

INFLUENCE OF DEATH CRITERIA ON THE X-RAY SURVIVAL CURVES OF THE FUNGUS, NEUROSPORA*

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Most recent papers on the effects of X-rays on microorganisms have been based on quantitative investigations, and the results usually have been expressed in the form of survival curves where the survival ratios are plotted as a function of the X-ray dosage. In general, attempts have also been made to correlate the findings with the predictions of the quantum hit theory. Originally derived in an elementary form by Crowther (1926) and Condon and Terrill (1927) from statistical considerations of the quantum nature of radiation, this theory has recently been elaborated by Glocker (1932) so as to be applicable to more complex biological systems.

The quantum hit theory has tacitly assumed that the concept of death is absolute and precise—that a distinction between the condition of life and death in a given organism can always be made. In determining survival ratios, one must first select some reliable criterion as to what constitutes survival. No theoretical significance can be attached to survival curves obtained in the absence of an accurate standard. For example, the traditional index of death adopted by bacteriologists is the failure of a cell to produce a macroscopic colony, or, in other words, the loss of the reproductive function.

The shape and meaning of bacterial killing curves have been discussed by Rahn (1932). It has been customary to draw such graphs on semilogarithmic paper.

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When data on the bactericidal effects of X-rays were graphed in this orthodox manner by Holweck and Lacassagne (1929, 1930 *a*) and Wyckoff (1930), straight lines were obtained. These were immediately interpretable in terms of the quantum hit theory with which they were in complete agreement, and meant that a bacterium was killed by the absorption of a single quantum. Since it was known from energy considerations that each bacterium had actually absorbed many quanta, the particular one effective in causing death was considered to have been absorbed in a so called sensitive volume. Such sensitive regions have been assigned values ranging from cell nuclear to molecular dimensions, depending on the experiment. Although no effects have been ascribed to the numerous quanta absorbed outside the sensitive volume, Wyckoff (1932) has recently had some misgivings about them.

Additional experiments to prove the correctness of the quantum hit theory were soon under way with yeasts as the test organisms. Life was found to continue even though the budding process was inhibited; furthermore, many cells were found budding one or more times before succumbing to apparent death. Although reproducible survival curves were obtained by Holweck and Lacassagne (1930 *b*), Wyckoff and Luyet (1931), and Glocker, Langendorff, and Reuss (1933), the adoption of arbitrary death criteria permitted no unique interpretations in terms of the theory. Rahn and Barnes (1933) in an experimental study of death criteria in yeast found their curves radically affected by the following selection of standards: reproduction, staining, rate of CO₂ production, and protoplasmic coagulation.

Very interesting in this connection are the results obtained by Zirkle (1932) on fern spores irradiated with alpha particles. Each of the three death criteria he used yielded a different value for the number of hits required to kill.

Of the several references known to the authors on the effects of X-radiation on fungi, only two contain quantitative data. Attempts by Leonian (1929) and Heldmaier (1929) were entirely negative; Holweck and Lacassagne (1930 *b*) found their material unsuitable for quantitative study; Nadson and Philippov (1925) report qualitative observations only. Studies on the control of agriculturally important fungi with X-radiation have been reported by Tascher (1933). Although most of his paper is devoted to a study of induced sector mutations, Dickson (1932) has published a killing curve for *Chaetomium cochliodes*, plotting number of injured spores against the dosage. The difficulties encountered by Luyet (1932) in his studies with the fungus, *Rhizopus nigricans*, are partially revealed in the following quotation: "Besides the giant spores without mycelia, others are found which, growing short mycelia before their development ceases, represent all transition stages toward normal cells. . . . The presence of swollen spores obviously makes it difficult to set up the accurate criterion of death necessary for obtaining significant survival ratios."

In inaugurating the researches reported in this paper, the authors were desirous of finding a fungus suitable for a quantitative study of

certain biological effects of X-rays. Two species of the ascomycetous fungus *Neurospora*, *N. sitophila* and *N. tetrasperma*, were selected. These are closely related species which do not hybridize freely, but as the former has uninucleate ascospores and the latter binucleate, some variation in the shape of their survival curves or in the rate of induced mutations was considered possible.

