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Report of Progress

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Cytological Studies

Cytological comparisons of fertile selections from gamma-irradiated T-type corn with male steriles, maintainers and restored steriles have not shown consistent differences in cytoplasmic constituents. However, selections of apical growing points from these lines are being reexamined for the small circular mitochondria which were observed in the T-sterile cytoplasm (last year's progress report).

Several different sterile cytoplasm corn lines and their fertility restored versions are being examined in thin sections for continuity of and possible paternal transmission of abnormal mitochondria (elongated and circular forms of mitochondria described in the 1972 and 73 progress reports). Whether cytoplasmic sterility factors in S-type corn are located in mitochondria (Rhoades, 1950) is still to be determined. In this connection recent studies in Neurospora crassa are of interest. The abnormal-1 cytoplasmic mutant controlling slow my al growth in N. crassa has been assumed to reside in mitochondria (Dia arkos et al., 1965). Kuntzel et al., (1973) have observed virus-lik articles in the mitochondria of abnormal-1 and suggest these particles ppress normal growth and normal respiration by infecting wild type mit ondria.

Cytological comparisons of cytopl mic male sterile and maintainer lines have been carried out on severa pecies other than corn (petunia, tobacco, sorghum, Crotalaria mucronat sugar beet, s lower, and Vicia faba). With the exception of Vicia no consistent differences in cytoplasmic constituents have been observed with either light or electron microscopy. In Vicia, cytoplasmic sterile tissues contain spherical cytoplasmic particles (previous progress reports) while the maintainers, if kept in isolation cages, do not contain such particles. The male sterility in

Vicia was assumed by Bond et al. (1966) to be controlled by a seed transmitted agent which they suggested might be a virus or defective virus. The cytoplasmic particles in Vicia faba resemble cytoplasmic particles in Epilobium (Anton-Lamprecht, 1965, 1966, 1967), Tropoelum (Ie, 1971, 1972), and Centaurea (Davey and Short, 1972). Cytological studies and transmission studies are continuing in Vicia faba in attempts to correlate the spherical cytoplasmic particles with male sterility.

Fertilization

Differences in the contribution of extranuclear nucleic acids by the gametes and/or differences in their fate in the zygote form the basis of non-Mendilian inheritance. Cytological studies of some gymnosperms have shown that the embryo may contain only paternal mitochondria and plastids (Biota orientalis, Chesnoy, 1969a,b), or only maternal mitochondria and plastids (Pinus laricio, Camefort, 1966), or a mixture of maternal and paternal mitochondria and only paternal plastids (Larix decidua, Camefort, 1968a,b). In some angiosperms the embryo may also contain cytoplasmic components of different origins. On the basis of genetic studies, biparental transmission of plastids has been demonstrated in Hypericum perforatum, H. acutum, several Oenothera species, and in Pelargonium zonale (Kirk and Tilney-Bassett, 1967). However, biparental transmission of plastids is very rare in Epilobium species (Michaelis, 1954) (Epilobium and Oenothera are members of the Onagraceae), and in many of the Gramineae (Kirk and Tilney-Bassett, 1967).

Cytological and genetic studies indicate that there is much to be learned about the processes which plants use to maintain cytoplasmic organelles at optimal levels. They also indicate that different mechanisms have been evolved for this maintenance.

In Pelargonium zonale, in which biparental plastid transmission occurs, Lombardo and Gerola (1968) observed many proplastids in the generative cells. Lombardo and Gerola also report that tube cells contained plastids, while generative cells of several angiosperms contained mitochondria but only in a few cases could proplastids be identified. We have encountered a similar situation in generative cells of corn and Crotalaria mucronata. Paternal plastids may not participate in zygote formation in some species because they are somehow excluded from generative nuclei in the pollen.

Nilsson-Tillgren and von Wettstein-Knowles (1970) studies of maternally inherited white tobacco plastids, have shown through production of haploid plants from pollen, that paternal plastids are functional at the first pollen mitosis. Most if not all the haploids were derived from the tube (vegetative) cells of the pollen.

