

GRANDPA'S BIOLOGY

Modern biology rests on two observations:

1. Every living organism is constituted by cells. Better: it's always initially constituted from a cell which, by successive divisions, gives birth to a multitude of other cells all possessing the same nucleus. This way of envisaging things is called "cellular biology".
2. By burning, every living organism releases carbon dioxide, water vapor, mineral salts and energy. It may thus be considered as the visible result of a multitude of chemical reactions, based on the carbon chemistry. This way of envisaging things is called "molecular biology".

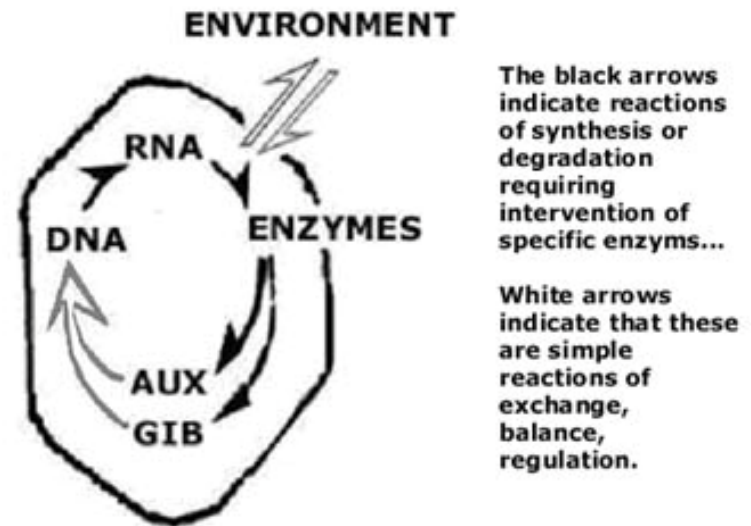
In recent decades, advances in research and new technologies brought in these two fields a huge number of data difficult to interpret, for now stored in databases. And the great challenge posed to the scientific community is to develop a new approach to biology, allowing us to benefit from all this data.

To meet this challenge, the solution unanimously proposed today is called "systems biology"..., biologists, computer scientists, mathematicians, physicists, biochemists and statisticians work together in order to better understand, thanks to computer and nanotechnology, the functioning of living systems, starting with the simplest of them: the cell ...

I am happy today introduce you to another approach to the problem. At once molecular and cellular, this approach rests on two techniques our scientists may not have used much of: reflection and good sense. Its name: "Grandpa's biology". Does it prefigure the biology of the future? It's simply what I hope...

Grandpa's biology represents a global approach to the functioning of the living organism, which differs from today's concept of biology by the fact that the notions of balance and regulation are considered as two sides of the same problem. It can be condensed as such:

A living organism is in good health (it passes then normally from egg to embryo, from embryo to youth, from youth to adulthood, old age and death) so long as the physiological activity of its cells is normal...



It is ill when this same physiological activity changes from the norm. And that for various reasons:

- reasons which are tied to the nature of certain genes, as in the case of genetic diseases,
- reasons which are tied to the nature of certain factors of the environment, as in the case of physiological diseases,
- reasons which are tied to the presence of pathological organisms (viruses, fungi, bacteria, animals), as in the case of infectious diseases.

With Grandpa's biology in effect, all that happens within a living organism is directly affected by a very precise hormonal balance. In the plants, the balance auxins/gibberellins (AUX/GIB). This balance conditions all reactions of synthesis and degradation by controlling the enzyme synthesis. In consequence, it conditions too in the short run the whole cells activity, in the long run the whole plant evolution. And that in function of the changes that occur with regards to the tissues, the age, the physiological state of the plant, with regards too to the environment conditions

What really happens? If you want to know more, consult the various pages of the site. It's free so long you are interested on a personal basis. If it is professional curiosity, You can write to us at the following address: labiologiedepapy@hotmail.fr.

SOME CONCEPTS TO RECALL

BIOLOGY

If I open my dictionary, I read the following: "As a life science, biology concerns all aspects of life, from biochemical reactions to life in society. Since the subject is very complex, every aspect of this study has been covered by specific fields of expertise: biochemistry, cytology, histology, physiology, genetics, etc., each of which has its own goals, methods and techniques. At present, under the heading of biology (general), only

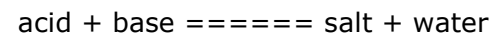
fundamental vital phenomena are considered, such as chemical constitution, structures and physiology of cells as well as the organisation and general functions of living beings". In brief, and seen from this angle, the approach to living organisms taken by biology today is based essentially on two observations:

1. a living organism burns, releasing carbon dioxide, water vapour, mineral salts (ashes) and energy. It can therefore be considered to be the visible result of a multitude of chemical reactions, based on carbon chemistry. When we take this approach, we do "molecular biology"...
2. a living organism is composed of cells. Even better: from the start it consists of one cell, which, through successive division, gives rise to a multitude of other cells which all have the same nucleus... Two cases can be envisaged here: After each cell division:
 - the "daughter cells" split and go their separate ways. This is a single-celled organism.
 - the "daughter cells" remain attached to each other, organise into a more or less complex system which is the only one capable of guaranteeing perpetuation of the species. This is a multicellular-organism. The approach which gives priority to this aspect is called- "cell biology".

THE CONCEPT OF CHEMICAL REACTION

According to the dictionary, "a chemical reaction is the transformation of one chemical species into another. It is characterised by an exchange of molecules, atoms, ions or electrons; the bonds between the atoms of molecules being formed must be more stable than those of the initial molecules".

"Some of these reactions do not require any initial external contribution of energy. They occur spontaneously both inside and outside the living organism. Non-specific and reversible, they lead to a balanced state which varies according to temperature and pressure". e.g.:



"Other chemical reactions require an additional external supply of energy and therefore require the intervention of specific catalysts. They are neither spontaneous nor reversible (at least not naturally). In the living organism, they are catalysed by enzymes, large molecules which are synthesised by cells and the activity subjected to environmental influence. Known as "enzymatic reactions", these are highly specific and are involved either in a process of synthesis (1) or breakdown" (2). e.g.:

1. $6\text{CO}_2 + 6\text{H}_2\text{O} + \text{energy} \rightarrow \text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2$
2. $\text{C}_6\text{H}_{12}\text{O}_6 + 6\text{O}_2 \rightarrow 6\text{CO}_2 + 6\text{H}_2\text{O} + \text{energy}$

N.B. In the approach to biology taken here (Grandpa's biology), the first type of reactions will be considered to be regulatory reactions, which seems quite logical because, in the world around us, a system which is not in balance has no other choice but to restore this balance or throw in the towel.