The genus *Neurospora* is well known from the papers of Shear and Dodge (1927), Dodge (1927, 1928, 1931), and Lindegren (1932, 1933). *N. sitophila* produces 8 unisexual, uninucleate, haploid ascospores in each ascus. A single spore culture gives a vegetative colony which is genetically homogeneous, for all the cells have been obtained from a haploid cell. Since these single spores are easy to isolate, this is a particularly favorable organism in which to study mutation rates in either control or irradiated cultures. Since the vegetative cultures are haploid, there is no problem of dominance for the vegetative characters; therefore, backcrossing is unnecessary. This may not be true in a study of reproductive characters. Sexual cultures of *N. sitophila* may be obtained readily by breeding the two sexually allelomorphic strains. *N. tetrasperma* produces 4 binucleate, bisexual, diploid spores in each ascus. If there is any dominance, the frequency of apparent variation should be much less. This organism is probably not so suitable for a study on the rate of mutation; however, it is more convenient for determining the effects of X-rays on sexual reproduction.

X-Radiation

As a source of low-voltage X-radiation, a water-cooled Mueller tube (the so called Grenz ray tube) was employed. In this particular form of tube, the filament leads extend through a hole in the center of the anticathode so that the focal source is annular in shape and inclined at an angle of 45° with respect to the utilizable X-ray beam. The 60 cycle alternating potential applied to both tube and filament was supplied by a manually controlled Wappler installation. Throughout the course of the experiments, the calibrated voltmeter on the primary of the high voltage transformer read 11 kv. (root mean square). The tube current was held constant at 8 milliamperes. Although no spectral analysis has been made of the quality of the radiation, it seems clear from the data of Glassner (1932) and Exner (1932) that over 95 per cent of the measured intensity was included in the wave length range from 1 to 2.5 Ångström units. The Lindemann glass window in the tube served as the only filter.

Dosage measurements in terms of the international roentgen were carried out with an open air ionization chamber and accessory apparatus previously constructed by one of the authors. Though similar in design to the instrument of Taylor and Stoneburner (1932), it differs in a number of structural features which have been described elsewhere by Uber (1933). Except in the germination experiments, the intensity used throughout was such as to give a measured dosage of 210 roentgens per minute. This value would be increased somewhat by scattered radiation.

Uniformity of X-ray intensity in a radial direction for the effective beam was found by ionization measurements to lie within the limits of the experimental error. Angular uniformity was secured by rotating the irradiated material continuously on a clinostat, the period of rotation being small compared to the exposure times. Since it was impossible to eliminate scattered radiation, an attempt was made to have its contribution to the total intensity a constant one over the area used. To accomplish this, the material was placed on a smooth agar surface which in turn was laid on a plane sheet of lead resting on the clinostat.

Material and Methods

Cultures of *Neurospora sitophila* and *N. tetrasperma* were kindly furnished us by Dr. B. O. Dodge of the New York Botanical Garden. Strains of the former bore the markings 56:8 A and 56:3 B, the latter 19.3 e. Both species produce an abundance of black ascospores which are discharged from their perithecia when mature. These spores are approximately 13–15 μ in diameter and 23–31 μ in length, the upper limits in each case being characteristic of *N. tetrasperma*. Conidia and microconidia are also produced. The prolonged heat treatment, to which the ascospores must be subjected in order to initiate growth processes, insures purity of the cultures by killing vegetative cells or contaminating organisms. This obviation of sterility precautions makes these ascospores very desirable test objects from the standpoint of experimental technic.

A suspension of ascospores, which had been discharged from mature perithecia, was made in distilled water. The dilution of the suspension was adjusted by trial so that when the surface of a circular agar disc was flooded the spores were well separated. The plain 3 per cent agar was 15 cm. in diameter and 3 mm. thick. After the surplus water had evaporated from the surface, this sheet of agar and its lead support were centered on the clinostat. The irradiation was carried out with the spores 21 cm. from the effective focal plane of the anticathode. Angular sectors of the agar disc were removed at the end of the several exposure intervals. When all of the exposures had been made the irradiated sectors and the controls were subcultured.

