Rapid Changes in Permeability of Cell Membranes to Water Brought About by Carbon Dioxide & Oxygen 1, 2

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Introduction

The object of this study was to examine whether certain factors which are regarded as inhibitors of the uptake of water by roots of intact and detopped plants [e.g. high CO₂ concentration, low temperature, anaerobiosis—see Kramer (5)] have a direct effect on the permeability of protoplasmic membranes to water.

Our experimental tissue was the hypocotyl of Helianthus annuus. We have observed the effect of such factors on the permeability of the cells toward water passing both in the inward and in the outward directions.

The rate of entry of water into uniform segments of tissue is a function of A, the difference in diffusion pressure deficit (DPD) between the cells and the external medium, and B, the permeability of the protoplasm to water. Since our interest lay in B, means had to be devised of keeping A as nearly constant as possible between treatments. We have met this requirement by ensuring that the DPD of the tissue was so large that any changes in DPD resulting from cellular processes during the period over which water uptake was measured will have been extremely small in comparison with total DPD. Observed changes in rate of water influx under these conditions must therefore be attributed to changes in the permeability of the protoplasmic membranes.

The observations on efflux of water were included in order to confirm beyond doubt that alterations in DPD were not responsible for the observed effects on water movement. Whereas a change in DPD would produce an opposite effect on efflux to that exerted on influx, a change in permeability would influence both efflux and influx in the same direction.

This paper presents evidence that CO₂ acts rapidly to reduce, and O₂ to increase, the permeability of cells to water.

Material & Methods

The sunflowers (Helianthus annuus L. var. Jupiter) were grown in sterile vermiculite, at 26 C, in darkness except for occasional red light. Segments 1 cm long were cut from the hypocotyls of 6-day-old seedlings at a distance of 1.5 cm below the crook.

The principle underlying the influx experiments was, first, to allow the segments to lose a considerable proportion of their water content by drying in air; then, to measure the rate of re-entry of water during the period immediately after their transfer to distilled water and long before they had regained their initial fresh weight. The segments were thus in a state of low turgor and high osmotic pressure, and fulfilled the requirement for a tissue of high DPD. The method had the additional advantage that the water movement was large enough to allow accurate measurements after very short intervals of time.

The procedure was as follows: After cutting, the segments were first washed in distilled water for 20 minutes. Samples of 16 segments (approx. 475 mg) were then carefully blotted with filter paper to remove surface moisture and were weighed on a torsion balance sensitive to 0.2 mg (Initial wt W₁). They were next dried in air at room temperature for approximately two hours, after which they were again weighed (W₂) and transferred to 25 ml of experimental solution contained in 100 ml Erlenmeyer flasks. A stream of air, CO₂, or O₂-free N₂ continuously stirred the suspending medium. In each case the gas stream had been led through the medium for at least 45 minutes before the introduction of the segments, and continued throughout the experiment. After the desired time interval the segments were blotted and weighed for a third time (W₃). Water uptake was expressed as [(W₃ - W₂) ÷ W₁] × 100.

In those experiments where water efflux was measured, the segments were first placed in distilled water for about half an hour. They were then blotted, weighed, and transferred to mannitol solutions. Their loss in weight under various experimental conditions was measured as described above.
conditions was followed by subsequent weighing. The tissue was never allowed to reach the point of plasmolysis.

All experiments were carried out at room temperature (25-28°C). The \( \text{N}_2 \) used in this investigation was first passed in the form of very fine bubbles through a meter-high column of \( K \) pyrogallate in order to free it of \( O_2 \).

**Results**

Of the four factors originally chosen for investigation in the present study—anaerobic conditions, \( \text{CO}_2 \) concentration, low temperature, and cyanide—only anaerobiosis and \( \text{CO}_2 \) provided clear evidence for a direct effect on the permeability of the tissue to water. The influence of low temperature was not significantly larger than could be satisfactorily accounted for by the known increase in viscosity of water with decrease in temperature. Cyanide at a concentration of \( 5 \times 10^{-3} \) M (the concentration reported by Brouwer (1) to cause immediate cessation of water uptake by intact bean roots) did not affect water influx into the cells for at least the first 20 minutes, or its efflux for at least 30 minutes. By contrast, the effects of \( \text{CO}_2 \) and of anaerobiosis were immediate and clearcut. This finding led us to investigate the influence of a fifth factor, one which has received far less attention in the literature on water uptake by intact roots—that of \( O_2 \) concentration.

