EFFECTS OF THE BIPYRIDYLIUM HERBICIDES PARAQUAT AND DIQUAT ON THE ULTRASTRUCTURE AND PHYSIOLOGY OF A DUCKWEED AND A BLUE-GREEN ALGA

by

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# ABBRE VIATIONS FOR FIGS. 1 - 132

C.	=	chloroplast
E	=	<b>cl</b> oroplast envelope
M	2	mitochondrion
Mp	=	microbody
N	#	nucleus
Pd	=	plasmodesmata
Pm	=	plasma membrane
R	=	ribosomes
S	=	starch grain
т	=	tonoplast
Th	-	thylakoid
V.	=	vacuole
W	E	cell wall

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Fig. 1 A cell of <u>Lemna gibba</u> following fixation with 2% KMnO4 for 60 min. Cellular preservation is poor. Note the irregular shapes of the chloroplasts, the abnormal arrangemets of their thylakoid systems and the incomplete preservation of the cellular membranes.

X 7,075



Fig. 2 Part of a mesophyll cell of L. gibba following fixation with 2% KMn04 for 60 min. Note the irregular shapes of the chloroplasts and the extensive breaks in the chloroplast envelopes. Note also the widespread intrathylakoidal swelling within the thylakoids. Preservation of the plasma membrane, the tonoplast and the outer mitochondrial membranes also appears to be incomplete.

X 10,000

Fig. 3 A chloroplast of <u>L</u>. <u>gibba</u> following fixation with 2% KMn04 for 60 min. The only visible fixation damage to the chloroplast is a slight localized intrathylakoidal swelling. However, the adjacent tonoplast is incompletely preserved.

X 20,550



Fig. 4 Part of a chloroplast of <u>L</u>. <u>gibba</u> following fixation with 2% KMn04 for 60 min. This micrograph illustrates the intermittent preservation of the plasma membrane and the tonoplast. Slight intrathylakoidal swelling is also visible within the chloroplast.

X 17,160

Fig. 5 Incomplete preservation of the tonoplast in a frond cell of L. gibba following fixation with 2% KMn04 for 60 min.

Fig. 6 Incomplete preservation of the chloroplast envelope and the plasma membrane in a frond cell of <u>L</u>. <u>gibba</u> following fixation with 2% KMn04 for 60 min.



Fig. 7 A cell of <u>L. gibba</u> following fixation with 2% KMm04 for 30 min. All structure is poorly preserved.



Fig. 8 A chloroplast of <u>L</u>. <u>gibba</u> following fixation with 2% KMnO4 for 30 min. Note the presence of slight intrathylakoidal swelling and occasional small breaks in the chloroplast envelope. Preservation of the plasma membrane is also incomplete.

X 20,700

Fig. 9 A chloroplast of L. gibba following fixation with 2% KMn04 for 30 min. Note the incomplete preservation of the plasma membrane, tonoplast and chloroplast envelope. Intrathylakoidal swelling is also evident.

X 20,900



Fig. 10 The remains of a chloroplast of Lemna minor following fixation with 1.2% KMn04 for 15 min. The chloroplast has ruptured allowing part of its contents to escape X 8,250

Fig. 11 A chloroplast of <u>L</u>. <u>minor</u> following fixation with 1.2% KMn04 for 15 min. The chloroplast is intact and intrathylakoidal swelling is very slight. Chloroplast fine structure was rarely so well preserved following this fixation regime. Note however, the incomplete preservation of both the plasma membrane and the tonoplast.

X 14,300

Fig. 12 A mesophyll cell of <u>L</u>. <u>minor</u> following fixation with 1.2% KMn04 for 30 min. Most of the chloroplasts are intact and lens-shaped and there is little intrathylakoidal ewelling. Other chloroplasts are irregularlyshaped. The mitochondria appear to be intact but the plasma membrane has not been preserved.

X 7,710



Fig. 13 Part of a mesophyll cell of <u>L</u>. <u>minor</u> following fixation with 1.2% KMn04 for 30 min. The chloroplasts are intact and only slight intrathylakoidal swelling is visible. The mitochondria also appear to be intact but preservation of both the plasma membrane and the tonoplast is incomplete.

X 11,200

Fig. 14 Chloroplasts of <u>L</u>. <u>minor</u> following fixation with 1.2% KMnO4 for 30 min. Note the small breaks in the envelopes of some of the chloroplasts (arrows). Note also the wavy appearance of the thylakoids both in the granal and intergranal regions.

X 19,750



Fig. 15 Part of a cell of <u>L. minor</u> following fixation with 1.2% KMm04 for 45 min. Variable degrees of intrathylakoidal swelling exist within the chloroplasts and the chloroplast envelopes are frequently ruptured. Note also the damage to the limiting membranes of the mitochondria and to the tonoplast

X 10,300

Fig. 16 Part of a mesophyll cell of <u>L. minor</u> following fixation with 1.2% KMn04 for 60 min. Note the extensive breaks in the chloroplast envelopes and the marked intrathylakoidal swelling. Note also the apparently intact but grossly swollen mitochondria.

X 9,200



Fig. 17 Chloroplasts of <u>L. minor</u> following fixation with 1.2% KMn04 for 60 min. Note the incomplete preservation of the chloroplast envelopes and the slight intrathylakoidal swelling.

X 13,050

- Fig. 18 Part of the previous micrograph in greater detail showing the breaks in the chloroplast envelopes and the intrathylakoidal swelling following fixation with 1.2% KMn04 for 60 min.
- Fig. 19 Part of a mesophyll cell of <u>L. gibba</u> following fixation of an entire frond with 2% KMn04 for 60 min. Preservation of the chloroplasts is improved although a slight amount of intrathylakoidal swelling persists and a small break can be seen in the envelope of one of the chloroplasts (arrow). Preservation of both the plasma membrane and the tonoplast is also improved but still incomplete

X 8,500



Fig. 20 A chloroplast of <u>L. minor</u> following glutaraldehyde/ KMn04 fixation. The chloroplast has an irregular shape but appears to remain intact. There is no evidence of intrathylakoidal swelling although slight swellings are evident between the paried membranes of the chloroplast envelope. Note also the incomplete preservation of the limiting membranes of the nearby mitochondria.

X 33,300

Fig. 21 A cell of <u>L. minor</u> following glutaraldehyde/KMN04 fixation. The condition of the chloroplasts is variable. Note also the incomplete preservation of the outer-mitochondrial membranes and the fragmented plasma membrane.

X 6,650



Fig. 22 Part of a mesophyll cell of <u>L</u>. <u>gibba</u> following gluteraldehyde/KMnD4 fixation. This micrograph shows lengths of intact plasma membrane and tonoplast. Damage to the chloroplast is visible in places however.

X 27,040

Fig. 23 A junction between two mesophyll cells of <u>L</u>. <u>gibbs</u> following glutaraldehyde/KMnO4 fixation. Note the preservation of both the plasma membrane and the tonoplast. This micrograph also reveals plasmodesmate linking the cytoplasm of the two cells.

X 58,760



Fig. 24 Part of a mesophyll cell of <u>L</u>. <u>gibba</u> following fixation with glutaraldehyde/ $0_{g}0_{4}$  in sodium cacodylate buffer (PH7.2). Note also the improved preservation of the thylakoid system. However, swelling between the paired membranes of the chloroplast envelopes can be seen in places. The intact portion of the plasma membranes visible in this micrograph is seen to follow en undulating course close to the cell wall and a break in the tonoplast is visible. Note also the low concentration of ribosomes in the cytoplasm.

X 34,810

Fig. 25 Part of a mesophyll cell of <u>L</u>. <u>gibba</u> following fixation with Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> in sodium cacodylate buffer (PH7.2). Note the swelling between the membranes of the chloroplast envelope. Breaks in the outer membrane of the envelope can be observed along the side of the chloroplast closest to the vacuole.