Single spore inoculations were made from the various sectors and controls with the aid of dissecting microscopes and small spatulas. From 100 to 150 single spore isolations were made for each interval, and each spore was inoculated into a 10 cm. test-tube. The nutrient medium was 0.5 per cent malt extract and 0.5 per cent glucose in 2 per cent agar.

The heat treatment administered to these agar slant cultures was carried out in a thermostat oven at 60°C. After exposure periods ranging from 1½ to 2½ hours, depending on the particular experiment, the cultures were transferred to the 25°C. incubation room.

Qualitative Results

Early experiments with *N. sitophila* were largely of an exploratory nature. In group germination studies of the irradiated ascospores, a marked delay in growth ranging from several hours to several days was at once apparent. Development of the spores in the higher dosage groups soon ceased, only short germ tubes having been produced. With successively shorter exposures, there was a progressive increase in the mycelial growth which finally obscured all observations. That the really interesting phenomena were being masked and that it would be necessary to adopt some such method as the one outlined in the preceding section were immediately evident.

Since each spore gave rise to an individual test-tube culture, the fate of each one could be easily followed. Most striking was the large percentage of abnormal cultures which developed from the irradiated spores while the controls showed almost complete uniformity; however, the abnormalities were practically continuous in gradation. Among the many mutant characteristics, the following may be mentioned: color variations in conidial masses, discoloration of agar substrate, abortive perithecia, non-conidial albino strains, unusual types of aerial mycelium, and the so called "wet" cultures. These qualitative observations parallel those of the earlier investigators on fungi.

Survival curves for *N. sitophila* were obtained too early in the evolution of our technic to be of quantitative value. In cases where the data are comparable, they corroborate the results obtained with *N. tetrasperma*.

Quantitative Results

Group Cultures.—The methods used in obtaining quantitative data on ascospore germination differ in several particulars from those outlined on previous pages. Higher dosage requirements necessitated shortening the distance from the anticathode to the irradiated material from 21 cm. to 9 cm. The approximate dosages for each of the

exposure periods, as given in Table I, are to be considered as minimal values. Upon removal from the X-ray beam, the original sectors of the agar disc holding the spores were placed in Petri dishes; at the conclusion of the series of exposures, these were heated for 2 hours in a thermostat oven at 62°C. The germination counts were made 12 hours later, the protrusion of as much as a mycelial beak being considered as an index of germination.

It will be noted from Table I, and from Table II as well, that control germinations under the conditions of the experiment were unusually uniform—ranging from 92 to 95 per cent. Series H and K differ in

TABLE I
Germination Data for X-Irradiated Ascospores of N. tetrasperma

X-ray exposure	Approximate dosage	Series H		Series K	
		No. counted	Germinated	No. counted	Germinated
<i>hrs.</i>	<i>roentgens</i>		<i>per cent</i>		<i>per cent</i>
0	0	244	93.5	804	95
1	70,000	228	91.7	545	92.4
2	140,000	217	76	347	85
2½	175,000			368	83
3	210,000	600	00	510	51
3½	245,500			588	34.5
4	280,000	168	46	653	82
5	350,000	373	93	446	26
6	420,000	203	94	788	2.5
7	490,000	174	79		
8	560,000	162	59		

that the spores in Series K were a month older; the latter were obtained from a culture aged 3 months. When presented in graphical form as Fig. 1, these results exhibit several striking features. Outstanding is the existence of a low minimum in the survival curve followed by a high secondary maximum. As shown by the composite growth curve which has been inserted for comparison, the normal growth process is inhibited long before the mechanism responsible for germination is destroyed. No relationship whatsoever seems to exist between germination and growth, other than that the former is necessarily a prerequisite of the latter.

