International Journal on Hands-on Science [ISSN (print): 1646-8937; (online): 1646-8945]
Received October 10, 2008; Accepted October 29, 2008.

Respiration and photosynthesis in context: Experiments demonstrating relationship between the two physiological processes

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Abstract. *The fact that plants are living organisms will be shown to children by simple experiments with the indicator bromthymol blue, which changes the colour from blue to yellow within one hour during respiration. Additionally to the use of indicators the fact that respiration and photosynthesis are opposite physiological processes can be demonstrated by an experiment measuring the electric potential between the respiration of peas and the photosynthesis of aquatic plants (Elodea canadensis) connected together with a voltmeter. The oxygen consumption during respiration develops a minus (-) pole and the oxygen production during photosynthesis a plus (+) pole.*

Keywords. Indicators, measurement of the electric potential, photosynthesis, respiration

1. Introduction

The most important metabolic processes in living organisms are respiration and photosynthesis [1]. Both are interconnected in many ways (Fig. 2). In a single plant cell, in lichens (Fig. 10), in plant galls (Fig. 11), in natural habitats and in ecosystems they are united in complex biological systems. At school photosynthesis and respiration often are taught separately. But on the other hand they belong together and one should not forget that they are correlated. The emergence concept of modern biology means that the whole, intact and complete system is more than the sum of the parts. From these considerations one may conclude that photosynthesis and respiration should be introduced in context [2]. This may be done in schools with the classic Priestley-experiment, but the problem is, that this experiment cannot be carried out in schools because one should not let die animals by this way (Fig. 9).

On the other hand animals are able to dissimilate and plants to assimilate in different separate organisms in natural habitats. In addition photosynthesis and respiration are opposite physiological pathways separated in isolated compartments like chloroplasts and mitochondria and maintain a metabolic equilibrium in a steady state in a plant cell. Chloroplasts and mitochondria have common features according to the endosymbiotic theory of evolution. They are surrounded by a double membrane layer and are able to synthesize ATP using a proton gradient after accumulation of protons between these two membranes according the concept of Peter Mitchell (Fig. 7). Halobacteria similarly transport protons outside the outer cell membrane comparable to chloroplasts and mitochondria. This outer membrane corresponds to the inner membrane of chloroplasts and mitochondria (Fig. 8).

We will present here an experiment showing the decrease of the pH - value in the surrounding medium when halobacteria are exposed to light. The acidification is due to the light driven proton transport from inside to outside.

Both physiological processes, photosynthesis and respiration, are not visible and only in the rarest cases detectable by our senses. The physiology knows however informative and elucidating experiments, which permit it to make important aspects of such life procedures visible.

Since photosynthesis and respiration both are opposite physiological pathways their effects may be compensated in some situations. In this case one cannot expect to detect any change of an indicator colour in the surrounding medium of a water plant. Alternatively photosynthesis and respiration can easily be studied together in a combination of a photosynthesizing O_2 producing system with an O_2 consuming system (Fig. 6) by a simple experiment measuring the electric potential between both [3].

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2. Methodology

In general results may be obtained by experimenting, by observing, comparing and combining. First, some experimental approaches will be presented and later on in the following further expanding aspects will be included into the considerations in order to elucidate the importance and advantage of the view of seeing photosynthesis and respiration in context.

2.1.1 Respiration of organisms: Basic process of life

Questions: How can one show that something lives? Is for instance life in branch pieces of shrubs in the winter time (Fig. 1)? Do plant roots live (Fig. 5)?

Material: 2 measuring cylinders 250 mL - Erlenmeyer flasks, 50 mL and 250 mL - Short test tubes – Drinking straw.

Chemicals: 1 L of saturated gypsum (calcium sulphate $CaSO_4$) solution coloured with bromthymol blue (0.1% in 20% ethanol) or phenolphthalein (1% in ethanol): Adjust the pH value with a diluted caustic soda solution, c(NaOH) = 0,01 mol L^{-1} in such a way that the colour change takes place immediately from yellow to blue or from red to colourless with minimal acid addition. Therefore solutions should be added drop by drop.

