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Circadian Sensitivity and Dosage-Rate Response to X-Irradiation in the Pink Bollworm[1,2]

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ABSTRACT

A laboratory culture of *Pectinophora gossypiella* (Saunders) was entrained on a photo-period of LD 14:10. Lights-on occurred at 0530 hours and lights-off at 1930 hours. At 3-hour intervals, beginning at 2400 hours throughout a 24-hour period, male and female moths were X-irradiated with 25 kr delivered at 1.0 kr per minute. In a separate study male and female moths were X-irradiated at 1500 hours with 25 kr at 4 different dosage rates.

A highly significant difference was observed between sexes of moths, time of X-irradiation, and dosage rates. Female moths at all times were less sensitive to X-irradiation than males, as measured by mean longevity. The time of minimum sensitivity to X-irradiation in both sexes occurred at 0600 hours, ½ hour after lights-on. Maximum sensitivity in both sexes occurred at ~~1200~~ 2100 hours, 1.5 hours after lights-off. It appeared that sensitivity to X-irradiation was correlated to moth activity.

As the dosage rate of X-irradiation was increased, the biologic damage also increased, expressed as reduced longevity. At lower dosage rates moths were able to recover and apparently repair radiation damage.

Experiments indicate that the pink bollworm, *Pectinophora gossypiella* (Saunders), may be controlled or eradicated through release of sterilized males in the field (Noble 1969). Sterilization studies have been carried out using both metepa, a chemosterilant, and gamma-radiation, of which the radiation seems to be the more practical for mass production of sterilized males. Ouye et al. (1964) determined the optimum sterilizing dosages for pink bollworms treated as pupae using gamma-radiation. Complete sterility in males was accomplished with 55 kr and in females with 40 kr. The longevity of adults emerging from 7-day-old pupae treated with 35 through 90 kr was significantly shorter than that of the control. Pupal sensitivity to gamma-radiation was dependent on age at time of treatment, the older pupae being less susceptible. In their study, no attention was given to the effect of treatment time during the day on the sensitivity of the moths to gamma-radiation, expressed as mean longevity.

The response of insects to photoperiod has been intensely studied in the past decade and Beck (1968) listed several reasons for this increased interest.

Insects, rats, and mice have been shown to vary in response to physiological stress factors in relation to the time of day in which the stress factor was administered or the photoperiodic regime in which the insects were reared. Pittendrigh (1961) reported a difference in response to excessive heat in 2 groups of *Drosophila*. His experiment was conducted 6 times throughout the day at 4-hr intervals and indicated that the shorter the photoperiod the less rapid the recovery at any time of day.

Several studies have indicated a circadian response to selected narcotics and insecticides (Beck 1963; Fernandez and Randolph 1966, 1967; Frudden and Wellso 1968; Gains and Mistric 1952; Keller et al. 1956; Polcik et al. 1964).

Pizzarello et al. (1963) found that rats X-irradiated in the morning lived more than 130 days, whereas those treated in the evening had a maximum survival time of only 13 days. Rugh et al. (1963), using mice, and Straube (1963), using rats, obtained what appeared to be completely negative results in a similar experiment.

Pizzarello et al. (1964) repeated their experiments using 2 strains of male mice maintained on an LD cycle of 12:12, lights-on at 0700. The mice were irradiated at 4-hr intervals and both strains were found to be more sensitive at 0200 than at any other time. They noted that the times chosen by Rugh et al. (1963) for their comparisons fell on periods of equal sensitivity on a cyclic curve.

