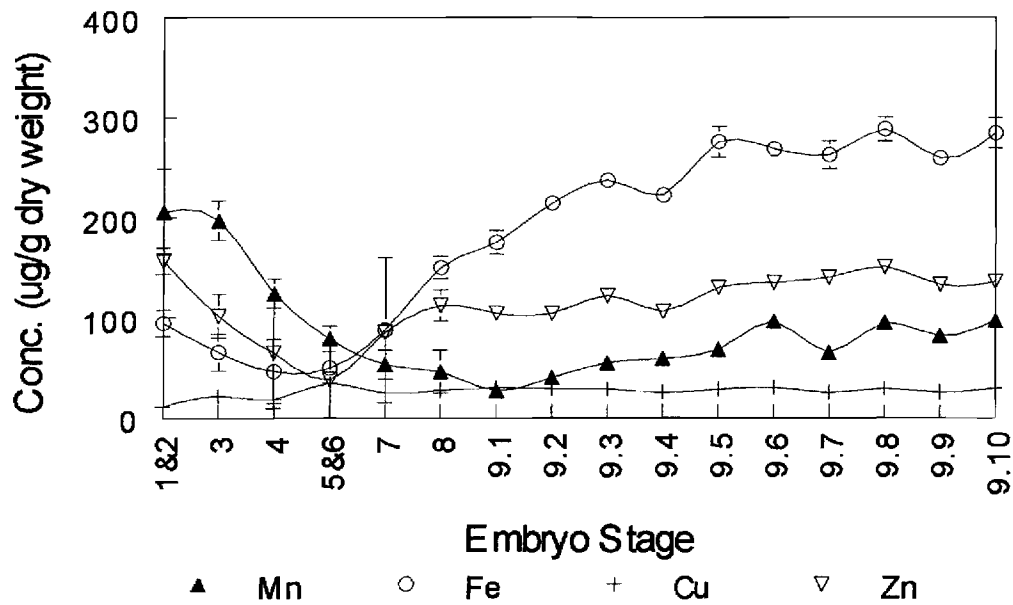
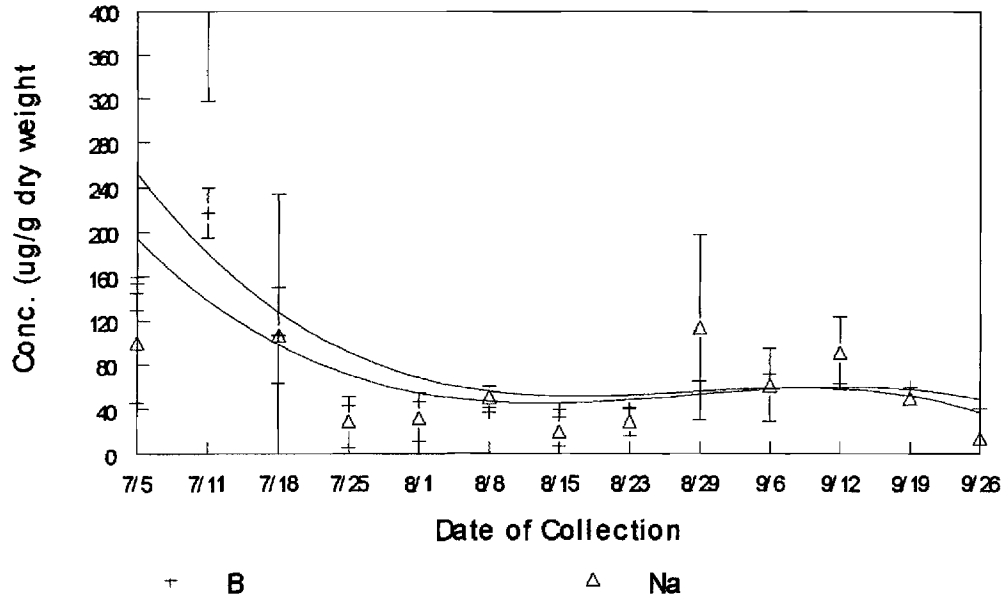


Figure 9.