► Preliminary Experiments: Experiments were first carried out to determine if the drying treatment impaired the capacity of the segments for subsequent water uptake. One such experiment is summarized in figure 1. The figure shows that there was little effect of drying on subsequent water uptake up to a point where the tissue had lost approximately 45% of its initial weight. Beyond this critical value, however, the curve for subsequent water uptake fell steeply. In the experiments to be reported in this paper preliminary water loss in the drying treatment was never greater than 45%.

► Effect of \( \text{CO}_2 \) and of Anaerobiosis on Water Influx: A typical experiment is summarized in figure 2. The hypocotyl segments in this experiment lost about 40% of their initial water content during the pre-treatment in air. They were then transferred to distilled water through which a stream of air, of \( \text{CO}_2 \), or of \( \text{N}_2 \), was bubbling. It will be seen (fig 2, Curves A & C) that the effect of \( \text{CO}_2 \) on the entry of water into the tissue was markedly inhibitory and that it was clearly visible within 3 minutes. The reduction in the amount of water taken up was 40% after 3 minutes, as compared with the control in aerated water: after 20 minutes it was 58%.

The inhibitory effect observed was not merely the result of lack of \( O_2 \). This may be seen by comparing Curve C in figure 2 with curve B. The latter gives the course of water uptake by segments under \( O_2 \)-free \( \text{N}_2 \). It will be noted that anaerobic conditions did in fact have a pronounced effect on the permeability of these segments to water and it is remarkable that this effect also was well marked after 3 minutes. The inhibitory effect of anaerobic conditions, however, could only account for approximately half that of \( \text{CO}_2 \).

The pH of the water through which \( \text{CO}_2 \) bubbled was 4.1: of that through which air bubbled 5.3. To assess to what extent the difference in pH might account for the results shown in figure 2, the influence of pH on water uptake by this tissue was examined. Segments which previously had been allowed to lose 40% of their water content were placed in water brought to pH 3.3, 5.3, or 7.3 with HCl or KOH. No differences in the rate of water uptake could be detected. A further experiment was moreover carried out which compared uptake by segments in water saturated by a \( \text{CO}_2 \) stream with that by segments in water adjusted to pH 4.1 with HCl and aerated. The inhibitory effect of \( \text{CO}_2 \) was as marked as in the experiment summarized in figure 2.

Since the DPD gradients set up by air drying of tissue might produce results different from DPD gradients set up by immersion in solutions of high osmotic concentration, another experiment was carried out in which the drying treatment was replaced by immersion in 0.4 M mannitol. This concentration of mannitol was approximately iso-osmotic with the tissue, which thus did not plasmolyse. Table I gives details of this experiment and shows that, under these conditions as well, \( \text{CO}_2 \) and anaerobiosis were clearly inhibitory. The experiment also serves to confirm that the inhibition was affecting the entry of water into the osmotic volume of the tissue, and not into its free space.

### Table I

**Effect of \( \text{CO}_2 \) & of Anaerobic Conditions on Influx of Water Into Segments of Sunflower Hypocotyl**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gain in wt* (% Initial wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>5.8</td>
</tr>
<tr>
<td>( \text{N}_2 )</td>
<td>4.2</td>
</tr>
<tr>
<td>( \text{CO}_2 )</td>
<td>1.3</td>
</tr>
<tr>
<td>Sig. diff. (P=0.05)</td>
<td>1.0</td>
</tr>
</tbody>
</table>

* The segments had first been placed in 0.4 M mannitol for 3 hours, during which they lost about 15% of their initial \( H_2O \) content. They were then transferred to \( H_2O \) through which \( \text{CO}_2 \), \( O_2 \)-free \( \text{N}_2 \), or air was bubbling. \( H_2O \) influx was measured after 30 minutes.