With increasing X-ray dosage up to 100,000 r., there is a progressive decrease in the length of the germ tube. This is in agreement with the observations of Dickson (1932) on *Chaetomium cochliodes* and Luyet (1932) on *Rhizopus nigricans*. It is difficult to determine just what constitutes germination at the higher doses. Spores receiving dosages around 150,000 r. produced short, swollen germ tubes. Upon closer examination these were found to contain coagulated protoplasm and were obviously dead. For dosages around 300,000 r. or more, the germ tubes were quite transparent as if filled with an aqueous solution. This is the region of the second maximum in the germination curve.

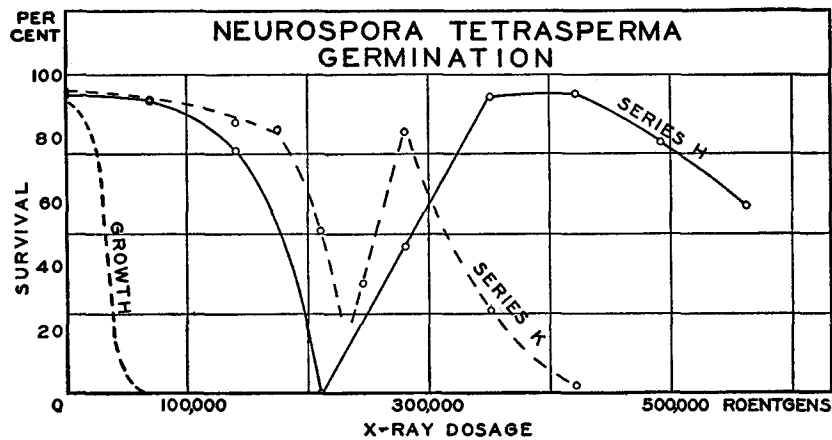


FIG. 1. Survival of irradiated ascospores using germination as a criterion of death. Note that growth ceases at a comparatively low dosage.

None of these germ tubes grew appreciably in length, and it seems probable that all these spores, even though producing germ tubes, were dead. It appears likely that the germination curve is dependent upon certain physical rather than physiological effects of the X-rays. The germination curve, then, probably represents the course of certain physical changes in a non-living system. Spore germination is not a criterion of the life of the cell; therefore, it cannot be used for obtaining data with which to test the quantum hit theory of lethal action. Luyet (1932) has used spore germination for such a criterion; from the above discussion this seems not to be justified.

Single Spore Cultures.—The data on single spore cultures of irra-

diated *N. tetrasperma* were obtained according to the methods already described in a previous section. The results are tabulated in Table II. Series E and G differ in regard to their heat treatment, the former being heated $1\frac{1}{2}$ hours and the latter $2\frac{1}{2}$ hours at 60°C . It was considered highly desirable to avoid all artificial distinctions in ascertaining the lethal effects produced. Consequently, only those criteria of death which appeared to have a real significance in the life

TABLE II
Single Spore Culture Data for X-Irradiated N. tetrasperma
(Series E Above; Series G Below)

Exposure time	Total No. of cultures	No. of cultures with					Cultures with	
		Normal perithecia	Mature ascospores	Perithecia absent or sterile	Positive growth	Growth absent	Positive growth	Mature ascospores
<i>hrs.</i>							<i>per cent</i>	<i>per cent</i>
0.0	100	87	88	4	92	8	92	88
0.5	100	84	87	1	88	12	88	87
1.0	60	38	49	2	51	9	85	82
1.5	149	31	73	35	108	41	72	49
2.0	150	7	30	80	110	40	73	20
3.0	149	0	2	20	22	127	15	1.3
4.0	100	0	0	1	1	99	1	0
5.0	97	0	0	0	0	97	0	0
0.0	100	93	93	1	94	6	94	93
0.5	100	79	79	2	81	19	81	79
1.0	99	77	88	3	91	8	92	89
1.33	145	63	98	20	118	27	81	68
1.66	150	33	64	74	138	12	92	43
2.0	149	3	19	105	124	25	83	13
2.5	100	1	4	28	32	68	32	4