X 31,100



Fig. 26 A chloroplast from a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following fixation with glutaraldehyde/0<sub>8</sub>0<sub>4</sub> in phosphate buffer (pH 6.8). Slight swelling between the paired membranes of the chloroplast envelope are still present but both membranes are intact. The plasma membrane and the tonoplast are largely intact, damage being visible only in small localized areas. Small plastoglobuli of the type seen within this chloroplast are found in most of the chloroplasts fixed in this way.

X 12,650

Fig. 27 Part of mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following fixation with glutaraldehyde/0<sub>8</sub>0<sub>4</sub> in phosphate buffer (pH 6.8). In two parts of the cell, the tonoplast has come away from the cytoplasm and become engulfed into the vacuole. Despite this, the tonoplast appears to be unbroken. X = 10,600



Fig. 28 Part of a cell of <u>S</u>. <u>oligorrhiza</u> following fixation with 3% glutaraldehyde/1% 0<sub>S</sub>0<sub>4</sub> in phosphate buffer (pH 6.8). The contents of the cell are separated by a relatively long distance from the cell wall but the organelles appear to have sustained little damage. Note the damaged plasma membrane and the low concentration of ribosomes in the cytoplasm.

X 19,800

Fig. 29 Part of a cell of <u>S. oligorrhiza</u> following fixation with 3% glutaraldehyde/1% 0<sub>8</sub>0<sub>4</sub> in phosphate buffer (pH 6.8) at 4<sup>0</sup>C. Very few ribosomes remain in the cytoplasm due probably to damage to the plasma membrane and the tonoplast.

X 21,100



Fig. 30 Portions of cells of <u>S. oligorrhiza</u> following fixation with 6% fjlutaraldehyde/1%0<sup>8</sup>0<sub>4</sub> in phosphate buffer (pH 6.8). Damage to the plasma membrane is evident in one of the cells

X 20,600

Fig. 31 Portions of cells of <u>S</u>. <u>oligorrhize</u> following fixation with 6% glutaraldehyde/1%0<sub>S</sub>0<sub>4</sub> in phosphate buffer (pH 6.8) Note the blistering of the tonoplast and the presence of membranous whorls close to the cell wall suggesting damage to the plasma membrane.

X 16,650



Fig. 32 Part of a mesophyll cell of <u>S. oligorrhiza</u> following fixation with 6% glutaraldehyde/1%0<sub>s</sub>0<sub>4</sub> in phosphate buffer (pH 6.8). The tonoplast has been destroyed and the cytoplasmic matrix has dispersed. The plasma membrane has also sustained damage and is separated from the cell wall by a relatively great distance. The cytoplasmic organelles appear little altered in spite of these changes. X 16,100



Fig. 33 A mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following fixation with 3% glutaraldehyde/1%0<sub>S</sub>0<sub>4</sub> in phosphate buffer (ph 6.8) Initial fixation with glutaraldehyde was carried out with relatively large segments of frond tissue. Note the much improved presentation of the plasma membrane and the tonoplast. The tonoplast of this cell is not entirely intact the breaks however being very small (arrows). The cytoplasmic organelles appear to have sustained no fixation damage and a high concentration of ribosomes can also be observed in the cytoplasm.

X 5,900


Fig. 34 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following fixation with 3% glutaraldehyde/1%0<sub>S</sub>0<sub>4</sub> in phosphate buffer (pH 6.8). This micrograph illustrates localized damage to plasma membrane

X 23.100

Fig. 35 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following fixation with 3% glutaraldehyde/1%  $0_80_4$  in phosphate buffer (pH 6.8). Note the slight blistering of both the plasma membrane and the tonoplast.

X 22,350



Fig. 36 Part of a mesophyll cell of <u>5</u>. <u>oligorrhiza</u> following fixation with 3% glutaraldehyde/1%0<sub>8</sub>0<sub>4</sub> in phosphate buffer (pH 6.8). The chloroplast envelope shows none of the types of damage caused by earlier fixation methods.

X 19.200

Fig. 37 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following fixation with 3% glutaraldehyde/1%0 $_{8}0_{4}$  in phosphate buffer (pH 6.8) The mitochondria reveal no signs of fixation damage.

X 38,550



Fig. 38 The nucleus of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following fixation with 3% glutaraldehyde/1%0 $_{804}$  in phosphate buffer (pH 6.8).

X 27,400

Fig. 39 Part of a mesophyll cell from a control frond of <u>S. oligorrhiza</u>. Chloroplasts are Lens-shaped and contain starch grains and small numbers of plastoglobuli within the stroma. The electron densities of the chloroplast stroma and the mitochondrial matrix are similar. Ribosomes are abundant in the cytoplasm Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 21,300



Fig. 40 Parts of mesophyll cells from a control frond of <u>S</u>. <u>cligorrhiza</u>. This micrograph illustrates the small localized areas of fixation damage often sustained by both the plasma membrane (lower cell) and the tonoplast (Upper cell).

Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 24,500

Fig. 41 Part of a chloroplast from the mesophyll of a control frond of <u>S. oligorrhiza</u> Glutaraldshyde/0<sub>8</sub>0<sub>4</sub> fixation

X 45,000



Fig. 42 Parts of mesophyll cells of <u>S</u>. <u>oligorrhize</u> following treatment in the light with 10 ppm diquat (cation) for 4 hours. No ultrastructural changes are visible at this stage.

Glutaraldehyde/ $0_{s}0_{4}$  fixation.

X 18,150

Fig. 43 Part of a chloroplast from the mesophyll of a frond of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 4 hour. No ultrastructural damage is visible although starch grains within the chloroplasts are few and very small. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 37,500



Fig. 44 Chloroplasts from the mesophyll of a frond of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 8 h. Although still intact, they have assumed a roughly circular shape and the thylakoids are displaced towards the periphery. Note the slight increase in the number of plastoglobuli within the chloroplasts. Note also the small electron dense areas occurring at intervals along the length of the chloroplast envelopes. A reduction in the electron density of the mitochondrial matrix is also visible at this time.

Glutaraldehyde/ $0_{s}0_{4}$  fixation

X 15,950

Fig. 45 This micrograph shows, in greater detail, the occurrence of electron dense deposits along the length of the chloroplast envelopes. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 28,250

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Fig. 46 Part of a mesophyll cell from a frond of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm. diquat (cation) for 8 hour. All mitochondria show reduced electron density of the matrix. Note how the irregularlyshaped mitochondria dove-tail with each other. Glutaraldehyde/0<sub>g</sub>0<sub>4</sub> fixation

X 41,550



Fig. 47 Part of a mesophyll cell from a frond of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 8 hours. Note the reduced electron density of the matrix of all mitochondria. The surrounding cytoplasm has a high concentration of ribosomes and adjacent portions of the plasma membrane and the tonoplast appear intact and undamaged. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

x 40,000

Fig. 48 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquqt (cation) for 8 hours. Note the many irregularly-shaped microbodies fitting closely together Glutareldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 30,850



Fig. 49 A chloroplast from a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 12 hours. The chloroplast is intact but the thylakoids are arched to one side. Slight intrathylakoidal swelling is also visible, particularly in the inter-granal region. Note also the build up of electron-dense material both within the chloroplast and at intervals along the length of the chloroplast envelope. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 25,200

Fig. 50 Part of a mesophyll cell of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 12 hours. The ultrastructural damage illustrated is more characteristic of that observed after only 8 hours. All membranes of the cellular organelles appear to be intact. Note however, the reduced electron density of the mitochondrial matrix and the irregularlyshaped and tightly-packed microbodies. Glutareldehyde/0\_0\_4 fixation

S 35,850



Fig. 51 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 16 hours. The arrangement of the thylakoids within the chloroplasts is disorganized and intrathylakoidal swelling is more marked. The chloroplasts appear to be intact although the paired membranes of the envelopes can seldom be resolved. Electron dense deposits are plentiful both within the chloroplasts and along the envelopes. This micrograph also illustrates the typical appearance of the mitochondria at this stage. Electron-dense deposits can be observed at intervals along the ill-defined outer membrane.