THE CONCEPT OF A CELL

We are accustomed to comparing a cell with a tiny chemical factory, the floor, walls and roof of which are represented by the cell walls..., walls whose permeability acts as doors, windows, chimneys and other waste disposal channels.

This factory is fully computerised. Its activity is governed by a sort of computer called the nucleus. In this nucleus there is a series of diskettes which are usually in pairs: the chromosomes.

These chromosomes hold more or less large quantities of encoded information, the genes; the code used is therefore called the "genetic code". These genes, which vary in number from a few hundred in the simplest organisms to several tens of thousands in the most complex organisms, therefore represent the organism's genetic programme:
- A programme which is transmitted from one generation to the next and is found in every cell in the organism (genes double in number each time a cell divides);

- A programme which, outside periods of cell division, conditions everything that happens in the cell including enzyme synthesis.

Outside periods of cell division, genes are generally inactive. They only become active at certain times, depending on the data provided by the cell (today we consider that certain hormones may play an important part). They therefore act as the nucleus' random access memory (RAM), which conditions all cell activity in the short term and the entire evolution of the organism in the long term.

DNA (DEOXYRIBONUCLEIC ACID)

We have compared the cell nucleus to a computer with its memory represented by genes. Genes consist of DNA. They vary in number from several hundred in the simplest organisms to 30 or 50 000 in humans. They carry all the information the organism needs to grow from a single cell (the egg) to the embryo, from the embryo to childhood, from childhood to adult status, to old age and death.

This DNA is in the form of long molecules organised in double strands, a bit like flies. These flies are rather special though because they have two types of buttons and button-holes, positioned on either of the strands of the double chain, but always matching: the famous Adenine/Thymine and Guanine/Cytosine groups, known as AT and GC, which are based on the attraction between a positively charged hydrogen ion (H⁺) and two negatively charged molecules.

Like all self-respecting flies, the double DNA chains are usually closed: the genes are then called inactive or repressed. Like all self-respecting flies, these same double chains open from time to time: the genes are then called active or derepressed. Why? Because on opening, enzymes called RNA-polymerases can synthesise a few molecules of RNA, a substance similar to DNA which we are going to talk about now.

RNA (RIBONUCLEIC ACIDS)

RNA is in the form of long molecules with one strand, replicas of one of the strands of the double DNA chains, from which they are synthesised. These newly synthesised molecules will migrate to the closest structures in the nucleus, the ribosomes, where the genetic information they carry will be decoded and physically transcribed into proteins.

Because its double chain structure is based on the existence of AT and GC groups, the DNA molecule (the cache memory of this nucleus-computer) is binary in nature, which it is difficult to ignore completely if we are to understand its mode of action (see later). The problem with RNA (this computer's RAM) is different, on the other hand. This is a single-strand molecule, characterised by a sequence of four molecules (A, T, G, C). e.g.:

DNA 117	ATGCCGTATAATGATCGTTTTCGATAATG TACGGCATATTACTAGCAAAGCTATTAC
RNA 117	TACGGCATATTACTAGCAAAGCTATTAC

PROTEINS AND ENZYMES

Proteins are large twisted, folded molecules consisting of twenty different amino acids. They have a primary, secondary and tertiary structure which makes them highly specific, from both a chemical and physiological point of view. They are classified in three categories depending on their size, solubility and the part they play in the organism: structural proteins, reserve proteins, active proteins or enzymes. These last ones interest us here.

Relatively small, soluble and mobile, enzymes are sort of organic catalysts. They each initiate a very specific chemical reaction inside the

cell. These are enzymatic reactions. They are always part of one of the many synthetic or respiratory processes, which, in the short term, will determine all the body's activity, and in the longer term, its entire evolution (growth, differentiation, reproduction, senescence, sensitivity or resistance to one disease or another). This is shown in the well-known diagram:

DNA ----- RNA ----- ENZYMES ----- CHEMICAL REACTIONS

It only remains to note something obvious here. For one of these synthetic or respiratory reactions to take place, two things are essential:

- the corresponding enzyme must obviously be present in the cell (in other words, it must have been synthesised by the nucleus);
- it must also be active.

An old philosophical principle states that "we act as we are", and enzymes, because of their convoluted structure, their size (relatively small) and their solubility (relatively high), are extremely sensitive to temperature, light, humidity, pH and obviously the nutrition reigning in the cell. Thus:

- an enzyme which is part of the cellulase group can only transform saccharose into cellulose if there is saccharose in the cell;
- it may be active at 25°C, inactive at 18 or 30°C;
- it may be active during the day and inactive at night, if it has a prosthetic group which is a colour pigment excited by certain wavelengths of light (this does not appear to be the case with cellulases but the principle still applies);
- it may be active when the body has enough water, and this activity may slow down or stop during dry periods;
- finally it may be active at pH 6 and inactive at pH 7.

All these phenomena which regulate enzyme activity are therefore essentially concerned with balance. We shall see that, in Grandpa's biology the same applies to events which control enzyme synthesis.

THE CONCEPT OF PLANTS HORMONES

If I open my dictionary once more, I can now read: "By analogy, substances produced by plants which are essential to their growth are known as plant hormones". This analysis of the problem given by our stout dictionary is a bit lightweight and it may be useful to add the following:

In the plant kingdom, there are two well known types of hormones: the auxins, produced by nitrogen metabolism, and the gibberellins produced by carbon metabolism. Simple molecules, characterised by an unsaturated cyclic nucleus, and relatively easy to synthesise, they are found commercially in the form of powders which are soluble in alcohol.

Auxins and gibberellins have two characteristics in common:

- they move around the plant and meet in all the plant tissues;
- they always act everywhere and at all levels, this action varying with the dose used, the age and physiological condition of the plant, environmental conditions and hormones already found in the tissues.

'Always' means during growth, differentiation, reproduction, senescence, sensitivity or resistance to this or that disease. 'Everywhere' means in the roots, stems, leaves, flowers and fruit. 'At all levels' means in basic and intermediate metabolism and in the plant phenotype.

