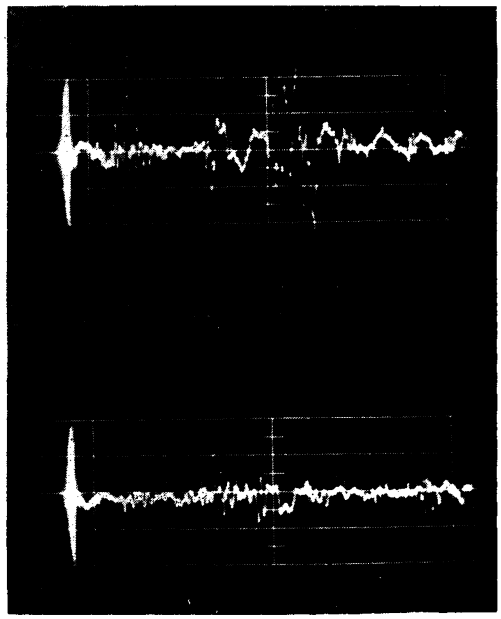

FIG. 1. Oscilloscope patterns of noises of internal infestation in wheat grains.

and removable cover 2½ in. thick, within which boxes of copper and Celotex surrounded the infested grain lying on the microphone. Records of wave patterns were made with a Tektronix oscilloscope and a Polaroid-Land camera attachment. Insects in the larval, pupal, and adult stages can be detected within the infested kernels, although the egg and extremely early stages of larval growth cannot. It appears that the larva must be nearly a week old before sufficient noise is produced for detection. In this research the stage of the infestation hidden within the grain kernels could be selected for study by means of x-ray techniques previously developed at this station (*3*).

Two distinct types of sound are associated with the larval and pupal stages, namely, a low-frequency scraping noise and a high-frequency tearing or rasping sound. From repeated observations it has been deduced that the low-frequency sounds are made by the movement of larva and pupa within the kernels, and the high-frequency sounds by the chewing of the endosperm of the grain by the larva. When several infested kernels are placed on the microphone, combinations of these frequencies may appear as shown in Fig. 1. Figure 1 was taken with the oscilloscope set at a sweep frequency of about 30 cps, and the insect sounds of both high and low frequencies are present. Thus in the upper trace the right and left ends show the low noise frequencies centering around 200 cps, characteristic of insect movement. Just to the right of the vertical scale in the center appears a high frequency burst of sound in the range of 1200–1500 cps. The frequency range of sounds due to internal insects appears to range from 200 to 8000 cps, although the lower limit has not been accurately determined. The voracious eating habits of the larval stage of rice weevil (*Sitophilus oryza* L.) has been clearly confirmed by this technique. It was also of interest to learn that, when the infested grains are disturbed in any way, the high-frequency sounds indicative of chewing usually cease and the low-frequency sounds, due apparently to movement, continue intermittently. After a short time the high-frequency sounds reappear. An experienced observer can estimate the approximate stage of development of the insect because the sounds are slightly different in the larval and pupal stages. This observation has suggested analysis of the recorded sound wave patterns as a means for differentiating developmental stages as well as physiological activities. Additional studies now in progress include evaluation of the method to determine numbers of infested kernels on the basis of cumulative recording of wave peaks, analysis of differences in sound characteristics of different species which infest grain internally, relationship of frequency of feeding and movement to stage of insect development, and determination of the influence of storage temperature and humidity on the nature, frequency, and periodicity of the sound patterns produced.

One practical application of this work is a means for the rapid evaluation of the effectiveness of fumigants whereby the normal delay of several weeks required for emergence of surviving insects, now necessary to determine fumigant efficiency, can be eliminated. A suitable sound detection device for performing such tests in mills and grain elevators is now under construction. Another application of the principle would be a means for monitoring grain within storage bins for infestation without sampling or removing the gain from the bins, in much the same manner as permanent thermocouple systems are now used for checking the heating of grain in storage.

References

1. BRAIN, C. K. *Annals Univ. Stellenbosch,* **2**, Sec. A, No. 2, 45 (1924).
2. MILNER, M., BARNEY, D. L., and SHELLENBERGER, J. A. *Science,* **112**, 791 (1950).
3. MILNER, M., LEE, M. R., and KATZ, R. *J. Econ. Entomol.,* **43**, 933 (1950).
4. POTTER, G. C., and SHELLENBERGER, J. A. *Cereal Chem.,* **29**, 223 (1952).
5. POTTER, G. C., SHELLENBERGER, J. A., and MILNER, M. *Trans. Am. Assoc. Cereal Chem.,* **10**, 181 (1952).

Manuscript received February 27, 1953.

Estrogenic Activity of Isoflavone Derivatives Extracted and Prepared from Soybean Oil Meal[1]

Edmund Cheng, Charles D. Story, Lester Yoder, W. H. Hale, and Wise Burroughs

Iowa Agricultural Experiment Station, Ames

Implantation of diethylstilbestrol (synthetic estrogenic substance) pellets under the skin of cattle and sheep stimulates live-weight gains and increased feed

[1] Journal Paper No. J-2262 of the Iowa Agricultural Experiment Station, Ames, Iowa Project 1208.

efficiency during the fattening period (*1–3*). Recently, naturally occurring estrogenic substances have been detected in varying amounts in various plant herbages, both pasture and hays, fed to cattle and sheep (*4–6*). Genistein (5,7,4′-trihydroxyisoflavone) has been suggested by Curnow and Bennetts as the chemical constituent responsible for estrogenic activity in one of these herbages, namely, subterranean clover (*7*). The glucoside of genistein, genistin (5,4′-dihydroxy-7-glucosidoisoflavone) has been shown by Walter (*8*) to be present in substantial amounts (0.1%) in soybean oil meal. Since soybean oil meal is used rather widely in livestock feeding, it appeared advisable to investigate the estrogenic activity of both genistin and genistein in ascertaining their probable significance in livestock feeding.

