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6. Environmental Stresses and Reproductive Biology of Loblolly Pine (*Pinus taeda* L.) and Flowering Dogwood (*Cornus florida* L.)

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We have long recognized that natural climatic shifts have influenced the development of plant and animal life on earth. These slow temperature fluctuations have resulted in either the extinction or the evolution of various species. However, human activities in the last century have so altered the chemical composition of the atmosphere that it is hypothesized that the process of climatic change has become more rapid. The resultant phenomenon, global warming, could greatly alter the existing vegetation regions on the planet.

The overall goal of most global change research with trees is to measure the impact of these environmental shifts and increased concentrations of the greenhouse gases-carbon dioxide and ozone-on individual species and ecosystems. Most of the research concerns the effects of pollutants and climatic change on seedlings and saplings. This study, however, examines changes occurring in young, yet reproductively active trees and focuses on one of the most sensitive phases of plant development, the reproductive cycle.

Some major environmental factors that are expected to change because of global warming are light intensity and quality, temperature, and moisture. These, in turn, are major factors affecting the various stages of flowering and fruiting in plants (Kramer and Kozlowski, 1979), namely floral bud initiation, flowering/pollen shed, pollination, gametophyte development, fertilization, and embryo/seed development. The effects of these climatic factors vary, depending on the stage of flower or fruit development. For example

1. Light. A high light intensity may increase the number of floral buds in *Tectonia* and *Pinus* (Nanda, 1962; Sarvas, 1962, at the same time favoring female over male flowers in *Acer, Juglans,* and a number of *Pinus* species (Hibbs and Fischer, 1979; Matthews, 1963; Giertych, 1977; Ryugo et al., 1980, 1985). Light quality also affects *Pinus* pollen germination (Dhawan and Malik, 198 1); germination is decreased by white light and enhanced by red light.
2. Temperature. High temperatures in late summer and fall favor the formation of floral buds over vegetative buds in various temperate tree species (Matthews, 1963; Jackson and Sweet, 1972; Menzel, 1983; Owens and Blake, 1985; Southwick and Davenport, 1986). These same temperatures in the following spring, however, may dry the pollination drop present in some species, and thus reduce pollination. Pollen tube growth is slowed or stopped by both high and low temperatures (Mellenthin et al., 1972; Griggs and Iwakiri, 1975; Sedgley, 1977; Sedgley and Annells, 1981; Staudt 1982; Sedgley and Grant, 1983).
3. Moisture. A heavy rain can adversely affect pollen dispersal in wind-pollinated species (Kramer and Kozlowski, 1979), yet can enhance fruit enlargement for cherries, grapes, peaches, pecans, and apples (Uriu and Magness, 1967; Goode and Ingram, 1971). La Bastide and Van Vredenburch (1970), Eriksson et al. (1975) and Fober (1976), Lindgren et al. (1977), Menzel (1983), Southwick and Davenport (1986), and Burgess (1972) all report that moisture stress may enhance floral initiation and seed-cone bud formation in tropical species, although Rehfeldt et al. (197 1) assert that any moisture deficit after cone initiation has an adverse effect. Finally, high relative humidity reduces electrolyte leakage and enhances pollen germination (Hoekstra and van der Wal, 1988; Yates and Sparks, 1989).

All of these three factors can be affected by air pollution, and now some studies have reported that acidity (Van Ryn et al. 1985), air pollutants CO_2, SO_2, and NO_2 (Wolters and Martens, 1987), and ultraviolet radiation (Pfahler, 1981) can affect in vitro pollen germination and tube growth.

Materials and Methods

The loblolly pine study area was on the campus of Mississippi State University. The seventeen-year-old open-grown trees were from a North Mississippi loblolly pine source and ranged in height from 7.3 to 10.0 m. The site had a mean annual maximum temperature of 23.3 °C, a mean annual minimum temperature of 11.2 °C, and a mean annual precipitation of 136.2 cm. The soil underlying the site was a Kipling silty *clay* loam; such soils are strongly acidic with slow permeability and, generally, available water capacity is high.