cycle of the organism were employed. These were germination, positive growth, mature ascospores, and the production of normal perithecia. Perithecia have been considered normal if they discharged their spores within 3 weeks after irradiation. In determining the cultures with mature ascospores, the non-discharging perithecia were crushed on cover-glasses and examined under the microscope. By "positive growth" is meant cultures whose mycelia covered the

surface of the agar substrate and/or which were capable of being sub-cultured. The selected groupings were found to possess well defined natural boundaries. Within each classification, however, continuous gradations existed. For example, the class "mature ascospores" includes those cultures which discharged spontaneously, those which contained a full complement of spores but did not discharge, and finally perithecia which contained relatively few mature spores. Similarly, under "perithecia absent or sterile" are grouped conidial and non-conidial cultures along with those having scleroid bodies, abortive perithecia, etc. The appearance of 4 non-perithecial cultures in the Series E controls is probably due to the unintentional culturing of small uninucleate spores. Since it was known that a small percentage of such spores appears in *N. tetrasperma*, an effort was made to select only the uniform binucleate variety. Even the binucleate spores may be unisexual in rare cases (see Lindegren, 1932). The final counts were made 30 days after the date of irradiation. That equally significant data might have been secured at a much earlier date was indicated by superficial counts made in the interim.

A graphical presentation of much of the data in Table II is furnished in Figs. 2 and 3, though in a slightly altered form. The survival ratios have been adjusted for the controls by setting the value of the latter at 100 per cent. In order that all the curves might be readily comparable with each other, the X-ray dosage in roentgens has been plotted as the abscissa in each case. In Figs. 2 and 3, each abscissa division corresponds to a time period of 1 hour or a dosage of 12,500 r. The germination curve, which for such low dosages is practically a straight line, has been sketched for comparison. No graphs have been drawn to represent the survival of normal perithecia since the values to be plotted for such curves are almost identical with the ascospore ratios for the initial periods of exposure; however, at the higher dosages the development of normal perithecia was curtailed very markedly. Thus while both nuclei of a spore might survive the irradiation process, injury sufficient to interfere with the automatic dispersal of mature ascospores from the perithecia was frequently sustained.

The dosages corresponding to 50 per cent survival for ascospore production and growth are approximately 20,000 and 30,000 roentgens,

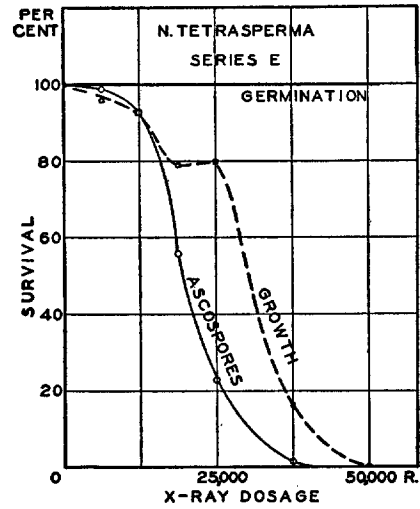


FIG. 2. Survival curves for irradiated ascospores, showing the dependence of shape on the choice of death criteria.

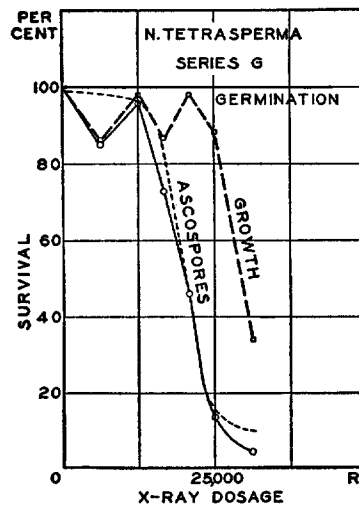


FIG. 3. Survival curves for irradiated ascospores. The broken line represents the ascospore production expressed as a percentage of the number of growing cultures.

respectively. Since two nuclei must be operative in sexual reproduction while only one may suffice for growth, one would expect from probability considerations some such relationship between these half-survival dosages. For example, if the probability of one nucleus in a spore being mortally injured by a given dosage is 0.5, the chance of both suffering the same fate is the product of the separate probabilities, or 0.25. This assumes that both nuclei are equally sensitive and that the theory of probability is valid when applied to the phenomenon of death induced by X-radiation. Though the above value fits the conditions in Fig. 2 rather well, the agreement with additional calculations made in the same manner is far from satisfactory in general.