Two studies have been reported involving circadian

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rhythms and X-irradiation of insects. Harker (1964) showed that X-ray dosages of 100–10,000 R have a phase-shifting effect on the running activity of *Periplaneta,* the magnitude of delay being directly related to dosage. Rensing (1969) reports a circadian rhythm in sensitivity to X-irradiation in *Drosophila melanogaster* Meigen. Using females of his wild strain on a photoperiod of LD 12:12 (lights-on at 0800), he obtained a 2-peaked rhythm of sensitivity. Maximum sensitivity, measured in death rate and survival time, occurred at lights-on and lights-off. Minimum sensitivity occurred 4 hr after lights-on and 6 hr after lights-off. The time of greatest sensitivity coincided with maxima of nuclear size in various tissues, presumed to correspond to the maxima of the nucleolus volume and hormone synthesis.

Villee (1946) demonstrated the effect of X-ray dosage rate on pupation of *Drosophila* larvae. An approximate dose of 7.0 kr given in 1.25 min caused much greater mortality before and after pupation than when administered over 90 min. He concluded that lethality depended not only on total dosage but also on dosage rate.

The purpose of this study was to demonstrate (1) that the time of day in which irradiation is administered has an effect on survival of adult pink bollworms and (2) a relationship between dosage rate and longevity.

METHODS AND MATERIALS.—*Rearing the Pink Bollworm.*—Pink bollworm moths were fed a 10% sucrose solution, and oviposition chambers were held in climate cabinets at 26.7°C, ca. 50% RH, and a photoperiod of LD 14:10, beginning at 0530 and ending at 1930 hr. Eggs on nonabsorbent, sterile cotton were removed from the oviposition chamber and placed in jars, sealed and held 4 days in the climate cabinet just described until hatched.

Newly hatched larvae were removed with a camel's hair brush and placed in 1-oz plastic individual-portion cups, 3–5 larvae/cup, containing the rearing medium developed by Shorey (1963). The cups were placed in cardboard flats and held in a rearing room at 26.7±1.5°C, 70±10% RH and constant light until the larvae reached the 3rd instar. They were then transferred to climate chambers with the described LD 14:10 photoperiod.

After pupation, the pupae were removed and placed in a ½-oz plastic cup and allowed to emerge into 1-pint mason jars in the LD 14:10 climate chambers. After emergence moths were collected daily by cooling to 4.4°C for 8 min and aspirating into oviposition chambers or treatment petri dishes.

Treatment and Evaluation Procedure.—One to 2 hr before irradiation, 24-hr-old moths, 25 of each sex, were chilled and aspirated into separate 8.75-cm plastic petri dishes, ventilated with 5.6-cm holes in the lids covered with mosquito netting. The dishes were then placed in the constant-light rearing room until treatment, irradiated, and returned to the rearing room for the duration of the study.

Chilling may be regarded as a stress factor that might elicit a variable physiological effect when applied at different times of day. In this study no unchilled control insects were used for separation of the irradiation and chilling effects. However the non-irradiated or control insects received the same degree of chilling as the irradiated insects and at the same time.

The X-ray source was a General Electric Maximar 400 therapy unit modified previously (Ware and Irwin 1969). The dosage rate of the unit was varied by altering voltage input. A petri dish containing 25–30 moths was placed on a 1 rpm rotating platform 4.8 cm from the X-ray tube in the oil displacer cone of the X-ray unit. Ozone produced during irradiation was removed by a pressure/vacuum pump at a displacement rate of 1.0 cfm.

