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PHOTOPERIODISM AND DORMANCY IN

SILVER MAPLE SEEDLINGS

(TITLE)

BY

SISTER MARIA REGIS, KILKENNY

B.A. in French, Manhattan College, 1956
M.A. in English, St. John's University, 1965

THESIS

SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
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1972

YEAR

I HEREBY RECOMMEND THIS THESIS BE ACCEPTED AS FULFILLING
THIS PART OF THE GRADUATE DEGREE CITED ABOVE

10 May 1972
DATE

15 May 1972
DATE

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INTRODUCTION

The primary purpose of this study was to investigate day length as a factor influencing dormancy in seedlings of Acer saccharinum L. One of the first to suggest that a specific role was played by light and darkness in the growth and development of plants was MacDougall (1903). However, it was not until Garner and Allard conducted their lengthy experiments in photoperiodism (1920, 1923), that interest was aroused. While their investigations did include some woody species: Liriodendron tulipifera, Acer negundo, Malus pumila and Rhus glabra, Garner and Allard were primarily concerned with the flowering response in herbaceous plants, but investigators have also observed definite effects on vegetative growth as well (Gevorkiantz and Roe, 1935; Pauley, 1954). There is a greater need for further studies, especially in regard to woody species.

The suggestion that further studies in woody species are necessary is not meant to convey the impression that nothing has been done in this area. Experiments have been carried out, and many investigators have shown that dormancy is controlled by day length (Vaartaja, 1957, 1959, 1960; Wareing, 1948, 1950, 1951).¹ For

1. Also see: Moshkov (1930); Bogdanov (1931); Jester and Kramer (1939); VanderVeen (1951); Olmstead (1951); Downs (1956); Kramer (1956); Waxman (1956); Phillips (1941); Nitsch (1957); Hamner (1957).

the greater number of species, short or decreasing photoperiods result in a stoppage of growth. A darker green leaf color is one of the first observed effects of short photoperiods (Garner and Allard, 1923; Olmstead, 1951; Downs, 1956). Populations within tree species with wide latitudinal occurrence in temperate and cold climates vary considerably in their photoperiodic responses (Vaartaja, 1959).

In their early investigations of the subject, Garner and Allard reported that after receiving supplementary light, Liriodendron tulipifera continued to grow continuously for a period of 18 months; Acer negundo grew very little under short days when compared to growth under normal days of summer; Malus pumila, when exposed to short days, made more rapid growth than when exposed to normal days. Subsequent observations (Gorter, 1955; Hoyle, 1955) have indicated that photoperiodic conditions have little or no effect in different varieties of apple. At present, it is commonly accepted that short days hasten dormancy in the seedlings of the majority of woody species, and it is delayed by long days.

Species which grow well into autumn possess a decided biological advantage if they are equipped with a photoperiodic mechanism by which the dormant condition is entered as late as possible, but before the arrival of the severe conditions of winter. Growing shoots manifest a far greater susceptibility to frost damage (Wareing, 1948; Moshkov, 1930; Olmstead, 1951). Dormancy brought about by the photoperiodic response includes leaf fall in deciduous species, and loss of green color precedes abscission.

Garner and Allard (1923) also showed that leaf fall was delayed with additional illumination. It has even been reported that leaf fall was delayed by street lights giving an effective illumination of only one foot candle (Matzke, 1936). Since leaf fall is associated with dormancy and an increased resistance to unfavorable conditions, the time of its incidence in relation to the onset of severe winter conditions must be an important factor in the adaptation of the species to a particular region.

In summary, the body of evidence which has been amassed indicates that day length affects growth duration and extension and time of leaf abscission in woody species.

In regard to the breaking of dormancy in spring, the length of day no longer acts as a limiting factor (Adams, 1925). It is the temperature that plays the limiting role (Kramer, 1956; Wareing, 1943, 1948, 1956a, 1956b). In addition, the breaking of dormancy in spring is, in many ways, dependent on low temperatures during the winter (Coville, 1920; Gustafson, 1938; Wareing, 1948, 1951, 1956; Vasil'yev, 1961; Olmstead, 1951; Olson-Nienstaedt, 1957).

While the previously mentioned facts indicate that a certain amount of study has been done in regard to photoperiodism in some woody species, it is obvious that additional study is necessary. For this reason, the present investigation was undertaken in the spring of 1971. Certain factors were most influential in determining the scope of the work. First, there was a definite time limit which was considered. For this reason, it was decided to work with only one species, Acer saccharinum which is known to be

a prolific seed bearer whose seeds germinate readily and grow quickly. In addition, it has a wide latitudinal and longitudinal range and no recognized taxonomical sub-species as does the otherwise suitable Acer rubrum. Some work has been done in other species of the genus (Garner and Allard, 1923; Jester and Kramer, 1939; Matzke, 1936; Wareing, 1948). No results have been reported concerning the silver maple. In studying this species, no effort was made to examine physiological processes. The primary concern was the effect of length of day on dormancy and leaf abscission in seedlings collected from sites at six different latitudes. When additional visible external differences were observed, they were recorded.

Should the reader be interested in a review of the literature dealing with the many facets of the subject of photoperiodism in plants, he is referred to Garner (1937), Burkholder (1936), Gevorkiantz and Roe (1935), Hamner (1944), Wareing (1948) and Murneek and Whyte (1948).

MATERIALS AND METHODS

Collection procedures

Silver maple seedlings used in this experiment were handled in the following manner. Seeds were collected in June, 1971, from Minnesota, Montreal, Plattsburg and Rock Island (for complete geographical locations, see table 1). In each instance, except sample one from Red Lake, the seeds were selected at random after they had fallen from trees native to the area. The seeds from the first sample, Red Lake, were taken from one tree before they had fallen. Seedlings were transplanted from Mississippi and Louisiana on July 12, while the ones from Oakland were collected on July 29. In every instance, the samples were brought to the greenhouse in Charleston, Illinois.

Germination procedures

Planting took place on June 21. Approximately one hundred seeds of each sample were placed in flats containing two inches of sand and one inch of sterilized soil. A quarter of an inch of sterilized soil was sprinkled on top of the seeds. The flats were placed in a sink containing about six inches of water. When the water had percolated through the soil so that it began to appear on the top, the flats were removed and placed under a bench in the greenhouse. After 72 hours, they were put on top

of the bench where they were exposed to the sun.

Transplanting procedures

After eight days, the seeds had germinated sufficiently for transplanting. Three inch clay pots were filled with sterilized soil, and one seedling was placed in each. Twenty three potted seedlings were put in each flat. As they were filled with the potted seedlings, the flats were placed in water and soaked. For the next 72 hours, the flats were kept in the shade. After this period, they were placed in the sun. This procedure was followed for each one of the seed samples.

The seedlings from the banks of the Pearl River in Mississippi and Louisiana were transplanted upon collection into three inch clay pots. In Charleston, they were re-potted into three inch clay pots containing sterilized soil. Seedlings collected from the farm near the Embarass River in Oakland were transplanted in a similar manner. All of the potted seedlings were put in flats, watered, and placed in the shade for 72 hours. After this, they were moved into the sun in the greenhouse.

Experimental procedures

All of the seedlings developed under greenhouse conditions until August 10. At that time, one half of the seedlings of each of the eight samples were planted outside, and the other half were placed in a section of the greenhouse where the minimum temperature was kept at 45°F. They received no supplementary illumination. (See table 2 for the exact number of seedlings in each of the sixteen samples.)

On September 22, and every two days thereafter until November 17, the number of leaves on each seedling of each of the sixteen samples was counted. A total of 29 counts was taken of leaf fall. (See tables 3 and 4 for total numbers.)

During this period, at three day intervals from September 22 until October 26, leaf discs were cut from each of the sixteen samples. Twenty discs were cut at random from each set of seedlings. The discs were placed in plastic bags and brought immediately to the laboratory and refrigerated.