The flowering dogwood study area was located in a natural stand on the Noxubee National Wildlife Refuge, approximately 25 km south of Starkville, MS. The trees ranged in age from thirty to fifty-two years and 4.3 to 18 m in height. Mean annual temperature and precipitation differed little from that of the pine site. The

underlying soil was a Smithdale sandy loam that was strongly acidic and moderately permeable; available water capacity was usually moderate.

Trees and branches were observed in the year preceding the study and only those that produced fruit or cones were used. Seven trees were selected at each site. Branch chambers constructed on the design of Teskey et al. (199 1) were used to enclose four test branches on each tree, and two branches per tree were used as ambient controls. The chambers were 1.48 m long and 0.58 m in diameter and were equipped with zippers to allow easy access to the branch. In the first year of the two year study (1993) the effects of temperature on loblolly pine flowering and fruiting were examined; in the second year (1994) 2x ambient CO, was added as a treatment to half of the chambered loblolly pine branches, and the temperature treatments on flowering dogwood began. The CO, treatment was added to the dogwood site in late summer of 1994. During the stage when temperature was the only treatment, centrally placed thermisters monitored the conditions of the following three treatments on each tree:

1. Ambient temperature on a chamberless branch.
2. Air temperature on a branch inside an unheated chamber (the reference temperature).
3. Air temperature on a branch inside a chamber heated 2 °C above the reference temperature.

Each of these three treatments was replicated on each test tree (six test branches per tree).

The addition of the CO, treatment in the second year yielded the following combinations in the four branch chambers on each tree:

1. 2x ambient CO,, reference temperature (U+ branches)
2. 2x ambient CO,, elevated (2 “C) temperature (H+ branches)
3. ambient CO,, elevated (2 “C) temperature (H- branches)
4. ambient CO,, reference temperature (U- branches)

Treatment branches were guyed to prevent damage in high winds. Branch chambers were attached at the top and bottom to a platform situated near each test tree as recommended by Teskey et al. (1991). Each chamber had its own one-half horse power industrial model blower mounted on the platform slightly below the level of the chamber. Air was blown through a plastic plenum, 16.5 cm in diameter and of varying length, into the chamber. Plenum diameters were individually adjusted with heavy twine or metal clamps to circulate roughly ten air changes per minute. Air flow was measured manually at installation with a Davis® Turbo-Meter mounted on a metal shaft and confirmed periodically throughout the study.

Omega® series 900 thermisters were placed in a monitored water bath to determine their accuracy prior to being placed on the branches. These temperature probes were found to be accurate to within ± 0.2 °C over a connecting cable distance of 30 m. Thermisters were placed in the center of each chamber and protected from the direct sun by an opaque plastic funnel. A PC computer, a

Keithly Metrabyte® Series 500 data logger and monitoring system, and a program developed by a cooperator in the project were used to monitor both temperature and CO_2. The program 1) activated the 750-watt open element heating units in the + 2 °C chambers if the temperature fell below the reference temperature of + 1.7 °C, and 2) shut the heaters off if the temperature rose above the reference temperature of + 2.1 "C. Temperatures were monitored by the computer in each chamber at the rate of thirty-five measurements per minute; average temperatures were automatically recorded every fifteen minutes throughout the day.

Large, refrigerated storage tanks at each site were the source for the CO_2. High-density polyethylene tubing delivered CO_2 from the tanks to the branch chambers. Flow from the tank to the chambers was controlled by a series of rotometers (one per chamber). A LI-6252 gas analyzer was used to measure CO_2 concentrations every hour; these readings were calibrated against readings taken every two weeks with a separate line that bypassed the normal intake system. The analyzer was calibrated once each week.

Throughout the study, measurements were made on the relative humidity and solar radiation levels both inside and outside the chambers to determine if any seasonal variations existed. Relative humidity was measured with a Jenway® Relative Humidity Temperature Meter (model 5075) and solar radiation with a YSI-Kettering@ radiometer (model 65).