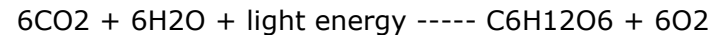
The most well-known auxin is indolacetic acid or IAA. The most well-known gibberellin is gibberellic acid or GA. Their structures and synthetic chains lead us to believe that they are close to animal serotonin and steroid hormones respectively.

THE SUGAR / NITROGEN RATIO

Young plants are low in sugars and rich in nitrogen, whereas old plants are poor in nitrogen and rich in sugars. Is this the result of natural selection, since young plants need large amounts of nitrogen for their growth? Are old plants naturally diabetic? I really don't know. Let's say

it's like that and leave it at that. What is certain is that this phenomenon conditions most methods of cultivation, in agriculture (in general) and in arboriculture (in particular), with the aim of obtaining the best possible harvest. Given that young trees do not produce fruit and that old trees produce less and less..., it is easy to conclude that trees need to be kept in a state of production, i.e. in a physiological condition which is midway between youth and age. In other words, we must be sure that their tissues are neither too rich nor too poor in sugars and neither too rich nor too poor in nitrogen.

When winter comes and the sap is no longer rising so fast, the tree grower takes his secateurs and starts pruning the trees. Does he need firewood? Not really. He does it because, by removing part of the branches, he also removes the leaves which would start growing on these branches in the spring. Leaves are green because they contain chlorophyll, a pigment which captures light energy to fuel sugar synthesis using carbon dioxide from the air which is absorbed at the stomata, and water from the ground absorbed by the roots. This takes place as shown in the well-known equation:



In fact, by pruning the trees more or less severely, the tree grower tries to control the tree's ability to synthesise sugars, depending on its age, physiological condition and the type of cultivation practised. He won't prune his trees the same way if he wants trees which go into production rapidly (small trees with a relatively short life) or bigger trees which start production later but live longer..., depending on whether the trees are young or old or whether the soil in which they are planted is more or less rich in nitrogen... etc.

Remember that for plants, like all other living organisms, sugars are not only the source of energy they need for physiological activity, but also the basic substances essential for the production of all other organic components (cellulose, lignin, fats, proteins, nucleic acids, etc.)

And then in spring, he adds fertiliser to the soil. Growth starts again. The roots must now find in the soil all the mineral elements the plants need, particularly nitrogen, an essential complement to sugars for the production of proteins, nucleic acids and other nitrogenous substances.

Not too much, but just enough for growth in a young plant and a good harvest once it is adult, or to rejuvenate an ageing tree.

I have included this paragraph on the importance of the sugar/nitrogen ratio in agriculture because Grandpa used it to study the influence of hormones on plant development.

GRANDPA'S HYPOTHESIS

This hypothesis concerns the mode of action of auxins and gibberellins, these mysterious substances known as "growth hormones" which act always, everywhere and at all levels, of which we have already spoken. It is summarised as follows:

"Auxins and gibberellins, plant hormones of which only the various mobile forms are known to us, once they reach their destination in the plant, are thought to act on DNA AT and GC groups, their role being to selectively open the double DNA chains (gene activation) by breaking the H+ bonds which provide stability. This can be expressed as follows: at a given moment in a given cell, the only active genes are those with a structure (AT/GC ratio) which matches the auxin/gibberellin equilibrium reigning at that moment in that cell".

THREE PRELIMINARY REMARKS

This hypothesis in no way prejudices the mode of action of other plant hormones, substances which are not chemically related to the two hormones envisaged here, and which therefore necessarily have a different activity.

Neither does it prejudice groups on which auxins and gibberellins are thought to act, or the fact that this action concerns the entire length of the DNA double chains rather than their initial part, known as an "operator" in molecular biology. It is strictly incapable of this and in any case, it is not important at present.

Finally, for convenience, from now on the term "hormones" will only apply to active forms of auxins and gibberellins, which are the substances concerned here. Their mobile forms will be called "free hormones".

WHY HAVE THIS HYPOTHESIS?

We have seen that the fact that a plant is treated with an auxin or a gibberellin causes deep modifications to its growth, differentiation, reproduction, senescence, sensitivity or resistance to disease of various kinds... , all phenomena result from a multitude of enzyme reactions. Like many others at the time, Grandpa thought that the action of these substances could not be explained without involving either all the enzymes or at least a large number of them.

However, there was something about this type of evidence that bothered the dear man: the contradiction between the non-specific nature of auxins and gibberellins and the specificity of enzymes. In the infinite diversity of enzymatic structures, how could the sites of action be located, these chemical groups which are always similar, in such a way

as to explain how non-specific hormone actions are performed at these locations?

He therefore came to the following conclusion: since direct action of auxins and gibberellins on enzymes is difficult to envisage, this action, since it must take place, must be indirect. Auxin and gibberellin should therefore act on substances with a non-specific structure, substances which must also reveal the infinite diversity of enzyme structures.

He thought that the choice was limited, in this case, and that it was necessary to go through DNA, its double helix structure and more precisely the AT and GC groups directly involved in enzyme synthesis.

This led to the previous hypothesis and the following diagrams to which we shall refer constantly from now on, because they form the basis of what we must refer to as "Grandpa's biology".

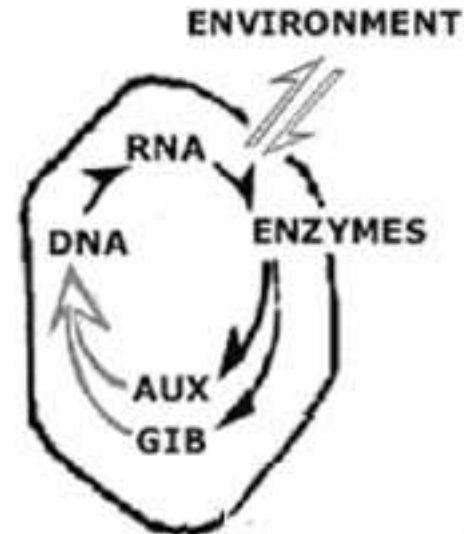
IF YOU HAVE THE CAT'S TAIL, YOU HAVE THE ENTIRE CAT...

For Grandpa, this hypothesis could only be right or wrong: it was right if the auxins and gibberellins really act on DNA AT and GC groups, their role being to control gene activity. It was wrong in any other case.