Each one of the sixteen samples was handled in the same way. From each plastic bag, ten discs were chosen randomly; they were weighed and the fresh weight recorded. The weighed discs were placed in a clean mortar, and 40 ml. of 80% (v/v) acetone was added. The tissue was ground without sand in a clean pestle. This was done for three to five minutes. Following this, the pulverized tissue was transferred to a Buchner funnel containing a pad of Whatman Number 1 filter paper. To remove any residue remaining on the pestle and in the mortar, both were rinsed with 80% acetone. This was put in the funnel and suction was used to extract all leaf pigment. If any green residue remained on the filter paper, it was removed to the mortar and ground again using the same procedure. When the plant tissue was devoid of chlorophyll, the filtrate was poured into a 100 ml. beaker, and the filter paper and tissue were discarded. The sides of the funnel and the original flask were rinsed with 10 ml. (more, if necessary) of 80% acetone which was then added to the filtrate. To calculate

the amount of chlorophyll present, the final volume of the filtrate was adjusted, in most instances, to 40 ml. by adding the necessary amount of 80% acetone.

Optical density was read and recorded at 652 nm. by using a Bausch and Lomb Spectronic 20 Spectrophotometer (after MacKinney, 1941). An 80% acetone solvent blank was used. The amount of chlorophyll present was calculated on the basis of milligrams of chlorophyll per gram fresh weight of leaf tissue extracted. Calculations were based on the following equation (after Arnon, 1949).

$$\frac{\text{Optical Density at 652}}{34.5} \times \frac{\text{Volume of the final filtrate}}{1,000 \times \text{Weight (fresh) of tissue}}$$

On January 7, the greenhouse samples were moved outdoors where they received approximately 800 hours of cold treatment. After this period, they were returned to the greenhouse. Following this, the farm and greenhouse samples were observed every two days in order to detect the first external signs of growth.

RESULTS

The seedlings which were planted on the farm and those in the greenhouse behaved similarly, and there was a noticeable time span in leaf abscission (figure 1). Between September 22 and November 17, 95% of the leaves from the seedlings which originated in Red Lake (1 & 2) had fallen (figures 2 & 3). During this same period, only 52% of the leaves from the Montreal sample had abscised (figure 4), while 68% of those from Plattsburg had fallen (figure 5). Of the sample from Rock Island, 56% of the leaves had fallen by November 17 (figure 6). At this time, only 29% of the leaves from Douglas County had fallen (figure 7), 16% from Mississippi (figure 8), and 8% from Louisiana (figure 9). By December 9, total leaf abscission had occurred in all the samples except those from Quebec, Mississippi and Louisiana. The few leaves which remained on the seedlings were a dark brown color.

With the abscission of the leaves in the silver maple seedlings, the green color at first became darker. Following this, there was a gradual loss of green color. However, while all of the samples turned the dark green color and then began to fade, only the samples from Red Lake (1 & 2), Montreal and Plattsburg showed any signs of changing to a predominantly red and red-orange color. The remaining samples exhibited only a waning of the dark green color.

On September 24, the chlorophyll content in the leaves from the Red Lake (1) sample was 4.8 mg. total chlorophyll/ g. fr. wt. tissue; at the same time, the chlorophyll content for Louisiana was 4.3. Approximately one month later, the chlorophyll content for Red Lake (1) was 1.2, and the Louisiana sample showed a content of 3.8. A complete record of the results of the chlorophyll assays is provided in tables 5 & 6.

The samples from Red Lake (1 & 2), Montreal and Plattsburg were much shorter in height and appeared to stop shoot growth earlier. The samples from Rock Island, Mississippi and Louisiana were the tallest at the end of the season. Those from Mississippi and Louisiana continued to grow aerially long after growth had ceased in the remaining samples. However, while there was a noticeable difference in growth above ground, all samples continued to grow below the ground as was evidenced by the increase in space taken by the roots.

Another noteworthy difference was observed in the size of the leaves. Those from Red Lake (1 & 2), Montreal and Plattsburg had the smallest leaves, while the leaves on the samples from Mississippi and Louisiana were the largest.

In the spring, there was a difference in the behavior of the greenhouse population and that of the one on the farm. After being returned to the greenhouse, the cold-treated seedlings that survived began growth after approximately 21 days. The percent of survival was as follows: 82% from Red Lake (1), 34% from Red Lake (2) and 34% from Montreal. There was no growth in any of the seedlings from Plattsburg, Douglas County, Mississippi

and Louisiana.

Leaf buds opened on the outdoor populations between April 21 and 23 which was the same time as the local populations. In the samples from Red Lake (1 & 2), Montreal and Rock Island, 100% survived. Only one seedling (5%) of the Plattsburg sample died, while 13% of the Mississippi sample lived. In the Louisiana sample, 50% survived. The populations from Louisiana and Mississippi were somewhat delayed, and began to open between April 23 and 26.

Most of the seedlings in both samples began growth terminally and laterally. However, in the seedlings grown outdoors, the samples from Montreal and Plattsburg grew terminally with very little lateral growth.

CONCLUSIONS

1. Figures 2 through 9 give clear indication that both samples, i.e. the seedlings on the farm and those in the greenhouse behaved in a similar manner. Where there were any differences in the rate of leaf fall during the course of the experiments, the end results were similar enough to warrant treating the two samples as one in the report of the results and in the discussion.
2. Day length is an influencing factor in the dormancy of silver maple seedlings.
3. The rate of leaf abscission differs according to the locality of the origin of the species. The northern samples lose their leaves with greater rapidity than the southern samples.
4. When the severe conditions of winter arrive, the northern samples appear to be completely dormant, and as a result do not suffer from winter conditions.
5. Southern seedlings continue to grow and are much taller at the end of the season when planted in a location farther north than their original environment.
6. Because they continue to grow much longer and are not dormant with the arrival of winter conditions, more southern samples appear to be killed.
7. Seedlings from northern localities tend to become dwarfed when planted in an environment farther south than their place of origin.
8. Chlorophyll content appears to fluctuate as a result of the variations in temperature as was determined by my own observations and the records of Ozark Airlines which supplied information concerning cloud cover and daily temperature.
9. Opening of the buds in spring does not appear to be a function of photoperiod. Rather, temperature seems to be the greatest influencing factor.

DISCUSSION

Figures 2 to 9 indicate clearly that the reaction of the farm and greenhouse samples was very similar. For this reason, when the results are discussed, they will apply to both samples.

As discussed in the introduction, silver maple seedlings, similar to many other woody species, manifest a photoperiodic sensitivity. With decrease in day length, this species makes a gradual transition from normal plant activity to a state of dormancy.

Certain definite external signs are obvious as silver maple seedlings undergo the gradual change from the active to the dormant state. At first, there is a noticeable change in the leaves from the normal green color to a much darker shade of green. Garner and Allard (1923) were the first to note such a general change, and Olmstead (1951) also reported specifically that sugar maple seedlings behave similarly. According to Garner and Allard (1923), this darkening of the leaves is due to photoperiod too long for etiolation but still below the optimum for flowering. They associate the dark green color with a condition of relative inactivity in the green portion of the plant. Each of the present samples showed this darkening followed by a waning of the green color in the leaves. In this study, an additional change is evident in the northern samples (Red Lake,

1 and 2, Montreal and Plattsburg). These samples gradually changed from a light green to a red, red-orange color. Wilson and Loomis (1967) attribute this change partly to heredity and partly to temperature and light. Why the remaining samples did not undergo this change may be due to the degree of dormancy. The samples from the most northern locality, Red Lake, Montreal and Plattsburg, were obviously very close to complete dormancy while the southern samples, Mississippi and Louisiana, were not. This may be a possible explanation as to why the samples from Rock Island, Douglas County, Mississippi and Louisiana showed very little red pigment even as late as November 17.

As the days became progressively shorter, the waning of the green color previously mentioned was paralleled by a gradual leaf abscission. Here, the behavior of the samples is noticeably different, and the rate of defoliation varied according to the locality of the origin of the seedlings. Those from the northern region lost their leaves much more rapidly than those from a region farther south. This variation in the rate of leaf abscission is normal for many woody species studied previously and reported by Wareing (1948); Pauley (1950); Pauley and Perry (1954); Kriebel (1957); and Vaartaja (1962). Figure 1 clearly depicts the decided difference in the behavior of the seedlings from the various localities.