Loblolly Pine Female Gametophyte UC5-1036 Microelements, 1994



S4PT6 Microelements, 1994

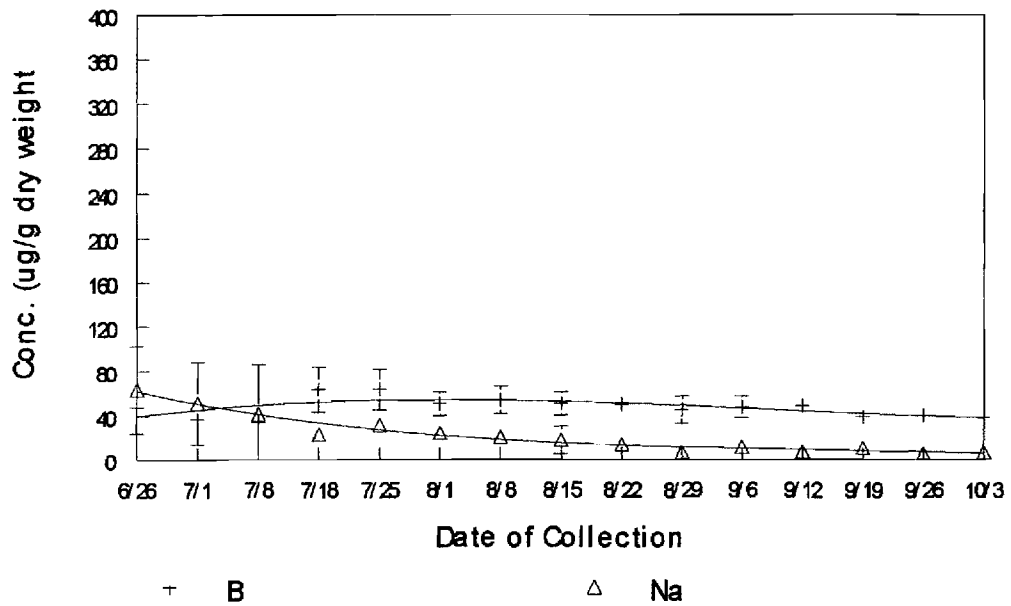
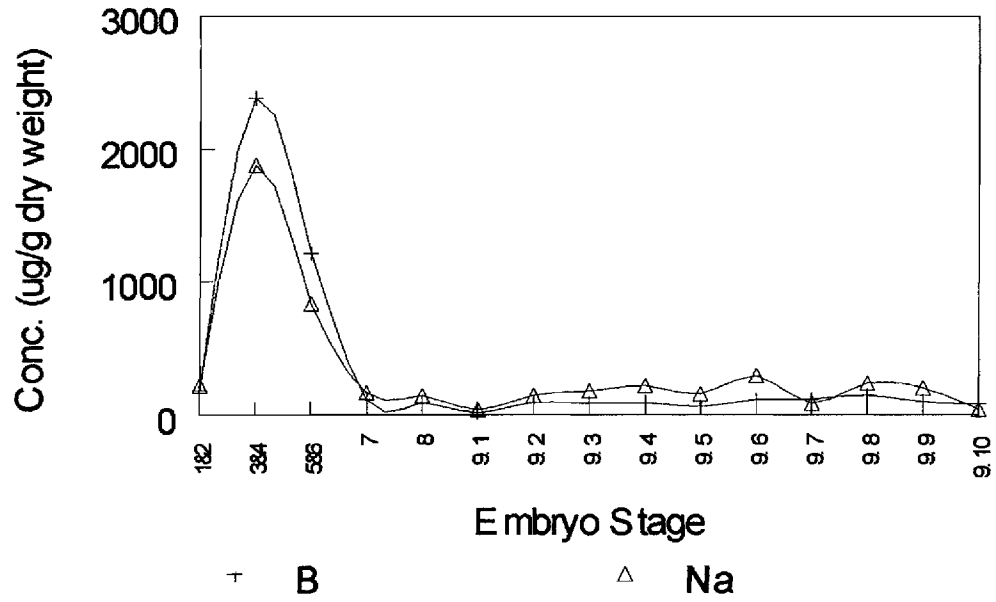


Figure 10.