If it was wrong, it would simply be another hypothesis to be discarded. If it was right, on the other hand, then it would be something really important, because it would lead to a better understanding, not only of the mode of action of the two main plant hormones, but also of cell regulation phenomena, cell function, plant function, concepts relating to health and sickness, different types of disease and doubtless many other things as well.

If the hypothesis proved correct, it could be said without much risk of being mistaken:

CONCERNING UNICELLULAR ORGANISMS



The black arrows indicate reactions of synthesis or degradation requiring intervention of specific enzymes...

White arrows indicate that these are simple reactions of exchange, balance, regulation.

1. At any given time in a given cell, only genes with a structure (AT/GC group ratio) matching auxin/gibberellin balance reigning at that time in that cell would be active. If this balance changes, these genes lose their active status to other genes with structures matching the new balance now found in the cell. This gives rise to the following:
 - everything that happens in a given cell at a given moment is directly linked to the auxin/gibberellin balance at that moment in that cell;
 - everything that happens in a given cell over a given time is directly linked to the evolution of the auxin/gibberellin balance in that cell during that same time.

2. Regulation of cell activity provided by these two hormones cannot be absolute. It concerns only enzyme synthesis, the activity of the enzymes in question being subject to the influence of the environment (the cell environment, itself influenced by the external environment).

3. Like any self-respecting metabolic operation, hormone balance results from complex enzyme activity (gibberellins are produced by carbon metabolism, auxins by nitrogen metabolism). It is therefore a sort of reflection of what is happening in the organism, considering its age, physiological status and environmental conditions...

4. This little nucleus computer does not regulate enzyme synthesis simply by following a predefined programme of events (the genetic programme). It takes into account information provided by the AUX/GIB balance in the evolution stage of the cell, its state of health and the environmental conditions.

CONCERNING MULTICELLULAR ORGANISMS

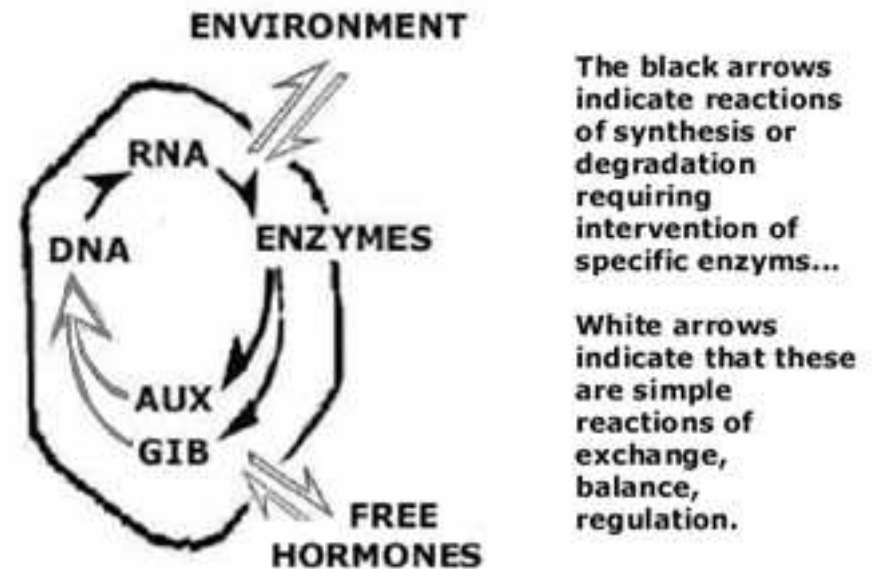
5. - everything that happens at a given moment in a given plant is directly linked to the auxin/gibberellin balance at that moment in each of this plant's cells.

- everything that happens in a given plant over a given time is directly linked to the evolution of the auxin/gibberellin balance in each of this plant's cells during that same time.

6. Unlike what happens in unicellular organisms, the hormone balance in the cell does not only reflect cell metabolism. It largely depends on free hormones, also, in its way, reflecting what happens in all the plant's other cells. These billions of tiny computers which are the cell nuclei and are strictly identical within the same plant, are connected together in a

form of network, just like the computers used by bank or business employees.

7. The roots therefore take into account the variations in temperature or light to which the aerial parts of the plant are subject. The stems or leaves react to changes in soil humidity, fertiliser supply or the damage done by a pathogenic fungus attacking the roots.



8. We can finally add this: every organism (uni- or multicellular) not meeting these conditions will be eliminated by natural selection, unless there are exceptional circumstances enabling it to bypass the problem (as in certain cases of symbiosis, parasitism or human intervention).

It remains now to discover how closely this theory matches reality. This leads to a new question: how can it be verified?

How to verify this hypothesis ?

To verify this hypothesis, Grandpa proposed the following experiment: a series of identical young plants would be split into batches of five or six individual plants. The first batch would then be treated with water, the others with solutions of auxin or gibberellin at equivalent and increasingly strong concentrations. He would then wait for long enough for the effects of treatment to be visible to the naked eye (it is better to avoid statistics), ensuring that all the plants received the same environmental conditions.

At the end of the experiment, leaves and roots would be taken from each batch for analysis... analysis of what? We shall see shortly. In any case, whether the hypothesis was right or wrong, the evolution of the control plants would show what would normally have happened in all these plants, in both basic metabolism (gene activity, RNA and enzyme synthesis) and intermediate metabolism (synthesis and breakdown of sugars, fats and proteins) or the plant phenotype (growth, differentiation, reproduction, etc.) There could be one of two results:

1. The hypothesis is right. The auxins and gibberellins really do act on DNA AT and GC groups, thereby controlling enzyme synthesis
 - Everything that happened in the control plants during this experiment was therefore linked to the evolution of a complex endogenous auxin/gibberellin balance;
 - The different treatments modified this natural auxin/gibberellin balance in favour of auxins or gibberellins, this modification being more intense the stronger the dose of treatment used;
 - And the results obtained clearly demonstrated that, when treatment with auxin is "white", treatment with gibberellin is "black", the reaction being more marked with increasingly strong doses.
2. The hypothesis is wrong. Auxins and gibberellins do not act on DNA AT and GC groups. What happened in the control plants is not linked to the evolution of endogenous hormone balance. IAA and GA treatments

do not alter this balance. A miracle would be needed to make the results obtained match the hoped for results.

