CORRELATION BETWEEN PHENOLOGY AND CALORIC CONTENT IN FOREST HERBS

BARBARA J. KIECKHEFER

University of Illinois, Urbana

Several phenological studies have been made in which factors such as breaking of dormancy, flowering, fruiting and seed dispersal of forest herbs have been recorded (Wolfe, Wareham and Scofield, 1949; Leopold and Jones, 1947; Deam, 1920-1952). Smith (1915) and Hopkins and Murray (1933) found that the time of occurrence of major events in plant development remained relatively constant from year to year. Lindsey and Newman (1956), on the other hand, found considerable variation for at least the first flowering dates of many herbaceous species in Indiana. They considered temperature to be the most important factor controlling time of flowering.

Closing of the canopy and leaf abscission cause considerable changes in the environment of the understory plants. Expansion of tree leaves causes a great reduction in sunlight, temperature fluctuation, precipitation, transpiration rate and wind velocity at the forest floor. These microenvironmental changes presumably cause readjustments in the physiological activity and phenological sequence of the plants in the lower synusia. Meyer and Anderson (1952) reported that exceptionally high respiration and assimilation rates occur in floral meristems and that various foods, inorganic compounds and water are translocated to developing flowers. They stated, however, that little is actually known about metabolic activities during flower and fruit production.

Golley (1958) compiled a list of caloric determinations for various plants from contributions by several investigators. However, none of these determinations were done on a phenological basis. Golley (1960) made caloric determinations on some of the species comprising an old field community in southern Michigan but found little seasonal variation. While considerable research is being done on the consumption and expenditure of energy in certain animal species during various phases of their life cycles, little or no research is being done on the energy dynamics of individual species of plants. The author is unaware of any studies relating changes in caloric content with plant development. The objective of this study was to determine whether any changes in energy content occur during the growing season, and if so, whether they are correlated with changes in plant phenology.

Methods

Native spring and early summer flowering herbs were collected from a 10 x 10 m plot in Trelease Woods (Section 1 T19N R9E) Champaign County, Illinois. The species collected included: *Claytonia virginica* (spring beauty), *Dicentra canadensis* (squirrel corn), *Trillium recurvatum* (purple trillium), *Osmorhiza* *longistylis* (sweet cicely) and *Hydrophyllum canadense* (waterleaf). Nomenclature is that of Jones (1950). Voucher specimens are on deposit in the University herbarium.

Only one plot was used in order to confine the collecting to a relatively uniform habitat. With reduced variation in habitat, variations in caloric content could be more easily correlated with phenological variation. Collections were made at approximately one week intervals from March 31 to June 1, 1960, and at approximately two week intervals from the latter date until August 3. Both roots and shoots were collected. The number of plants collected depended upon the species and upon the phenological condition. Approximately 50 g (fresh weight) samples were collected for each species. After extraneous material was removed and roots were washed to remove soil, the samples were oven dried for about 48 hours at 80° C and ground twice in a Wiley mill (20 mesh/in).

Caloric content determinations were made using a Parr adiabatic oxygen bomb calorimeter. Two determinations were made on each sample (except where widely divergent results were obtained, in which case a third determination was made). Values were not corrected to an ash-free weight basis. Average caloric values for carbohydrates (4100 cal/g), proteins (5700 cal/g) and fats (9400 cal/g) obtained from Fruton and Simmons (1953) were used as a standard for the interpretation of experimental data.

A slightly modified form of Nessler's Procedure (Umbreit, Burris and Stauffer, 1957) was used for analysis of total nitrogen content. Duplicate 4mg samples were digested in Nessler tubes by adding 1 ml of 20% H_2SO_4 (no copper selenite added) and heating on an electric plate for about an hour. After cooling for at least a minute, 2 drops of 30% H_2O_2 were added, and heating was resumed for 15 minutes. Cooling, adding of H_2O_2 and heating were repeated a second time in order to completely clear the samples. When the tubes had cooled to room temperature, 20 ml of deionized water were added and the samples stirred vigorously. Stirring was repeated after addition of 4 ml of 4N KOH and again after addition (by blowing) of 2 ml Nessler's reagent. This solution was diluted to 35 ml with deionized water and allowed to stand for 15 minutes. Optical density was measured with a spectrophotometer at 490 mμ. These readings were then converted to mg protein/g dry weight (Nx6.25).