Test objects: Roots of the wild flower plants from gardens, tufts of grass, Petty Spurge (*Euphorbia peplus*) or Annual Mercury (*Mercurialis annua*). The plants are loosened with root with the help of a small grave shovel carefully from the ground and washed off afterwards with tap water. - Finger-long grey, brown or red branch pieces (Fig.1) of Black Elder (*Sambucus nigra*), Red Dogwood (*Cornus sanguinea*) with crust pores ("lenticells") as well as green branch pieces of Golden Bell (*Forsythia spp*.), Jews Mantle (*Kerria japonica)*, Blackberry (*Rubus fruticosus*) or Rose (*Rosa spp.*) without crust pores ("lenticells").

All branch pieces were well washed off with tap-water before the experiment.

Procedure: The plants are given with the roots to the measuring cylinders filled with test solution. The branch pieces are also placed in test tubes with test solution. A measuring cylinder with test solution without plant served as control. One also can place recognizably dead branch pieces or roots into the solutions for comparison. The results with the branch pieces are noted in a table (Table 1), in that the colour of the branches, the occurrence of crust pores or "lenticells" and the time until to the colour change is registered. Subsequently, one can give the comparison solutions in Erlenmeyer flasks and blow with the help of a drinking straw carefully to exhaust bubbles into the solutions.

2.1.2 Respiration and photosynthesis: A cycle as in nature

Question: Respiration and photosynthesis are the most important physiological life processes in nature. They are opposite physiological pathways, so that they can be joined to a cycle (Fig. 2). Thus oxidants (O_2) are consumed by respiration and set free again by photosynthesis. The opposite direction of both processes may be demonstrated by indicator changes or with the help of a potential measurement in the following experiment. How may be demonstrated in a model experiment based on the consumption and formation of oxidizing agents (oxygen O_2) that respiration and photosynthesis are included in a natural cycle (Fig. 6)?

Material: Voltmeter - Measuring clips (alligator clips) with cables - U-bend with porous glass -frit - Graphite electrodes - Lamp (200-250 Watts).

Chemicals: Sulphate - rich mineral water.

Test objects: For respiration: germinated seeds, e.g. peas (*Pisum sativum*) in mineral water or alternatively yeast suspension *(Saccharomyces cerevisiae*) in glucose solution (1%). For photosynthesis: Aquatic plant, e.g. Canadian Waterweed (*Elodea spp*.) in mineral water.

Procedure: Respiration is prepared in the left part of the U-bend, photosynthesis in the right part. Connect the voltmeter and note the voltage levels. After 10 min the photosynthesis is started by exposition with the light of a lamp.

2.1.3 Sun and life: light driven proton transport by halobacteria

Question: Life needs necessarily energy of the sun. This energy is first taken up by plants. The primary step is the transformation of light energy into electric energy in certain biomembranes, which contain photoreceptors (e.g. chlorophyll). The moved electrons provide the operating proton pumps with energy. By this light-driven proton transport H^+-ions are transported outward

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into the surrounding medium. The resulting proton gradient at the external membranes is used to synthesize ATP (chemiosmosis). How can the light driven proton export in the external medium be experimentally demonstrated?

Material: pH value measuring instrument with electrode for automatic recording (e.g. Cassy system, pH box No. 524035, Leybold,Hürth,Germany) - 250 mL Erlenmeyer flasks – graduate measuring pipette 5 and 10 mL.

Chemicals: 1L saline solution, c(NaCl) = 4 mol L^{-1}.

Test objects: Suspension culture of halobacteria (*Halobacterium halobium*) in 4 mol L^{-1} solution of common salt NaCl or alternatively suspension culture of blue bacteria (blue-green "algae ", cyanobacteria) in tap - water. Procurement reference: One may get Halobacteria from journeys to salt lakes (e.g. Dead Sea), but also from research institutes.