► Efflux of Water From Tissue to Environment: Table II gives details of a typical experiment. It will be seen that efflux of water from the tissue into the solution was reduced by \( \text{CO}_2 \) treatment, and that the reduction (25%), though less than that observed in the influx experiments, was statistically significant. The figures for the segments under \( \text{N}_2 \), however, were not appreciably different from those for the aerated tissue.
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Table II

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Loss in wt* (% Initial wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>After 15 min</td>
</tr>
<tr>
<td>Air</td>
<td>7.9</td>
</tr>
<tr>
<td>N₂</td>
<td>7.4</td>
</tr>
<tr>
<td>CO₂</td>
<td>6.0</td>
</tr>
<tr>
<td>Sig. diff. (P=0.05)</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* The segments had first been placed in distilled H₂O for 20 minutes. Their loss in weight was followed after transfer to 0.6 M mannitol through which CO₂, O₂-free N₂, or air was bubbling.

Reversibility of Effects Produced by CO₂ and by Anaerobiosis: Figure 3 summarizes an experiment carried out to test whether the observed inhibition of water influx was reversible. The standard of comparison for the treated segments after they had been returned to aerated water presented a problem, since the rate of water uptake by control segments continuously decreases. This difficulty was met by assessing total water uptake after 24 hours, by which time the rate of uptake has declined to an extremely low level. The segments were allowed to lose water as usual in air, and were then transferred to water through which either CO₂ or N₂ was bubbling. After periods of treatment ranging from 30 to 120 minutes.

In all cases 3 hours elapsed between the initial weighing of the segments and their transfer to H₂O. The control segments spent this 3 hour period in saturated air. The other segment samples remained in saturated air for varying lengths of time before being exposed to laboratory air at 26°C for from 0.5 to 3 hours. Subsequent H₂O influx was measured over a 24 hour period.

Fig. 2 (center). The effect of CO₂ and of anaerobic conditions on influx of water into segments of sunflower hypocotyl. The segments had previously lost approx. 40% of their initial water content while drying in air at room temperature. The dotted line, D, indicates the level of water uptake equivalent to recovery of initial weight.

Fig. 3 (bottom). Extent of reversibility of the CO₂ and anaerobic effects.

Curves A and B: Progress curves for the influx of water into segments of sunflower hypocotyl during treatment with CO₂ or N₂.

Curves C and D: The relationship between final weight of segments after 24 hours and length of treatment with the gas.

The segments had first lost approx. 35% of their initial H₂O content while drying in air at room temperature. They were then placed in water through which CO₂ or O₂-free N₂ was bubbling. After treatment with the gas they were transferred to distilled water.

Each point represents the mean either of duplicates (figs 1 & 3) or of triplicates (fig 2). Their range is indicated where this extended beyond the symbol drawn.

Fig. 1 (top). The relationship between the amount of H₂O lost by sunflower hypocotyl segments during the drying treatment and subsequent H₂O influx after transfer to distilled H₂O.

Fig. 2. (center). The effect of CO₂ and anaerobic conditions on influx of water into segments of sunflower hypocotyl. The segments had previously lost approx. 40% of their initial water content while drying in air at room temperature. The dotted line, D, indicates the level of water uptake equivalent to recovery of initial weight.

Fig. 3 (bottom). Extent of reversibility of the CO₂ and anaerobic effects.

Curves A and B: Progress curves for the influx of water into segments of sunflower hypocotyl during treatment with CO₂ or N₂.

Curves C and D: The relationship between final weight of segments after 24 hours and length of treatment with the gas.

The segments had first lost approx. 35% of their initial H₂O content while drying in air at room temperature. They were then placed in water through which CO₂ or O₂-free N₂ was bubbling. After treatment with the gas they were transferred to distilled water.

Each point represents the mean either of duplicates (figs 1 & 3) or of triplicates (fig 2). Their range is indicated where this extended beyond the symbol drawn.
the segments were blotted, weighed, and transferred to distilled water for 24 hours less the period of the treatment. They were then weighed again. The control segments spent the entire 24 hours in distilled water.