Pollen and seeds were collected by hand; pine cones and flowering dogwood drupes were placed in paper bags identified by a code specifying both tree and treatment. Similarly labelled plastic petri dishes were used for pollen collection, which, in the pine trees, began on the third day of pollen shed. Pollen on the test branch was dusted into the labelled petri dish and thoroughly mixed. This pollen mixture was used for all tests of pollen moisture content, viability, and differential scanning calorimeter (DSC) studies. Flowering dogwood is an entomophilous species, however, which it proved to be very difficult to remove the sticky pollen from the anthers. This problem was resolved by removing entire anthers with the adhering pollen for testing, drying and storage.

All of the seeds produced by the test branches were collected. Loblolly pine cones were picked when color and specific gravity tests on cones on adjacent branches indicated ripeness. Flowering dogwood fruit were picked when they changed color (from green to red). Seeds were logged into a sample record as soon as they arrived at the laboratory.

The pine cones were placed in paper sacks in the laboratory under ambient conditions until they had completely opened. Seeds were separated with hand screens, dewinged by hand, and floated to remove empties. The pine seeds were placed in small, clear plastic bags containing water and soaked overnight. The water was then removed, and the the bags sealed and placed in cold storage (4 "C) for twenty-eight days prior to germination. This is a standard pregermination treatment for loblolly pine. Any extra cleaned seeds were air dried to 10% moisture content, placed in labelled containers, and stored at 4 "C. The flowering

dogwood drupes were depulped in a laboratory blender or by hand, spread to dry, and then stratified for germination tests. The seeds were placed in the same type of bags containing a damp paper towel and stored at 4 °C for at least ninety days as a pregermination treatment. Any extra seeds were air-dried to 10% moisture content and stored at 4 °C in labelled containers.

Upon arrival at the laboratory, some of the freshly collected pollen mixture was immediately tested for viability and moisture content. Pollen quality was evaluated by germination tests and the DSC, a thermal analyzer that measured the energy associated with melting lipids and water in the pollen grains. For moisture-content determination, pollen samples were weighed to the nearest 0.0001 g on an electronic balance, dried at 104 °C in a convection oven for two hours, cooled at room temperature for one minute and reweighed. Excess pollen was dried, tested again for viability, and stored at − 20 °C for future use. If the pollen did not need to be dried (i.e. if moisture content was already 10% or less), the viability was not retested prior to storage at − 20 "C.

Fresh pollen samples collected from each treated or control loblolly pine branch were germinated for seventy-two hours in a Brewbaker-Kwack solution (Brewbaker and Kwack 1963) containing 10% sucrose (Sowa et al. 1991). Pollen samples from flowering dogwood were germinated for seventy-two hours in a Brewbaker-Kwack solution containing 20% sucrose. There were four replicate vials for each sample. Vials were placed in a shaking water bath set at 25 °C to allow both temperature control and aeration while pollen grains were germinating. Germination counts were made on 400 pollen grains per vial for each sample collected from the tree. A pollen grain was considered germinated when the pollen tube length was at least two times the diameter of the grain.

DSC studies were performed with a Perkin Elmer® DSC-7 to determine the effects of temperature and CO, on pollen desiccation and chilling sensitivity. Scans were made on whole pollen grains to find if shifts in peaks or glass formations were detectable.

Seed quality was evaluated by germination tests and seed weight. For seed weight, two 25-seed samples from each treatment branch were weighed to the nearest 0.0001 g. These samples were then used in the standard germination test. Seeds germinated on moist blotters for twenty-eight days in Stults® water-curtain germinators, on a cycle of eight hours of light at 30 °C and sixteen hours of dark at 20 °C (Association of Official Seed Analysts, 1993). Temperature and relative humidity of the germinators was recorded on a hygrothermograph. Temperature was checked daily and adjusted if necessary. Light during the 30 °C cycle was provided by cool-white fluorescent bulbs at levels of at least 500 lux and not more than 1000 lux. Germination was counted three times weekly, and all ungerminated seeds were cut at the end of the test to identify dormant, empty, and dead seeds. On count days, trays were moved up one shelf space and rotated 180" to minimize any effect of temperature stratification or light inequity in the germinator. Germination values were expressed as percentages.