Genetic investigations and breeding studies in corn have shown that whatever the mechanisms controlling uniparental transmission of sterility factors might be, they are stable and efficient. The "reversions" to fertility in some plants of T-type sterile single crosses (Rogers, 1954) were probably induced by restorer genes in the pollen parent (Tx173D) of the single cross (previous progress reports). However, the appearance of some fertile and partially fertile plants in S-type sterile lines (Jones, 1956; Singh and Laughnan, 1972) may be the result of paternal transmission of normal cytoplasmic factors into egg cells containing sterility factors. Some of the difficulty in "difficult to sterilize" cytoplasms in corn and other species may result from occassional paternal transmission of cytoplasmic factors. The control of such paternal transmissions of cytoplasmic factors would not necessarily reside in the pollinators, but could reside in occasional breakdowns in the mechanisms excluding paternal cytoplasmic constituents in the egg or young zygotes.

We have developed, I think, adequate skills in the techniques of preparing germinating pollen and unfertilized ovules for electron microscopy. A large part of our cytological studies have involved comparisons of egg cells and ovules from sterile and maintainer lines of various species on the assumption that whatever the loci and distribution of sterility factors in the plant might be, the sterility factors will be in the egg cell. Markers are necessary for cytological studies of the fertilization process in relation to cytoplasmic inheritance. We have obtained several kinds of markers: Cytoplasmically inherited abnormal plastids in tobacco (Edwardson, 1965), petunia (Edwardson, unpublished), sorghum (Karper, 1934), corn (Rhoades, 1943), abnormal mitochondria in corn, and spherical cytoplasmic inclusions in Vicia faba. The use of the inclusions in Vicia for studies of fertilization is still impeded by our inability to get seed set in this species. The major problem in utilizing the marker plastids for fertilization studies has been the lack of or limited sectoring of the abnormal plastids in inflorescences. I think the production of larger populations will enable us to select inflorescences and large sectors of inflorescences containing only abnormal plastids for reciprocal crosses with normals. Also, we have plantlets developing from tobacco callus derived from variegated portions of stems and petioles. Selecting yellow-green plantlest for grafts onto normals should allow us to obtain yellow-green inflorescences for reciprocal crosses with normals. We have requested, but not yet received, seed of a maternally inherited mutant in tobacco which contains both cytologically abnormal plastids and mitochondria.

The modes of plastid transmission are of particular interest since plastids have been indicated as the sites of cytoplasmic sterility factors in Oenothera (Schwemmle et al., 1938; Stinson, 1960; Stubbe, 1964), and in Epilobium (Michaelis, 1969).

The Datura Q-Virus

The effects of Datura Q-virus infection (Blakeslee, 1921, a,b) for many years were regarded as mimicing cases of cytoplasmic male sterility. Mimicry, was assumed because the causal agent of the disease was graft-transmitted (mechanical transmission trails had been unsuccessful, and no vectors are known). However, reports of gene-cytoplasm interaction controlled sterility being graft-transmitted (Bianchi, 1967; Frankel, 1956, 1962; Curtis, 1967; Edwardson and Corbett, 1961; Kinoshita, 1971) suggested that cytoplasmic inheritance rather than virus infection might be involved in the Datura Q-virus induced syndrome. Virus-like particles had not been observed in gene-cytoplasm sterility controlling systems or in Datura Q-virus infected material. This was no doubt due in part to the absence of cytological studies on Datura Q-infected tissues. However, for an embarrassingly long period of time my electron microscope investigations of Datura Q-virus infected leaf tissues showed only the occasional occurrence of fibrous cytoplasmic inclusions which did not look like virus particles. We have been unable to extract these fibers from infected tissues.

D. E. Purcifull and I cooperated in transmitting the Datura Q-virus to Datura and other species by mechanical methods. Electron microscopy of apical growing points and young leaflets of Q-infected Datura stramonium and tobacco has revealed the presence of aggregated small spherical virus particles often associated with fibrous inclusions. In Datura the virus particles are confined to the cytoplasm, in tobacco they occur in both cytoplasm and nuclei. Properties of tobacco streak virus described by Fulton (1967, 1971) and Brunt (1968) suggested that the Datura Q-virus and tobacco streak virus might be related. Electron microscopy of tobacco streak virus infected tobacco and Datura stramonium apical growing points and young leaflets show aggregated virus particles and fibrous inclusions very similar to those induced by the Datura Q-virus. Immunodiffusion tests (in cooperation with D. E. Purcifull) show that antigens of the two viruses give reactions of identity. The Datura Q-virus is a strain of tobacco streak virus.