The zigzag nature of the growth curve in Fig. 3 may be spurious, such as would result from sampling or other error. It is certainly brought about by a cause other than that immediately responsible for the processes of sexual reproduction. Support for such a view is seen in the shape which the ascospore curve for Series G assumes when the survival ratios are based on the number of positive growth cultures instead of on the total number of tubes inoculated. This transformation gives the broken curve in Fig. 3, a curve which is regular in shape and approximately congruent with the corresponding one in Fig. 2. Most of the data graphed in Figs. 2 and 3 are unsuitable for an analysis on the basis of the quantum hit theory due to irregularities in the shape of the curves. If this anomalous behavior is real, and due, perhaps, to the interaction of several destructive phenomena, the theory is not strictly applicable unless the gross survival curves can be resolved into their component parts. If one plots the standard theoretical survival curves corresponding to the effective number of hits required to kill (see Condon and Terrill, 1927), and compares them with the ascospore curve in Fig. 2, one obtains the value 9 for the best fit. From energy considerations it is now possible to calculate the so called sensitive volume of the spores. Assuming the effective wave length to be 1.5 Ångström units, the specific gravity 1.1, the ionizing energy in volts per ion pair 35, and making other customary assumptions as to absorption coefficients, etc. (see Wyckoff, 1930), one finds for the supposed sensitive volume a diameter of 0.5μ . Recalling that the ellipsoidal spores have the minor and major axes 15μ and

31 μ respectively, one has a reference basis for comparison. In the absence of precise data on nuclear dimensions, it seems reasonable to assume that the sensitive region in volume corresponds to the chromatin or perhaps to a single chromosome. Since the X-rays were neither monochromatic nor filtered, any attempt to arrive at a more accurate picture of the lethal process with the data at hand would be manifestly unjustifiable.

DISCUSSION

Just as the life of a cell demands the presence and functioning of several distinct structures, so death processes may be induced by the destruction or injury of any one or all of these necessary cell entities. Hence one would not anticipate *a priori* a simple killing curve. That many survival curves do possess an elementary shape is probably attributable to the fact that some cell structures are much more sensitive than others to lethal agents. In his studies with fern spores, Zirkle (1932) has shown that the nuclear elements are much more susceptible to alpha radiation injuries than the extranuclear ones, but that sufficient interference with the functions of the latter will also destroy life. In fact, Zirkle found that the shape of his survival curves could be completely altered by changing the orientation of the spores with respect to the source of radiation—thus rendering the nuclei more or less accessible to the alpha particles. While such radical effects are not to be expected under the conditions of the present experiment, it is well to bear the possibility in mind when employing low voltage X-rays.

The fact that germination—defined as the protrusion of microscopically detectable material from the spore—can withstand extremely high dosages compared to the mechanism involved in growth, indicates that the two processes are quite dissimilar or at least proceed at very different rates. It is known that the continued growth of an organism requires a nucleus, although cytoplasm may live for some time without the benefit of such a structure. Increasing degrees of cytolysis under prolonged X-radiation might therefore account for decreasing mycelial development with exposure time. It is very probable, too, that germination phenomena are intimately associated with structural changes in the spore membrane. The question of

permeability assumes importance in connection with osmotic phenomena. That X-radiation renders cell membranes more permeable to many solutions has been established by several investigators (see Kovacs, 1928). Diffusion coefficients likewise increase.

Along with these considerations, it is interesting to read the recent papers of Nakashima (1926) and Rajewsky (1930) on the effects of ultraviolet light and X-rays in coagulating pure proteins *in vitro*. They obtained a rhythmic curve for the number of protein particles observable in the ultramicroscope when plotted as a function of dosage. Just how far such a picture may be applicable, if at all, to the data on *Neurospora* germination cannot be foretold.