Glutaraldehyde/ $0_0_A$  fixation

## X 25,300

Fig. 52 Part of a mesophyll cell of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 16 hours. This illustrates the more advanced disruption of fine structure occasionally observed at this stage. Note the marked intrathylakoidal swelling and the separation of part of one of the chloroplast envelopes from the rest of the chloroplasts. Note also the patchy reduction in the electron density of the microbodies. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 34,050



Fig. 53 A mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 20 hours. The chloroplasts are devoid of starch grains. In most of the chloroplasts the thylakoid system is arched away from the cell wall. Some of the chloroplasts remain intact while others have sustained breaks in their envelopes. Plastoglobuli are abundant within most chloroplasts. Both the plasma membrane and the tonoplast are extensively damaged.

Glutaraldehyde/0 0, Fixation

X 4,230

Fig. 54 A chloroplast from the mesophyll of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 20 hours. This chloroplast is representative of those which had sustained the least amount of ultrastructural damage at this time. The arrangement of the thylakoids is disorganized but the chloroplast is still intact. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 21,200



Fig. 55 Chloroplasts from the mesophyll of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 20 hours. These are representative of those chloroplasts which had sustained the greatest amount of ultrastructural damage at this time. Note the swollen, disorganized remains of the thylakoids the greatly reduced electron density of the stroma and the breaks in the chloroplast envelopes.

X 11,300

Fig. 56 A microbody and the remains of a mitochondrion from a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> treated in the light with 10 ppm diquat (cation) for 20 hours. The mitochon-drion is grossly swollen and contains little matrix material. In contrast, the adjacent microbody is less severely damaged. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 35,600



Fig. 57 Part of a mesophyll cell from a control frond of <u>S. oligorrhiza</u>. Glutaraldehyde/0 0 fixation

X 30,000

Fig. 58 Part of the cytoplasm of a mesophyll cell from a control frond of <u>S</u>. <u>oligorrhiza</u>. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 27,850



Fig. 59 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> which has sustained no ultrastructural damage following treatment in the light with 10 ppm diquat (cation) for 6 hours. Glutaraldehyde/0<sub>s</sub>0<sub>4</sub> fixation

X 43,300

Fig. 60 A chloroplast from the mesophyll of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 6 hours. The thylakoid system is arched towards one side of the chloroplast. No intrathylakoidal swelling is evident although there is a slight reduction in the electron density of the loculi of the thylakoids. The chloroplast is intact although a break can be seen in the outer membrane of the envelope (arrow). At this time the plasme membrane and the tonoplast show no signs of damage. Glutaraldehyde/0\_0\_4 fixation



Fig. 61 A chloroplast from the mesophyll of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 6 hours. Note the irregular arrangement of the thylakoids and the reduction in electron density of the loculi. The plasma membrane and the tonoplast remain intact.

 $Glutaraldehyde/0_0_A$  fixation

X 20.850

Fig. 62 The appearance of some mitochondria and microbodies from the mesophyll of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 6 hours. The mitochondria are generally intact but appear to have lost the bulk of their matrix substance. Microbodies have retained much of their internal substance but have sustained considerable damage to their limiting membranes. Both the plasma membrane and the tonoplast are unbroken although blistering of the tonoplast is visible. Gluteraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 40,800



Fig. 63 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 6 hours. No ultrastructural damage of any kind is visible at this time.

Glutaraldehyde/0 0 fixation

X 43,300

Fig. 64 Part of a mesophyll cell of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 12 hours. A slight degree of intrathylakoidal swelling can be seen. Note that parts of the envelopes of some of the chloroplasts can no longer be resolved. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 24,500



Fig. 65 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment with 10 ppm diquat (cation) for 12 h. This micrograph illustrates the appearance of the most badly damaged chloroplasts at this time. Glutaraldehyde/0<sub>S</sub>0<sub>4</sub> fixation.

X 23.350

Fig. 66 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 12 h. Note the great reduction in the electron density of the mitochondrial matrix. Areas are visible where the outer mitochondrial membrane cannot be resolved (arrow). A cluster of microbodies can be seen showing no sign of the loss of internal substance. Lack of resolution of parts of their limiting membranes are nonetheless evident (arrow). Note also the incomplete chloroplast envelope and the slight intrathylakoidal ewelling.

Glutaraldehyde/ $0_80_4$  fixation

X 64,300



Fig. 67 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 12 hours. The mitochondria appear to be intact but have lost much of their matrix material. Flutaraldehyde/0<sub>S</sub>0<sub>4</sub> fixation

X 30,000

Fig. 68 Part of a mesophyll cell of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 12 hours. The microbodies reveal characteristically less damage than do the mitochondria. Note also the damage sustained by the plasma membrane and the tohoplast. Despite damage to these membranes there is little dispersal of the cytoplasm. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 30,000


Fig. 69 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 12 hours. The chloroplast is intact but has a rounded appearance with the thylakoids arched away from the cell wall. No intrathylakoidal swelling is visible although there is a slight reduction in the electron density of the thylakoid loculi. The mitochondria are intact but appear to have lost most of their matrix substance. The microbody appears to have sustained no such loss of internal substance. Note the blistering of the tonoplast. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 20,560

Fig. 70 A chloroplast from a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 12 hours. Note its somewhat rounded shape and the undulating arrangement of the thylakoids within the stroma. Note also the many breaks in the tonopl<sub>a</sub>st Glutaraldehyde/0<sub>e</sub>0<sub>4</sub> fixation

X 17,700



Fig. 71 A chloroplast from the mesophyll of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 12 hours. Note once again the alterations to both the shape of the chloroplast and the arrangement of the thylakoids. Both the plasma membrane and the tonoplast are extensively damaged and breaks can be seen in the outer membrane of the chloroplast envelope. Glutaraldehyde/0<sub>s</sub>0<sub>4</sub> fixation

X 18,650

Fig. 72 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 12 hours. Note the apparent loss of substance from the matrix of the mitochondria and the loss of resolution of parts of the outer mitochondrial membranes. Note also the apparent destruction of the part of the tonoplast adjacent to the mitochondria. Glutaraldehyde/0<sub>g</sub>0<sub>d</sub> fixation

X 56,250



Fig. 73 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 18 hours. Note the advanced degenerative changes in the chloroplasts. Intrathylakoidal swelling is more marked and there is a reduction in the electron density of the stroma. This micrograph also shows extensive destruction of the plasma membrane and the tonoplast. Glutaraldehyde/0<sub>S</sub>0<sub>4</sub> fixation

X 18,750

Fig. 74 Part of a mesophyll cell of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 18 hours. The chloroplasts are rounded and show considerable disorganization of the thylakoid system. Starch grains are present within the chloroplasts but much of the stroma material appears to have been lost. Most of the matrix material also appears to have been lost from the mitochondria but little substance appears to have been lost from within the microbodies Glutareldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 14,060



Fig. 75 The remains of a chloroplast from the mesophyll of <u>5. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 18 hours. Note the marked intrathylakoidal swelling, the disorganized arrangement of the thylakoids, the destruction of the envelope and the reduced electron density of the stroma. Damage to the plasma membrane and the tonoplast is also severe. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 21,250

Fig. 76 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 18 hours. The chloroplasts are grossly swollen and contain the remains of the thylakoid system and large, apparently empty spaces. Starch grains can also be seen within the chloroplasts. Isolated pieces of the thylakoid system indicate that the disintegration of some of the chloroplasts has already taken place.

Glutaraldehyde/0\_0\_ fixation

X 10,560



Fig. 77 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> which has sustained comparatively little ultrastructural damage following treatment in the light with 10 ppm paraquat (Cation) for 18 hours. The chloroplasts illustrated have a similar appearance to those exposed to paraquat for only 12 hours. They are intact but have a rounded shape. No intrathylakoidal swelling is visible although the arrangement of the internal membrane system is abnormal, the thylakoids following a somewhat undulating course throughout the stroma. Note also the density of the matrix of the mitochondria. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 13,450



Fig. 78 Part of a mesophyll cell of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 18 hours. Note the irregularities in both the shape of the chloroplast and the arrangement of the thylakoids. Very slight intrathylakoidal swelling is also visible. A cluster of microbodies is present between the chloroplasts. Unlike the nearly mitochondria, these organelles do not appear to have lost any of their internal substance. Cytoplasmic ribosomes are plentiful despite the destruction of much of the tonoplast.

Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 20,400



Fig. 79 Part of a seriously damaged mesophyll cell of <u>5</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 18 hours. Intrathylakoidal swelling is advanced and the stroma has a reduced electron density. Adjacent parts of the plasma membrane and tonoplast are still intact and cytoplasmic ribosomes are still plentiful.

Glutaraldehyde/ $0_{s}0_{4}$  fixation

X 13,900

Fig. 80 A chloroplast from a more seriously damaged mesophyll of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 18 hours. Intrathylakoidal swelling is less marked than in the previous micrograph but breaks can be seen in the chloroolast envelope. Note ribosomes are plentiful in the cytoplasm despite damage to both the plasma membrane and the tonoplast. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 19,750



Fig. 81 A chloroplast from a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 24 hours. The chloroplast appears to be intact but the paired membranes of the envelope cannot be properly resolved. Within the chloroplast the thylakoid system is totally disorganized and little of the stroma material remains. The plasma membrane and the tonoplast are totally disrupted and the distribution of the cytoplasmic ribosomes has become patchy. Glutaraldehyde/0<sub>e</sub>0<sub>4</sub> fixation

X 19,400

Fig. 82 Part of a mesophyll cell of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 24 hOurs. The chloroplast has ruptured leaving behind the disorganized swollen thylakoids and the loosely scattered remains of the stroma. Note the remains of the plasma membrane and the dispersed cytoplasmic ribosomes. Also visible between the two chloroplasts are structures which are possibly the remains of mitochondria or microbodies. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 9,500



Fig. 83 The remains of a chloroplast from a mesophyll cell of <u>S. oligorrhiza</u> following treatment in the light with 10 ppm diquat (cation) for 24 hours. The chloroplast envelope is almost totally destroyed leaving behind small amounts of stroma material and the thylakoids many of which are grossly swollen. Glutaraldehyde/0<sub>0</sub>0<sub>4</sub> fixation

X 15,000

Fig. 84 A chloroplast from a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 24 hours. The chloroplast appears to be intact but is swollen and grossly irregular in shape. Within the chloroplast some stroma material remains together with the abnormally arranged thylakoids which exhibit only slight intrathylakoidal swelling. Very little of either the plasma membrane or the tonoplast remains intact.

Glutaraldehyde/0\_0\_ fixation

X 14,500



Fig. 85 Part of a mesophyll cell of S. oligorrhiza following treatment in the light with 10 ppm paraquat (cation) for 24 hours. Some of the chloroplasts have ruptured allowing fragments of the thylakoid system to escape. Glutaraldehyde/0<sub>5</sub>0<sub>4</sub> fixation

X 21,100

Fig. 86 A chloroplast from a mesophyll cell of <u>5</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 24 hours. The chloroplast envelope is ruptured. Within the remains of the envelope are the twisted and swollen thylakoids and loosely dispersed stroma material.

Glutaraldehyde/0\_04 fixation

X 16,300



Fig. 87 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> following treatment in the light with 10 ppm paraquat (cation) for 24 hours. The chloroplasts have lost considerable portions of their envelopes. The thylakoids are irregularly arranged and variable degrees of intrathylakoidal swelling are visible. Note also the cluster of small, badly damaged organelles. These are probably the remains of mitochondria and microbodies. Glutaraldehyde/ $0_{\rm s}0_A$  fixation

X 14,815



Fig. 88 Part of a mesophyll cell from a control frond of <u>S. oligorrhiza</u> after exposure to complete darkness for 140 hours. No starch grains are present but note the accumulation of plastoglobuli within the stroma. Note also the relatively long membrane which almost bisects the mitochondrion. Such membranes were not normally observed within the mitochondria of lightgrown fronds.

Glutaraldehyde/ $0_80_4$  fixation

X 27,800

Fig. 89 Plastoglobuli in a chloroplast of a mesophyll cell from a control frond of <u>S</u>. <u>oligorrhiza</u> after exposure to complete darkness for 140 hours. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 94,800



Fig. 88 Part of a mesophyll cell from a control frond of

<u>S. oligorrhiza</u> after exposure to complete darkness for 140 hours. No starch grains are present but note the accumulation of plastoglobuli within the stroma. Note also the relatively long membrane which almost bisects the mitochondrion. Such membranes were not normally observed within the mitochondria of lightgrown fronds.

Glutaraldehyde/ $0_{s}0_{4}$  fixation

X 27,800

Fig. 89 Plastoglobuli in a chloroplast of a mesophyll cell from a control frond of <u>S. oligorrhiza</u> after exposure to complete darkness for 140 hours. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 94,800



Fig. 90 Part of a mesophyll cell from a control frond of <u>S</u>.

oligorrhiza after exposure to complete darkness for 140 hours. Note the membranous structure in the centre of the mitochondrion

Glutaraldehyde/ $0_{s}0_{4}$  fixation

X 70,200



Fig. 91 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> after treatment with 10 ppm diquat (cation) in complete darkness for 140 hours. Plastoglobuli have accumulated in the chloroplast stroma but there is little evidence of any ultrastructural damage. The plasma membrane has become widely separated from the cell wall but this was also observed in some control cells.

Glutaraldehyde/ $0_{g}0_{4}$  fixation

X 22,000

Fig. 92 A chloroplast in a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> after treatment with 10 ppm diquat (cation) in complete darkness for 140 hours. The chloroplast envelops cannot be resolved in some areas and the tonoplast has become separated from the cytoplasm in the region of the chloroplast.

Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 18,150



Fig. 93 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> after treatment with 10 ppm diquat (cation) in complete darkness for 140 hours. The chloroplast has become rounded in appearance and its thylakoids have been displaced towards the vacuole. Note also the reduction in the electron density of the stroma, the disappearance of the tonoplast and the unusually dense nature of the cytoplasm. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 24,600

Fig. 94 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> after treatment with 10 ppm diquat (cation) in complete darkness for 140 hours. Note the clusters of microbodies and the greatly reduced electron density of the mitochondria. Note also the damaged plasma membrane and the dense appearance of the cytoplasm.

Glutaraldehyde/0,0, fixation

X 23,600



Fig. 95 A chloroplast in a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> after treatment with 10 ppm paraquat (cation) in complete darkness for 140 hours. There appears to be some damage to the outer membrane of the chloroplast envelope (arrows). Glutaraldehyde/0<sub>S</sub>0<sub>4</sub> fixation

X 27,200

Fig. 96 Parts of cells in the mesophyll of <u>S</u>. <u>oligorrhiza</u> after treatment with 10 ppm paraquat (cation) in complete darkness for 140 hours. The chloroplasts have irregular shapes and their envelopes have sustained variable degrees of damage. Note also the invagination of the tonoplast into the vacuole in one of the cells. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 18,150



Fig. 97 Part of a mesophyll cell of <u>S. oligorrhiza</u> after treatment with 10 ppm paraquat (cation) in complete darkness for 140 hours. The chloroplast is swollen and its stroma has a greatly reduced electron density. There is a slight amount of intrathylakoidal swelling and swelling is also visible between the paired membranes of the chloroplast envelopes.

Glutaraldehyde/ $0_{s}0_{4}$  fixation

## X 21,300

Fig. 98 Part of a mesophyll cell of <u>S</u>. <u>oligorrhiza</u> after treatment with 10 ppm paraquat (cation) in complete darkness for 140 hours. Note the irregular shape of the mitochondrion and the low electron density of its matrix. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 54,800


Fig. 99 An apparently undamaged chloroplast in frond tissue exposed to 500 ppm diquat (salt) for 1 hour. in the light. The plasma membrane and the tonoplast also appear to be undamaged.