There appears to be a close relationship between the rate of leaf fall, the normal number of frost free days and the date when the particular sample may have become less active above

ground (table 7). In their natural environment, the seedlings from Red Lake would have begun their transition from an active to a dormant state on or about September 20 after a comparatively short frost free period of 120 days. In addition to the shortening of the length of day, this early date for the inception of dormancy coupled with the short period of possible growth may be the explanation for the fact that by November 17, these samples had lost 95% of their leaves by abscission.

The natural populations in Montreal and Plattsburg should have begun the transition from activity to dormancy between September 30 and October 5 after approximately 140 frost free days. By November 17, the Montreal sample had lost only 56% of its leaves, and at the same time the Plattsburg sample has lost 65% of its leaves. While the difference is not really significant, the fact that the Plattsburg sample was taken from a location 65 miles south of the Montreal sample would lead an investigator to expect a more rapid rate of defoliation from the latter sample. The very slight difference may be a genetic one, or it may be due to the fact that the climate of Montreal is more of a maritime one in which natural selection might possibly be an additional factor influencing the abscission rate. However, there seems to be no clear explanation for the slight variation in the Montreal sample.

In referring again to Figure 1, it is obvious that the remaining samples were consistent in the rate at which abscission occurred. As the seed sources progressed from a more northern locality to a

southern one, there was a decided difference in the date on which the change from the active to the dormant state should have begun. In its natural habitat, the Rock Island sample would have begun the transition on October 10 after a frost free period of approximately 160 days. Since only 56% of the leaves had fallen by November 17, it is quite possible that complete dormancy had not been reached by this time. Douglas County annually enjoys approximately 20 additional frost free days over Rock Island and should be expected to enter dormancy somewhere near October 15. By November 17, only 29% of the leaves from the Douglas County sample had abscised. Undoubtedly, these seedlings were more active than the ones from Rock Island. Since all of the seedlings were grown in Charleston which is very close to Douglas County, the close similarity of the natural environment of the original sample and the location at which the experiment was conducted may be a possible explanation for the significant difference between the Rock Island sample and the one from Douglas County.

The most prolonged activity is obviously in the samples from Mississippi and Louisiana which in their natural environment would have enjoyed a frost free period of 220 and 280 days respectively. Only 16% of the leaves from the Mississippi sample had abscised, while abscission of the leaves from the Louisiana sample was a mere 8% by November 17. This suggests that the length of day becomes less important in regulating dormancy in samples of silver

maple with a more southern origin. In these samples, the number of frost free days may be more influential.

The location of the origin of the seedlings is also closely paralleled with the cessation of stem elongation and leaf initiation. In this study, seeds from a northern source were planted farther south and those from the south were planted farther north. No further growth of stems or leaf initiation was noted after October 1 in the samples from Red Lake (1 & 2), Montreal and Plattsburg. Rock Island and Douglas County had ceased elongation by October 8. Mississippi and Louisiana samples continued not only stem elongation but also leaf initiation. As a result of this, the seedlings from the northern locality were dwarfed in comparison to those from Mississippi and Louisiana which were the tallest by the end of the season. Other investigators have also reported this relationship between ultimate height and period of growth in seedlings which are planted farther south or north of their natural environment (Kriebel, 1957; Vaartaja, 1962; Moshkov, 1933, 1934, 1935; Sylven, 1942).

As a result of this, by the time that the severe winter conditions had set in, the northern samples were almost completely dormant while those from the south were still active above ground. Thus, the former did not suffer from the extreme cold, and the latter might possibly be killed by the frost conditions because they were only partially dormant. That this killing from frost does take place in southern samples when planted in a more northern location has been reported by Bogdanov (1931). Even

though the northern samples were completely dormant and the others only partially so, all of the samples continued to remain quite active below the ground as was evidenced by the increase in space taken by the roots. Garner and Allard (1923) also reported this continued activity below the ground after the aerial portions were partially or completely dormant.

The smaller leaves on the samples from Red Lake (1 and 2), Montreal and Plattsburg may be due to the short frost free period in their natural environment or it may be genetically controlled. Whatever the explanation, the leaves on the samples from Mississippi and Louisiana had the largest leaves at the end of the season.

Mention has already been made of the changes in leaf coloration. Another interesting point concerning pigmentation is concerned with chlorophyll content. Despite some variations which existed on September 24 in all healthy and actively growing samples, the chlorophyll content ranged from 4.8 mg. total chlorophyll/g. fr. wt. tissue for Red Lake (1) sample to 4.3 for Louisiana. Approximately one month later, the content for the same samples ranged from 1.2 for Red Lake to 3.8 for Louisiana. The decrease in chlorophyll content is closely paralleled by the decrease in temperature. There also seems to be some relationship between the degree of dormancy and the amount of chlorophyll content.

The fact that there was a difference in the time at which leaf foliage began to appear in the spring indicates that photoperiod is not the determining factor in the breaking of dormancy. The

greenhouse samples, after a chilling period, started growth much earlier than the farm samples. The factor that was different between the two populations was temperature. No doubt, there were other internal physiological factors, but temperature seems to be the dominant external factor which regulates the breaking of dormancy in spring.

The majority of the seedlings in the outdoor population broke dormancy while a large percentage of the samples in the greenhouse did not. Since all other factors were equal except the length of the wintering period, it seems feasible to conclude that some greenhouse samples did not receive adequate chilling.

While the results of this study clearly indicate that dormancy in silver maple seedlings is controlled by photoperiod, there is no suggestion that, by extrapolation, the same conclusion might be made for the mature tree (Wareing 1956).

APPENDIX

Figure 1 shows the percent of leaves which fell at each location. It represents the average between the greenhouse and the farm. Day lengths are for the Charleston area.