This work has been very satisfying. The Datura Q-virus has been rescued from obscurity, it is not mentioned in recent textbooks on virology, some puzzling aspects of the Q-virus syndrome have been clarified. We have improved our eye and our technique in searching for this small spherical virus. At present, small spherical viruses are not the most popular candidates for cytological studies. One aspect of the cytological investigations of the Datura Q-virus is dismaying. The procedures of fixation, dehydration, embedding, sectioning, and staining which rendered the aggregated virus particles visible in growing points and young leaflets, did not make the particles visible in leaf tissues exhibiting Q-virus symptoms. Infective virus was present in these more mature leaves since such leaves provided the source of inoculum in successful mechanical transmission trials. I assume that virus particles could not be distinguished from ribosomes in these leaves, and that they could be detected in young tissues because they formed aggregates. This is not encouraging for cytological studies of some sterility factors which may reside in small spherical virus-like particles, particularly if the particles do not aggregate.

As a result of our experiences in studying various pathogens with the electron microscope I am confident that we can detect sterility factors residing in unaggregated rickettsias, mycoplasms, anisometric virus particles, as well as isometric virus particles if they are larger than 30 nm in diameter. We have detected unaggregated pangola stunt virus particles in the cytoplasm of infected hosts, these particles have a diameter of about 70 nm (Schank and Edwardson, 1968; Schank _et al_. 1972).

Mutants Controlling Resistance to Helminthosporium maydis

Miller and Koeppe (1971) reported that the _H. maydis_ pathotoxin induced marked swelling and loss of phosphorylating ability in isolated T-sterile corn mitochondria. They suggested the toxin might adversely affect mitochondria in living tissue. However, Arntzen _et al_. (1973) have concluded that mitochondria in living tissue are not the primary site of the pathotoxin's action. We are attempting to obtain from T-type corn lines mutants controlling resistance to race-T of _H. maydis_. Irradiation, and chemical mutagens are being applied to T-sterile corn to induce mutations in cytoplasmic factors controlling susceptibility to _H. maydis_; June varieties (from which the original T-cytoplasm was selected) are being examined for resistance to _H. maydis_.

In previous progress reports selections from gamma irradiated T-sterile corn have been described which are as resistant to _H. maydis_ infection as are corn lines in normal cytoplasm and as fertile as normal corn lines. The fertile-resistant selections have been outcrossed on T-sterile lines. The completely male sterile-susceptible progeneis obtained from these crosses strongly indicate that cytoplasmic mutation(s) have been induced rather than nuclear mutations involving genes controlling _H. maydis_ resistance or fertility restoration. Whether it is possible to alter cytoplasmic susceptibility to _H. maydis_ without altering cytoplasmic T-sterility is unknown but we hope to obtain such alterations from mutagen treatments and from selections in T-cytoplasm populations. The induction of cytoplasmic male sterility by X-irradiation has been reported in tomato

(Lindstrom, 1933), and by gamma irradiation in sugar beet (Kinoshita and Takahashi, 1966; Kinoshita and Nagao, 1968; Kinoshita, 1971). The loci of the mutated cytoplasmic factors are not known in any of these cases. The induction of maternally inherited plastid abnormalities, has been reported from X-irratiation treatments of Arabidopsis thaliana (1962).

Another approach to obtaining cytoplasmic male sterility and resistance to H. maydis is the application of mutagens to corn lines containing normal cytoplasm. The different types of cytoplasmic male sterility in corn presumably originated in normal cytoplasm through infections, or more likely through mutations in cytoplasmic nucleic acids. Various chemical mutagens have been reported to induce mutations in nucleic acids of cytoplasmic organelles: Plastid mutations have been induced in Euglena with N-methyl-N-nitro-N-nitrosoguanidine (McCalla, 1965), and with nalidixic acid (Ebringer, 1970), and in Chlamydomonas with ethidium bromide (Flechtner and Sager, 1973); Ethidium bromide has also been reported to induce mutations in yeast mitochondria (Goldring et al., 1970). In angiosperms maternally inherited plastid mutations have been induced in tobacco (Dulieu, 1967) and petunia (Cornu, 1970) with ethyl methane sulfonate, and in Oenothera (Kutzelnigg, 1972) with N-methyl-N-nitro-N-nitrosoguanidine.