The director, who did not believe in miracles, agreed. The equipment then had to be chosen. Grandpa had no preferences. If the hypothesis was right, it should be verified in every case and under all kinds of conditions. The director would therefore decide. His choice was to use a variety of tomato called Supermamide, sensitive to fusariosis, from which he could obtain selected, homogenous seeds, and one pathogenic fungus, *Fusarium lycopersici*. This was a plant physiopathology laboratory, so the hypothesis would not be verified by growth but by the evolution of host/parasite relations.

FIRST TESTS

One month later, the plants were ready. Grandpa went to work, but without any great success at first. It was summer and successive experiments only gave mixed results. One month before the photos were taken, all the plants were absolutely identical. They had reached the "4-6 leaves" stage and were ready for the experiment. They were depotted and their roots were carefully washed. The plants in the first photo were then simply repotted: these were the control plants, free of any disease. The plants in the second photo were inoculated by soaking the roots in a suspension of fusarium spores before repotting. *Fusarium* is a fungus which normally lives in the soil. It penetrates plants via the

roots and gradually colonises all the other organs via the sap carrying vessels. This is a particularly suitable means of access: progress is easy, food is choice and abundant... so the method of inoculation described above was most appropriate. The next day, diseased and healthy plants were split into nine separate batches and each batch was treated by spraying the leaves as follows:



GA - IAA

- the first batch with water, in other words, no treatment at all, to avoid modifying the plant's natural hormone balance. These are the plants shown in the centre of each photo, which are called "control plants".
- the others with increasing doses of IAA, the best-known auxin (towards the right starting from the control plant) and GA, the most representative gibberellin (towards the left starting from the same control plant) so as to alter the plants' natural auxin / gibberellin balance more and more strongly, in favour of either auxins (right-hand plants) or gibberellins (left-hand plants).

Result: Although the influence of the various treatments on the phenotypic aspect of healthy plants was not surprising (it matched the descriptions found in the literature in every way, photo n° 1), although it may also confirm the hypothesis in the case of diseased plants treated with gibberellin (left-hand plants in photo n° 2, the visible effects of disease were attenuated until they had completely disappeared), on the other hand it raised serious questions in the case of plants inoculated with auxin, the various treatments remaining apparently without effect on the evolution of the disease (right-hand plants in the same photo).

However, at the end of October, things changed. The last experiment finally gave the expected results. It was autumn. Environmental conditions were less favourable to plant growth as well as development of the parasite. Even though the GA treatments (left-hand plants in photo n° 4) still showed better tolerance to the disease, IAA treatments (right-hand plants) now also showed that they were effective in promoting the fusarium attack.



GA - IAA

One incident occurred: during these last experiments: one plant inoculated with fusarium was accidentally contaminated with tobacco mosaic virus. Whereas the other plants in the same batch displayed classic symptoms of fusariosis (small size, leaf epinasty), the plant which had been secondarily inoculated with tobacco mosaic virus only differed from the healthy controls in the mosaic leaf symptoms (yellow and green patches juxtaposed, reminiscent of a mosaic). One month later, the leaves suddenly turned yellow and fell off. The sap carrying vessels

in the stems and roots had turned brown, which is characteristic of plants suffering from fusariosis.

PLANT EVOLUTION

Encouraged by these results, Grandpa now decided to push the envelope a little further; but in which direction? Studying what happens at basic levels (DNA, RNA, enzymes) was not really in his field. He therefore decided to work with the intermediate metabolism, more specifically, by studying nitrogen and sugar concentrations in the plants... which, with a bit of luck, would not be too difficult. We know that sugar and nitrogen levels in plant tissues evolve according to the plant's age and physiological condition. It is easy to imagine that this evolution of the environment in which the parasite is developing is the cause of the phenomena observed. In 1953, R.W. LEWIS summarised the situation in these words: "The possible combinations of Foods present plus the capacity of the parasite to respond differently to these combinations account for the varying degrees of resistance and susceptibility not explicable in other terms. One can imagine combinations of metabolites that would permit any intermediate condition between complete resistance and complete susceptibility". So this was the direction chosen for research to continue.

I am not going to describe this laborious work in details. If you are interested, you can contact the director of the INRA who will, I'm sure,

be delighted to go through the archives to give you the information you want. Let's just say that this work, seen from the point of view of Papy's biology, from what was known about the evolution of sugar/nitrogen ratios according to the age of the plants and data provided at the time in the literature, on the hormone content of various plant organs, led to the following conclusions:

- IAA treatment slowed the natural development of leaves and accelerated that of roots (it makes leaves younger and roots older);
- GA treatment had the opposite effect in every case, which was more marked the higher the dose of hormone given. e.g.

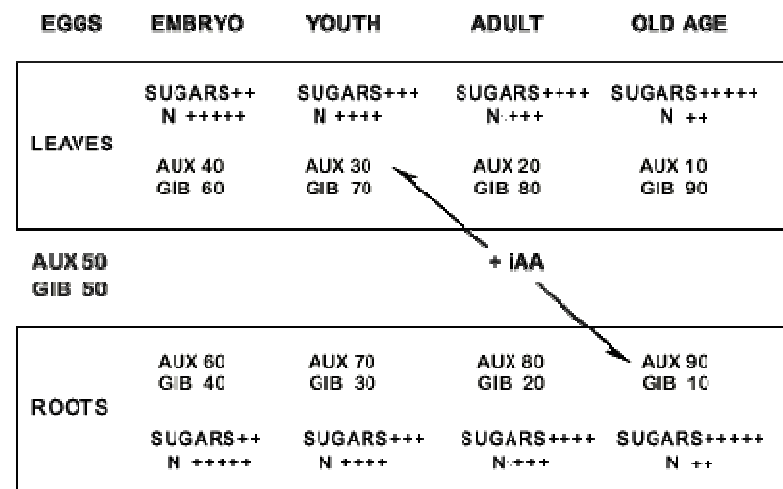
Concentration of sugars and nitrogen in a control serie, assayed at the beginning and end of the experiment, expressed in mg per gram of dry material

TREATMENTS		LEAVES	ROOTS
IAA		25 ----- 43	20 ----- 36
0	SUGARS	25 ----- 53	20 ----- 25
GA		25 ----- 64	20 ----- 16
IAA		52 ----- 44	33 ----- 30
0	NITROGEN	52 ----- 42	33 ----- 31
GA		52 ----- 40	33 ----- 32

These observations are not surprising. They confirm the data given in the literature, according to which senescence is reflected:

- in the leaves, by hypoauxinia (in terms of balance, a decrease in auxin concentration linked to an increase in gibberellin concentration);
- in the roots, by hyperauxinia (in terms of balance, an increase in auxin concentration linked to a decrease in gibberellin concentration).