Procedure: As much material is given with a pipette from the halobacteria stock culture to an Erlenmeyer flask filled with saline solution that straight all light is absorbed. The cells from halobacteria are illuminated with a lamp. The pH value is measured in the medium by an electrode connected with an instrument for graphical registration. The measured values are noted and the measuring curve is projected to the wall (Fig. 8).

3. Results

3.1 Experiments

3.1.1 Respiration of organisms: Basic process of life

Observation: Within 1 hour, often already after approximately 20 minutes, it may be observed a clear colour change of blue to yellow with bromthymol blue and of red to colourless with phenolphthalein in direct proximity of the roots or the branch pieces. With the green branches (Jews Mantle, Rose or Blackberry) the colour change takes place later than with branches with "lenticells". The colour change is substantially faster, if one gives breathing air with a drinking straw to the solutions.

Explanation: In all cases acid is excreted over the surface by the living plant parts. To a large extent this is due to carbonic acid or carbon dioxide, which is set free by the respiration. Additionally in the calcium sulphate solutions Ca^{2+} -ions were exchanged against H^{+} - ions. Thus the colour change takes place more rapidly in solutions with calcium sulphate salt (Fig. 5).The green branches produce fewer carbon dioxide CO_2 quantities than the other branches with crust pores. Partially photosynthesis may compensate the respiration effect. The crust pores ("lenticells") are to be interpreted as structures in connection with the respiration and the gas exchange (structure function relationships in biology). As respiration and ion exchange are physiological processes, these experiments are suitable for the demonstration of life phenomena. The experiment with the exhausted air bubbles points out the comparison of human respiration with the respiration of plants and underlines the importance of the respiration for all organisms including humans.

3.1.2 Respiration and photosynthesis: A cycle as in nature

Observation: An electric potential is to be determined between both sides, which increases due to the living processes of the participating organisms during the experiment. When photosynthesis is started a clearly stronger increase of the measured electric potential is observed.

Explanation: The oxygen on both sides is differently used by the respiration. Therefore the output potential begins to change. When photosynthesis is started oxygen is produced, so that on the side of the photosynthesis a positive pole develops. In the respiration part the oxygen dissolved in the water is consumed. This may be the onset of fermentation and perhaps even of the production of reducing agents (H_2S, NH_3). Thus a negative pole forms. The changes of the potential are therefore direct consequences of physiological events. They are based on physiological processes such as oxygen consumption and oxygen production (Fig. 6).

3.1.3 Sun and life: light driven proton transport by halobacteria

Observation: It is recognizable from the course of the curve that the pH value drops slightly during illumination within 1 hour.

Explanation: Under the effect of the light electrons are moved (see solar - pocket calculators). With the kinetic energy of the electrons protons from the cells of the

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halobacteria are exported (light - driven proton transport).Thus the concentration of hydrogen ions (H^+-ions) rises in the medium and the pH value decreases (Fig.8). It may be of interest to emphasize that the pH value in the external medium rises with the photosynthesis of eukaryotic green plants and algae in contrast to the situation of halobacteria because of the consumption of carbon dioxide or carbonic acid and other acids. On the other hand in eukaryotic plants the light driven proton transport in chloroplasts corresponds to the light driven proton transport in halobacteria. The protons are pumped there into the gap between the two membranes (lumen of the thylacoids) and cannot be proven therefore in the external medium.

4. Discussion: comparison and combination

The relationship of photosynthesis and respiration is introduced in school books by the well known Priestley experiment (Fig. 9). Here a new approach arising from a direct observation on the living object is offered. The comparison of branches with and without crust pores and the colours of the epidermis lead to a better understanding of structure-function relationships of plant surfaces adapted on photosynthesis or respiration (Table 1). In most cases the green branches of shrubs did not have crust pores and in contrast to this observation crust pores occurred on brown, red or grey coloured branches. The conclusion is that green parts maintain photosynthesis and also obtain the oxygen by this way and that the others get the oxygen through the crust pores from the air.

The fact that respiration and photosynthesis are opposite physiological processes can be demonstrated by simple experiments with the indicator bromthymol blue or phenolphthalein (Fig. 3, 4, 5).