Though no quantitative environmental data were obtained, generalized observations of climatic conditions were recorded along with observations of phenological condition of the species collected. The latter included observations of the time and amount of vegetative growth, flower bud initiation, flowering, fruit development and vegetative drying. A record was also kept of the initiation, the gradual development and the complete closing of the tree canopy.

Phenological Observations

The first observations of the study

area were made on March 29. Although a heavy snow had just melted three days previously, all of the species mentioned above had broken dormancy. In fact, *Claytonia, Hydrophyllum* and *Osmorhiza* had already penetrated the leaf litter. By March 31, some *Dicentra* were beginning to appear above the litter. Abundant etiolated stems of *Claytonia* were found below the humus, and flower buds were already present. *Trillium* ranged in height from 4 to 6 cm, *Osmorhiza* from 2 to 3 cm.

On April 3 about 10% of *Claytonia* flowers were open with the remaining buds showing pink color. Only a small amount of vegetative growth occurred in all species between March 29 and April 8 due to abnormally low temperatures (Illinois State Water Survey data, March - April, 1960). By this date, however, large buds were present on *Trillium*. *Claytonia* was in full bloom on April 14 and continued to be so for about 10 days. Although flower buds were visible on *Dicentra* on the 14th, full bloom was not attained until the 20th. Flowering had just begun in *Trillium,* while considerable leaf expansion had occurred in *Osmorhiza* and *Hydrophyllum*. Leaf buds were just beginning to expand in the canopy, but leaf development was almost completed on buckeye seedlings and saplings.

By April 27 most *Dicentra* and *Claytonia* were through blooming, while *Trillium* was in full flower. *Osmorhiza* and *Hydrophyllum* continued vegetative growth. *Hydrophyllum,* rather than *Claytonia* and *Dicentra,* now formed the predominant herbaceous cover.

Although *Dicentra* produced very few fruits, those which were formed were mature on May 4. Leaves of this species were turning yellow on this date, and many new corms were observed. The fruits of *Claytonia* were nearly all mature, and the petals of many *Trillium* flowers had dropped. Flower buds were present on about 50% of *Osmorhiza*. The leaves of *Hydrophyllum* had attained a height of about 45 cm. In areas where *Hydrophyllum* was sparse, *Laportea canadensis* had become very predominant in the herbaceous layer.

During the week from May 4 to May 11, little change took place due to cold, wet weather. However, *Claytonia* seeds were dispersed; *Dicentra* leaves were dying; most *Trillium* were through blooming. The tree canopy began to close during this week. By May 18 the canopy was almost completely developed, *Dicentra* and *Claytonia* had disappeared from the herbaceous layer and a few *Hydrophyllum* were in bloom, although flower buds were not yet visible on many individuals.

On May 25 the canopy was fully developed. *Laportea* was 0.7 to 1 m in height, and yellowing was observed in *Trillium*. By June 1 *Trillium* was drying. The majority of *Osmorhiza* were in fruit by the latter date, while approximately 90% of *Hydrophyllum* were in bud.

The author was not in Urbana after June 1. Therefore, no further field observations of phenological condition were recorded. However, while preparing samples for grinding, it was noted that fruits of *Osmorhiza* were green on June 23 and had matured by July 7.

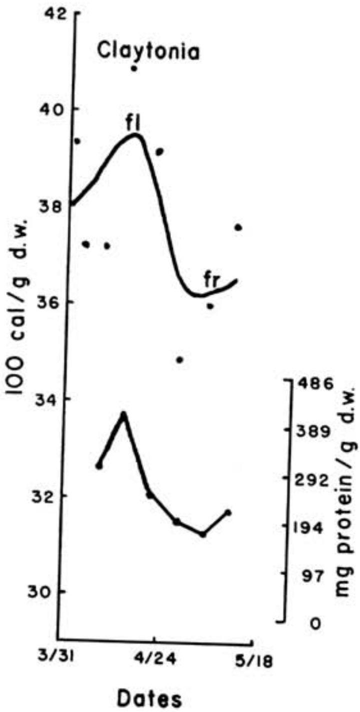

FIG. 1.—Caloric and protein determinations for *Claytonia virginica*, (fl-flowering, fr-fruiting).