All this may appear complicated, so here is a diagram giving all this data to help understand why IAA treatment can both "rejuvenate" the leaves and "age" the roots.



This diagram is based on the following principle: every plant starts as a single cell in which there can only be one auxin/gibberellin balance and one sugar/nitrogen ratio. In view of the previous results, it may now be thought that this auxin/gibberellin balance experiences a double evolution from the first cell divisions:

- evolution in favour of gibberellins in cells which will give rise to the aerial parts of the plant;
- evolution in favour of auxins in cells which will give rise to roots.

The gradual growth of the plant's aerial parts from egg to embryo, embryo to youth, youth to adulthood, old age and death would then correspond to a gradual reading of half the genome, linked to the evolution of hormone balance in favour of gibberellins and leading to the hypoauxinia revealed in old leaves.

The gradual growth of the roots from egg to embryo, etc. would correspond to a gradual reading of the other half of the genome, linked to the evolution of hormone balance in favour of auxins and leading to the hyperauxinia revealed in old roots.

HOST PARASITE RELATIONS

The host-parasite relations observed between the plant and the fusarium on the one hand and the plant and tobacco mosaic virus on the other still has to be explained, the conjunction of these phenomena theoretically providing an understanding of what happened in the case of the plant inoculated with both parasites.

If you look at diagrams on cell function, it can be considered that a plant is in good health if its physiological activity is normal and that it is sick if this same activity deviates from normal for various reasons:

- reasons linked to the nature of certain genes in the event of genetic diseases,
- reasons linked to the type of environment in the event of physiological diseases,
- reasons linked to the presence of a pathogenic agent (animal, bacterium, fungus, virus) in the event of infectious diseases.

It is the third type of disease which interests us, the approach preferred by Papy, in this case based on the following idea: the plant represents the environment in which the fungus and the virus developed:

- an environment which evolves, this evolution being altered by appropriate hormone treatments;
- an environment, the evolution of which will be affected by the two parasites.
- an environment, the evolution of which will also affect the two parasites

This led to the following two facets of the studies:

- the influence exerted by the parasite on the plant-host, particularly on the evolution of sugar and nitrogen concentrations in the leaves and roots during experiments, depending on IAA and GA treatments of course, and in relation with the data provided by the literature on the hormonal imbalance of diseased tissues;
- the influence exerted by the plant-host, in other words the influence of the environment in which the fungus and virus develop, on the evolution of the two parasites.

One more remark: according to the literature available at the end of the 1960's:

- fusariosis was thought to cause hyperauxinia in leaves and hypoauxinia in roots. In view of the results obtained from plants free of disease (chap. 8), it should therefore slow the evolution of the sugar/nitrogen ratio in diseased plants;
- the tobacco mosaic virus was thought to cause hypoauxinia in leaves. It should therefore accelerate the physiological evolution of these organs;
- no information on what happens to roots in this case could be found, nor on gibberellin concentration in diseased tissues, whether infected by the virus.

ACTION OF FUSARIUM ON THE PLANT

Evolution of sugars and nitrogen concentrations in healthy and diseased leaves assayed at the start and end of the experiment in a "fusariosis" series, expressed in mg per gram of dry materia. I e.g.

Sugar concentrations in healthy and diseased leaves

GA	+++	14	72	14	46
GA	+	14	69	14	53
-	-	14	60	14	72
IAA	+	14	48	14	88
IAA	+++	14	43	14	60

Corresponding nitrogen concentrations

GA	+++	57	24,4	57	35,7
GA	+	57	24,8	57	30,5
-	-	57	26,0	57	29,0
IAA	+	57	28,7	57	28,6
IAA	+++	57	33,0	57	32,2

Remark n° 1: In control leaves, the sugars concentration increases with the age of the plant, whereas the N concentration falls, these two phenomena being all the more sensitive the higher the dose of GA, and less sensitive the higher the dose of IAA. This appears to be normal, in view of the results already obtained.

Remark n° 2: In diseased plants treated with GA (the least affected), these same sugars and nitrogen concentrations evolve in the same way as in corresponding healthy plants, but more slowly. This leads to the idea that the diseased leaves are younger and richer in auxins than the corresponding healthy leaves (see remark 1). This also matches the data given in the literature (leaves of plants suffering from fusariosis are characterised by hyperauxinia).

Remark n° 3: In untreated plants and plants treated with IAA (the most affected), the reverse is observed. Sugars concentrations assayed at the end of the experiments in diseased leaves, which were expected to be lower than sugars concentrations in control leaves, were found to be

distinctly higher. As for nitrogen concentrations, which were expected to be slightly higher, they were found to be virtually the same.

In the end, these results indicate:

- that initially the fusariosis slows the natural evolution of diseased leaves, since these leaves are physiologically younger than the control leaves (plants treated with GA);
- that the reverse then occurs, with diseased leaves suddenly ageing (untreated plants and plants treated with IAA).

Why does the diseased plant first show signs of rejuvenation? Why does it then suddenly age? If you want to know the answer to these existential questions, read the next chapter.

N.B. Nitrogen was assayed here using Kjeldahl's method, which takes into account any organic nitrogen in the tissues: ammonium ions, amino acids, nucleic acids, proteins of all kinds etc. which necessarily involves slow evolution of this element in the plant. The same does not apply to sugars, the method used only taking free sugars into account (sugars found in large molecules: starch, cellulose, lignin, nucleic acids, etc. are not assayed), which gives a much faster evolution of this element.

ACTION OF THE PLANT ON FUNGUS

Through curiosity, Grandpa went on to study the behaviour of fusarium in an artificial liquid medium. A simple medium, in which he varied sugar (glucose) and nitrogen (ammonium salt) concentrations in opposite ways, as occurred in the plant. This gave the following results:

MONDAY			INOCULATION		
TUESDAY					
WEDNESDAY					
THURSDAY					
FRIDAY					
SATURDAY					
SUNDAY					
MONDAY					
TUESDAY					
WEDNESDAY					
THURSDAY					
FRIDAY					
SATURDAY			END		

During the first few days, the fungus developed normally in all the media, with the cultures displaying a white, fluffy appearance. On the morning of the fourth day there was a surprise: the four flasks containing medium n° 1 (the richest in sugar and poorest in nitrogen) had turned bright red overnight, the others retaining their white appearance. On the morning of the fifth day, a second surprise was found: the four flasks containing medium n° 2 had also turned bright red. And night after night, medium to medium, the same thing happened. The last medium, very poor in sugar and rich in N, was the only exception to the rule. It retained its white appearance until the end of the experiment.