In our hands it changes the colour from blue to yellow within 1 hour during respiration of whole plants or of plant roots (Fig. 5). One can accelerate this change by the addition of calcium sulphate $CaSO_4$ because of cation exchange between the plant surface and the medium. The opposite colour change from yellow to blue occurs when plants exhibit photosynthesis (Fig. 4).

In addition to the use of indicators the electric potential between respirating peas and photosynthesizing aquatic plants (*Elodea canadensis*) was measured with a voltmeter. The oxygen consumption during respiration develops a minus (-) pole and the oxygen production during photosynthesis a plus (+) pole (Fig. 6).

The context of respiration and photosynthesis may be extended on the basis of other biological examples: respiration and photosynthesis in ecological systems (Fig. 2), in a plant cell (Fig. 7), in lichens (Fig. 10), in plant galls and in leafs of horse-chestnut (*Aesculus hippocastanum*) after infestation with larvae of the moth *Cameraria ohridella*. These examples show clearly the photosynthesis and respiration in context in addition to well known experiments with plants and other organisms [4,5}.

Table1: Survey of properties of selected shrub branches with and without crust pores

Respiration and Photosynthesis in Context

Object Bough	Bark Colour	Surface	Lenti-cells	Photosynthesis	Respiration
Forsythia	grey-brown		++		
Sambucus	grey		+		
Cornus	red		+		
Kerria	green		–		
Rosa	green		–		
Fargesia (Bamboo)	green		–		

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Table 1 demonstrates that objects without crust pores have a green surface and should be able to perform photosynthesis and to produce oxygen O_2.The others which are not green show crust pores. In this case the oxygen is taken up by the pores from the surrounding air.

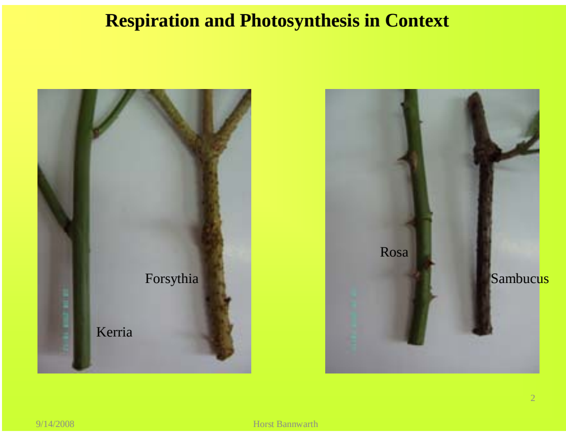

Figure 1: Relationships concerning structure and function of photosynthesis and respiration

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In figure 1 is shown green branch pieces without crust pores ("lenticells") of Jews Mantle (*Kerria japonica), left, or* Rose (*Rosa spp.*), right, were compared with grey or brown pieces of branch of Golden Bell (*Forsythia spp.*), left, or Black Elder (*Sambucus nigra*), right, with crust pores ("lenticells").

In every case the green photosynthesizing plant parts had no crust pores whereas the respirating parts showed a lot of "lenticells" (Table 1).

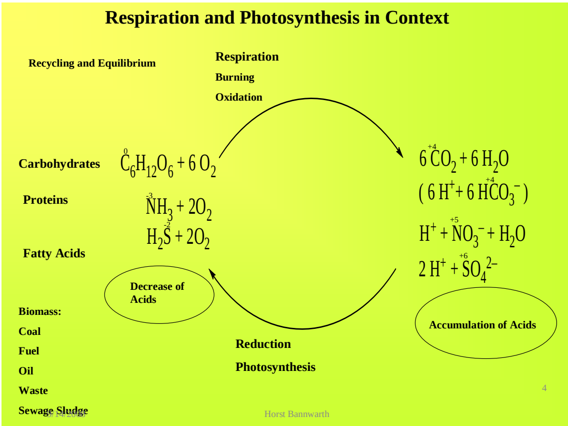

Figure 2: Photosynthesis and respiration in context

Photosynthesis and respiration represent chains of redox – reactions in a steady state or in a dynamic equilibrium running in opposite directions (Fig. 2). During photosynthesis carbon, nitrogen and sulphur are reduced and acid (H^+-ions) are consumed. During respiration carbon, nitrogen and sulphur are oxidized an acid is produced. This leads to an acidification of the environment. Burning augment this process. This means that burning should be reduced and photosynthesis reinforced by human actions.