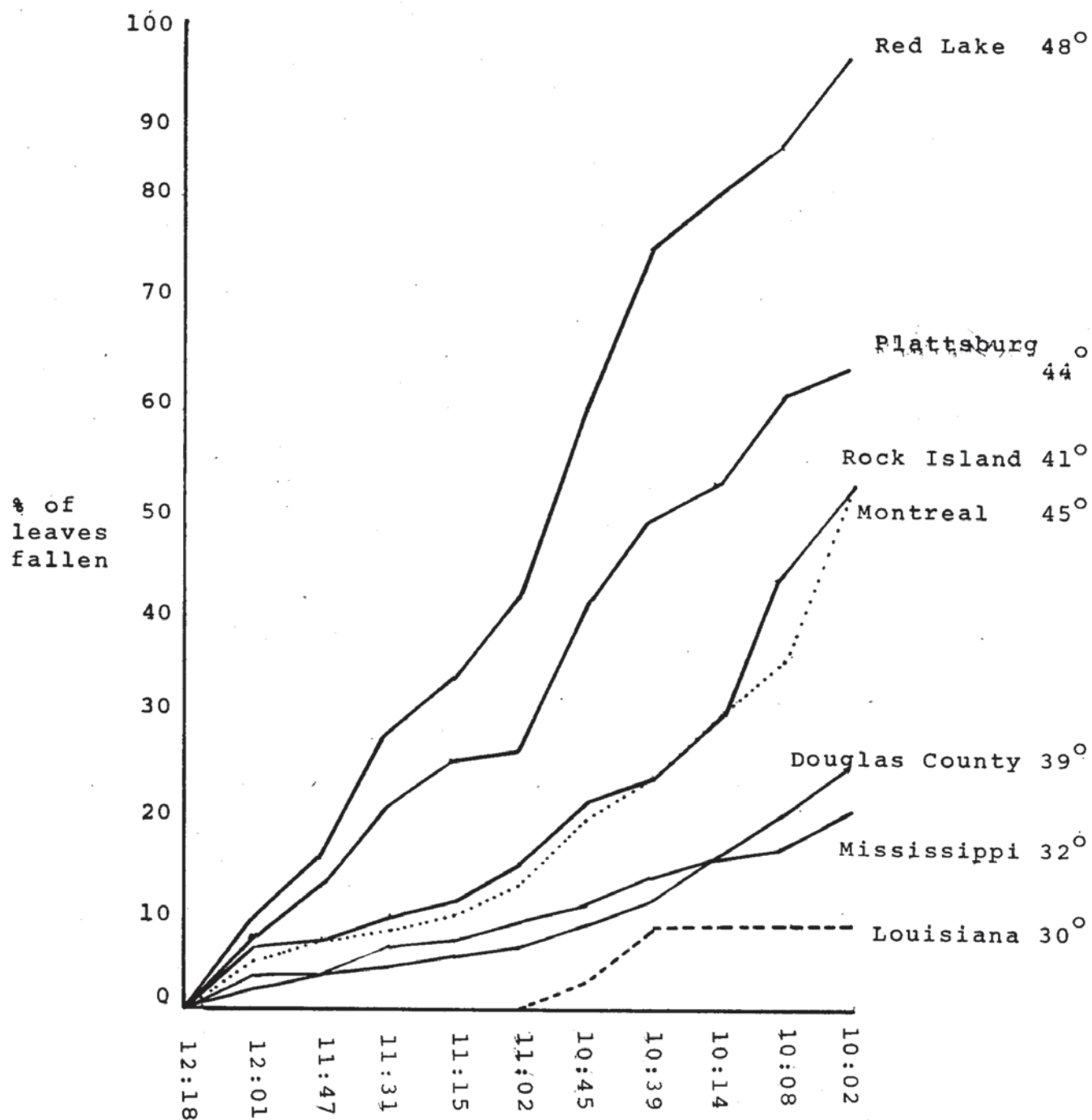


FIGURE 2

Figure 2 shows leaf fall for Red Lake (1) sample. The broken line represents the greenhouse sample and the solid line represents the farm results. Day length is shown for the Charleston area and the date represents the date of the same length of day in the natural environment of the sample.

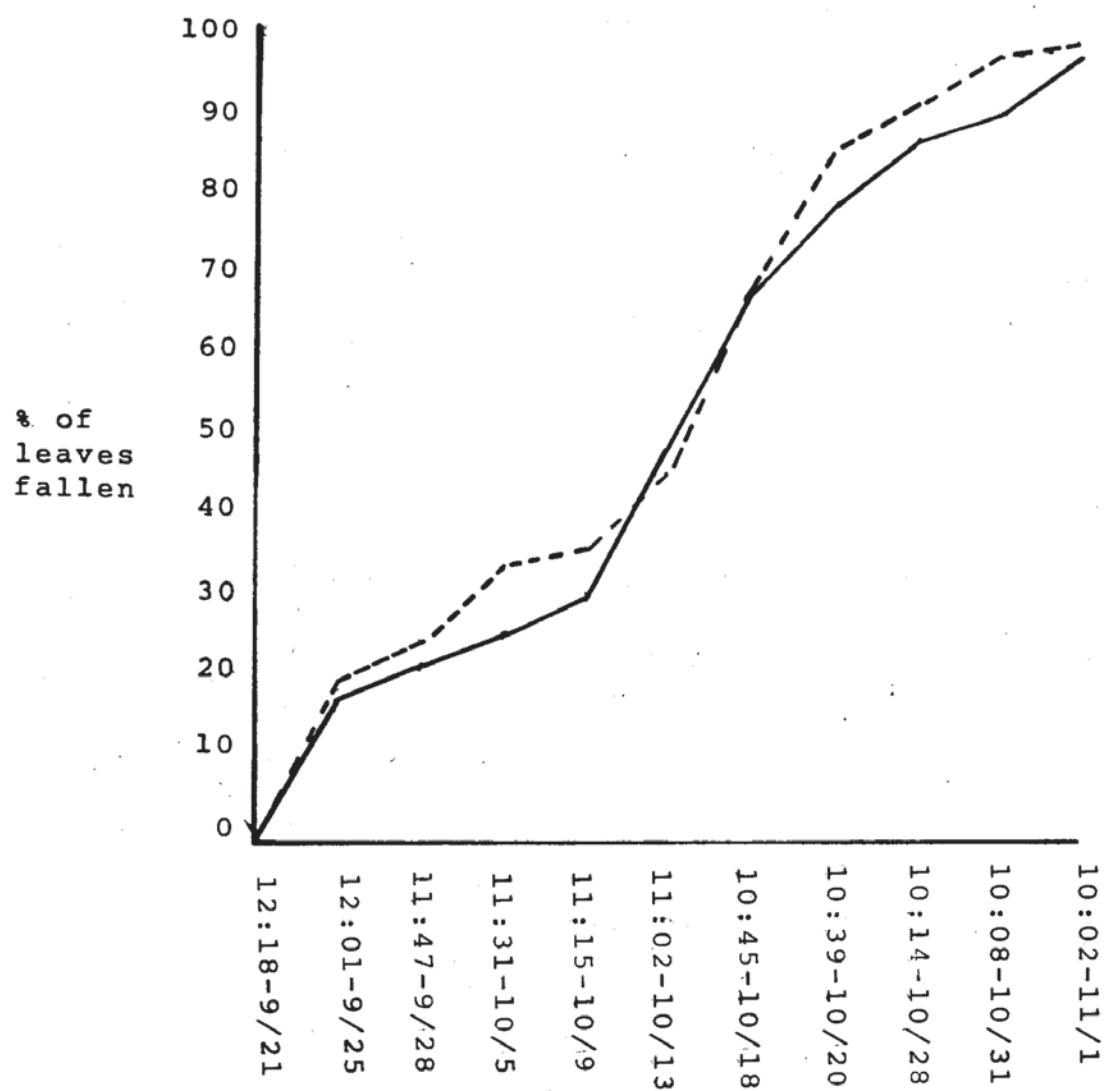


FIGURE 3

Figure 3 shows leaf fall for Red Lake (2) sample. The broken line represents the greenhouse sample and the solid line represents the farm results. Day length is shown for the Charleston area and the date represents the date of the same length of day in the natural environment of the sample.