Glutaraldshyde/ $0_{s}^{0}_{4}$  fixation

X 27,600

Fig. 100 A chloroplast in frond tissue exposed to 500 ppm diquat (salt) for 1 hour in the light. Note the slight intrathylakoidal swelling and the changes in the stroma between the thylakoids. The plasma membrane and the tonoplast appear to have remained undamaged. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 23,950



Fig. 101 A chloroplast in frond tissue exposed to 500 ppm diquat (salt) for 1 hour in the light. Intrathylakoidal swelling is more advanced but the chloroplast, the plasma membrane and the tonoplast appear to remain intact. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 23,100

Fig. 102 A chloroplast in frond tissue exposed to 500 ppm. diquat (salt) for 1 hour in the light. The internal structure of the chloroplast is completely disorganized and portions nearby of the tonoplast have disintegrated. Gluteraldehyde/ $0_80_4$  fixation

X 27,950



Fig. 103 Part of a cell from a frond of <u>S</u>. <u>oligorrhiza</u> after exposure to 500 ppm diquat (salt) for 1 hour in the light. Note the reduced electron density of the matrix of the mitochondrion Gluteraldehyde/0<sub>s</sub>0<sub>4</sub> fixation

X 46,900

Fig. 104 Microbodies in frond tissue after exposure to 500 ppm diquat (salt) for 1 hour in the light. The microbodies appear to have lost little of their internal substance although parts of their limiting membranes seem to be absent.

Gluteraldehyde/ $0_80_4$  fixation

X 46,750



Fig. 105 Part of a mesophyll cell from a frond of <u>S. oligorrhiza</u> exposed to 500 ppm diquat (salt) for 1 hour. in the light. The mitochondria and microbodies do not appear to have sustained injury despite damage to the nearby chloroplasts. Gluteraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 35,750

Fig. 106 Part of a mesophyll cell from a frond of <u>S</u>. <u>oligorrhiza</u> exposed to 500 ppm. diquat (salt) for 1 hour in the light. The mitochondria show little sign of injury despite damage to nearby chloroplasts. Gluteraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 42,950



Fig. 107 The absence of ultrastructural damage in mesophyll cells after the exposure of fronds to 500 ppm diquat (salt) for 1 hour in the dark. Gluteraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

X 13,650



Fig. 108 Part of a mesophyll cell from a frond after exposure to 500 ppm. diquat (salt) for 1 hour in the dark. There are no signs of ultrastructural damage. Gluteraldehyde/0<sub>s</sub>0<sub>4</sub> fixation

X 21,750

Fig. 109 The nucleus of a mesophyll cell from a frond after exposure to 500 ppm diquat (salt) for 1 hour in the dark. Gluteraldehyde/0 0 fixation

X 23,100



Figs. 110 - 114 illustrate the ultrastructural damage sustained by fronds of <u>S</u>. <u>oligorrhiza</u> following exposure to 500 PPM diquat (salt) for 15 hours in the light. All gluteraldehyde/0<sub>s</sub>0<sub>4</sub> fixation. Fig. 110 Note the complete destruction of the plasma membrane and the tonoplast.

X 5,670

Fig. 111 Note the reduction in the electron density of the thylakoids and in increased electron density of the stroma

X 30,750



Fig. 112 Note that the chloroplast appears to remain intact despite having undergone drastic ultrastructural change.

X 18,750

Fig. 113 A chloroplast which has lost the greater part of its envelope. Note also the patchy distribution of densely staining cytoplasm.

X 22,000



Fig. 114 This micrograph reveals empty-looking structures which probably represent the remains of mitochondria and microbodies.

X 38,750



FIGS. 115 - These micrographs illustrate the range of ultra-119 structural changes which had occurred in fronds of <u>S. oligorrhiza</u> after exposure to 500 ppm. diquat (salt) for 15 h. in the dark.

All glutaraldehyde/0\_0, fixation

Fig. 115 The shape of many of the chloroplasts and the arrangement of their thylakoids have become irregular. Gaps are frequently observed between the tonoplast and the cytoplasm and rounded membranous structures between the cytoplasm and the cell wall are suggestive of extensive damage to the plasma membrane. Note also the reduced electron density of the matrix of the mitochondria.

X 8,750



Fig. 116 The thylakoids have become arched to one side of the chloroplast but there is no evidence of intrathylakoidal swelling. One of the mitochondria has become irregularly shaped and all show considerable reduction in the electron density of the matrix. There appears to have been no loss of material from within the microbody but its shape has become slightly altered. Note also the dense appearance of the cytoplasm.

X 26,300

Fig. 117 Note the irregular shape of the chloroplast and the presence of intrathylakoidal swelling. Note also the loss of material from within the mitochondrion and the apparent loss of the tonoplast.

X 19,750



Fig. 118 Note especially the variety of shapes assumed by the mitochondria and also their reduced electron density. Note also the clumping together of the irregularly shaped microbodies.

X 18,650

Fig. 119 A mitochondrion showing only a slight loss of electron density in the matrix. The spherical membranous inclusion within the organelle is similar to structures often observed within the mitochondria of control fronds at this time.

X 58,270



Figs. 120 - Parts of <u>S</u>. <u>oligorrhiza</u> frond mesophyll cells following 123 incubation in a medium containing DAB without hydrogen peroxide.

All glutaraldehyde/ $0_{s}0_{4}$  fixation

Fig. 120 The electron-opaque reaction product is localized within the microbodies.

X 35,500

Fig. 121 Another microbody which is packed with the darklystaining reaction product.

X 35,500



Fig. 122 Deposition of electron-opaque material is seen in the cell wall. However, there is no appreciable deposition in the chloroplast or the cytoplasm.

X 21,100

Fig. 123 The electron-opaque reaction product is visible in the cell wall in the region of the middle lamella, but not in the cytoplasm, the chloroplasts or the mitochondrion.

X 37,500



Fig. 124 Part of a <u>S</u>. <u>oligorrhiza</u> frond mesophyll cell following incubation in a medium containing DAB and hydrogen peroxide as substrate. Very dense deposits of the reaction product are seen without the microbody and the cell wall. Electron-opaque deposits are also seen scattered throughout the cytoplasm but not within the chloroplasts.

Gluteraldehyde/0\_0, fixation

X 30,000

Fig. 125 Part of a <u>S</u>. <u>oligorrhiza</u> frond mesophyll cell following incubation in a medium containing DAB and hydrogen peroxide as substrate. There is no appreciable deposition of reaction product within the chloroplasts but a considerable build-up has occurred within the microbody. The microbody appears to have ruptured which may account for the electron-opaque deposits present in the surrounding cytoplasm. Glutaraldehyde/ $O_aO_a$  fixation

X 53,330



Fig. 126 Part of a mesophyll cell of an ageing, pale green frond of S. oligorrhiza. The chloroplast appears to be intact although there may be some damage to the envelope. Within the chloroplast an accumulation of starch and plastoglobuli has begun. Note also the ruptured tonoplast and the low concentration of ribosomes in the cytoplasm. Glutaraldehyde/0<sub>8</sub>0<sub>4</sub> fixation

## X 22,100

Fig. 127 Part of a mesophyll cell of an ageing, pale green frond of <u>S. oligorrhiza</u>. Note the large starch grains which fill a considerable part of the chloroplast. Small plastoglobuli are also present in the strome but the chloroplast appears to remain intact. Note also the scarcity of cytoplasmic ribosomes and the twisted remains of the tonoplast. Glutaraldehyde/O<sub>p</sub>O<sub>A</sub> fixation

X 15,200

Fig. 128 A chloroplast in an ageing, yellow frond of <u>S</u>. oligorrhiza. The envelope has been lost and the starch grains and plastoglobuli have increased in size. Note the slight intrathylakoidal swelling. Glutaraldehyde/0<sub>s</sub>0<sub>4</sub> fixation

X 18,750

Fig. 129 Part of a mesophyll cell of an ageing, yellow frond of <u>S</u>. <u>oligorrhiza</u>. The chloroplast has become almost completely filled with starch grains and plastoglobuli and now has an irregular shape. Note the absence of considerable portions of the chloroplast envelope.