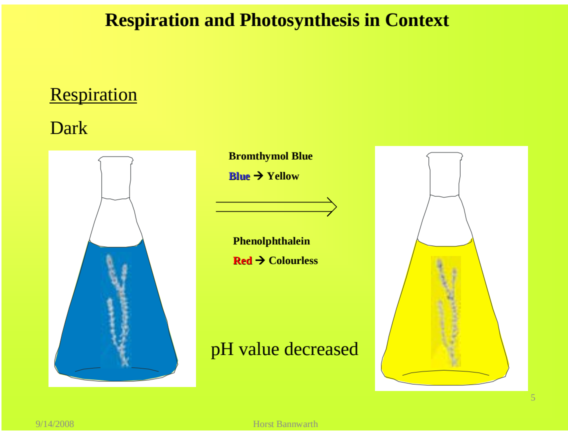

Figure 3: Respiration of plants

The production of acid (Fig. 2) during respiration can be demonstrated by the use of indicators. For instance bromthymol blue changes the colour from blue to yellow (Fig. 3).

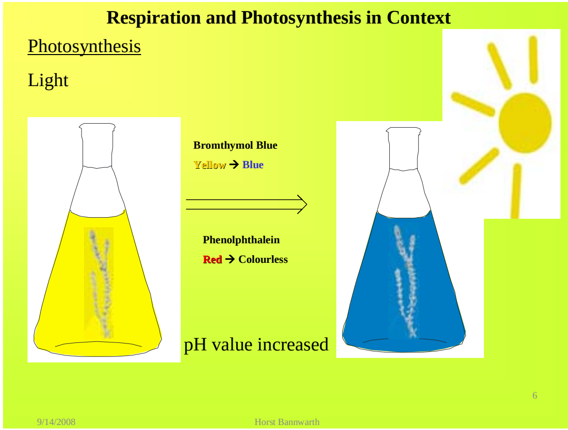

Figure 4: Photosynthesis of plants

The consumption of acid during photosynthesis of plants can also be demonstrated by the use of the same indicator. But in this case the colour of bromthymol blue changes from yellow to blue in the presence of light (Fig. 4).

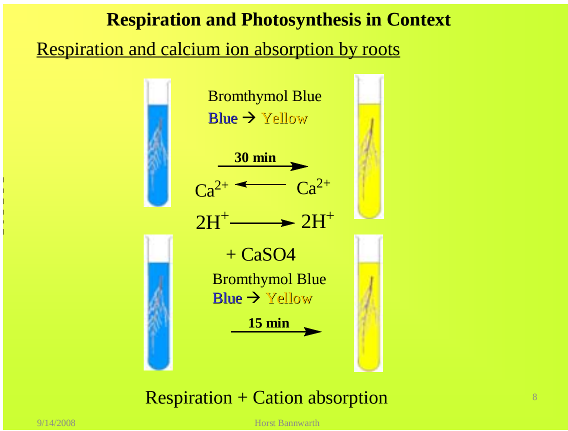

Figure 5: Roots: Respiration and cation exchange

Respiration of roots with and without the addition of gypsum $CaSO_4$.During respiration the pH value is decreased since carbonic acid CO_2 or H_2CO_3 is produced (Fig. 5). This decrease is indicated by the change of the colour of the indicator. It is faster in the presence of $CaSO_4$.This is due to the exchange of Ca^{2+}-ions against H^+-ions or protons by the plant roots.