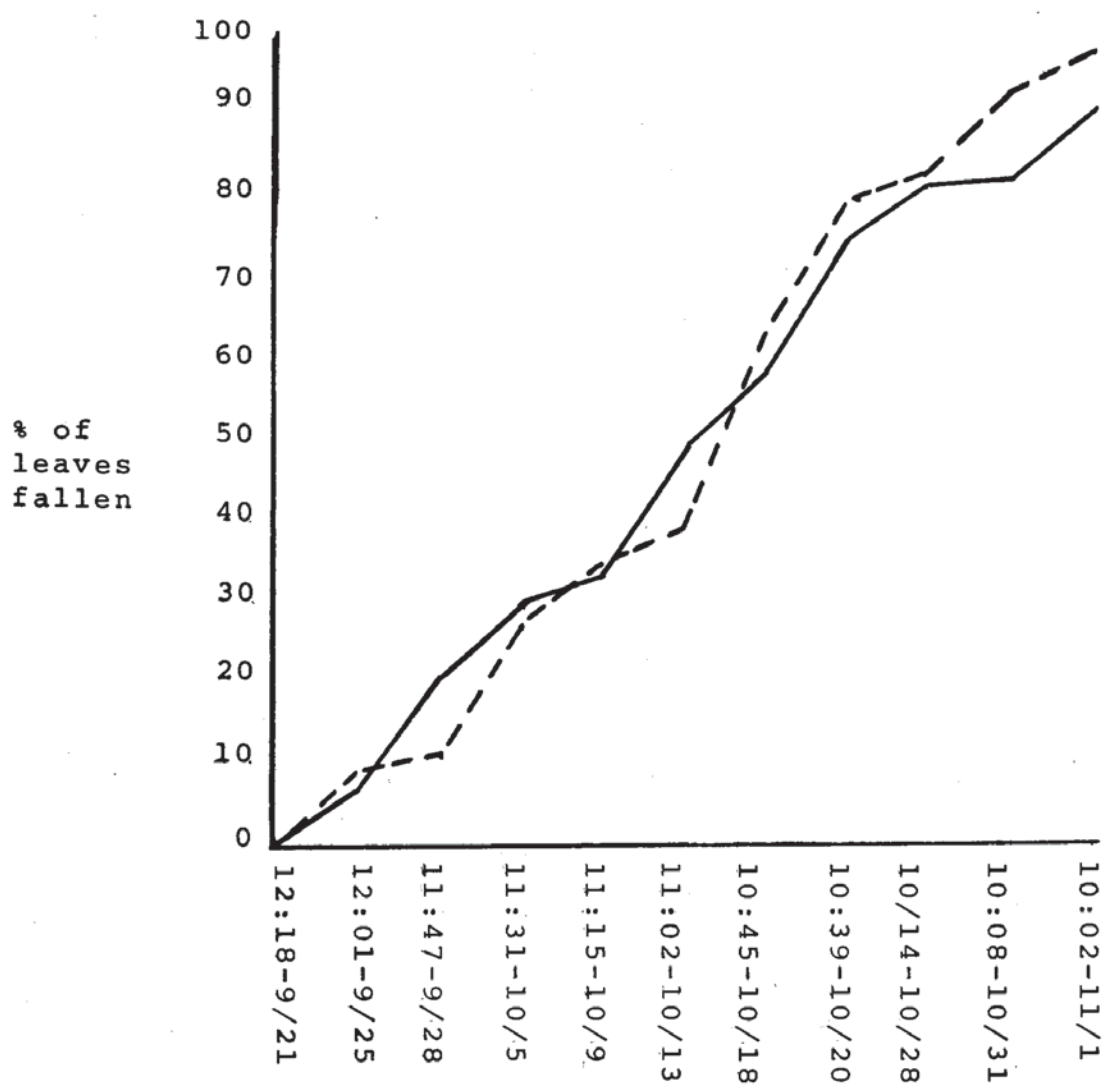


FIGURE 4

Figure 4 shows leaf fall for the Montreal sample. The broken line represents the greenhouse sample and the solid line represents the farm results. Day length is for the Charleston area and the date represents the date of the same day length in the natural environment of the sample.

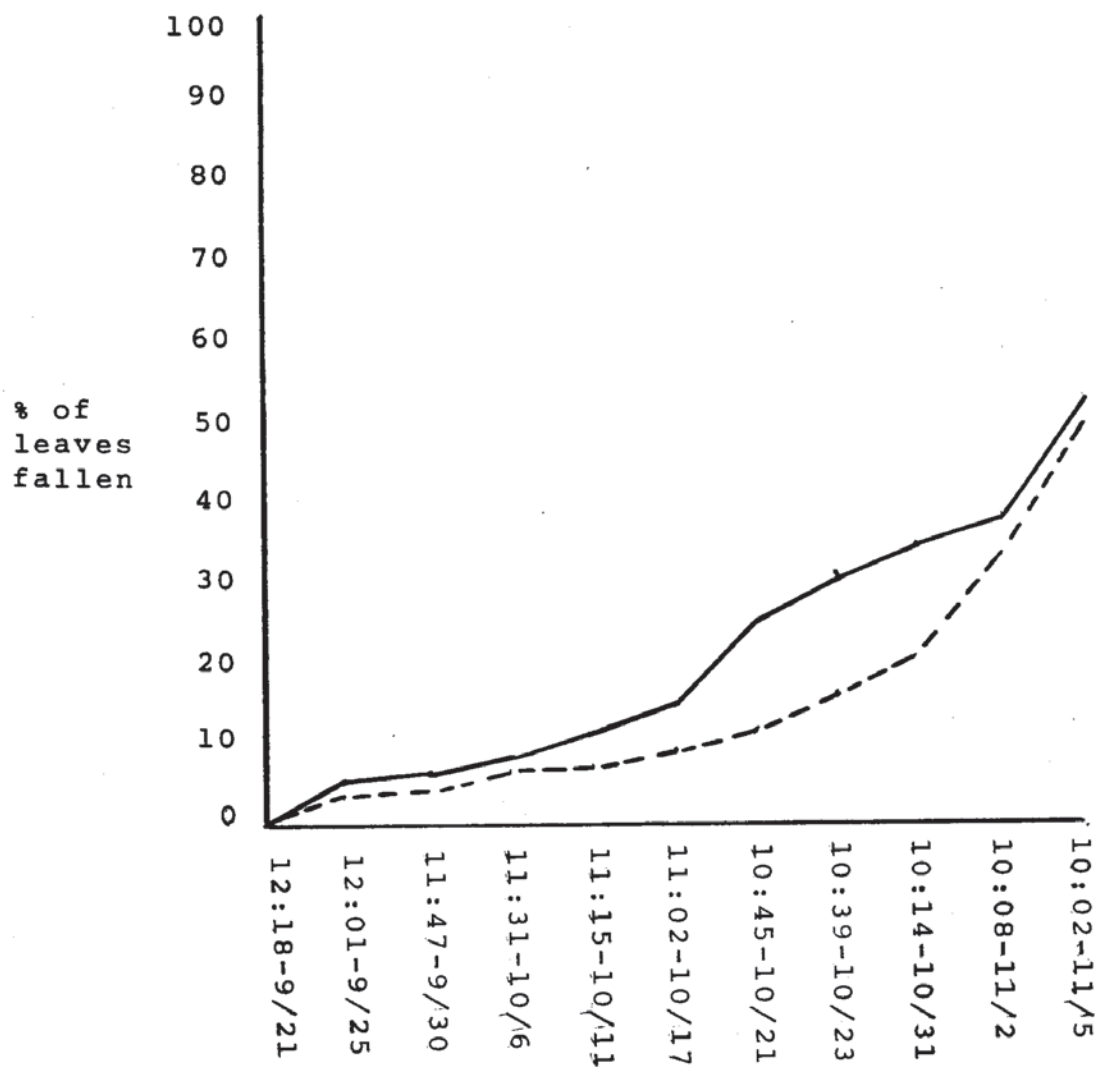


FIGURE 5

Figure 5 shows leaf fall for the Plattsburg sample. The broken line represents the greenhouse sample and the solid line represents the farm results. Day length is shown for the Charleston area and the date represents the date of the same day length in the natural environment of the sample.