Loblolly Pine Embryo Analysis UC5-1036 Microelements, 1994



S4P T6 Microelements, 1994

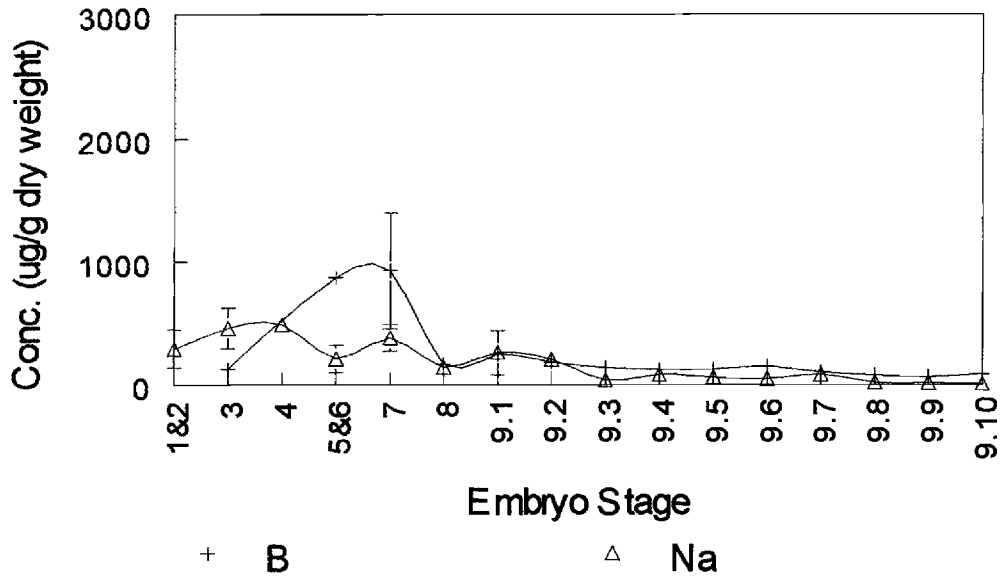
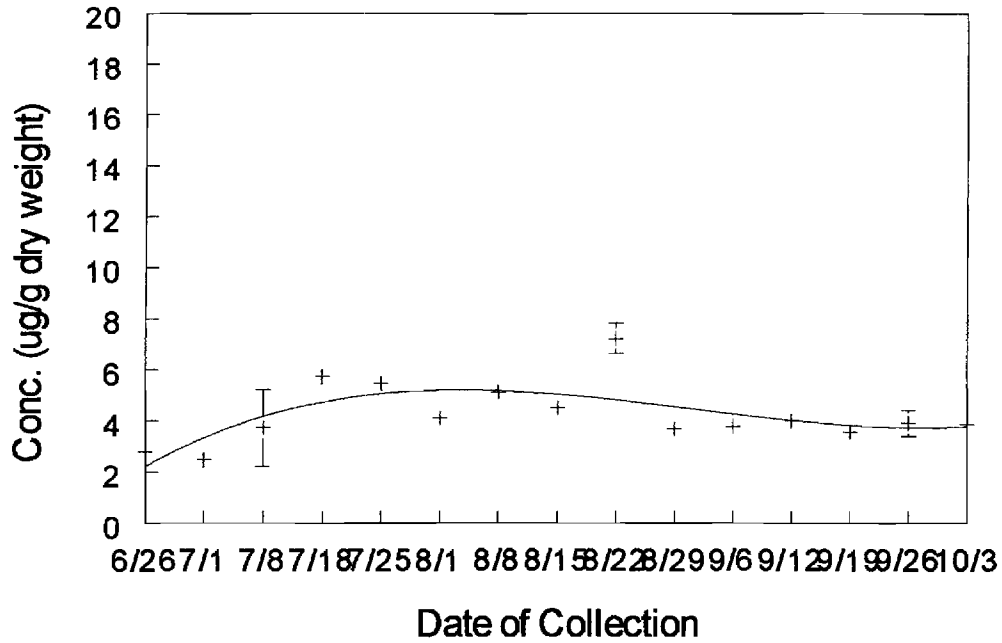


Figure 11.

Loblolly Pine Female Gametophyte S4PT6 Nickel, 1994



Loblolly Pine Embryo Analysis S4PT6 Nickel, 1994