Glutaraldehyde/0,04 fixation

X 16,170



Figs. 130-132 The ultrastructure of cells of fully senescent, white fronds of <u>S. oligorrhiza</u>. All glutaraldehyde/0<sub>S</sub>0<sub>4</sub> fixation

Fig. 130 This micrograph illustrates the empty appearance of cells viewed at this stage. X 7,300

Fig. 131 Membranous fragments which were often found lying close to the cell wall in cells at this stage.

X 59,500

Fig. 132 This micrograph illustrates the type of large oval granule which was viewed in a relatively small number of cells after the complete description of the chloroplasts.

X 47,500



## ABBRE VIATIONS FOR FIGS. 133-210

A	=	akinete
Cg.	=	cyanophycin granule
ε	=	envelope
н	=	heterocyst
Lg.	=	lipid granule
N	=	nuclear region
РЬ	=	Polyhed <b>ral</b> body
Pg	*	polyglucoside granule
Pm	=	plasma membrane
рр	=	polyphosphate granule
S	-	sheath
Vc	2	vegetative cell
W	-	cell wall
Fig. 133 A low power light micrograph showing filaments of <u>A. cylindrica</u> grown under continuous illumination in nitrogen-free culture solution. Note the regular occurrence of heterocysts along the lengths of the filaments. Developing akinetes can be observed adjacent to a number of the heterocysts.

X 490

Fig. 134 A medium power light micrograph showing filaments of <u>A. cylindrica</u> grown under continuous illumination in nitrogen-free culture solution. Note the occurrence of large granules within the akinetes. Granules are also common within the vegetative cells but are seldom visible within the heterocysts.

X 970



Fig. 135 The normal appearance of young light-grown vegetative cells of A. cylindrica from nitrogen-free medium after fixation with 2% aqueous KMn04. The plasma membrane and the thylakoids are easily visible, the latter occupying a peripheral position within the cells. The irregularlyshaped areas of low electron density in the central region of the cells represent nuclear material. Small polyglucoside granules are present in large numbers around the thylakoids and, in much smaller numbers, throughout the rest of the cytoplasm. Lipid granules are less numerous and occur close to the periphery of the cells. Polyhedral bodies are seen especially in the central region of the cells while, occupying a more peripheral position, cyanophycin granules occur as round or oval electrontransparent structures. No ribosomes are visible in the cytoplasm following fixation with KMn04.

X 30,600



Fig. 136 The appearance of a vegetative cell of <u>A</u>. <u>cylindrica</u> after fixation with 1% O<sub>S</sub>O<sub>4</sub>. The cytoplasm appears densely granular while the plasma membrane and the thylakoids can only be discerned with difficulty. X 30,000

Fig. 137 The appearance of a vegetative cell of <u>A</u>. <u>cylindrica</u> after double fixation with 3% glutaraldehyde and 1%  $0_{s}0_{4}$ . Note the sheath microfibrils external to the cell wall. Little detail of the membranous components of the cell can be discerned.

X 37,000



Fig. 138 The peripheral region of part of a vegetative cell of <u>A</u>. cylindrica. The four layered nature of the cell wall (LI - LIV) is evident. Notice the crinkly appearance of the plasma membrane. At two points the plasma membrane appears to be continuous with the thylakoids. KMn04 fixation

X 87,100

Fig. 139 The peripheral region of part of a vegetative cell of <u>A</u>. <u>cylindrica</u> after fixation with  $1\% 0_80_4$ .

X 107,100

Fig. 140 A vegetative cell of <u>A</u>. <u>cylindrica</u> after double fixation with 3% glutaraldehyde and  $1\% \circ_{s} \circ_{4}$ . The plasma membrane and the thylakoids are difficult to discern. In addition to the ribosomes and polyglucoside granules, an assortment of larger granules are present.

X 19,950



Fig. 141 A heterocyst of <u>A</u>. <u>cylindrica</u>. Note the thick envelope external to the heterocyst cell wall. This envelope comprises three layers; an outer fibrous layer (F), a middle homogeneous layer (H) and an innermost laminated layer (L). Note also the reticulate arrangement of the thylakoids. the cuclear regions are less conspicuous than in vegetative cells and there is an absence of large granular inclusions in the cytoplasm. However, numerous polyglucoside granules are present indicating that this is a relatively young heterocyst. KMn04 fixation

X 39,500



Fig. 142 A senescent heterocyst of <u>A</u>. cylindrica. This heterocyst has been sectioned in a plane passing through the pore channel (P). Note that in this region the thick envelope of the heterocyst is absent. KMn04 fixation

X 22,960

Fig. 143 A heterocyst of <u>A</u>. <u>cylindrica</u> in which the thylakoids are concentrated towards the polar regions where they assume a honeycomb-like configuration. Note that in this heterocyst polyglucoside granules are scarce. KMn04 fixation

X 15,860



Fig. 144 An akinete and vegetative cell from a colony of <u>A. cylindrice</u>. The akinete is considerably larger than the vegetative cells and is completely surrounded by a thick protective envelope. The arrangement of the thylakoids within the akinete is similar to that found in actively-growing vegetative cells. Note the accumulation of cyanophycin granules. Polyhedral bodies are also numerous. KMn04 fixation

X 15,200

Fig. 145 A transverse section through an akinete of <u>A</u>. <u>cylindrica</u> Note the thick envelope and the prominent cyanophycin granules. KMn04 fixation

X 22,200



Fig. 146 A germinating akinete from an ageing colony of <u>A. cylindrica</u>. The cell has started to divide. Note the slight intrathylakoidal swelling. Polyglucoside granules are plentiful but little cyanophycin remains. KMn04 fixation

X 29,000



Fig. 147 A control vegetative cell of <u>A</u>. <u>cylindrica</u> at zero time. Notice the characteristic crinkled appearance of the plasma membrane and the close opposition of the paired thylakoid membranes. Polyglucoside granules are numerous and the only polyhedral body visible is distinct in outline. Notice also the clear distinction between the nuclear regions and the surrounding cytoplasmic matrix. KMnD4 fixation

X 40,650



Fig. 148 The typical appearance of vegetative cells of <u>A</u>. <u>cylindrica</u> after treatment in the light with 10 ppm diquat (salt) for 60 min. No ultrastructural changes are visible at this stage. KMn04 fixation

X 32,550



Fig. 149 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 80 min. No ultrastructural damage is visible at this stage but there is a noticeable reduction in the number of polyglucoside granules in the cytoplasm. KMn04 fixation

X 36,300

Fig. 150 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 100 min. Notice the almost complete absence of polyglucoside granules in the cytoplasm. However there is no evidence of ultrastructural damage to any part of the cell. KMn04 fixation

X 28,100



Fig. 151 Vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 120 min. The plasma membrane appears to be intact and the membranes of the thylakoids remain close together. The cytoplasm is less granular than in control cells but large numbers of ribsomes remain.

OsO4 fixation

X 41,850



Fig. 152 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 120 min. Many ultrastructural changes are visible at this time. The plasma membrane has lost its characteristic crinkled appearance and has become ruptured in places (arrows). In addition the nuclear areas are no longer visible, the cytoplasmic matrix having a more homogeneous electron density. Notice also that the polyhedral bodies are less distinct in outline at this time. This micrograph also reveals the presence of two unusual whorls of membranes close to the periphery of the cell.