We have been treating seed of T-sterile inbreds (Fla F6T, Fla F44T) and their normal counterparts (Fla F6, Fla F44) with N-methyl-N-nitro-N-nitrosoguanidine, nalidixic acid, ethidium bromide, and ethyl methane sulfonate. Since Fla F44 restores fertility to most of the sterile cytoplasm accessions we assume that induced steriles would probably by T-type. Progeny of partially male sterile and tasselless Fla F44 plants treated with ethyl methane sulfonate produced normal offspring. The progeny of Fla F44 sterile (ethidium bromide treated) X Fla F44 control will be studied for transmission of sterility. Progeny of all treated lines are being observed for alterations in fertility (Fla F6, Fla F44) and susceptibility to H. maydis (Fla F6T, Fla F44T) in the treated, S_1, and BC_1 generations. We plan to increase the numbers of treated seed, and the size of the S_1 and BC_1 progenies in order to increase our chances of detecting cytoplasmic factor mutations.

Other Studies

Studies of the inheritance of dominant nuclear male sterility genes and their suppressors in S-cytoplasm corn are continuing. The effect of dominant sterility genes in EK (Early King) and PS (Pride of Saline) cytoplasms will be studied since those types of cytoplasm appear to be similar to the S-type. Asexual transmission of cytoplasmic male sterility factors is being attempted in sterile-maintainer grafts in sunflower.

Present State of Knowledge

Cytoplasmic male sterility is being used, or has the potential for being used to exploit heterosis in a large number of plant species. The inheritance of fertility restoration is well understood in a majority of these species. However, location of the cytoplasmic sterility factors, their transmission, and the mechanisms under their control are not at all well understood. In T-cytoplasm corn there exist cytoplasmic factors which govern susceptibility to race-T of Helminthosporium maydis, but whether these factors are the sterility factors or different factors is unknown. The existance of cytoplasmic factors controlling susceptibility to infection is a very important problem for plant breeders, pathologists, and geneticists.

The very basis of cytoplasmic inheritance are the mechanisms controlling uniparental transmission of cytoplasmic organelles. These mechanisms are not known or not well understood in the great majority of angiosperms. There is evidence that cytoplasmic sterility factors in some species (petunia, sugar beet, sunflower, Vicia faba) are not located in organelles, however, little is known about the nature of these factors, their location in the cytoplasm or the mechanisms which exclude them from being pollen transmitted.

There is a great need for additional information which could lead to better understanding of what we are working with in breeding programs, and of what cytoplasmic inheritance involves at the cellular level as well as in the developing plant.

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Literature Cited

Anton-Lamprecht, I. 1965. Electron microscopical evidence of unusual structures in the cytoplasm of some plasmotypes of Epilobium hybrids. J. Ultrastr. Res. 12:624-633.

Anton-Lamprecht, I. 1966. Elektronenmikroskopische Untersuchungen an Plasmonabanderungen von Epilobium-Bastarden. Zeits. Vererbungslehre 98:257-296.

Anton-Lamprecht, I. 1967. Elektronenmikroskopische Untersuchungen an Plasmonabanderungen von Epilobium-Bastarden. Protoplasma 64:267-296.

Arntzen, C. J., D. E. Koeppe, R. J. Miller, and J. H. Peverly. 1973. The effect of pathotoxin from Helminthosporium maydis (race T) on energy linked processes of corn seedlings. Physiol. Plant Pathol. 3:79-89.

Bianchi, F. 1967. Transmission of male sterility in Petunia by grafting. Genen et Phaenen 8:36-43.

Blakeslee, A. F. 1921a. An apparent case of non-Mendelian inheritance in Datura due to a disease. Proc. Nat. Acad. Sci. 7:116-118.

Blakeslee, A. F. 1921b. A graft-infective disease of Datura resembling a vegetative mutation. J. Genetics 11:17-35.

Bond, D. A., J. L. Fyfe, and G. Toynbee-Clarke. 1966. Male sterility in field beans (Vicia faba L.) III. Male sterility with a cytoplasmic type of inheritance. J. Agric. Res. (Cambridge). 66:359-372.

Brunt, A. A. 1968. Tobacco streak (TSV). Glasshouse Crops Res. Inst. Ann. Report. 104.

Camefort, H. 1966. Observations sur les mitochondries et les plastes d'origine pollinique apre leur entree dans une oosphere chezle Pin noir (Pinus larichio Poir. var. austriaca=Pinus nigra Arn.). Comptes Rendus Ser. D. 263:959-962.