Males and females were treated separately, 1 group

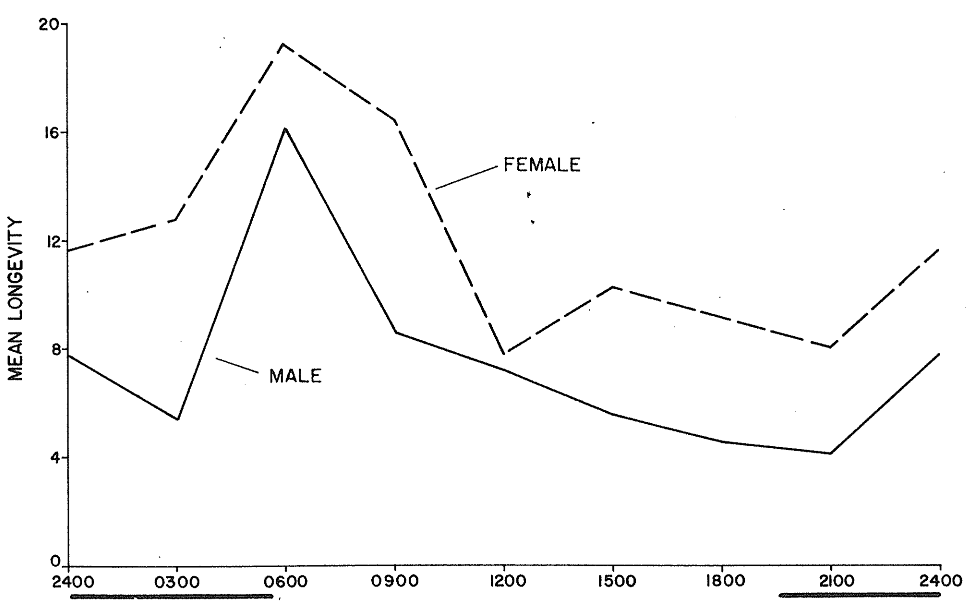

FIG. 1.—Longevity in days of moths X-irradiated as 1-day-old adults at 3-hr intervals with 25 kr (1.0 kr/min). Bar indicates dark portion of photoperiod.

Table 1.—Combined longevity of male and female moths X-irradiated as 1-day-old adults at 3-hr intervals with 25 kr (1 kr/min).

Time of irradiation	Mean longevity[a] in days
0600	17.642 a
0900	12.490 b
2400	9.676 c
0300	9.075 cd
1500	7.835 cde
1200	7.460 cde
1800	6.790 de
2100	6.065 e

[a] Any 2 means followed by the same letter are not statistically significant at the 5% level of significance as determined by least squares method.

before and 1 after the hour, alternating this position with each of the 4 replications, 25 moths/replicate. Two separate experiments were run for the circadian rhythm and dosage rate responses. To determine a circadian rhythm in sensitivity, a sample of 1-day-old moths was exposed to 25 kr at 1.0 kr/min, at a given time of day. Eight times were used at 3-hr intervals beginning at 2400 hr.

The test for the 0300-hr group was actually conducted at 0600 hr. The clock in the climate chamber was advanced by 3 hr 4 days before the 0300 irradiations were administered to insure entrainment by the shift in phase of the new photoperiod. The 0300 moths were removed from the climate chamber at 2200 hr the evening before, transferred to petri dishes, and placed in the rearing room. Therefore, the moths irradiated at 0300 hr were in the petri dishes 8 hr before treatment. This was the only deviation from the desired 1- to 2-hr holding period, and it was done as a matter of convenience.

Four dosage rates were administered at 1500 hr to determine the relationship between dosage rate and sensitivity. The experiment was replicated 4 times, 25 of each sex/replicate, for each treatment time and dosage rate. Four nonirradiated treatments paralleled the dosage rate test at 1500 hr.

After treatment, each dish was chilled 5 minutes at 4.4°C to facilitate moth handling. Moths were individually aspirated into 1-oz plastic cups which were sealed with a paper lid. A hole was punched through the center of the lid to accommodate a 2-ml feeding vial filled with adult food, 10% sucrose solution. The cups were placed in cardboard flats and held in the rearing room. The moths were examined daily, and mortality was recorded.

The longevity of each moth was recorded as days beyond treatment. Mean longevity of each replication was computed and punched on electronic data cards which were programmed in a standard analysis of variance.

RESULTS AND DISCUSSION.—*Circadian Sensitivity to X-Irradiation.*—Adult pink bollworm moths of both sexes showed an extreme variation in longevity after X-irradiation (Fig. 1) and at all treatment times the females were less sensitive to X-irradiation. Only at 1200 hr was there any close similarity in mean longevity.