KMn04 fixation

## X 38,700

Fig. 153 This micrograph shows part of the previous cell in greater detail. Note how the plasma membrane no longer has a crinkled appearance. KMn04 fixation

X 130,050



Fig. 154 A dividing cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 150 min. This micrograph illustrates a more extensive breakdown of the plasma membrane. An unusual membranous inclusion is seen close to the region of invagination on one side of the cell. Note also the homogeneous nature of the cytoplasmic matrix and the reduced sharpness in the outline of the polyhedral bodies. Despite these changes, the paired membranes of the thylakoids remain closely opposed. KMn04 fixation

X 32,200

Fig. 155 This micrograph shows part of the previous cell in greater detail. Note the areas in which part of the plasma membrane has been destroyed. Close to one such area an inclusion comprising whorled membranes is present. KMn04 fixation

X 55,500



Fig. 156 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 150 min. Note the complete destruction of the plasma membrane. The remnants of this membrane can be seen close to the periphery of the cell. Whorls of membranes are also visible in the peripheral regions of this cell. KMn04 fixation

X 35,550

Fig. 157 This micrograph illustrates part of the previous cell in greater detail. Close to the periphery of the cell tiny fragments of the disrupted plasma membrane can be seen. At this stage the cell wall and the thylakoids are still intact. KMn04 fixation

X 90,000



Fig. 158 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 150 min. Note the destruction of the plasma membrane and the separation of the cytoplasm from the cell wall. OsO4 fixation

X 43,650

Fig. 159 This micrograph shows the peripheral region of part of the previous cell in greater detail. Note the fragmented remains of the plasma membrane. Os04 fixation

X 90,000



Fig. 160 A vegetable cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 180 min. Deterioration of fine structure is further increased. Although still intact, the paired membranes of the thylakoids have started to swell apart. KMn04 fixation

X41.100

Fig. 161 Vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 300 min. All of the cells exhibit intrathylakoidal swelling although the thylakoids remain intact. The cells also show a considerable reduction in the electron density of the cytoplasm indicating a loss of much of the cytoplasmic matrix. KMn04 fixation

X 16,020



Fig. 162 Vegetative cells of <u>A. cylindrica</u> treated in the light with 10 ppm diquat (salt) for 300 min. Considerable intrathylakoidal swelling is visible although there is little evidence of fragmentation of the thylakoids. Damage to the cell walls is also evident at this time and almost all of the cytoplasmic matrix has been lost. KMn04 fixation

## X 33,500


Fig. 163 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 160 min. The plasma membrane is totally disrupted. Note also how the thylakoids have begun to fragment. Only slight interthylakoidal swelling is evident at this stage. KMn04 fixation

X 43,200

Fig. 164 A dividing vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 180 mith. Many of the small fragments of thylakoids have swollen forming rounded vesicles. KMn04 fixation

X 33,000



Fig. 165 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 200 min. The normal arrangement of the thylakoids has been completely lost, the thylakoid membranes being reduced to a mass of tiny, rounded vesicles. Note also the small breaks in the cell wall.

KMn04 fixation

X 49,350



Fig. 166 A dividing vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for **300** min. The cell wall has been extensively damaged. The cytoplasmic matrix has been lost leaving the swollen and disorganized remains of the thylakoids. Note that in this cell only part of the thylakoid system has formed small vesicles. KMn04 fixation

X 37,700



Fig. 167 a - h Membranous inclusions in vegetative cells of <u>A</u>. cylindrica treated in the light with 10 ppm diquat (salt). These structures were present in peripheral regions of many treated cells after the onset of damage

to the plasma membrane.

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KMn04	fixation	a	×	94,500
		ь	x	141,750
		C	×	111 <b>,6</b> 00
		d	×	120,000
		8	x	115,400
		f	×	115,400
		9	x	197,600
		h	x	120,000



Fig. 168 Vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 160 min. The plasma membrane has sustained extensive damage in all cells. Note the wide variation between cells in the extent of ultrastructural damage to other cellular structures. KMn04 fixation

X 17,400



Fig. 169 Part of a filament of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 120 min. The heterocyst appears structurally undamaged despite drastic changes in the adjacent vegetative cells. KMn04 fixation

X 16,350

Fig. 170 This micrograph shows in greater detail the junction of the heterocyst with one of the adjacent vegetative cells illustrated in the previous micrograph. Note the remains of the plasma membrane of the vegetative cell and the intact, crinkled plasma membrane of the heterocysts. Intrathylakoidal swelling is only evident within the vegetative cell while within the heterocyst the paired membranes of the thylakoids remain in close opposition. Note also the reduced electron density of the cytoplasmic matrix within the vegetative cell. KMn04 fixation

X 32,700



Fig. 171 Part of a filament of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 160 min. Note the advanced ultrastructural damage sustained by the vegetative cells. By comparison, the heterocyst shows only slight signs of injury. Although the plasma membrane of the heterocyst appears to be still intact, it is less crinkled than in untreated heterocysts. Note also the presence of membranous inclusions not normally found in control heterocysts. KMn04 fixation

X 22,100

Fig. 172 Part of a heterocyst and vegetative cell of <u>A. cylindrica</u> treated in the light with 10 ppm diquat (salt) for 120 min. Note that the plasma membrane has sustained many breaks in both the vegetative cell and heterocyst. KMn04 fixation

X 40,990



Fig. 173 A heterocyst of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (salt) for 180 min. Note the destruction of the plasma membrane. However the paired membranes of the thylakoids remain closely opposed and appear to be undamaged. KMn04 fixation

X 27,700

Fig. 174 This micrograph shows part of the previous heterocyst in greater detail. Breaks in the plasma membrane can easily be seen. Note also the undamaged appearance of the thylakoids. KMn04 fixation

X90,000



Fig. 175 A heterocyst of <u>A</u>. <u>cylindrica</u> from a colony exposed in the light to 10 ppm diquat (salt) for 48 hours. Note the loss of the plasma membrane and all of the cytoplasmic matrix. The thylakoids are twisted and many membranous vesicles are present around the periphery. KMn04 fixation

X 22,500

Fig. 176 A senescent heterocyst of <u>A</u>. <u>cylindrica</u> from a control colony. The plasma membrane and the cytoplasmic matrix have been lost leaving a twisted mass of thylakoids and numerous membranous vesicles around the periphery.

0\_0\_ fixation

X 31,650



Fig. 177 Akinetes and vegetative cells of <u>A. cylindrica</u> from a colony treated in the light with 1 ppm diquat (salt) for 48 hours. The akinetes appear to be structurally undamaged at this time. Nearby vegetative cells have been reduced to little more than swollen thylakoids enclosed within the remains of their cell walls. The spaces between the cell walls and the outer envelopes of the akinetes possibly represent artefacts due to shrinkage. Such spaces were often observed in control akinetes.

KMn04 fixation

X 9,650

Fig. 178An akinete of A. cylindrica from a colony treated<br/>in the light with 1 ppm diquat (salt) for 48 hours.<br/>The akinete appears undamaged despite complete destruction<br/>of adjacent vegetative cells.<br/>KMnD4 fixation<br/>X 14,500<br/>The inset shows the peripheral region of part of the<br/>akinete in greater detail

X 41,325



Fig. 179 An akinete of <u>A</u>. <u>cylindrica</u> from a control colony. The space between the cell wall and the outer envelope was found in many akinetes and is possibly an artefact caused by shrinkage during preparation. KMn04 fixation

X 19,075



Fig. 180 Parts of untreated vegetative cells of <u>A</u>. cylindrica exposed to darkness for 300 min. All of the cellular membranes are still intact although there is evidence of slight intrathylakoidal swelling in some places. Note the lack of polyglucoside granules in the cytoplasm. Otherwise, cell structure is similar to that observed in control vegetative cells exposed to continuous illumination KMn04 fixation

X 39,000

Fig. 181 Parts of vegetative cells of <u>A</u>. <u>cylindrica</u> treated with 10 ppm diquat (salt) in complete darkness for 300 min. The cells appear undamaged and similar to those exposed to darkness for the same length of time in the absence of diquat. KMn04 fixation

X 41,850



Fig. 182 Parts of untreated vegetative cells of <u>A</u>. <u>cylindrica</u> after growth under continuous illumination. Note the large number of polyglucoside granules in the cytoplasm around the thylakoids. KMn04 fixation

x 55,000

Fig. 183 Parts of vegetative cells of <u>A</u>. <u>cylindrica</u> after exposure to complete darkness for a period of 18 hours. Note the absence of polyglucoside granules in the cytoplasm. The prolonged darkness has also caused considerable intrathylakoidal swelling in places. KMn04 fixation