Camefort, H. 1968a. Sur l'organisation du nucleoplasme dans les proembryons tetranuclees du Larix decidua Mill (Larix europea D. C.) et l'origine des mitochondries et des plastes de l'embryon chez cette espece. Comptes Rendus Ser. D. 266:88-91.

Camefort, H. 1968b. Cytologie de la fecondation et de la proembryogenese chez quelques Gymnospermes. Bull. Soc. bot. Fr. 115:137-160.

Chesnoy, L. 1969a. Sur l'origine du cytoplasme des embryons chez le Biota orientalis Endl. (Cupressacees). Comptes Rendus Ser. D. 268: 1921-1924.

Chesnoy, L. 1969b. Sur la participation du gamete male a la constitution du cytoplasme de l'embryon chez le Biota orientalis Endl. Rev. Cytol. et Biol. Veg. 32:273-294.

Cornu, A. 1970. Sur l'obtention de mutations somatiques apres traitments de graines de Petunias. Ann. Amelior. Plantes 20:189-214.

Curtis, G. J. 1967. Graft transmission of male sterility in sugar beet (Beta vulgaris L.). Euphytica 16:419-424.

Davey, M. R., and K. C. Short. 1972. Spherical bodies in the cytoplasm of Centaurea cyanus L. Protoplasma 75:199-203.

Diacumarkos, E. G., L. Garnjobst, and E. L. Tatum. 1965. A cytoplasmic character in Neurospora crassa. The role of nuclei and mitochondria. J. Cell Biol. 26:427-443.

Dulieu, H. 1967. Sur les differente types de mutations extracucleaires induites par le methane sulfonate d'ethyl chez Nicotiana tabacum. Mutation Res. 4:177-189.

Ebringer, L. 1970. The action of nalidixic acid on Euglena plastids. J. Gen. Microbiolol. 61:141-144.

Edwardson, J. R. 1965. Gene control of non-Mendelian variegation in Nicotiana tabacum. Genetics 52:365-370.

Edwardson, J. R., and M. K. Corbett. 1961. Asexual transmission of cytoplasmic male sterility. Proc. Nat. Acad. Sci. 47:390-396.

Flechtner, R., and R. Sager. 1973. Ethidium bromide induced selective and reversible loss of chloroplast DNA. Nature New Biol. 241:277-279.

Frankel, R. 1956. Graft-induced transmission to progeny of cytoplasmic male sterility in petunia. Science 124:684-685.

Frankel, R. 1962. Further evidence on graft induced transmission to progeny of cytoplasmic male sterility in Petunia. Genetics 47:641-646.

Fulton, R. W. 1967. Purification and some properties of tobacco streak and Tulare apple mosaic viruses. Virology 32:153-162.

Fulton, R. W. 1971. Tobacco streak virus. Commonwealth Mycol. Descriptions of Plant Viruses #44.

Goldring, E. S., L. I. Grossman, D. Krupnick, D. R. Cryer, and J. Marmur. 1970. The petite mutation in yeast. Loss of mitochondrial deoxyribonucleic acid during induction of petites with ethidium bromide. J. Molec. Biol. 52:323-335.

Ie, T. S. 1971. Electron microscopy of developmental stages of tomato spotted wilt virus in plant cells. Virology 43:468-479.

Ie, T. S. 1972. Cytoplasmic particles in Tropaeolum majus. Planta 106:227-236.

Jones, D. F. 1956. Genic and cytoplasmic control of pollen abortion in maize. Brookhaven Symp. in Plant Breeding 9:101-112.

Karper, R. E. 1934. Maternal inheritance of chlorophyll in sorghum. J. Heredity 25:48-54.

Kinsohita, T. 1971. Genetical studies on the male sterily of sugar beets (Beta vulgaris L.) and its related species. J. Fac. Agr. Hokkaido Univ. Sapporo 56:437-541.

Kinoshita, T., and S. Nagao. 1968. Use of male sterily in triploid sugar beets. Proc. XII Int. Cong. Genet. 232-233.

Kinoshita, T., and M. Takahashi. 1966. Inheritance of a pollen sterility induced by the irradiation. Bull. Sugar Beet Res. Suppl. 7:40-42.

Kirk, J. T. O., and R. A. E. Tilney-Bassett. 1967. The Plastids. W. H. Freeman and Co. San Francisco.