Combining all treatment times, the variation between male and female longevity was significantly different at the 1% level as determined by analysis of variance. Combining the male and female longevities for each time of day, the variation between the longevity for the 8 times of day was also significantly different at the 1% level. No significant interaction between sex and treatment time was found using the 5% level of significance.

After the sexes were combined, the mean longevities for the times of treatment were arranged in a decreasing order (Table 1). Treatments at 0600 and 0900 hr had extremely high mean longevities significantly different, at the 5% level, from the other means and from each other. The treatment at 2100 hr had the lowest mean longevity and was significantly different from all morning treatments. Morning treatments between 2400 and 0900 hr had higher means than treatments between 1200 and 2100 hr, though the significance of this difference was not examined.

Mean longevity can be used to determine significant differences between treatments of X-irradiation at different times of day, but this method of interpretation might leave unnoticed the differences in the times required to reach these means. Percent mortality was computed for 3-day intervals after the day of treatment for each time of day and sex (Fig. 2, 3).

Males varied widely in mortality 3 days after X-irradiation (Fig. 2). On days 6 and 9 the variations were still quite large, although the relative positions of the treatment groups were unchanged. With increasing time, the mortality of all treatment groups came closer together with the exception of the 0600 treatment

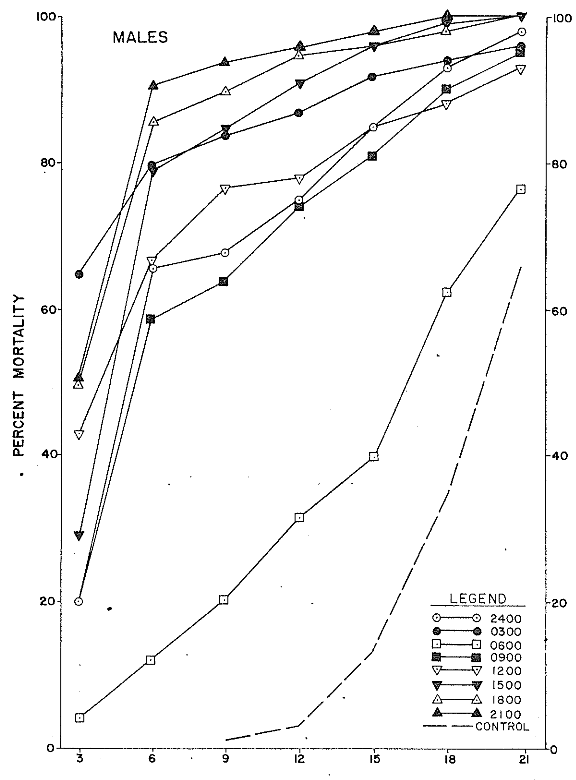

FIG. 2.—Male mortality after X-irradiation with 25 kr (1.0 kr/min) at 8 times of day.

(Fig. 2), which showed a near linear progression from days 3 to 21. All other groups increased rapidly to at least 59% by day 6. The 0600 treatment at all times had higher mortality than the control group, but with only 10.5% separating them on day 21, whereas the next closest treatment group was 16.5% away.

The 0300 treatment resulted in the highest mortality on day 3. This group was passed on day 6 by the 1800 and 2100 hr treatments which maintained the highest mortality and lowest mean longevity. The 1500 hr treatment showed the greatest increase in mortality from day 3 to day 6. The treatments at 2400, 0900, and 1200 hr had very similar mortality throughout the experiment. For males the results of the first 6–9 days after treatment appeared to be the most significant, since variation between groups was greatest. The significance of this information lay in the fact that, if these males were X-irradiated to produce sterilization, the moths with the least mortality after 9 days would be the most effective in inseminating fertile females with sterile sperm.