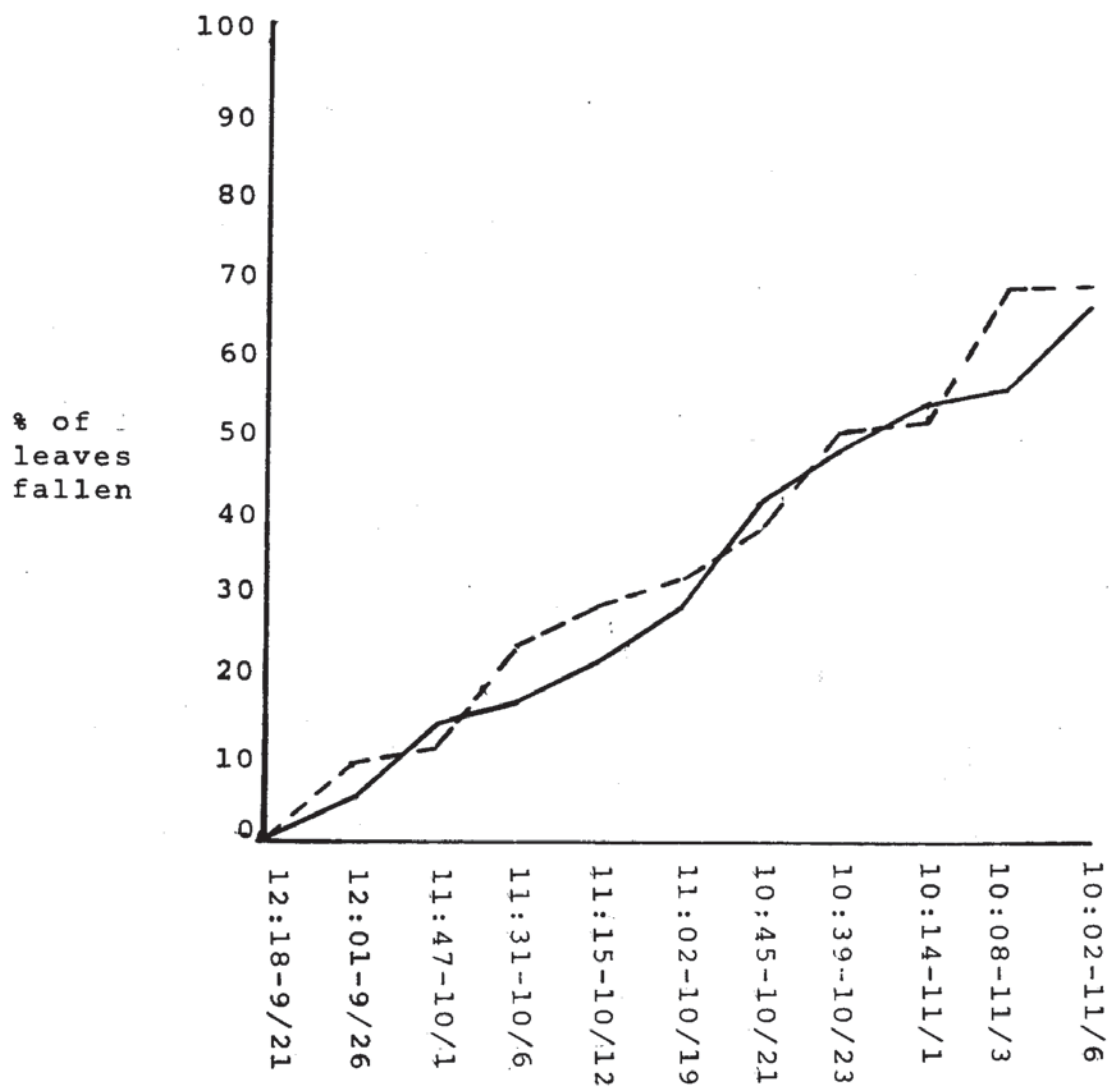


FIGURE 6

Figure 6 shows leaf fall for Rock Island. The broken line represents the greenhouse sample and the solid line represents the farm results. Day length is shown for the Charleston area, and the date represents the date of the same day length in the natural environment of the sample.

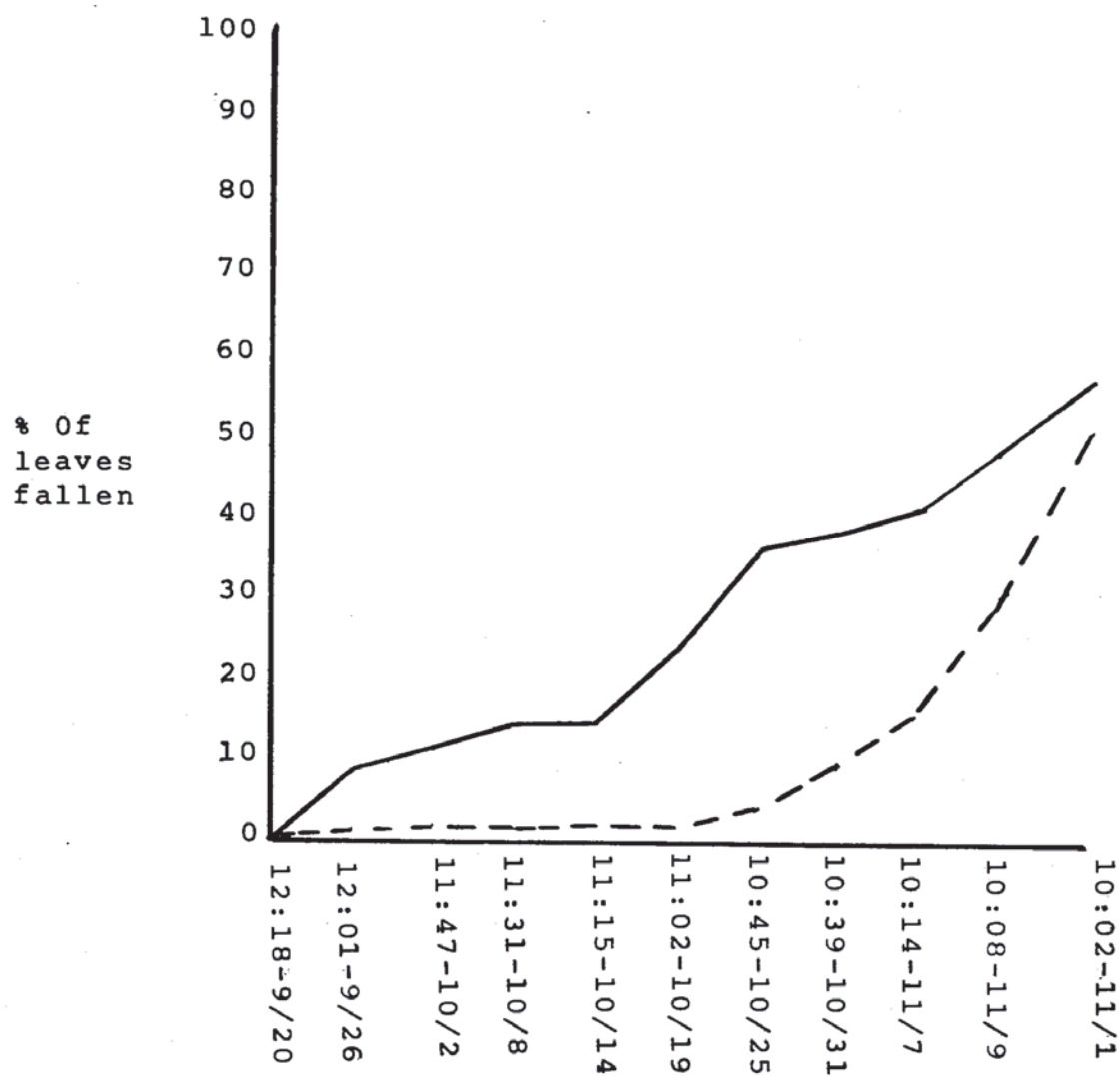


FIGURE 7

Figure 7 shows leaf fall for Douglas County. The broken line represents the sample from the greenhouse and the solid line represents the farm results. Day length is shown for the Charleston area, and the date represents the date of the same length of day in the natural environment of the sample.