Kuntzel, H., Z. Barath, I. Ali, J. Kind, and H. H. Althaus. 1973. Virus-like particles in an extranuclear mutant of Neurospora crassa. Proc. Nat. Acad. Sci. 70:1574-1578.

Kutzelnigg, J. 1972. Mutationsversuche mit N-methyl-N-nitro-N-nitrosoguanidine an Oenothera hookeri. Radiation Bot. 12:63-75.

Lindstrom, E. E. 1933. Hereditary radium-induced variations in the tomato. J. Heredity 24:129-137.

Lombardo, G., and F. M. Gerola. 1968. Cytoplasmic inheritance and ultrastructure of the male generative cell of higher plants. Planta 82:105-110.

McCalla, D. R. 1965. Chloroplast mutagenesss: Effect of N-methyl-N-nitro-N-nitrosoguanidine and some other agents in Euglena. Science 148: 497-499.

Michaelis, P. 1954. Cytoplasmic inheritance in Epilobium and its theoretical significance. Adv. Genetics 6:287-401.

Michaelis, P. 1969. Uber Plastiden-Restitutionen. Cytologia 34:1-115.

Miller, R. J., and D. E. Koeppe. 1971. Southern corn leaf blight: Susceptible and resistant mitochondria. Science 173:67-69.

Nilsson-Tillgren, T., and P. von Wettstein-Knowles. 1970. When is the male plastome eliminated? Nature 227:1265-1266.

Pfahler, P. 1967. In vitro germination and pollen tube growth of maize pollen I. Calcium and boron effects. Canadian J. Bot. 45:839-845.

Rhoades, M. M. 1943. Genic induction of an inherited cytoplasmic difference. Proc. Nat. Acad. Sci. 29:327-329.

Rhoades, M. M. 1950. Gene induced mutation of a heritable cytoplasmic factor producing male sterility in maize. Proc. Nat. Acad. Sci. 36:634-635.

Rogers, J. S. 1954. Breeding for pollen restorers. Proc. 9th Ann. Hybrid Corn Ind. Res. Cong. 85-93.

Schank, S. C., and J. R. Edwardson, 1968. Cytological examination of pangolagrass (Digitaria decumbens Stent) infected with stunt virus. Crop Sci. 8:118-119.

Schank, S. C., J. R. Edwardson, R. G. Christie, and M. A. Overman. 1972. Pangola stunt virus studied in pangolagrass and Digitaria hybrids. Euphytica 21:344-351.

Schwemmle, J., E. Haustein, A. Strum, and M. Binder 1938. Genetische und Cytologische Untersuchungen an Eu-Oenotheren I-IV. Zeits. Ind. Abst. Vererb. 75:358-800.

Singh, A., and J. R. Laughnan. 1972. Instability of S male-sterile cytoplasm in maize. Genetics 71:607-620.

Stinson, H. T. 1960. Extranuclear barriers to interspecific hybridization between Oenothera hookeri and Oenothera argillicola. Genetics 45:819-839.

Stubbe, W. 1964. The role of the plastome in evolution of the genus Oenothera. Genetica 35:28-33.

Bibliography of Publications Associated with

A. E. C. Contract No. AT-(40-1)-2583

Edwardson, J. R. and Corbett, M. K. 1961. Asexual transmission of cytoplasmic male sterility. P.N.A.S. 47:390-396.

Edwardson, J. R. 1962. Cytoplasmic inclusion in T-type cytoplasmic male sterile corn. Am. J. Bot. 49:184-187.

Edwardson, J. R. 1965. Radiosensitivity in maize seed influenced by cytoplasmic factors. Radiation Botany 5:53-59.

Edwardson, J. R. 1965. Gene control of non-Mendelian variegation in *Nicotiana tabacum*. Genetics 52:365-370.

Edwardson, J. R. 1966. Electron microscopy of cytoplasmic inclusions in thin sections of cells infected with rod-shaped viruses. Am. J. Bot.53:359-364.

Warmke, H. E. and Edwardson, J. R. 1966. Use of potassium permanganate for preserving virus particles in plant tissues. Virology 28:693-700.

Warmke, H. E. and Edwardson, J. R. 1966. Electron microscopy of crystalline inclusions of tobacco mosaic virus in leaf tissue. Virology 30:45-57.

Edwardson, J. R., Purcifull, D. E. and Christie, R. G. 1966. Electron microscopy of two small spherical plant viruses in thin sections. Canadian J. Bot. 44: 821-826.