Females showed extreme variation in mortality from 6 to 21 days (Fig. 3). On day 3 the treatment groups were tightly clumped with the exception of the 1200 group, which had the lowest mean longevity and the highest mortality on days 3, 6, 9, and 15. The lowest increase in mortality occurred in the 0600- and 0900-hr groups, with the 0600 group being the lowest. Treatment at 0600 resulted in higher mortality than the controls at all times, but the difference on day 21 was only 3%. On day 21, groups treated in the afternoon (1200 through 2100 hr) had mortalities ranging from 94 to 97%, whereas the groups treated in the morning (2400 through 0900 hr) had mortalities ranging from 61 to 86%. This trend separating morning, afternoon, and evening treatments was established and maintained on day 9. This is in complete agreement with the earlier statement that morning treatments permit a greater mean longevity than do evening treatments.

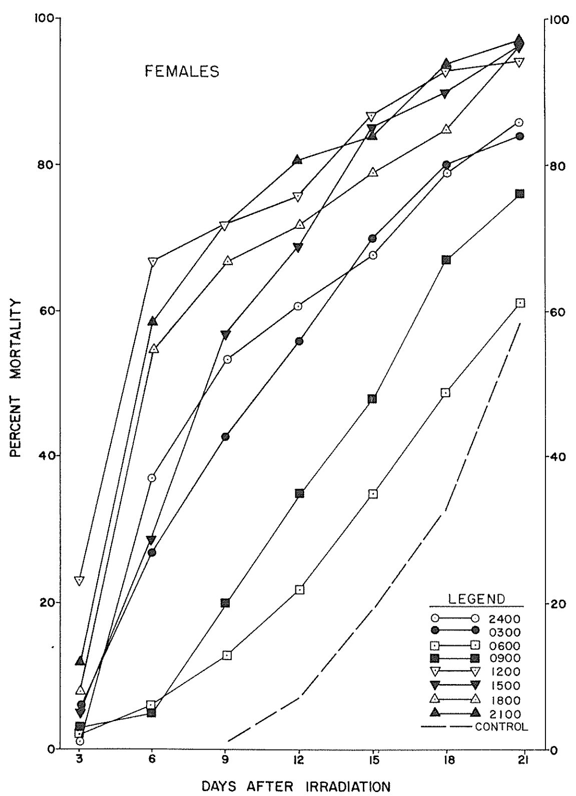

FIG. 3.—Female mortality after X-irradiation with 25 kr (1.0 kr/min) at 8 times of day.

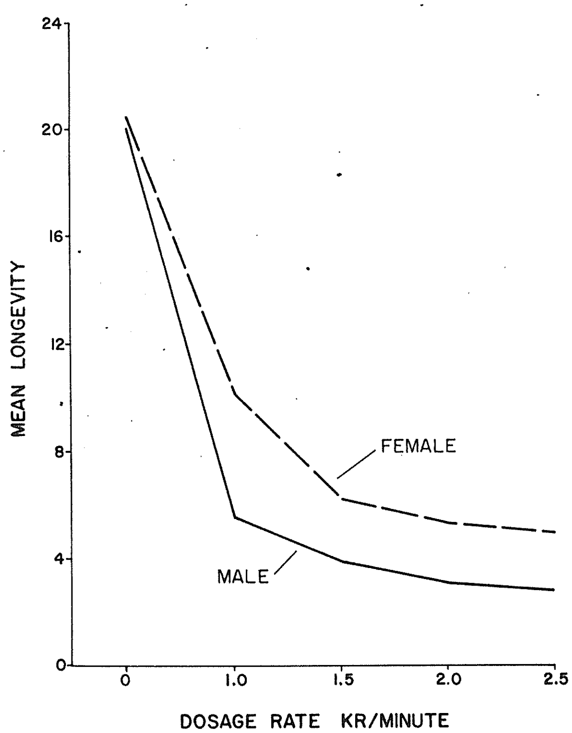

FIG. 4.—Male and female longevity in days after X-irradiation with 25 kr at 4 dosage rates.