Comparison of the curves A & B in figure 3, which depict the course of water influx during the gas treatment, shows that, as usual, influx was much slower under CO₂ than under N₂. Curves C and D relate the final weight after 24 hours to length of treatment with the gas. It will be seen that the values for water uptake by tissues treated for 30 minutes with either CO₂ or N₂ are not appreciably different from those for the controls. Thus the effects of treatment for 30 minutes or less with CO₂ or N₂ appear to be fully reversible. It is further apparent from figure 3 that irreversible effects followed treatment for longer than 30 minutes with either gas.

Effect of Presence of O₂ on Degree of CO₂ Inhibition: A further experiment investigated whether the presence of O₂ would affect the CO₂ inhibition, since in all previous experiments treatment with CO₂ had of necessity involved absence of O₂. It was decided to compare the effect of 80 % CO₂ + 20 % O₂ with that of 80 % CO₂ + 20 % N₂. It was necessary to include a N₂ control as well as an air control in this experiment in order to be able to allow for the known inhibitory effect of anaerobiosis.

Table III
Influence of Gas Mixtures on Water Influx Into Segments of Sunflower Hypocotyl

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gain in wt* (%)</th>
<th>% Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Air</td>
<td>16.4</td>
<td>13.5</td>
</tr>
<tr>
<td>B 100 % N₂</td>
<td>8.0</td>
<td>52</td>
</tr>
<tr>
<td>C 80 % CO₂ + 20 % N₂</td>
<td>8.0</td>
<td>52</td>
</tr>
<tr>
<td>D 80 % CO₂ + 20 % O₂</td>
<td>13.1</td>
<td>20</td>
</tr>
<tr>
<td>E 100 % CO₂</td>
<td>7.4</td>
<td>55</td>
</tr>
<tr>
<td>Sig. diff. (P=0.05)</td>
<td>1.5</td>
<td></td>
</tr>
</tbody>
</table>

* The segments had first lost approximately 40 % of their initial H₂O content while drying in air. They were then transferred to distilled H₂O through which the various gas mixtures were bubbling. H₂O influx was measured after 8 minutes.

Details of the experiment are given in table III. The overall reduction in amount of influx produced by 80 % CO₂ + 20 % N₂ was 52 %. Under anaerobic conditions the reduction was 18 %. The inhibition directly attributable to the CO₂ in the CO₂ + N₂ treatment, after allowance has been made for the anaerobiosis involved, is therefore 34 %. (It is here assumed that the N₂ and CO₂ effects are additive). The inhibition produced by 80 % CO₂ + 20 % O₂ was only 20 %. The presence of O₂ thus appears to have countered in some measure the inhibitory effects of CO₂.

Table IV
Effect of O₂ Concentration on Water Influx Into Segments of Sunflower Hypocotyl

<table>
<thead>
<tr>
<th>O₂ Concentration (%)</th>
<th>Gain in wt* (%) Initial wt</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>11.9</td>
</tr>
<tr>
<td>20</td>
<td>15.8</td>
</tr>
<tr>
<td>40</td>
<td>19.1</td>
</tr>
<tr>
<td>60</td>
<td>17.9</td>
</tr>
<tr>
<td>100</td>
<td>17.1</td>
</tr>
<tr>
<td>Sig. diff. (P=0.05)</td>
<td>1.4</td>
</tr>
</tbody>
</table>

* The segments had first lost approx. 38 % of their initial H₂O content while drying in air. They were then transferred to H₂O through which the various O₂-N₂ mixtures were bubbling. H₂O influx was measured after 10 minutes.

Treatment with 100 % CO₂ brought about a 55 % reduction in the rate of water entry. Again subtracting the 18 % attributable to the anaerobic conditions, the inhibition caused by the CO₂ itself appears to have been 37 %. Since the inhibition due to the CO₂ factor in the CO₂ + N₂ treatment was assessed at 34 %, it appears that reducing the CO₂ concentration by 20 % did not appreciably lower the degree of inhibition.

Effect of O₂ Concentration on Water Influx: In view of the inhibiting effect of lack of O₂ on water influx, it was decided to investigate the effect of varying the concentration of O₂. Table IV gives details of such an experiment. It may be seen that raising the O₂ concentration above that of air did in fact significantly increase water influx into the tissue. The maximum effect was obtained with 40 % O₂. It is possible that O₂ concentrations in excess of 40 % caused a decline in permeability to water, though, as may be seen from the table, this decline was not statistically significant.

