The temperature t_θ of the thermistor may then be computed from the equation

$$t_\theta = [a/(b + \log R_\theta)] - c, \quad (8)$$

where a, b, and c are constants characteristic of the particular thermistor and are determined for each thermistor by prior calibration.

By using a four-decade Wheatstone bridge and a sensitive galvanometer with this circuit, it has been found possible to obtain a measurement precision in the field with a probable error of less than ± 0.01°C.

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Comparison of Two Methods of Analysis of Rate of Leaf Initiation in *Zea mays* L.

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A method of growth analysis that, until recently, has been little emphasized is the determination of rate of leaf initiation. It has been used in this laboratory in the study of corn morphogenesis. The technique (*1*) involves the following steps: (i) in each sample, each corn seedling is dissected and the number of leaves produced to date is recorded; (ii) the data are seriated according to leaf stage; and (iii) the average number of days from the date of planting (or from date of pollination, if embryogeny is under consideration) to mid-point of (leaf) stage X is computed. We refer to this as the *mid-point* method. A curve representing data (marked X) from a current experiment on corn-seedling growth thus treated is shown by the solid line in Fig. 1. This method (*1, 2*) has been used because it permits arithmetical calculation of the duration of each plastochron (leaf stage) by use of the formula,

$$t_X = \tfrac{1}{2}(x_2 - x_1) + \tfrac{1}{2}(x_3 - x_2),$$

in which X is the plastochron number, and x_1, x_2, and x_3 represent the time values from planting to mid-points of three successive stages.

A basic assumption in the utilization of this method is that the rate of leaf initiation is linear. Although this may be true in special cases, generally it is not true. Also, the method has some rather rigorous biostatistical requirements that may not always be attained in the average experiment. These requirements are that (i) the samples be taken at equal time intervals, (ii) the samples be of equal size, and (iii) at any initial or final sampling no more than one leaf stage be represented. Concerning the last point, when a sample comprises a range of two or more leaf stages, it may be assumed that only the tardy plants of the previous stage have been sampled at the time of initiation of the experiment, and only the more precocious plants of the latest stage have been sampled at the time of termination of the experiment. Thus, their time values contribute to longer and shorter calculated durations of the stage, respectively, than is actually the case.

An alternative method of calculation of rates of leaf initiation (*3*) has been used in a study of corn embryogeny. In this method, the average leaf stage per sampling date is determined; this is referred to as the *stage-per-day* method. It differs from the mid-point method by omitting seriation. The same data treated in this fashion are shown graphically as circles in Fig. 1. A comparison of the two curves shows agreement in general trends but differences in detail, because the second method is more sensitive to changes in rates. These differences are of considerable biological significance, but they are evident only if sampling intervals are sufficiently brief. Thus, the extremely long duration of plastochron 10 is obscured by the mid-point method because it distributes some extreme time values between plastochrons 9 and 11. Furthermore, the graph demonstrates the discrepancy between the two methods at the upper end of the curves. The mid-point method suggests an increase in the terminal rate of leaf initiation, whereas day-by-day analysis of the same data gives no indication of this. A similar discrepancy would have been noted at the beginning

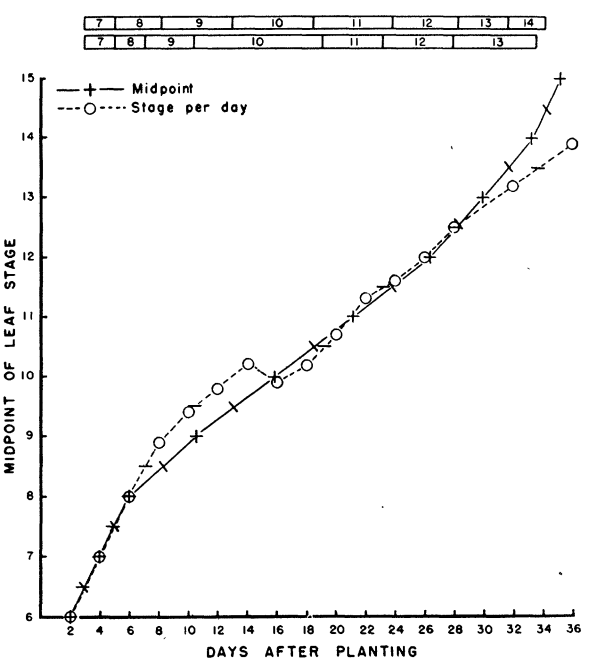

Fig. 1. Rate of leaf initiation of corn seedlings as determined by the stage-per-day method (broken line and circles) and the mid-point method (solid line and x's). Diagonal ticks on the solid line and the upper bar at the top of the figure indicate the duration of successive plastochrons as calculated by the mid-point method. Horizontal ticks on the broken line and the lower bar at the top of the figure represent the duration of successive plastochrons as determined graphically by the stage-per-day method.

of the experiment if some of the embryos of the strain used had been of a different leaf stage.