Germination tests were conducted according to the official rules of the Associa-

Table 6.1. Data Quality Objectives for Biological Variables*

Variable	Units	Technique	Range	Quantitative Limits	Precision (CV)	Accuracy (%)	C
Vegetative/reproductive buds	**	visual count	n/a	O-10	15		
female/male buds	**	visual count	n/a	0--.5	15		
Pollen moisture	%	electronic balance	O-200	5-50	5	95	
pollen germination	%	visual count	n/a	O-100	15	---	
Lipid/water peaks[1]	deg. C	Calorimeter	- 196-100	- 100-40	5	95	
seed weights[2]	g	electronic balance	O-200	.5--1.5	10	99	
seed per cone	#	visual count	n/a	5-50	5	---	
seed germination	%	visual count	n/a	O-100	5		

*Variables were measured annually.

**These values are reported as proportions.

1 These measurements were taken only on the first year's pine pollen; because differences were not found, they were discontinued. The sticky dogwood poll be used for such tests.

2 Pine seed weights were not taken in 1993.

tion of Official Seed Analysts, 1993 revision. Under these rules, a "normal" seedling was a "seedling possessing those essential structures that are indicative of its ability to produce a plant under favorable conditions."

Counts of male, female, and vegetative buds were made every spring for two years. Ratios of female/male buds and vegetative/flowering buds were determined for each treatment branch. Date and time of pollen shed and cone/fruit harvest was also recorded. Statistical comparisons between years were not made, because each year's treatment may affect flowering in subsequent years. Quality control information is presented in Table 6.1.

Results and Conclusions

Loblolly pine. The comparative time at which pollen shed began was very predictable over the three-year period of the pine study. Pollen shed started three to eleven days earlier on heated (H) chambered branches than on ambient branches and one to eight days earlier in H than in unheated (U) chambers. Pollen shed on the H branches stretched over a seven to twenty-one day period; on the U branches the pollen shed period was five to thirteen days; on ambient branches the pollen shed period was nine to fourteen days. This suggests a very strong influence of heat sums on time and duration of pollen shed (Boyer 1973, 1978) in loblolly pine. The effects of CO, on the date of pollen shed were negligible, and the beginning of pollen shed varied little on CO, branches within a treatment.

A higher number of male strobili were found on ambient branches than on those in chambers in all three years of the study (Table 6.2). Although there were no

Table 6.2. 1993 to 1995 Male and Female Strobili Counts and Cones Harvested at the Loblolly Pine Site

		Chambers				
Characteristic[1]	Year	H+	H−	U+	U−	Ambient
# Female strobili	1993	—	36		31	96
	1994	1	0	0	0	4
	1995	5	0	0	0	38
#Male strobili	1993	—	2182		2118	2646
	1994	83	7[2]	91	143	348
	1995	1577	9413	1992	1714	5569
# Male strobili/cluster	1993		223	—	205	227
	1994	16	2	11	2	57
	1995	136	84	160	146	458
Total # cones harvested	1993		42		63	42
	1994	3	24	5	14	55
	1995	—	—	1	—	2
Average cone length (cm)	1993	—	8.7	—	8.8	9.1
	1994	10.82	9.43	9.13	8.9	8.9
	1995	—		8.5	—	8.6

[1] Only the temperature treatment was in effect in 1993.
[2] Significantly different from ambient (p = 0.05).
[3] Significantly different from H + (p = 0.05).