Fig. 2 and 3 indicate that irradiation of either sex at 0600 hr within the described photoperiod results in the greatest longevity and the lowest rate of mortality increase. Treatment of both sexes at 2100 hr resulted in the lowest longevity and the most rapid rate of mortality increase in males. Females exhibited the most rapid rate of mortality increase from the 1200 hr irradiations.

Interpreting whole body irradiation data and circadian rhythms is extremely difficult because of possible tissue-specific rhythms which might be masked in the process. Rensing (1969) attributed the increase in sensitivity to X-irradiation in *Drosophila* to corresponding rhythmical changes in cell structures and processes. From this he inferred that the nucleolus volume and hormone synthesis are also at a maximum.

No physiological data, as those just reported, have been reported for the pink bollworm. However, Minis (1965) reported an oviposition rhythm in female pink bollworm moths entrained on a photoperiod of LD 14:10. Oviposition began 1 or 2 hr before lights-off (1730 in the photoperiod of this study), reached a peak 2.5 hr after lights-off (2200) and tapered off until 2 hr before lights-on (0330) when

Table 2.—Longevity of moths X-irradiated as 1-day-old adults with 25 kr at various dosage rates.

Dosage rate (R/min)	Sex	Mean longevity in days	Combined mean longevity[a] in days
Control	M	20.09	
Control	F	20.50	20.295 a
1000	M	5.53	
1000	F	10.14	7.832 b
1500	M	3.81	
1500	F	6.19	5.000 c
2000	M	3.02	
2000	F	5.25	4.135 c
2500	M	2.76	
2500	F	4.92	3.840 c

[a] Any 2 means followed by the same letter are not statistically significant at the 5% level of significance as determined by least squares method.

all oviposition ceased. Lukefahr and Griffen (1957) observed that pink bollworm mating occurred between 0200 and 0500 hr but that 80% of oviposition took place between 1800 and 2400.

Both sexes of the pink bollworm were nocturnal, with male activity probably correlating closely with female oviposition. Activity began at, or slightly before, lights-off and was suppressed at lights-on and through the photophase. Maximum sensitivity to X-irradiation corresponded to Minis' (1965) findings that maximum activity occurs 2.5 hr after lights-off. Minimum sensitivity to X-irradiation occurred 0.5 hr after lights-on at the time when activity was first suppressed. Thus occurrence of a circadian rhythm in sensitivity to X-irradiation is probably dependent on the corresponding circadian rhythm in activity and a general rhythm in cellular processes.

Dosage Rate Response to X-Irradiation.—Fig. 4 presents results of the dosage rate study. Dosage rates of 1000, 1500, 2000, and 2500 R/min of X-irradiation, with a total dosage of 25,000 R, and a control, were administered to 24-hr-old males and females at 1500 hr. The result was a gradually decreasing mean longevity with increasing intensity of irradiation (Table 2 and Figure 4).

Combining the results of all dosage rates, differences between the mean longevity of both sexes were found to be significantly different at the 1% level of significance by an analysis of variance. Combining the results for both sexes, differences between the control and 4 dosage rates were also found to be significantly different at the 1% level. No significant interaction was found between sex and dosage rate at the 5% level. Both the control and 1.0 kr/min treatments were found to be significantly different from the 3 other dosage rates at the 5% level as determined by the least squares method. The control and 1.0 kr/min treatments were also significantly different from each other.

The evidence presented in this study supports the results of Villee (1946) and Clark (1956). Pizzarello and Witcofski (1967), generalizing on the effect of dosage rate, explained that the effect will be lessened the longer the time required to deliver a given dose of radiation, and they presented the reasoning behind this phenomenon.

REFERENCES CITED

Beck, S. D. 1963. Physiology and ecology of photoperiodism. Bull. Entomol. Soc. Amer. 9 (1): 8–16.