Experiments With Root Tissue: Since the influence of soil CO₂ and of lack of soil O₂ on the uptake of water by intact and detopped plants must primarily be exerted on roots, it was of interest to examine whether the effects reported above were also detectable in root tissue. The very fine, much-branched character of sunflower roots made them less suitable for this purpose than the roots of peas. Table V gives details of an experiment carried out with the roots of etiolated 5-day-old seedlings of Pisum sativum var. Alaska. Re-entry of water was more rapid than was the case with hypocotyl tissue (probably owing to the lack of cuticle and the small diameter of the segments) so that after 3 minutes the segments had regained nearly 50 % of the water they had lost during the drying period. Table V shows that within 3 minutes CO₂ treatment had produced a marked effect, the reduction in amount of water entering being 48 %. No significant effect of anaerobiosis was observable.
Effect of discs

21.0

No appreciable results obtained in rate of the investigation permeability of cells to plants [notably Kramer (4); Jackson (2)] have observed that a high concentration of CO₂ around the roots reduces the amount of water taken up by the plant. Our results confirm that an alteration in the permeability of the osmotic barriers is the probable explanation, and extend the earlier observations by showing that the effect is very quickly manifested. The celerity of this response—it was well marked within 3 minutes—makes it unlikely that CO₂ affects the membranes indirectly through an effect on metabolism.

The experiment on reversibility showed that, if exposure to CO₂ is limited to 30 minutes or less, the effects are fully reversible. It is to be assumed that this was the case for all the effects observed in this investigation since the experiments were in general of less than 30 minutes' duration. We were thus dealing with the classical narcotic action of CO₂ (3). The irreversible effects that followed longer exposures culminated in the destruction of the semipermeability of the membranes, as may be deduced from the fact that 2 hours' treatment produced a final weight of tissue 14% below the initial weight. This is approximately the figure for the loss in fresh weight observed when hypocotyl tissue is brought to the point of incipient plasmolysis in mannitol solution. It therefore appears that the CO₂ treated tissue had lost all turgor and that its membranes had lost their semipermeable properties.

The effect of anaerobiosis on water uptake by intact roots has been much less emphasized in the literature than that of CO₂. Kramer (4) noted a small inhibitory effect (10%) whereas Chang and Loomis (2) observed no reduction in water uptake as a result of bubbling N₂ through their culture solutions. The evidence provided here for an effect of anaerobic conditions on membrane permeability in sunflower hypocotyl tissue seems unmistakable. As with CO₂, the effect was apparent within 3 minutes, again suggesting that it was not exerted via metabolism. In contrast to CO₂, however, anaerobic conditions only had a significant influence on influx into hypocotyl segments. No effect was observed with root tissue. It is just possible that lack of CO₂ does, in fact, affect root tissue but that this has not been detectable owing to the greater speed with which this tissue recovers its initial fresh weight after drying. The curve for water uptake against time flattened out within 5 minutes, after which differences in slope between treatments would have been very difficult to detect. Therefore, if for some reason the anaerobic effect only becomes apparent after 5 minutes in root tissue, instead of within 3 minutes as in hypocotyls, it would have been missed.

The effect of up to 30 minutes' treatment with N₂, as with CO₂, was fully reversible. In contrast with CO₂ injury, however, the irreversible effects which followed longer treatment seem to have related principally to growth, since the final weight of the segments after 24 hours was never significantly less than their initial weight.

In addition to the effects of lack of O₂ discussed above, our experiments showed that raising the O₂ concentration above that of air increased the permeability of the membranes to water. The optimum O₂ concentration appeared to be about 40%. An interesting feature was the diminution in the degree of CO₂ inhibition when O₂ was present. This could not be explained simply on the basis of the promotive effect of O₂ on water influx as compared with anaerobic conditions. Reappraisal of table III from a different point of view—this time considering the anaerobic treatments as the controls—brings out this point very clearly. Comparing A and B we note that 20% O₂ increased permeability by 21%. When 80% CO₂ was present in the gas mixture, however, 20% O₂ increased permeability by 65% (comparing C & D). Thus part of the action of O₂ related to the action of CO₂.