Recent studies by Stubbe (1933) on the variation of mutation rates in *Antirrhinum majus* with X-ray dosage may possibly have a bearing on this problem. Over a wide range of X-ray wave lengths, Stubbe found with increasing dosage that the mutation rate increased to a maximum, then decreased to a minimum, and finally increased again.

SUMMARY

1. When ascospores of *Neurospora tetrasperma* were irradiated with 11 kv. X-rays, the single spore cultures obtained displayed a wide variety of mutated forms.
2. Control germinations of ascospores showed uniform behavior, ranging from 92–95 per cent germination.
3. The shape of the survival curves was found to be a function of the criterion of death. The following criteria were used: germination, growth, production of mature ascospores, and the production of normal perithecia.
4. The germination survival curve exhibited a rhythmic variation with dosage. Germination is not a significant criterion of death.
5. Half-survival dosages for growth and ascospore production were approximately 30,000 and 20,000 roentgens, respectively.
6. Multiple hit-to-kill relations were found on the basis of the quantum hit theory; no accurate analysis was possible.
7. The studies indicate that ascospore death does not result from a single well defined reaction, but rather from the integrated effects of several deleterious processes initiated by the radiation.

LITERATURE CITED

- Condon, E. U., and Terrill, H. M., 1927, *J. Cancer Research*, **11**, 324.
Crowther, J. A., 1926, *Proc. Roy. Soc. London, Series B*, **100**, 390.
Dickson, H., 1932, *Ann. Bot.*, **46**, 389.
Dodge, B. O., 1927, *J. Agric. Research*, **35**, 289; 1928, **36**, 1; 1931, *Mycologia*, **23**, 1.
Exner, F. M., 1932, *Am. J. Cancer*, **16**, 1275.
Glassner, O., 1932, *Radiology*, **18**, 713.
Glocker, R., 1932, *Z. Phys.*, **77**, 653.
Glocker, R., Langendorff, H., and Reuss, A., 1933, *Strahlentherapie*, **46**, 517.
Heldmaier, C., 1929, *Z. Bot.*, **22**, 161.
Holweck, F., and Lacassagne, A., 1929, *Compt. rend. Acad.*, **188**, 197; 1930 a, *Compt. rend. Soc. biol.*, **103**, 60; 1930 b, *Compt. rend. Acad.*, **190**, 524.
Kovacs, K., 1928, *Strahlentherapie*, **30**, 77.
Leonian, L. H., 1929, *Phytopathology*, **19**, 753.
Lindegren, C. C., 1932, *Bull. Torrey Bot. Club*, **59**, 85, 119; 1933, **60**, 133.
Luyet, B. J., 1932, *Radiology*, **18**, 1019.
Nadson, G., and Philippov, G., 1925, *Compt. rend. Soc. biol.*, **93**, 473.
Nakashima, J., 1926, *Strahlentherapie*, **24**, 1.
Rahn, O., 1932, *Physiology of bacteria*, Philadelphia, P. Blakiston's Son and Co., Inc.
Rahn, O., and Barnes, M. N., 1933, *J. Gen. Physiol.*, **16**, 579.
Rajewsky, B., 1930, *Biochem. Z.*, **227**, 272.
Shear, C. L., and Dodge, B. O., 1927, *J. Agric. Research*, **34**, 1019.
Stubbe, H., 1933, *Z. indukt. Abstammungs-u. Vererbungslehre*, **64**, 181.
Tascher, W. R., 1933, *J. Agric. Research*, **46**, 909.
Taylor, L., and Stoneburner, C., 1932, *Bureau Standards J. Research*, **9**, 769.
Uber, F. M., 1933, *Rev. Scient. Inst.*, **4**, 649.
Wyckoff, R. W. G., 1930, *J. Exp. Med.*, **52**, 435; 1932, *J. Gen. Physiol.*, **15**, 351.
Wyckoff, R. W. G., and Luyet, B. J., 1931, *Radiology*, **17**, 1171.
Zirkle, R. E., 1932, *J. Cellular and Comp. Physiol.*, **2**, 251.