1968. Insect Photoperiodism. Academic Press, New York. 288 p.

Clark, A. M. 1956. Genetic effects of X-rays in relation to dose-rate in *Drosophila*. Nature (London) 177: 787.

Fernandez, A. T., and N. M. Randolph. 1966. The susceptibility of house flies reared under various photoperiods to insecticide residues. J. Econ. Entomol. 59 (1): 37–39.

1967. A photoperiodic effect on the daily susceptibility of the house fly to Trichlorfon. Ibid. 60 (6): 1633–5.

Fisher, R. W. 1967. Diel periodicity in sensitivity of *Tetranychus urticae* to Dicofol. Can. Entomol. 99: 281–4.

Frudden, L., and S. G. Wellso. 1968. Daily susceptibility of house flies to malathion. J. Econ. Entomol. 61 (6): 1692–4.

Gains, J. C., and W. J. Mistric, Jr. 1952. Effects of environmental factors on the toxicity of certain insecticides. Ibid. 45 (3): 409–16.

Harker, J. E. 1964. The Physiology of Diurnal Rhythms. Cambridge Univ. Press. 114 p.

Keller, J. C., G. C. LaBrecque, H. C. Chapman, and A. N. Davis. 1956. Seasonal variations in susceptibility of salt marsh mosquito larvae to insecticides. Mosquito News 16: 20–21.

Lukefahr, M. J., and J. Griffin. 1957. Mating and oviposition habits of the pink bollworm moth. J. Econ. Entomol. 50 (4): 487–90.

Minis, D. H. 1965. Parallel peculiarities in the entrainment of a circadian rhythm and photoperiodic induction in the pink bollworm, p. 333–43. *In* J. Aschoff [ed.] Circadian Clocks. North-Holland Publishing Co., Amsterdam.

Noble, L. W. 1969. Fifty years of research on the pink bollworm in the United States. USDA Agr. Handb. 357. 62 p.

Ouye, M. T., R. S. Garcia, and D. F. Martin. 1964. Determination of the optimum sterilizing dosage for pink bollworms treated as pupae with gamma radiation. J. Econ. Entomol. 57: 387–90.

Pittendrigh, C. S. 1961. On temporal organization in living systems. Harvey Lect. 56: 93–125.

Pizzarello, D. J., and R. L. Witcofski. 1967. Basic Radiation Biology. Leo Febiger. Philadelphia. 301 p.

Pizzarello, D. J., R. L. Witcofski, and E. A. Lyons. 1963. Variations in survival time after whole-body radiation at two times of day. Science 139: 349.

Pizzarello, D. J., D. Isaak, K. E. Chua, and A. L. Rhyne. 1964. Circadian rhythmicity in the sensitivity of two strains of mice to whole-body radiation. Ibid. 145: 286–91.

Polcik, B., J. W. Nowosielski, and J. A. Naegele. 1964. Daily sensitivity rhythm of the two-spotted spider mite, *Tetranychus urticae*, to DDVP. Ibid. 145: 405–6.

Rensing, L. 1969. Ein circadianer Rhythmus der Empfindlichkeit gegen Röntgenstrahlen bei *Drosophila*. Z. Vergl. Physiol. 62: 214–20.

Rugh, R., V. Castro, S. Balter, E. V. Kennelly, D. S. Marsden, J. Warmund, and M. Wollin. 1963. X-rays: are there cyclic variations in radiosensitivity? Science 142: 53–56.

Shorey, H. H. 1963. A simple artificial rearing medium for the cabbage looper. J. Econ. Entomol. 56: 536–7.

Straube, R. L. 1963. Examination of diurnal variation in lethally irradiated rats. Science 142: 1062.

Villee, C. A. 1946. Some effects of X-rays on development in *Drosophila*. J. Exp. Zool. 101: 261–80.

Ware, G. W., and C. K. Irwin. 1969. Conversion of a therapy X-ray unit for insect irradiation. J. Econ. Entomol. 62: 721–2.