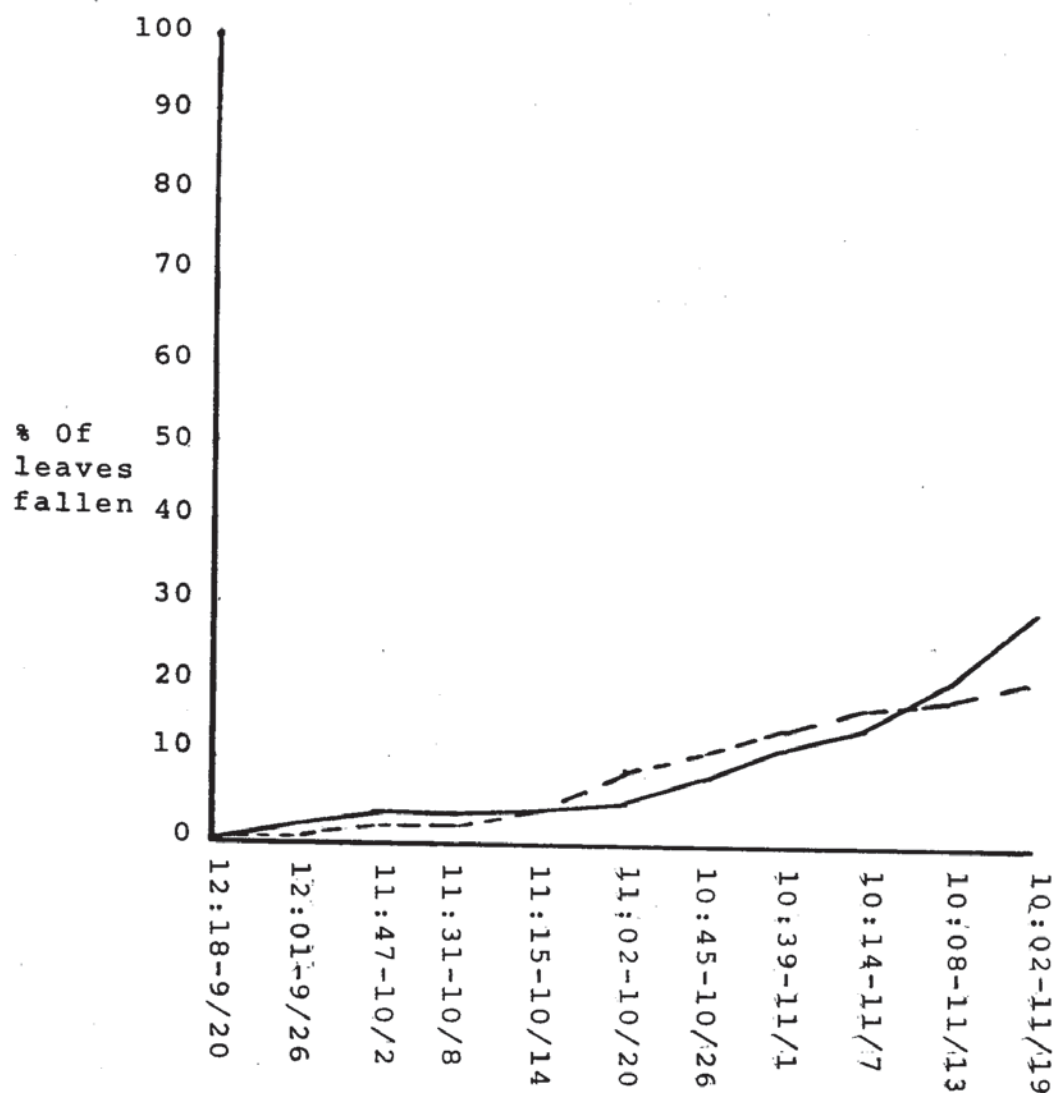


FIGURE 8

Figure 8 shows leaf fall for the Mississippi sample. The broken line represents the greenhouse sample and the solid line represents the farm results. Day length is shown for the Charleston area and the date represents the date of the same length of day in the natural environment of the sample.

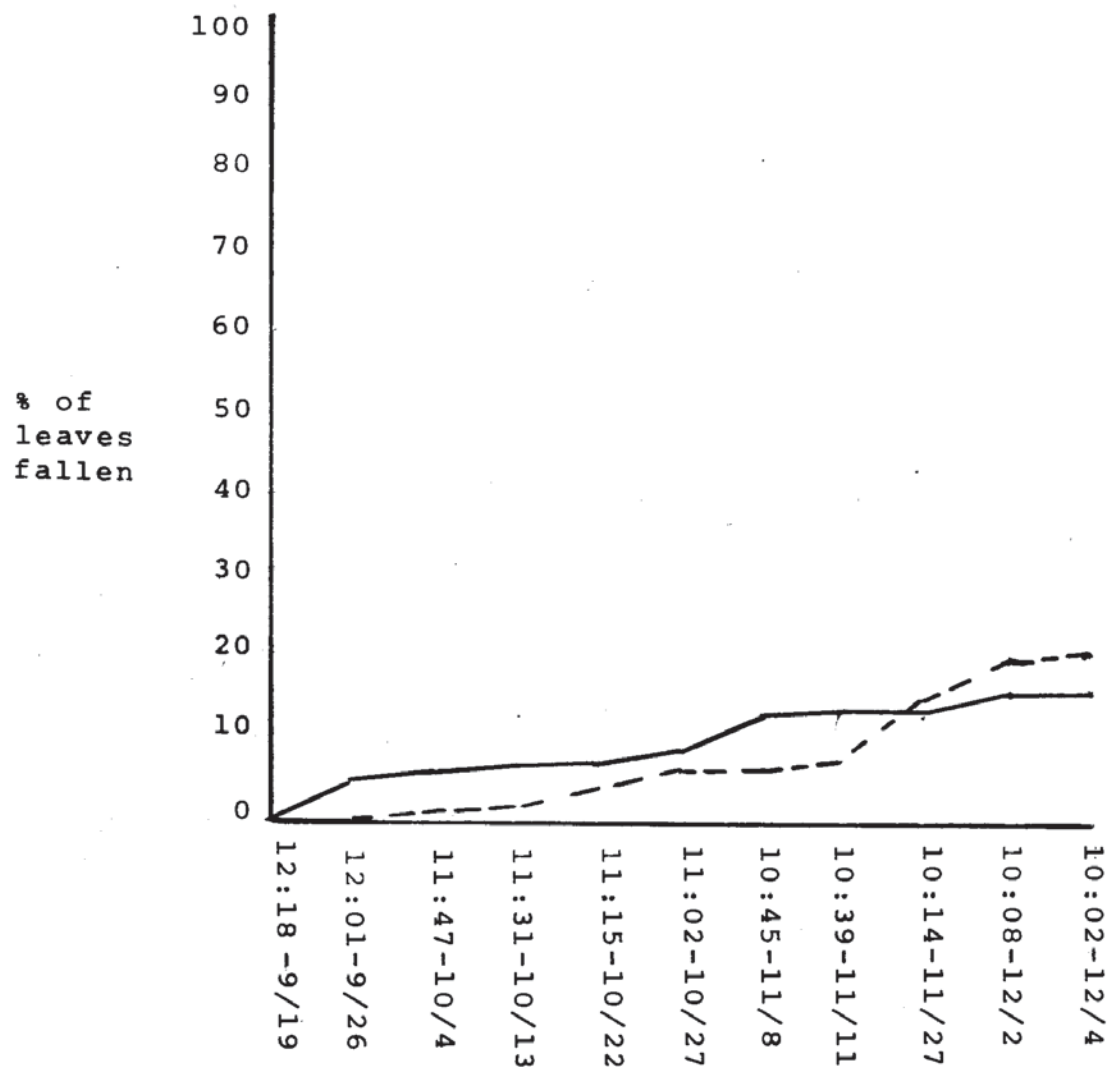


FIGURE 9

Figure 9 shows the leaf fall for the Louisiana sample. The broken line represents the greenhouse sample and the solid line represents the farm results. Day length is shown for the Charleston area, and the date represents the date of the same length of day in the natural environment of the sample.