X 66,300



Fig. 184 A control dark pre-treated vegetative cell of <u>A</u>. <u>cylindrica</u> 60 min. after the commencement of illumination. Note the re-appearance of polyglucoside granules in the cytoplasm KMn04 fixation

X 29,400

Fig. 185 Control dark pre-treated vegetative cells of <u>A. cylindrica</u> 120 min. after the commencement of illumination. Note the further accumulation of polyglucoside granules in the cytoplasm KMn04 fixation

X 28,100



Fig. 186 A dark pre-treated vegetative cell of <u>A</u>. <u>cylindrica</u> 60 min. after the commencement of illumination in the presence of 10 ppm diquat (salt). Note the absence of polyglucoside granules in the cytoplasm. Note also the absence of ultrastructural damage to the cell. KMn04 fixation

X 36,900

Fig. 187 Dark pre-treated vegetative cells of <u>A</u>. <u>cylindrica</u> 120 min. after the commencement of illumination in the presence of 10 ppm diquat (salt). Polyglucoside granules have yet to re-appear despite the absence of any visible ultrastructural damage to the cells. KMn04 fixation

X 28,800



Fig. 188 Vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm paraquat (cation) for 60 min. There is not ultrastructural damage visible at this stage. Note that polyglucoside granules are still numerous in the cytoplasm. KMn04 fixation

X 31,500

Fig. 189 Vegetative cells of <u>A. cylindrica</u> treated in the light with 10 ppm paraquat (cation) for 90 min. There are still no signs of ultrastructural damage. Note however, the reduced number of polyglucoside granules in the cytoplasm.

KMnO4 fixation

X 31,500



Fig. 190 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm paraquat (cation) for 120 min. The plasma membrane has been destroyed and the polyglucoside granules have disappeared from the cytoplasm. In addition the polyhedral bodies and the nuclear areas have a more diffuse appearance than hitherto. Note also the presence of two membranous inclusions in the top right hand corner of the cell. KMn04 fixation

X 19,500

Fig. 191 Parts of vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm paraquat (cation) for 120 min. Note the breaks in the plasma membranes of both cells. Note also that where the plasma membrane is intact it has lost its characteristic small undulations. The thylakoids appear to be undamaged at this stage. KMn04 fixation

X 48,450



Fig. 192 A vegetative cell of <u>A</u>. cylindrica treated in the light with 10 ppm paraquat (cation) for 150 min. This cell is representative of those which had sustained the least amount of ultrastructural damage at this time. The plasma membrane is reduced to many fragments close to the periphery. Early stages of intrathylakoidal swelling are evident. Note also the fuzzy outlines of the polyhedral bodies and the homogeneous appearance of the gytoplasm. KMn04 fixation

X 38,400

Fig. 193 Part of a dividing cell of <u>A. cylindrica</u> treated in the light with 10 ppm paraquat (cation) for 150 min. This micrograph is typical of the appearance of the most drastically altered vegetative cells at this time. Little of the cytoplasmic matrix remains and intrathylakoidal swelling is more marked. Note that fragmentation of the thylakoids and subsequent vesicle formation has also occurred.

KMn04 fixation

X 38,200


Fig. 194 Vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm paraquat (cation) for 180 min. Little of the cytoplasmic matrix remains. The thylakoids are swollen and often fragmented, forming rounded vesicles. The remains of the thylakoids are confined within the cell walls which have sustained only small breaks. KMn04 fixation

## X 27,000

Fig. 195 The remains of a vegetative cell of <u>A. cylindrica</u> treated in the light with 10 ppm paraquat (cation) for 180 min. In this cell the cell wall has sustained a break sufficiently large to allow some of the contents to escape. KMn04 fixation

X 29,800



Fig. 196 The typical appearance of vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (cation) for 60 min. Note the slightly reduced numbers of polyglucoside granules in the cytoplasm. No ultrestructural damage is visible at this time. KMn04 fixation

X 28,510

Fig. 197 The typical appearance of vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (cation) for 90 min. There is a reduced number of polyglucoside granules in the cytoplasm but no visible ultrastructural damage. KMn04 fixation

X 32,700



Fig. 198 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (cation) for 90 min. This micrograph illustrates more advanced symptoms observed in a relatively small number of cells at this time. Note the plasma membrane which has lost its characteristic small undulations. Also note that the nuclear areas are no longer visible in the central region of the cell. Other parts of the cell remain unchanged.

KMn04 fixation

x 41,100



Fig. 199 Parts of vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (cation) for 120 min. It can be seen that the plasma membrane has sustained many breaks. Note also the reduced definition of the polyhedral bodies and the disappearance of the nuclear areas. The thylakoids appear unchanged at this time. KMnD4 fixation

X 39,400

Fig. 200 A vegetative cell of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (cation) for 150 min. This cell is representative of those which had sustained the least amount of ultrastructural damage at this time. The cell is still intact although the plasma membrane is now reduced to small fragments close to the periphery of the cytoplasm. Slight intrathylakoidal swelling is also visible. KMm04 fixation

X 40,800



Fig. 201 The remains of a vegetative cell of <u>A. cylindrica</u> treated in the light with 10 ppm diquat (cation) for 150 min. This micrograph is representative of the appearance of those cells which had undergone the greatest degenerative changes by this time. Intrathylakoidal swelling is considerable and almost all of the cytoplasmic matrix has escaped through extensive breaks in the cell wall. KMn04 fixation

X 39,500

Fig. 202 The typical appearance of vegetative cells of <u>A</u>. <u>cylindrica</u> treated in the light with 10 ppm diquat (cation) for 180 min.

KMn04 fixation

X 39,500



Fig. 203 Senescing vegetative cells of <u>A. cylindrica</u> from a large blue-green colony. Note the extensive intrathylakoidal swelling and the elmost total absence of polyglucoside granules.

KMnO4 fixation

X 22,870

Fig. 204 A senescing vegetative cell of <u>A. cylindrica</u> from a large blue-green colony. Considerable intrathylakoidal swelling is evident in parts of the cell. All other parts of the cell appear to be undamaged and polyglucoside granules are still plentiful. KMn04 fixation

X 27,600



Fig. 205 A senescing vegetative cell of <u>A</u>. <u>cylindrica</u> from a large blue-green colony. Note the marked intrathylakoidal swelling throughout the cell. All other parts of the cell appear to be undamaged and polyglucoside granules are present in reduced number. KMnD4 fixation

X 34,600

Fig. 206 A senescing vegetative cell of <u>A</u>. <u>cylindrica</u> from a yellow-green colony. Note the extensive intrathylakoidal swelling and accumulation of lipid granules. Polyglucoside granules are still present although in reduced numbers. KMn04 fixation

X 27,285



Fig. 207 A senescing vegetative cell of <u>A. cylindrica</u> from a yellow-green colony. The thylakoid system is totally disorganized. Note also the accumulation of lipid granules within the cell. Despite these changes, the polyglucoside granules are present in the cytoplasm and the plasma membrane is still intact and has its usual crinkled appearance. KMnD4 fixation

X 41.400

Fig. 208 A senescing vegetative cell of <u>A</u>. <u>cylindrica</u> from a yellow-green colony. This cell is similar in appearance to the previous cell. In addition, this micrograph reveals the presence of a structure bearing a striking resemblance to a prolamellar body (arrow) KMn04 fixation

X 40,050



Fig. 209 A senescent vegetative cell of <u>A</u>. <u>cylindrica</u> from a yellow-green colony. In this cell the plasma membrane has broken down and many breaks have appeared in the cell wall. Note also the accumulation of lipid granules. KMn04 fixation

X 29,260

Fig. 210 Fully senescent vegetative cells of <u>A</u>. <u>cylindrica</u> from a yellow-green colony. The cell walls have disintegrated allowing the escape of fragments of the disorganized remains of the thylakoid system. KMn04 fixation

X 26,790