significant differences between treatment branches and controls in 1993, this was not the case in 1994 and 1995. In both of these years, branches in heated chambers with ambient CO, (H-) had significantly fewer male strobili than did ambient branches. A significant difference also existed between unheated branches with ambient CO, (U-) and ambient branches in 1994. This latter distinction is questionable, however, because the trend wasn't repeated in 1995. Also, male strobili were so scarce on all branches in 1994 that there was great difficulty in finding them amongst the foliage. This drop in numbers makes strobili formation data from that year, and hence the differences among the treatments observed during that time period, of questionable value. The reduction in male strobili in 1994 was not considered the result of either temperature or CO,-treatment because the numbers on ambient branches were also greatly reduced, and also because the strobili numbers rebounded in 1995. However, there was more than a twenty-fold reduction in strobili in chambers compared to an eight-fold reduction on ambient branches, which may indicate a chamber effect. Although it has been reported that hot, dry weather during the period of bud formation favors floral buds over those that are vegetative (Owens and Blake 1985), the very favorable conditions in 1993 produced very few male strobili; and in 1994, which had triple the normal amount of the rainfall and lower than average temperatures, yielded a twenty-fold increase in male strobili. The effect of the poor reproductive bud formation in 1994 is reflected in the bud ratio data shown in Table 6.3. A large number of male strobili

Table 6.3. Bud Ratios from the Pine and Dogwood Sites

Site	Treatment	Year	Vegetative/Reproductive	Female/Male
Pine	Heated	1993	0.21	0.016
		1994	6.36[1]	0.011
		1995	0.25	0.002
	Unheated	1993	0.17	0.016
		1994	5.26[1]	0.000
		1995	0.19	0.000
	Ambient	1993	0.18	0.03 1
		1994	0.55	0.011
		1995	0.16	0.007
Dogwood	Heated	1994	0.17	
		1995	0.46	
	Unheated	1994	0.15	
		1995	0.46	
	Ambient	1994	0.19	
		1995	0.70	

[1] Significantly different from ambient ($p = 0.05$).

Table 6.4. Pollen Germination for Three Years of Loblolly Pine and One Year of Flowering Dogwood

	Pine			Dogwood
Treatment	1993	1994	1995[1]	1994
Heated	85.2	2.2	53.7	
Heated + CO,		14.1	59.0	0.1
Unheated	54.3	0.4	30.7	
Unheated + CO,		2.7	65.9	0.6
Ambient	75.1	1.2	28.2	0.4

[1] These are preliminary figures; pollen has been germinated but counts are not yet complete.

being formed normally results in a low vegetative/reproductive bud ratio, as indicated by the 1993 and 1995 figures. The poor showing in 1994 resulted in significant differences between chambered and ambient vegetative/reproductive bud ratios. The female/male bud ratios did not differ significantly from year to year.

Pollen moisture at shedding averaged 7.0% on all branches tested in 1993 and 6.5% in 1995. None of the 1994 pollen was used for moisture determinations because pollen was scarce and germination tests were considered a higher priority. Pollen germination varied from year to year but generally was unaffected by chamber, temperature, or CO, (Table 6.4). Even though germination of 1995 pollen from ambient branches was lower than that from treated branches, it could not be assumed that this was a treatment effect because there was great variability in germination from ambient branches and the differences were not significant. The pollen from the few strobili present in 1994 was of very poor quality, and germination was negligible in all treatments. Pollen counts for 1995 indicate that pine pollen germination appears to have rebounded. DSC scans of pine pollen from the various treatment branches indicated no change in peak melting and enthalpy values for either lipids or water in the pollen. These results support the conclusion that pollen quality was not altered by treatment.