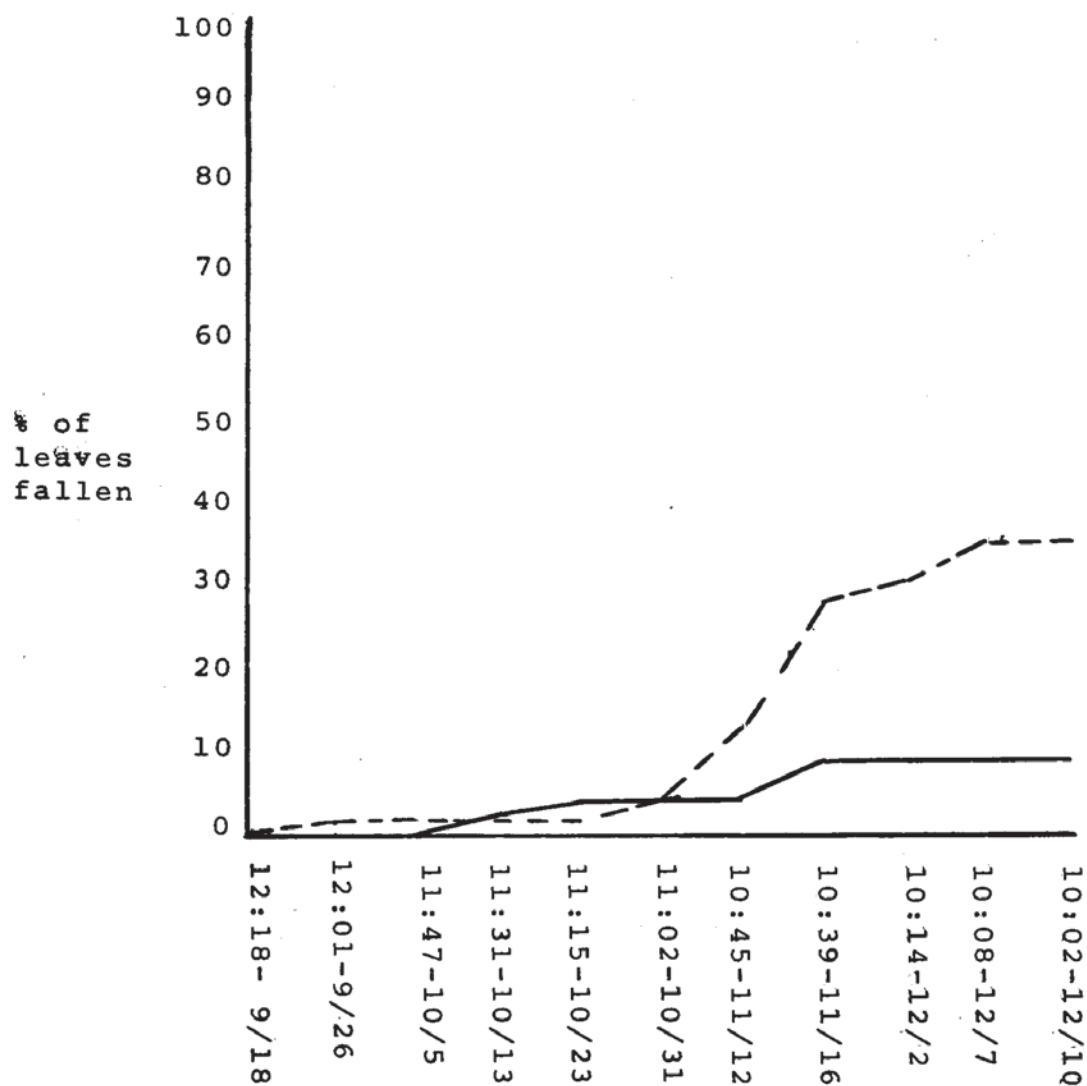


TABLE 1 contains the exact geographical locations of the eight samples used in this study.

City	State	County	Latitude	Longitude
Red Lake (1 and 2)	Minnesota	Beltrami	48.02N.	96.04W.
Montreal	Quebec	Chambly	45.31N.	73.30W.
Plattsburg	New York	Clinton	44.40N.	73.30W.
Rock Island	Illinois	Henry	41.31N.	90.27W.
Oakland	Illinois	Douglas	39.45N.	88.15W.
Terry	Mississippi	Hinds	32.15N.	90.27W.
Bogalusa	Louisiana	Tangipahoa	30.48N.	89.52W.

TABLE 2 contains the number of seedlings in each sample.

Location	Farm	Greenhouse
Red Lake (1)	23	23
(2)	23	23
Montreal	22	23
Plattsburg	21	23
Rock Island	17	22
Oakland	18	24
Terry	7	7
Bogalusa	2	2

TABLE 3 contains the total number of leaves counted in the farm samples. These are the numbers on which the graphs are based.

Location	Date	9/22	9/26	10/2	10/8	10/14	10/20	10/26	11/1	11/7	11/13	11/17
Red Lake	1	237	197	187	178	166	119	77	49	29	22	5
	2	285	264	229	203	191	161	117	70	51	48	23
Montreal		357	338	332	329	319	304	269	248	233	222	169
Plattsburg		297	281	251	246	232	215	173	149	137	131	100
Rock Island		177	162	158	153	152	134	114	109	104	90	67
Douglas County		169	165	165	161	161	161	155	148	143	135	122
Mississippi		104	99	98	97	97	95	90	89	89	86	86
Louisiana		24	24	24	24	22	22	19	17	17	17	17

TABLE 4 contains the total number of leaves counted in the greenhouse sample. These are the numbers on which the graphs are based.

Location	Date	9/22	9/26	10/2	10/8	10/14	10/20	10/26	11/1	11/7	11/13	11/17
Red Lake	1	253	204	190	177	163	135	82	38	21	6	2
	2	273	251	223	198	181	167	102	58	47	18	6
Montreal		403	391	385	380	377	366	353	337	319	267	198
Plattsburg		376	342	303	288	269	252	225	184	175	120	120
Rock Island		356	356	352	352	349	348	343	319	295	234	189
Douglas County		348	342	336	336	334	321	310	299	295	288	287
Mississippi		138	138	138	135	135	130	122	110	106	106	104
Louisiana		77	76	76	76	76	72	65	57	57	55	54

TABLE 5 contains the results of the chlorophyll assays for the farm samples, expressed in milligrams of chlorophyll/gram fresh weight.

Date	Red Lake(1)	(2)	Montreal	Plattsburg	Rock Island	Douglas Co.	Mississippi	Louisiana
9/24	4.8	2.9	6.1	3.3	16.6	17.1	1.7	3.6
9/27	6.4	4.1	4.0	3.2	8.2	9.7	4.6	5.1
10/1	6.8	5.5	4.4	3.6	5.2	3.1	5.8	7.7
10/5	2.3	2.4	2.6	2.2	2.6	2.9	2.7	2.3
10/9	2.1	2.0	1.8	2.0	2.3	3.1	2.7	3.0
10/13	1.5	1.5	2.3	2.2	2.6	1.5	2.8	1.8
10/16	1.3	1.8	1.5	1.1	2.4	2.1	2.4	2.6
10/20	.8	1.0	1.9	1.0	2.0	1.3	2.2	2.7
10/23	.9	1.5	1.8	1.3	2.0	1.7	2.2	2.6
10/26	1.0	1.9	1.8	1.8	2.9	2.4	2.5	3.2

TABLE 6 contains the results of the chlorophyll assays for the greenhouse samples, expressed in milligrams of chlorophyll/gram fresh weight.

Date	Red Lake(1)	(2)	Montreal	Plattsburg	Rock Island	Douglas Co.	Mississippi	Louisiana
9/24	4.8	4.7	4.5	3.6	5.5	4.3	8.1	5.1
9/27	7.1	15.8	5.6	4.7	4.6	9.6	8.4	24.1
10/1	2.9	2.8	3.4	1.3	3.3	3.8	3.3	2.3
10/5	2.6	3.0	2.9	2.9	3.8	4.6	4.0	3.7
10/9	2.1	2.8	2.2	2.2	3.6	3.7	3.0	3.4
10/13	2.1	2.6	2.3	2.0	3.0	3.0	3.5	3.5
10/16	2.0	2.3	3.0	2.5	3.9	4.2	4.1	3.6
10/20	1.4	2.1	2.2	2.4	3.1	3.7	3.8	4.5
10/23	1.7	1.7	2.3	2.4	3.4	3.5	2.9	3.7
10/26	1.3	2.0	3.1	2.6	4.4	4.1	3.8	4.3

TABLE 7 gives the approximate number of frost free days in the natural environment of each sample. It also contains the date of the first frost. (Data taken from United States Department of Agriculture Yearbook).

Location	Frost Free Period	Date of First Frost
Red Lake (1 & 2)	120 days	September 20
Montreal	140 days	September 30- October 5
Plattsburg	140 days	September 30- October 5.
Rock Island	160 days	October 10
Douglas County	160-180 days	October 15
Mississippi	220 days	November 5
Louisiana	280 days	November 20

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