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The Hands-on Science Network' 2008

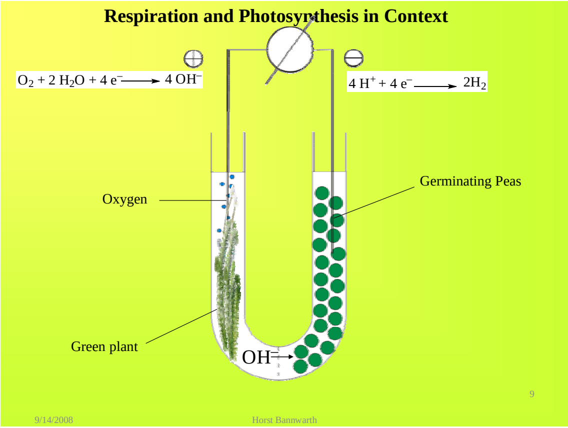

Figure 6: Photosynthesis and Respiration interconnected: Electric potential measurement

In figure 6 is illustrated the experimental combination of photosynthesis (left) with respiration (right).

Photosynthesizing water plants produce oxygen O_2 and respirating peas consume oxygen O_2. By this reason on the left side a plus pole and on the right side a minus pole is developed**.**

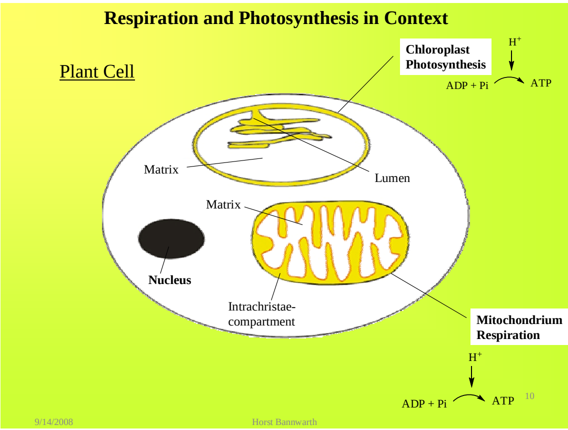

Figure 7: Chloroplasts and mitochondria: Compartments and ATP synthesis

Protons are pumped by the kinetic energy of the moved electrons into the gap between the two membranes (lumen of the thylacoids) in chloroplasts and in mitochondria and cannot be detected therefore in the external medium. This can be done with halobacteria. During the reflux of protons ATP is synthesized according to the chemiosmotic theory (Fig. 7).

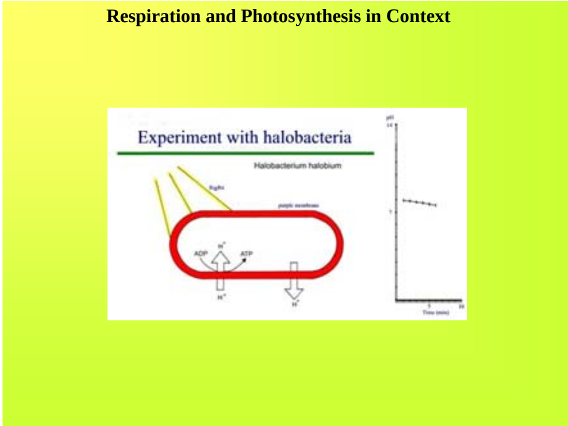

Figure 8: Halobacteria: Light driven proton transport

Under the effect of the light electrons are moved. With the kinetic energy of electrons protons are exported from the cells of the halobacteria (light - driven proton transport).Thus the concentration of hydrogen ions (H^+-ions) rises in the medium and the pH value decreases (Fig. 8). During the reflux of protons ATP is synthesized (left). The light driven proton transport in halobacteria corresponds to the light driven proton transport in chloroplasts in eukaryotic plants. The pH value drops slightly with illumination of the culture containing halobacteria in the course of 1 hour (right).

