

APPENDIX 2

The Analysis of Plant Growth

To gain a more complete understanding of plant growth and development, it is first essential to obtain clear and concise descriptions of changes that occur over time. Classically, plant growth has been analyzed in terms of cell number or overall size (i.e., mass). However, these measures tell only part of the story.

Growth in plants is defined as an irreversible increase in volume. The largest component of plant growth is cell expansion driven by turgor pressure. During this process, cells increase in volume many fold and become highly vacuolate. However, size is only one criterion that may be used to measure growth.

Growth also can be measured in terms of change in fresh weight—that is, the weight of the living tissue—over a particular period of time. However, the fresh weight of plants growing in soil fluctuates in response to changes in water status, so this criterion may be a poor indicator of actual growth. In these situations, measurements of dry weight are often more appropriate.

Cell number is a common and convenient parameter by which to measure the growth of unicellular organisms, such as the green alga *Chlamydomonas* (FIGURE A2.1). In multicellular plants, however, cell number can be a misleading growth measurement because cells can divide without increasing in volume. For example, during the early stages of embryogenesis, the zygote subdivides into progressively smaller cells with no net increase in the size of the embryo. Only after it reaches the eight-cell stage does the increase in volume begin to parallel the increase in cell number. Another example of the lack

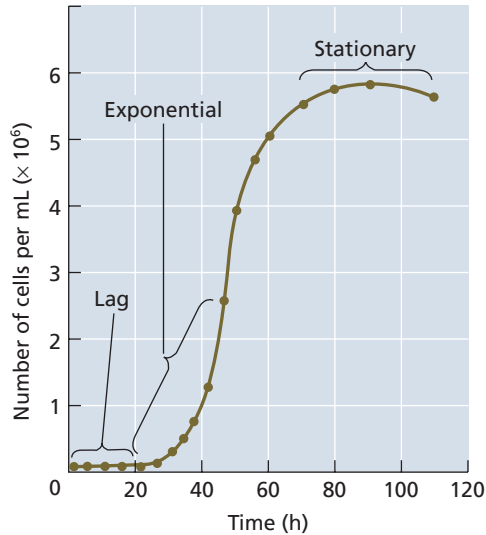


FIGURE A2.1 Growth of the unicellular green alga *Chlamydomonas*. Growth is assessed by a count of the number of cells per milliliter at increasing times after the cells are placed in fresh growth medium. Temperature, light, and nutrients provided are optimal for growth. An initial lag period during which cells may synthesize enzymes required for rapid growth is followed by a period in which cell number increases exponentially. This period of rapid growth is followed by a period of slowing growth in which the cell number increases linearly. Then comes the stationary phase, in which the cell number remains constant or even declines as nutrients are exhausted from the medium.

of correlation between cell number and growth occurs under the influence of environmental stress, which typically affects cell division and cell elongation differentially.

In this brief overview, we will discuss both the classical definitions of growth and a more recent approach, termed **kinematics**, which views growth and the related problem of cell expansion in terms of motions of cells or “tissue elements.” As we will see, the advantage of the kinematic approach is that it allows one to describe the growth patterns of organs mathematically in terms of the expansion patterns of their component cells. These quantitative and localized descriptions of growth provide a useful perspective from which to develop models for underlying mechanisms that control growth.

Growth Rates and Growth Curves

As we have seen, growth can be defined as an increase in volume or mass or cell number. Several kinds of growth rates are used in plant physiology. The absolute growth rate (g) is the time (t) rate of change in size (s)

$$g = \frac{\Delta s}{\Delta t}$$

The relative growth rate (r) is the absolute growth rate divided by the size

$$r = \frac{1}{s} \frac{\Delta s}{\Delta t}$$

If Δs is small relative to s ,

$$r \approx \frac{\Delta \ln s}{\Delta t}$$

“Growth curves” are data on size or weight or dry weight (“biomass”) versus time. The slope of the growth curve represents the growth rate at an instant in time. For building models, or characterizing seasonal patterns, simple global functions such as a straight line, an exponential curve, or a logistic (S-shaped) curve are often fit to growth curves. Growth rates can be obtained by analytic differentiation of the global fits. However, physiologists are usually concerned with mechanistic explanations. Local fits and local derivatives evaluated over short distances along the growth curve are often valuable in physiological work.