Microscopic study of the various media showed that in media very rich in sugar, the fusarium developed in an unusual way. Although it normally takes the form of long overlapping filaments (the mycelium), in this case it was in the form of small round, isolated cells, giving it the appearance of a yeast, the little fungus which transforms sugars into alcohol and is so useful to our winegrowers. And that is how, without really intending to, Grandpa may have found the answer to the previous two questions.

Question n° 1: why is the plant inoculated with fusarium initially rejuvenated?

Fusarium is a so-called "imperfect" fungus. It is imperfect because the only method of reproduction it is known to have is vegetative reproduction. This makes it unclassifiable, since fungi are classified according to their method of reproduction.

Microscopic observation then led to the next question: is this imperfect fungus related to yeasts? In other words, does it have a habit of transforming sugars into alcohol in the same way as yeasts do?

- which would explain the low level of sugars in diseased plants and the involuntary rejuvenation imposed by the parasite.
- which would also explain why plants treated with GA and rich in sugar, seem to adapt to its presence better than plants treated with IAA.

Question n° 2: why did the plant age suddenly later on?

Following the advice of the laboratory director, Grandpa also extracted and purified the pigment responsible for this sequence of surprises. This pigment was doubtless already known because it gives old cultures of fusarium a red-violet colour which is so routine that it goes unnoticed. This pigment, when purified, crystallises into long sharp needles. It has obvious colloidal properties: soluble in acid (it then takes on this red colour we mentioned), it flocculates in an alkaline medium, taking on a much less attractive purple colouring. In the end, a powerful oxidation-reduction agent synthesised by the ageing fungus, may be the source of

"vascular symptoms of fusariosis", the brown colouring taken on by the sap carrying vessels in plants suffering from fusariosis.

By contributing to the breakdown of conducting vessels in roots, this pigment may prevent these organs from functioning normally, thereby depriving the plant of N. By contributing to the breakdown of these same vessels in the stems, does it promote the increase in sugar concentrations observed in the leaves of the most affected plants, the sugars synthesised in these organs being unable to migrate normally to other tissues? Since the plant or fungus cannot be asked, Grandpa had to be content to leave a question mark hanging over these possibilities, which was perfectly logical.

Remark: One thing is certain: the media used above were much less rich in sugar at the end than at the beginning of the experiment, growth of the fungus alone being inadequate to explain the intensity of the reaction. Grandpa tried to perform alcohol assays, but did not manage to assay anything at all.

ACTION OF THE VIRUS ON THE PLANT

What we just said about fusarium and its real or imagined propensity to transform sugars into alcohol will, in any case, be useful in understanding the tomato/TMV (tobacco mosaic virus) relationship.

Like all viruses, TMV consists of a nucleic acid and a protein envelope, two constituents which are very rich in N (160 mg per gram of dry material). To be able to grow, it has to find large quantities of nitrogen in its plant-host tissues. For example, it is estimated that in certain leaves affected by the virus, 80% of the assayed nitrogen is viral nitrogen.

This leads to two unavoidable consequences:

- N assays of diseased plants don't mean much, from our point of view here, since the method used assays not only plant tissue nitrogen but also viral nitrogen;
- tissues affected by the virus can be expected to be richer in sugars than corresponding healthy tissues, due the fall in nitrogen content in plant tissues resulting, logically, in proliferation of the virus and thus explaining the hypoauxinia characterising diseased leaves. e.g.

1. Sugars concentrations in healthy and diseased leaves in a "mosaic" series, assayed at the beginning and end of the experiment, expressed in mg/g of dry material

GA	25	64,8	25	71,4
-	25	53,0	25	59,7
IAA	25	42,6	25	47,3

2. Corresponding nitrogen concentrations

GA	52	40,6	52	40,5
-	52	42,2	52	41,7
IAA	52	43,7	52	44,0

In the control series, the concentration of sugars in the leaves increases with the age of the plant and N concentration falls. As usual, IAA slows and GA accelerates the process. The concentrations of sugar assayed at the end of the experiment in sick leaves were higher than those of the control leaves. N concentrations were apparently equivalent. Considering the presence of the virus, nitrogen concentrations corresponding to plant tissues alone are therefore doubtless lower than N concentrations in control leaves, which matches the expected results (see previous paragraph).

ACTION OF THE PLANT ON THE VIRUS (TMV)

To explain the influence of the plant on the virus, Grandpa would find it difficult to use the same approach as was used for fusarium. A virus does not develop in an artificial environment. It is an absolute parasite which cannot survive outside the living organism, and he does not see how to explain what happened starting from the tomato/TMV relationship.

He therefore chose a different host: tobacco. A plant which he knew and which seemed particularly appropriate to this type of study. The laboratory director and most of his team were studying the influence of temperature on host/parasite relations between tobacco and this same virus (hence the accidental contamination with TMV of a plant infected with fusariosis).

Tobacco has large lanceolate leaves covered with hairs. If a glass rod is dipped into a suspension of the virus and the epidermis of a leaf is rubbed gently with the rod in question, some of the hairs break, allowing the virus to penetrate the plant. Two different cases are then possible:

- Case n° 1. The virus multiplies gently and steadily. It starts by invading the leaf, then gradually colonises the whole plant, including the roots. Without causing any particular reaction, other than white or yellow patches on the surface of the leaves (which is why this disease is called a mosaic), slower growth and poor quality leaves which lose all commercial value. The plant is said to be sensitive or belonging to a variety sensitive to tobacco mosaic virus.