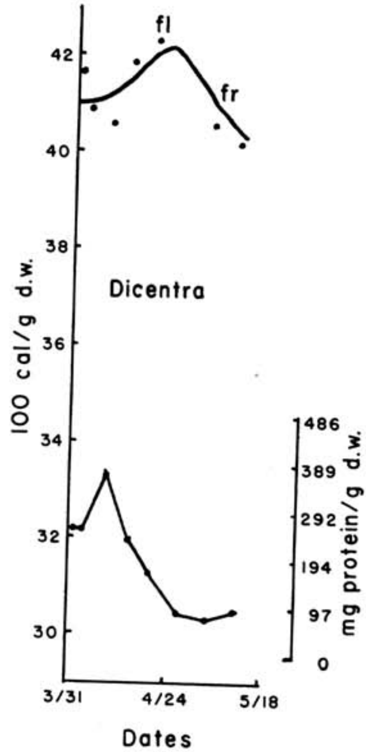

FIG. 2.—Caloric and protein determinations for *Dicentra canadensis.*

RESULTS AND DISCUSSION

Caloric content was found to vary with plant development. *Claytonia virginica* (Fig. 1) and *Dicentra canadensis* (Fig. 2) showed a peak in caloric content at maximum flowering, while the peak for *Trillium recurvatum* (Fig. 3) occurred at maximum flower bud development (general curves were drawn from sight inspection). Caloric values for *Claytonia* dropped from 4100 cal/g at flowering to 3500 cal/g two weeks after flowering. By the time of vegetative die back, caloric content rose to about 3750 cal/g. *Dicentra* and *Trillium* showed a continuous drop in caloric content following peak values. *Dicentra* dropped from 4250 cal/g at flowering to about 4000 cal/g at vegetative die back, while *Trillium* dropped from 4200 cal/g at maximum floral bud development to about 3950 cal/g at dormancy. *Osmorhiza longistylis* (Fig. 4) and *Hydrophyllum canadense* (Fig. 5), on the other hand, showed a continuous increase in calories per gram over the entire growing season. Caloric values for *Osmorhiza*

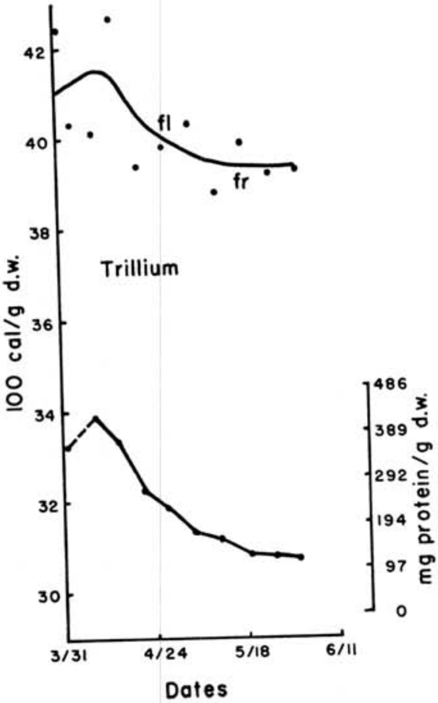

FIG. 3.—Caloric and protein determinations for *Trillium recurvatum*.

increased from about 3500 cal/g at the beginning of the season to approximately 4100 cal/g at the end. Values for *Hydrophyllum* rose from 3250 cal/g to 3800 cal/g followed by a slight drop (to 3700 cal/g) at the end of the summer.

Corrections of caloric values to an ash-free weight basis were made on limited amounts of material at a later date. A rise in caloric values occurred in every case, but the general seasonal pattern was maintained. The extent to which the values increased varied with the species.

The variation in time of maximum caloric content between the species could possibly be explained by inherent differences. Differences in the manner and time of floral development may be associated with differences in time of peak caloric content in *Claytonia, Dicentra* and *Trillium. Claytonia* produces flowers and leaves on the same stem. Flower buds are already present when the stem appears above the litter. The same is true for *Trillium* except that only one large flower bud is produced per plant. *Dicentra* differs in that flowers are produced on a scape when vegetative growth is nearly complete. *Claytonia, Dicentra* and *Trillium* grow, reproduce and die back within two months, while vegetative growth occurs for a much longer period of time in *Osmorhiza* and *Hydrophyllum*. The ratio of organic to inorganic matter probably increases due to continuous vegetative growth. This could account for the continuous rise in caloric content in the latter two species during the growing season.