Female strobili in the H chambers began expanding eight to nine days prior to those in U chambers and twelve to eighteen days before those on ambient branches. This growth period, encompassing stages 3 through 5 (Bramlett and O'Gwynn 1980), lasted from six to sixteen days. Although female strobili formation appeared to be adversely affected by the presence of the chamber, significant differences did not exist between treatment and ambient branches (Table 6.2). The few female strobili that formed in chambers in 1994 aborted after pollen shed; however, the few number that formed on all fourteen of the ambient branches that year were not enough to make significant differences among the treatments. An interesting abnormality was observed in 1995 on Tree 6 in a H - and a U - chamber. Several bisexual cones were found on the terminal and first order laterals of these branches; the lower half was male, the upper half formed a female conelet. Pollen was shed from the male portion of the strobili, and, after the

bottom half dried, the female conelets were shed along with their male counterparts. This phenomenon was not observed on any other branches of Tree 6, nor on any of the other test trees, nor on any other trees in the stand. In prior studies, this condition has been seen in *Pinus* by Lanner (1966) and in ***Cupressus*** by Lev-Yadun (1992) but not in loblolly pine. Lev-Yadun suggested that the abnormalities that were found in ***Cupressus sempewirens*** were the result of changes in the normal hormonal balance of the tree.

The 1994 cones harvested from H + branches were significantly larger than those that developed on ambient, H −, and U+ branches (Table 6.2). However, they also averaged significantly fewer full seeds/cones than those from ambient and H- branches, as did those from H+ vs H- branches in 1993 (Table 6.5). Unfortunately, the absence of cones from heated chambers in 1995 and the few number of cones on unheated branches prevented the determination that this characteristic was definitely the result of the applied treatments.

The percent of full seeds in the 1994 cones from heated chambers dropped from the 1993 average, stayed about the same in cones from unheated chambers, and also dropped in ambient branch cones (Table 6.5). This variability may have been because of the reduced amount of pollen produced that year. Germination of full seed was over 90% regardless of treatment.

Flowering dogwood. The average number of flowers per inflorescence, which varied little from year to year or among treatments, was 20.5 (Table 6.6). To date,

Table 6.5. 1993 to 1995 Seed Data from the Loblolly Pine Site

		Chambers				
Characteristic[1]	Year	H+	H−	U+	U−	Ambient
Total # seeds	1993	—	4779		7387	4612
	1994	65	782	243	749	3067
	1995	—	—	19	—	178
Average # seeds/cone	1993	—	113.8	—	117.3	109.8
	1994	21.7	24.1	48.6	53.5	56.8
	1995	—	—	19.0		89.0
Total # full seeds	1993	—	2661	—	4805	3166
	1994	16	151	136	439	1328
	1995	—	—	13	—	83
Average # full seeds/cone	1993	—	63.4	—	76.32	75.1
	1994	5.32	6.3[3]	27.2	31.4	24.6
	1995	—	—	13.0	—	41.5
% Germination	1993	—	94.4	—	95.1	98.3
	1994	93.8	95.4	100.0	97.9	99.1
	1995	—	—	38.5		36.1
Seed weight (mg)	1994	28.1	24.7	30.9	25.9	20.1
	1995	—		19.8		21.6

[1] Only the temperature treatment was in effect in 1993; 1995 cone data not statistically analyzed because the sample size was too small.
[2] Significantly different from ambient (p = 0.05).
[3] Significantly different from H+ (p = 0.05).

Table 6.6. 1994 to 1995 Flower, Fruit, and Seed Data from the Flowering Dogwood Site

Characteristic'	Year	Chambers H+	Chambers H-	Chambers U+	Chambers U-	Ambient
# Inflorescences	1994	—	515'	—	425	258
	1995	443	377	209	201	383
#Flowers	1994	—	10626	—	8962	5341
	1995	8120	9064	4067	4131	7359
# Flowers/Inflor.	1994	—	20.6	—	21.1	20.7
	1995	18.3	24.0	19.5	20.6	19.2
#Fruits	1994	—	1212	—	1009	938
	1995	57	77	37	83	98
#Fruits/Cluster	1994	—	3.9	—	3.1[1,2]	3.9
	1995	1.3'	1.4'	1.2'	1.9'	2.5
Average fruit wt. (g)	1994	—	0.55'	—	0.48[1,2]	0.32
	1995	0.41'	0.48	0.37	0.36	0.29
Average fruit diam. (mm)[3]	1994	—	8.63'	—	8.14[1,2]	7.01
	1995	7.94'	8.41'	7.47'	7.59'	7.04
Average seed length (mm)	1994	—	9.12'	—	8.86[1,2]	8.08
	1995	7.91	7.88	7.94	8.19	7.90
Average seed diam. (mm)	1994	—	5.09'	—	4.89[1,2]	4.57
	1995	4.85	4.94'	4.88	4.86	4.70
% Germination	1994	—	39.4	—	50.5'	31.5