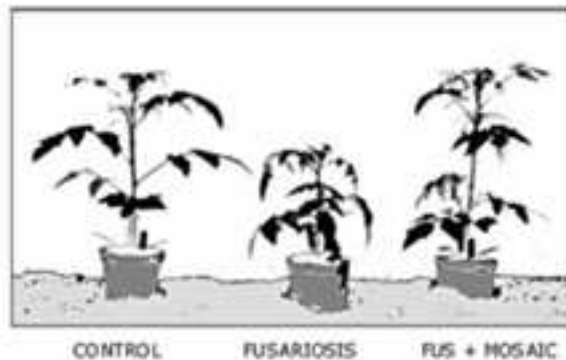
- Case n° 2. Three days after inoculation, at the places where the virus penetrated, small, brown, necrotic marks develop, about two millimetres in diameter. These marks are surrounded by a yellow halo in which the virus can be detected. However, this virus, relieved of its protein envelope, is in a latent condition, incapable of invading the neighbouring tissues, which remain green and healthy. This takes place at normal temperature, because if the plant is placed in a temperature of 30°C, the inactive virus starts to multiply and now invades the neighbouring tissues. This activity will last as long as the plant is at 30°C. If the plant is returned to a 20° C environment, the colonised tissues undergo necrosis with a yellow halo developing around the edges of the necrotic zones. This halo contains the virus, incapable of invasion etc. This plant is said to be hypersensitive to tobacco mosaic.

What is interesting about this story, is that there are three events taking place here, which normally succeed each other: green leaf, yellow leaf, dead leaf. The virus clearly finds a suitable environment in hypersensitive leaves, so that it multiplies very fast, so fast and so intensively that:

- the colonised tissues, literally emptied of their nitrogen, die of old age and undergo necrosis;
- the neighbouring tissues, also involved, are already so poor in nitrogen that they turn yellow, reveal a state of advanced senescence, explaining why the viral particles which have started to multiply cannot complete their growth. This is our TMV, half constituted and having lost all infectious ability, obliged to wait until an external event (such as an increase in temperature) stimulates the plant rejuvenation. With its compulsory corollary: a resurgence in nitrogen metabolism.

Is it really necessary to say that Grandpa collected several tobacco plants which nobody needed, to study their reaction to auxin and gibberellin and compare the N content of plants at 30°C with that of plants left at 20°C?

PLANT SUBJECTED TO ATTACK BY BOTH FUNGUS AND VIRUS.



Reminder: During one experiment, a plant inoculated with fusarium was accidentally contaminated with tobacco mosaic virus. Although the other plants in the same batch displayed the classic symptoms of fusariosis, the plant secondarily inoculated with the virus could not be distinguished externally from the healthy controls, other than by mosaic leaf symptoms. One month later the leaves suddenly turned yellow and fell off; the stems and roots displayed vascular symptoms of fusariosis.

The previous results logically lead to an interpretation of the case of the plant subjected to a double attack by both fungus and virus, as follows: at first, the fusarium tends to change the auxins/gibberellins balance of the aerial organs in favour of auxins (hyperauxinia), but the TMV favours gibberellins (hypoauxinia), and the host's physiological balance is maintained in a state close to that of the controls. With its evolution thus disturbed, the fusariosis only reaches a certain level of severity one month later. The artificially maintained equilibrium is broken, the effects of the fusariosis now reinforce those of the mosaic virus (hypoauxinia in both cases), leading to leaves turning yellow and falling off, evidence of accelerated senescence... (basic metabolism).

Or in other words: at first, the fusarium tends to delay the natural evolution of sugars/nitrogen ratios in the leaves, the virus accelerates it and the host's physiological balance is maintained in a condition close to that of the controls. Thus disturbed in its evolution, the fusariosis only reaches a certain level of severity one month later. The artificially maintained equilibrium is broken (increase in sugars/nitrogen ratios in both cases), leading to leaves turning yellow and falling off, evidence of accelerated senescence (intermediate metabolism).

Or in yet other words: at first, the fusarium tends to delay the plant's general evolution, TMV accelerates it and the host's physiological balance is maintained in a state close to that of the controls. Thus disturbed in its evolution, the fusariosis only reaches a certain level of severity one month later. The artificially maintained equilibrium is broken, the effects of fusariosis now reinforcing those of the mosaic virus (accelerated physiological evolution of the plant in both cases), leading to leaves turning yellow and falling off, evidence of accelerated senescence... (phenotypic aspect)

CONCLUSION

Biology is a science. It can be defined as all of human knowledge acquired in a specific field. It began with "zero" knowledge and moves towards a sort of "absolute" knowledge... and is now somewhere on the long, difficult path leading to this absolute.

This science studies living beings. Living beings are composed of cells. The path leading to this knowledge must pass logically through the following stages:

- discovery of the existence of cells...
- study of their different components and the role they play...
- study and understanding how cells work, in the case of uni- and multicellular organisms...
- understanding of the normal functioning of complex organisms, and their gradual transition from egg to embryo, from embryo to youth, from youth to adulthood, old age and death...
- the notion of disease (abnormal function of the organism, host/parasite relations, etc.).

And a question arises: where are we today? - If the first step mentioned above (the discovery of the existence of cells) dates back to 1665 with the development of a rudimentary microscope by Hooke ..., the true cell theory, stating that living organisms only consist of cells, was created in 1839 with the work of Schleiden and Schwann.

- The second stage (study of the different cell components and the role they play...) has made in recent decades tremendous progress through the development of genomics, transcriptomics, proteomics, metabolomics and regulomics (I hope to forget nothing...).

- This second stage seems now sufficiently advanced in order to allow the scientific community to engage with enthusiasm the in steps 3, 4 and 5 mentioned above, thanks to new approaches titled integrative biology, systems biology, predictive biology, synthetic biology and theoretical biology ..., approaches involving biologists, computer scientists, mathematicians, biochemists, physicists and statisticians,

whose ambition is to understand and predict the normal or abnormal functioning of cells, complex organisms or ecosystems, according to the environments and their interactions. A very long-term work, requiring huge resources.

In this context, one can seriously ask the following question: Is Grandpa's biology simple or simplistic? or in other words: Is Grandpa wrong by considering that notions of balance and regulation are two sides of the same problem, or did he set obviously a fact of life that the scientific community will one day be obliged to consider?

To you to form your opinion. In any case, " grandpa's biology" is only now the point of view of Grandpa, a retired agronomist who in his youth spent several years in a laboratory of the INRA, his function being then to study the mode of action of plant growth hormones in the case of host/parasite relation existing between a variety of tomato and a pathogenic fungus.

JOSEPHINE

PS. I have called this document "The biology of Grandpa" because Grandpa is my father, and in order to permit to his grandchildren, with all the children in the world, to say, one day, studying their lessons: That's Grandpa's biology!"