Total protein was also found to vary with phenological development in *Claytonia, Dicentra* and *Trillium*. Maximum protein content in *Claytonia* (Fig. 1) occurred at maximum flowering. Total protein dropped from about 412 mg/g dry weight at flowering to 170 mg/g a week before complete die back. A slight rise in protein occurred during the last week resulting in a curve similar to that for caloric content. Apparently the rise in caloric values is related to a relative increase in protein content. The latter may be due, in *Claytonia,* to continued production of vegetative and floral structures until flowering occurs.

The peak in caloric content for

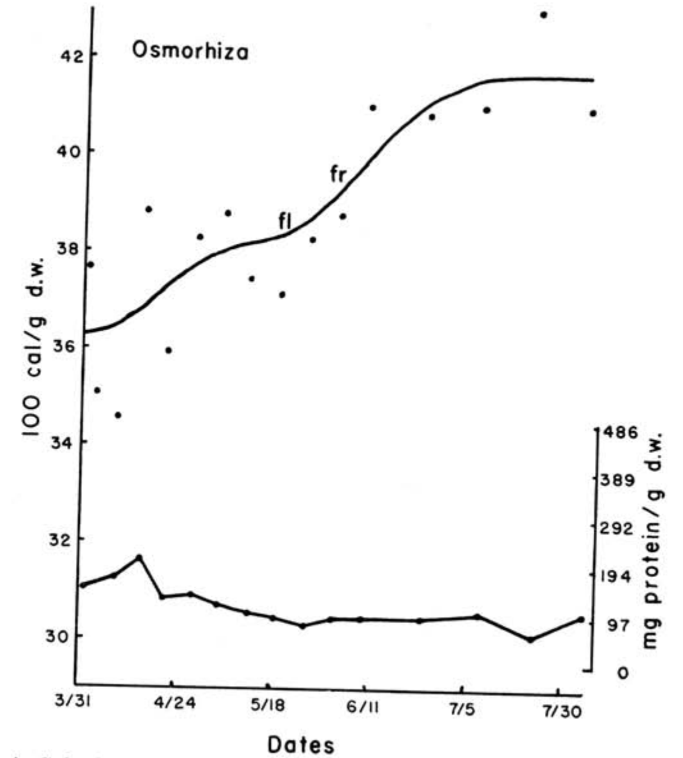

Fig. 4. Caloric and protein determinations for *Osmorhiza longistylis.*

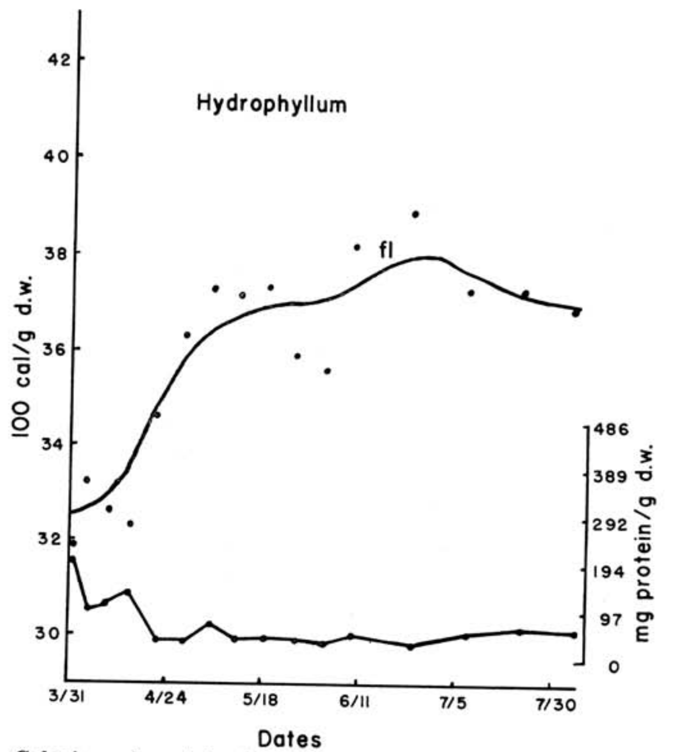

Fig. 5. Caloric and protein determinations for *Hydrophyllum canadense.*

Trillium lagged slightly behind the peak in protein content. The latter occurred during maximum vegetative and floral bud development. Total protein dropped continuously from the peak of about 435 mg/g to about 122 mg/g at vegetative die back. It appeared that most tissue production and differentiation was completed somewhat before flowering and that further growth was due to expansion of existing cells. The relative increase in amount of total protein during production and differentiation of vegetative and floral structures could account for most of the increase in caloric content. However, the higher caloric values in *Trillium* probably indicate formation of some lipids.