[1] Significantly different from ambient ($p = 0.05$).
[2] Significantly different from H ($p = 0.05$)
[3] All possible comparisons of 1995 fruit diameters are significantly different from each other except U+ vsu-.

the only significant difference in flower production was between the number of inflorescences produced on H vs ambient branches in 1994.

There was no difference in the number of fruits formed per cluster on H and ambient branches in 1994 (Table 6.6); however, there were significantly fewer fruits per cluster on unheated branches than on either the heated or ambient branches. An interesting fact to note, however, is that a steady progression in both fruit size (weight and diameter) and seed size (length and diameter) was found, with the largest occurring on H branches and the smallest on ambient branches. This may be a result of the extension of the growing season in the heated and unheated chambers. Flowers opened ten days sooner and fruits were collected seven days later from chambered branches than from non-chambered branches. Germination differed little between H and ambient branches, but seeds from U branches had an inexplicably higher percentage of germination than those on ambient branches.

The number of fruits per cluster was significantly fewer on all chambered vs ambient branches in 1995 (Table 6.6). A difference also existed between U + and U - branches but not between H + and H branches, suggesting that the addition of CO, was not a consistently significant factor in increased fruit production. Again, fruits from the chambered branches were significantly larger in diameter;

however, only those from H^+ branches were also significantly heavier. In 1995, increases in fruit size did not result in increased seed size. The larger fruits did not produce larger seeds even though flowers opened five days sooner and fruits were harvested seven days later on chambered branches. The question of whether or not the extended growing season was responsible for the increase in fruit size in 1995 is complicated by the reduction in number of fruit per cluster. The size increase may simply be the result of reallocation of carbohydrate and nutrient resources to a fewer number of developing fruit rather than to the extension of the growing season by the temperature enhancement.

Dogwood pollen was first collectable from heated, and then from the unheated and ambient branches. Within a treatment, collection occurred over a four to seventeen day period. Collection of dogwood pollen proved to be difficult; in 1994, collection of the pollen was attempted without removing any of the flower structures. In 1995, whole anthers were collected and used in germination and moisture tests. Average moisture was 12%, a relatively low figure when considering that anther tissue was present. However, it is believed that moisture content was greatly reduced by the length of time occurring between collection and time of arrival in the laboratory. Dogwood pollen germination was very low in 1994 (Table 6.4), and, despite changes to the growing medium, the low counts were repeated in 1995. Sucrose in the Brewbaker-Kwack (1963) medium had been reduced to 15% for 1995 dogwood pollen germinations in the hope that this might correct the problem. Our inability to isolate significant quantities of dogwood pollen in either 1994 or 1995 precluded the planned DSC experiment. The DSC tests were set up to examine moisture and lipids in pure pollen and could not be done with the anther tissue present.

Results to date indicate that temperature plays a role in reproductive structure formation and in both fruit and cone development. Although the results in pine are somewhat overshadowed by possible chamber effects and by an overall reduction in female reproductive structures, there are still marked changes in both male and female strobili phenology and in cone size. The chambers did not have a deleterious effect on flower bud formation in dogwood. Inflorescence and flower counts were always higher on chambered branches than on ambient branches, flowering phenology was noticeably changed, and fruits were larger. At this time, the effect of CO, on the reproductive biology of both species appears negligible.

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