Commercial soybean oil meal (solvent process) was extracted with methanol according to the method of Walter (*8*). Genistin was isolated as pale yellow, thin rectangular plates having a melting point of 256° C. Upon hydrolysis of genistin with hydrochloric acid in methanol, genistein was obtained. It was crystallized from hot 60% ethanol as white rectangular rods having a melting point of 298° C.

The estrogenic activity of these compounds was determined by the mouse uterine weight method described in detail in an earlier paper (*6*). The chemicals under study were either fed directly to immature female mice or were injected subcutaneously. Since neither genistin nor genistein is soluble in water, they were injected as their sodium salts. Six mice were used in each group, and the treatments were given once daily for 4 days. The mice were sacrificed 24 hr after the last treatment, their uteri dissected, fixed in Bouin's fluid, and weighed. The results obtained are presented in Table 1.

Feeding 2.5 and 5.0 mg of either genistin or genistein per day per mouse resulted in increased uterine weights. Injecting genistein at 1- and 2-mg levels respectively also increased uterine weights consistently over the corresponding weights of control animals. Whereas the injection of 1 mg of genistin did not have a measurable effect, the injection of 2 mg proved quite effective. It should be noted that these responses are similar to those due to the injection of 0.02–0.04 μg respectively of diethylstilbestrol, as shown in Table 1. The estrogenic activity of genistein can accordingly be estimated as approximately equivalent to 1/50,000 the activity of diethylstilbestrol. Genistin activity on a weight basis was slightly lower than that of genistein. However, the two compounds appeared to have approximately equal activity on a molecular basis.

Experiments are in progress with fattening lambs to determine whether the estrogenic activity of genistin as found in soybean oil meal is as beneficial as the estrogenic activity of stilbestrol, which has been shown experimentally to be valuable in lamb feeding. Although the estrogenic activity of genistin per unit of weight is small compared with that of diethylstilbestrol, the relatively large amount of genistin present in soybean oil meal, coupled with its presence in small amounts in certain hays, suggests the likelihood that the amounts present in certain cattle and sheep rations may be sufficiently large to exert major beneficial influences.

TABLE 1

ESTROGENIC ACTIVITY OF GENISTIN AND GENISTEIN

Group	No. of mice	Treatment*	Av uterine weight, mg
1	6	Normal control	9.7 ± 2.8
2	6	Feeding genistin, 2.5 mg	12.9 ± 4.4
3	6	Feeding genistin, 5.0 mg	39.8 ± 8.9
4	6	Injecting genistin, 1 mg	9.2 ± 1.7
5	6	Injecting genistin, 2 mg	14.6 ± 3.6
6	6	Feeding genistein, 2.5 mg	21.6 ± 13.4
7	6	Feeding genistein, 5.0 mg	22.6 ± 4.6
8	6	Injecting genistein, 1 mg	13.2 ± 2.4
9	6	Injecting genistein, 2 mg	17.0 ± 7.3
10	6	Injecting stilbestrol, 0.02 μg	13.2 ± 2.6
11	5	Injecting stilbestrol, 0.04 μg	18.3 ± 6.9

* Treatment was given daily for 4 days.

References

1. ANDREWS, F. N., *et al. J. Animal Sci.*, **8**, 578 (1949).
2. JORDAN, R. M. *Ibid.*, 383 (1950).
3. DINUSSON, W. E., *et al. Ibid.*, **9**, 321 (1950).
4. CURNOW, D. H., *et al. Australian J. Exptl. Biol. Med. Sci.*, **26**, 271 (1948).
5. LEGG, S. P., *et al. Biochem. J.*, **46**, xix (1950).
6. CHENG, E., *et al.* In press (1953).
7. CURNOW, D. H., and BENNETTS, H. W. Abstract of papers from 6th International Grassland Congr. (1952).
8. WALTER, D. D. *J. Am. Chem. Soc.*, **63**, 3273 (1941).

Manuscript received February 26, 1953.

The Reduction by Reactivating Light of the Frequency of Phenocopies Induced by Ultraviolet Light in *Drosophila melanogaster*

Max Perlitsh and Albert Kelner

Brandeis University, Waltham, Massachusetts

Phenocopies (*1*), or abnormalities of adults simulating mutations, are readily induced in *Drosophila melanogaster* by irradiating eggs, larvae, or pupae with ultraviolet light (*ca.* 2600 A) (*2, 3*). Visible and near-ultraviolet light (3600–4900 A) prevents ultraviolet-induced killing or mutation in bacteria and other organisms (*4, 5*). Such reversal of ultraviolet effects has been found in various organisms (see [*5*] for references) including, recently, *Drosophila,* where reactivating light lowered the incidence of ultraviolet-induced lethal mutations (*6*).

It became of interest to determine whether phenocopies induced by ultraviolet light in *Drosophila* were also affected by reactivating light, especially since the induction of phenocopies was perhaps a more complex phenomenon than those studied before.