Some other advantages of the stage-per-day method are not as obvious. Each of the first seven circles in Fig. 1 represents a sample of five plants, and each of the remaining circles represents a sample of 10 plants per harvest. The sample size for the x's, however, ranges from 3 to 29, being a function of the duration of the stage. It follows that, except in cases of linear-growth rates, equal sample size cannot be attained in the mid-point method. Nor is there any possibility of determining somewhat more subtle changes in growth rate within a stage except by classification into morphological substages (*4*). Although the mid-point method requires samples of equal size and sampling at equal time intervals, these conditions do not have to be met in the stage-per-day method, since the data from one sample are not combined with those from the others.

As stated earlier, an advantage of the mid-point method is that it permits mathematical determination of the duration of stage, whereas this must be determined graphically in the stage-per-day method. However, depending on the precision desired, points can be obtained close enough to one another to assure accurate determination in the latter case. Horizontal ticks on the broken line of the graph and the lower bar at the top of Fig. 1 represent duration of plastochrons 7–13 thus ascertained.

References

1. E. C. Abbe and B. O. Phinney, *Am. J. Botany* **38**, 737 (1951).
2. E. C. Abbe and O. L. Stein, *ibid.* **41**, 285 (1954).
3. O. L. Stein, master's thesis, Univ. of Minnesota (1952).
4. O. L. Stein and E. C. Abbe, *Proc. Minn. Acad. Sci.* **17**, 97 (1949).

7 May 1954.

Estrogenic Activity of Some Isoflavone Derivatives*

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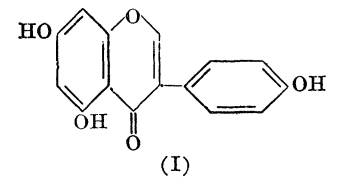
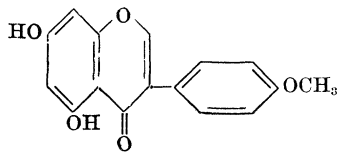
Interest in natural estrogenic compounds present in livestock feeds has been stimulated by the beneficial results obtained from the addition of diethylstilbestrol to cattle rations (*1*). The presence of estrogenic substances in subterranean clover interfering with the breeding performance in sheep (*2*) has led to the isolation of an isoflavone derivative, genistein, as one of the active substances (*3*). Cheng *et al.* (*4*) reported that genistein, as well as the glucoside of genistein, known as genistin, was estrogenic as detected by the mouse uterine response procedure. The estrogenic activity of genistein has further been confirmed by Carter *et al.* (*5*).

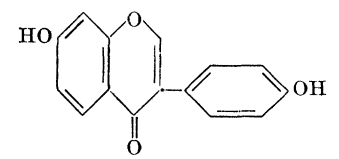
Since there are several known isoflavone compounds present in natural plant material, it is of interest to determine which of these compounds are estrogenic. Chemical synthesis of four isoflavone derivatives, genistein (I), biochanin A (II), daidzein (III), and formononetin (IV), has been completed in this laboratory (*6*). Their structural formulas are shown here.



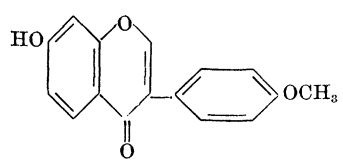
(I)



(II)



(III)



(IV)

Unpublished data in this laboratory indicated that both synthetic and naturally occurring genistein have equally potent estrogenic activity. Consequently, only synthetic isoflavone compounds were tested in the present experiment. These compounds were fed to mice at a level of 1.25 mg/g of ration in testing estrogenic activity using the uterine response technique (*7*). The respective isoflavone compounds were first dissolved in ethanol, then mixed with the basal ration, and the ethanol was evaporated from the completely mixed ration. Since the mice consumed an average of 2 g of diet daily, the average intake of the respective compounds was 2.5 mg daily over the experimental period of 4 days. The results of this experiment are presented in Table 1.

It is readily apparent that each of these isoflavones is estrogenic in nature. Daidzein appears to be the most active substance. Genistein and biochanin A have approximately equal activity. Formononetin showed