Purcifull, D. E., Edwardson, J. R. and Christie, R. G. 1966. Electron microscopy of intracellular aggregates in pea (*Pisum sativum*) infected with clover yellow mosaic virus. Virology 29:276-284.

Edwardson, J. R. 1966. Cylindrical inclusions in the cytoplasm of leaf cells infected with tobacco etch virus. Science 153:883-884.

Purcifull, D. E. and Edwardson, J. R. 1967. Watermelon mosaic virus: Tubular inclusions in pumpkin leaves and aggregates in leaf extracts. Virology 32: 393-401.

Zettler, F. W., Christie, R. G. and Edwardson, J. R. 1967. Aphid transmission of virus from leaf sectors correlated with intracellular inclusions. Virology 33: 549-552.

Edwardson, J. R. and Warmke, H. E. 1967. Fertility restoration in cytoplasmic male sterile petunia. J. Heredity 53:195-196.

Edwardson, J. R. 1967. Cytoplasmic male sterility and fertility restoration in *Crotalaria mucronata*. J. Heredity 58:266-268.

Schank, S. C. and Edwardson, J. R. 1968. Cytological examination of pangolagrass (Digitaria decumbens Stent) infected with stunt virus. Crop Science 8:118-119.

Edwardson, J. R., Purcifull, D. E. and Christie, R. G. 1968. Structure of cytoplasmic inclusions in plants infected with rod-shaped viruses. Virology 34: 250-263.

Purcifull, D. E. and Edwardson, J. R. 1968. Nuclear crystals in Zinnia and other non-solanaceous plants infected with tobacco etch virus. Phytopathology 58: 532-533.

Zettler, F. W., Edwardson, J. R. and Purcifull, D. E. 1968. Ultramicroscopic differences in inclusions of Papaya mosaic virus and Papaya ringspot virus correlated with differential aphid transmission. Phytopathology 58:332-335.

Christie, S. R. and Edwardson, J. R. 1968. A disassemblable microstrainer for biological specimens. Stain Tech. 43:122-123.

Purcifull, D. E., Edwardson, J. R. and Christie, S. R. 1968. Agregated filaments in extracts from Lupinus infected with watermelon mosaic virus. Virology 35: 478-482.

Christie, S. R., Edwardson, J. R. and Zettler, F. W. 1968. Characterization and electron microscopy of a virus isolated from Bidens and Lepidium. Plant Disease Reporter 52:763-768.

Edwardson, J. R. 1968. Inclusion bodies. First Int. Congress of Plant Pathology 53 (Abstract).

Edwardson, J. R. 1968. Nature of cytoplasmic factors inducing male sterility. Proceedings 12th Int. Congress of Genetics 226-227. (Abstract).

Edwardson, J. R. and D. E. Purcifull. 1970. Turnip mosaic virus induced inclusions. Phytopathology 60:85-88.

Edwardson, J. R., D. E. Purcifull, F. W. Zettler, R. G. Christie, and S. R. Christie. 1970. A virus isolated from Desmodium canum: Characterization and electron microscopy. Plant Disease Reporter 54:161-164.

Purcifull, D. E., J. R. Edwardson, and S. R. Christie. 1970. A morphological comparison of inclusions induced by tobacco etch and potato Y virus. Phytopathology 60:779-782.

Zettler, F. W., N. J. Foxe, R. D. Hartman, J. R. Edwardson, and R. G. Christie. 1970. Filamentous viruses infecting dasheen and other araceous hosts. Phytopathology 60:983-987.

Edwardson, J. R. 1970. Cytoplasmic male sterility. Botanical Review 36:341-420.

Edwardson, J. R. 1971. Studies on cytoplasmic male sterility. Report 26th Southern Corn Improvement Conference, 2-6.

Schank, S. C., J. R. Edwardson, R. G. Christie and M. A. Overman. 1972. Pangola stunt virus studied in Pangolagrass and Digitaria hybrids. Euphytica 21:344-351.

Edwardson, J. R., F. W. Zettler, R. G. Christie and I. R. Evans. 1972. A cytological comparison of inclusions as a basis for distinguishing two filamentous legume viruses. Gen. Virolcgy 15;113-118.

Purcifull, D. E., J. R. Edwardson, and R. G. Christie. 1973. Rapid staining procedures for detecting plant virus inclusions. Second International Congress Plant Pathology.