Elucidation of the mechanism by means of which CO₂ and O₂ induced these rapid changes in the permeability of the cell membranes to water must await further study.

Table V

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Gain in wt* (% Initial wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Air</td>
<td>22.0</td>
</tr>
<tr>
<td>N₂</td>
<td>21.0</td>
</tr>
<tr>
<td>CO₂</td>
<td>11.4</td>
</tr>
<tr>
<td>Sig. diff. (P=0.05)</td>
<td>5.6</td>
</tr>
</tbody>
</table>

* Each tissue sample consisted of 21 one-centimeter segments removed at a distance of 2 cm from the root tip. The segments lost approximately 40% of their initial water content while drying in air. They were then transferred to distilled H₂O, through which CO₂, O₂-free N₂, or air was bubbling. H₂O influx was measured after 3 minutes.

Experiments were also carried out with 1 mm-thick discs of the root storage tissue of Daucus carota. The results obtained were similar to those reported for pea roots—i.e., marked inhibition by CO₂, no appreciable effect of anaerobic conditions.

Discussion

Owing to the special conditions of the experiments on water uptake reported in this paper, alterations in rate of water movement are interpretable as a direct measure of changes in the permeability of the cells. The investigation has therefore demonstrated that an excess of CO₂ can induce rapid changes in the permeability of cells to water. Proof that permeability, and not DPD, was the property affected is provided by the fact that CO₂ had a depressant effect on both influx and efflux of water.

Earlier workers experimenting with whole or detopped plants [notably Kramer (4); Kramer & Jackson (6); & Chang & Loomis (2)] have observed that a high concentration of CO₂ around the roots reduces the amount of water taken up by the plant. Our results confirm that an alteration in the permeability of the osmotic barriers is the probable explanation, and extend the earlier observations by showing that the effect is very quickly manifested. The celerity of this response—it was well marked within 3 minutes—makes it unlikely that CO₂ affects the membranes indirectly through an effect on metabolism.

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Elucidation of the mechanism by means of which CO₂ and O₂ induced these rapid changes in the permeability of the cell membranes to water must await further study.
Summary

I. Influx of H₂O into segments of hypocotyl of Helianthus annuus L. has been observed. The segments had first been allowed to lose approx. 40% of their H₂O content by drying in air at room temperature. Reasons are given for interpreting the initial rate of influx under these conditions as a measure of the permeability of the cell membranes to H₂O. The drying treatment was shown not to impair the capacity of the segments for subsequent H₂O uptake.

II. Efflux of H₂O was measured after segments which had previously been immersed in distilled H₂O were transferred to mannitol solutions.

III. Treatment with CO₂ markedly reduced the rate of movement of water passing both into and out of the cells. The effect was clear-cut after 3 minutes. It was shown that inhibition was not due to lack of O₂; nor to the lowered pH of the medium.

IV. Anaerobiosis also reduced the rate of H₂O influx within 3 minutes, though to a lesser extent than did CO₂.

V. The depressant effects of CO₂ and anaerobiosis were also apparent when immersion in mannitol solutions was substituted for the drying treatment in the influx experiments.

VI. The effects of treatment for 30 minutes or less with either CO₂ or O₂-free N₂ were fully reversible. Irreversible effects followed longer treatment which, in the case of CO₂, led to the destruction of the semipermeability of the cell membranes.

VII. Increasing the O₂ concentration to 40% was found to increase H₂O influx. The presence of O₂ in the gas stream also countered to a marked extent the inhibitory effects of CO₂.

VIII. Experiments on segments of roots of pea seedlings, and of carrot root storage tissue, revealed an inhibitory action of CO₂ on H₂O influx similar to that observed for hypocotyl tissue. No effect of anaerobiosis could be detected.

IX. It is concluded that CO₂ acts rapidly to decrease, and O₂ to increase, the permeability of cells to water.

Acknowledgment

We wish to thank our colleague, Dr. D. Cohen, for many helpful discussions.

Literature Cited