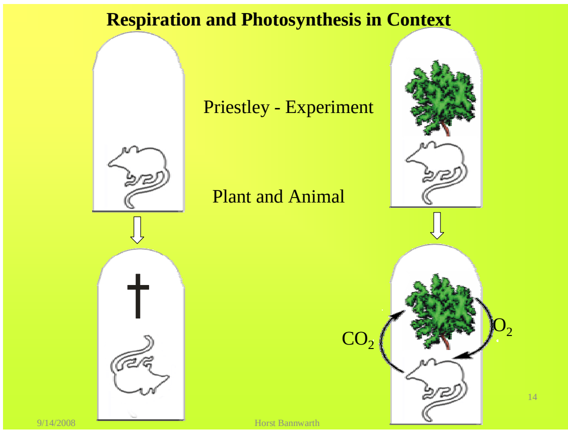

Figure 9: Priestley-experiment

Joseph Priestley (1733 - 1804) described the dependency of animal life from plant metabolism by simple experiments (Fig. 9). This experiment is often used in schools for the introduction of the relationship of photosynthesis and respiration in order to clarify the dependency of heterotrophic animals and autotrophic plants from each other. The disadvantage of this

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approach according to Priestley is that this experiment cannot be carried out in schools.

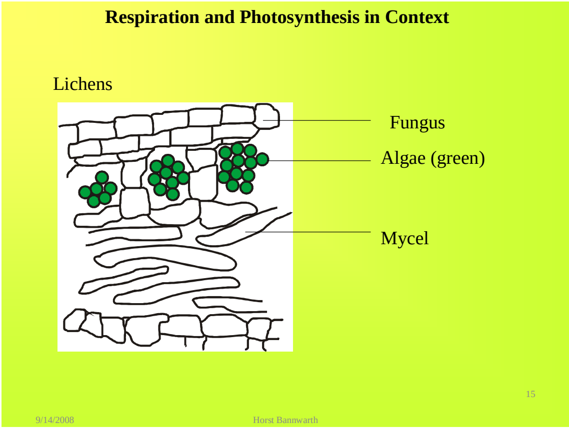

Figure 10: Lichens - symbiosis between alga and fungus

The heterotrophic organism in this case is the fungus which provides carbon dioxide and obtains vice versa oxygen and organic compounds from the autotrophic alga (Fig. 10). The alga on the other hand is protected by the fungus by retaining water and keeping wet. Photosynthesis of algae and respiration of fungus are integrated in a living system.

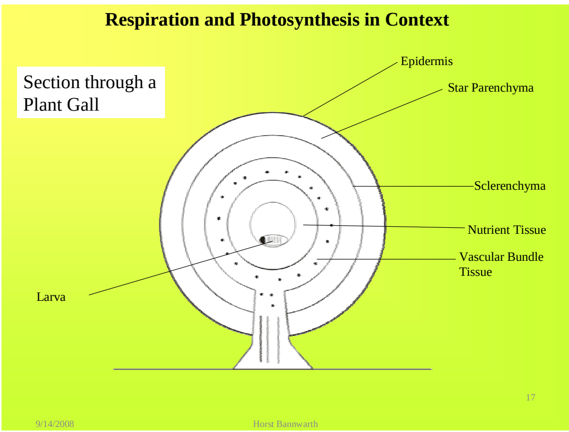

Figure 11: Plant galls - cooperating organisms

The advantage is clearly on the side of the insect larva. It is uncertain that the tree leaf profits from hosting the insect (Fig. 11). But it should be emphasized that this is not a parasitic relationship because the insect larva does not normally damage the leaf of the tree. Moreover the plant offers all the animal needs for life and development. This situation is completely different from a parasitic relationship, especially since the plant supports and sustains the foreign animal organism and does not attack it. In this case plant photosynthesis and animal respiration are integrated in an emergent biological system.

5. Conclusions

1. Instructions about photosynthesis and respiration can be combined by simple experiments.
2. By these experiments it could be clarified that both are opposite physiological processes.
3. The idea of emergency in the modern natural sciences is promoted by the concept of regarding photosynthesis and respiration in context.
4. Structure-function-relations of photosynthesis and respiration may be better understood by a common treatment.
5. The inclusion of chemical and physical aspects into a new concept of emergence may favour the biological understanding.

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