In *Dicentra* the peak in protein content occurred, as in *Trillium,* during maximum vegetative and floral bud development. Protein content dropped continuously from a maximum of about 365 mg/g at this time to about 90 mg/g at vegetative die back. A lag, greater than that in *Trillium,* occurred between peak protein content and peak caloric content. Leaf and flower bud production and differentiation were completed well before flowering. Petiole and scape length increased to the time of flowering, but leaves simply unfolded and flower buds expanded for a period of at least a week prior to flowering. Increase in total protein appeared, therefore, to be related to the period of maximum tissue formation and differentiation. Since the lag between maximum protein and maximum caloric content was considerable, relative increase in the amount of protein probably did not account for the peak in caloric content in *Dicentra.* Lipid formation may account for the peak, a possibility which is supported by the relatively high caloric values.

The initial values for caloric and protein content for *Claytonia, Dicentra* and *Trillium* were well above the values at vegetative die back. Thus, some conversion of carbohydrates to proteins or lipids must take place in the bulbs, corms or root systems of these species during the dormant period.

Protein content per gram in *Osmorhiza* and *Hydrophyllum* did not vary with plant phenology. Both species had values close to 97 mg/g throughout the growing season. Therefore, rise in caloric content can not be attributed to a rise in protein content. If an increased organic to inorganic matter ratio occurs, the increase must be in the form of carbohydrates or fats. Since the total rise in caloric content was great, increased lipid formation is probably the causal factor. Caloric values for the last collection date were well above initial values for both of these genera. However, drying had not occurred in either species when collection ceased. A drop in caloric values would probably occur with the shedding of fruits and the drying of leaves.

An analysis of variance was run on this factorial experiment in a completely randomized design. The results show that caloric values for different species, for different dates and for the effect of species upon dates were significant at the 1% level.

Summary

Three species of spring flowering herbs (*Claytonia virginica, Dicentra canadensis* and *Trillium recurvatum*) and two species of summer flowering herbs (*Osmorhiza longistylis* and *Hydrophyllum canadense*) were studied to determine whether changes in plant development are correlated with changes in caloric and protein values. The plants were collected from a 10 x 10 m plot in Trelease Woods (Section 1 T19N R9E) Champaign County, Illinois, at 1 to 2 week intervals from March 31 through August 3, 1960. Time of maximum vegetative growth, flower bud development, flowering, fruiting and vegetative die back were recorded. Notes were also taken on canopy development and on general climatic conditions. Caloric determinations were made on oven dried, ground samples using a Parr adiabatic oxygen bomb calorimeter. A modified form of Nessler's Procedure was used to determine total nitrogen content. The results were then converted to total protein content.

A correlation was found between time of maximum leaf and flower development and peak caloric content in *Claytonia, Dicentra* and *Trillium.* These species grow, flower, fruit and die back in a two month period. Caloric values for *Osmorhiza* and *Hydrophyllum* rose almost continuously throughout the growing season. These species are physiologically active during most of the summer and, thus, there appears to be no correlation between peak caloric values and time of maximum growth.

The curves for caloric and protein content were quite similar in *Claytonia* and *Trillium;* both reached a peak at flowering. The peak in protein content occurred well ahead of the peak in caloric content in *Dicentra,* while in *Osmorhiza* and *Hydrophyllum,* protein remained constant (and low) during the entire growing season. Since no significant increase in protein was found in the latter two species, increased lipid content may account for the rise in caloric values. Some conversion of carbohydrates to proteins or fats must occur during dormancy in the corms, bulbs or root systems of *Claytonia, Dicentra* and *Trillium* in order to bring caloric values back to their initial value by the following spring.

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