Cell division and cell expansion are independent processes that are often synchronized during development

For multicellular plants, many aspects of growth relate to newly formed cells produced by meristematic tissues. Growth associated with these cells is neither uniform nor random. For example, newly formed cells in apical meristems first enlarge slowly, but later expand more rapidly as they are displaced into sub-apical regions. The resulting increase in cell volume can range from several-fold to one hundred-fold, depending on the species and environmental conditions. The predictable and site-specific ways these derivatives expand is closely linked to the final size and shape of the primary plant body. In essence, the total growth of the plant can be thought of as the sum of the local patterns of cell division and expansion.

In many plant axes, progressively formed cells go through similar patterns of expansion during their displacement through the meristem into adjacent growth zones. In such cases, the final axis length is related to the number of cells produced. In some cases, however, cell division is not closely correlated to organ growth or growth rate. Cell division, the partitioning of an existing cell into two initially smaller cells, must be considered as physically independent of cell expansion. The two processes may be either synchronized or uncoupled. Also the two processes can be affected differently by environmental stress. Kinematic analysis (see below) allows us to see the cell size profile as the result of the concurrent processes of cell division and expansion. Recent studies have combined kinematic analysis, flow cytometry, and microarray analysis to characterize cell cycle regulation during the growth process of leaves and roots of *Arabidopsis* (Beemster et

al. 2005). Genome-wide microarray analysis allowed cell cycle genes to be categorized into three major classes: constitutively expressed, proliferative, and inhibitory. Comparison with published expression data corresponding to similar developmental stages in other growth zones and from synchronized cell cultures supported this categorization and enabled identification of over 100 proliferation genes. Other genes independently regulate the expansion process (e.g., Zhu et al. 2007).

The production and fate of meristematic cells is comparable to a fountain

Moving fluids such as waterfalls, fountains, and the wakes of boats can generate specific forms. The study of the motion of fluid particles and the shape changes that the fluids undergo is called **kinematics**. The ideas and numerical methods used to study these fluid forms are useful for characterizing plant growth. In both cases, an unchanging form is produced even though it is composed of moving and changing elements.

An example of an unchanging form composed of changing and displaced elements in plants is the hypocotyl hook of a dicot, such as the common bean (FIGURE A2.2). As the bean seedling emerges from the seed coat, the apical end of the hypocotyl grows in a curved form, a hook. The hook is thought to protect the seedling apex from damage during growth through the soil. During seedling growth (in soil or dim light) the hook migrates up the stem, from the hypocotyl into the epicotyl and then to the first and second internodes, but the form of the hook remains constant.

If we mark a specific epidermal cell on the seedling stem located close to the seedling apex, we can watch it as it flows into the hook summit, then down into the straight region below the hook (see Figure A2.2). The mark is not crawling over the plant surface, of course; plant cells are

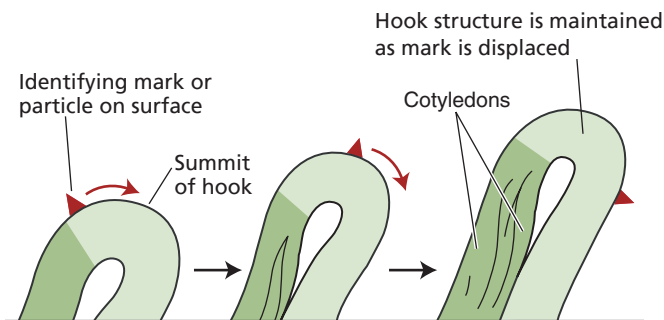


FIGURE A2.2 The dicot hypocotyl hook is an example of a constant form composed of changing elements. The hooked form is maintained over time, while different tissues first curve and then straighten as they are displaced from the seedling apex during growth. If a mark is placed at a fixed point on the surface, it will be displaced (indicated by the arrow), appearing to flow through the hook over time. (After Silk 1984.)

cemented together and do not experience much relative motion during development. The change in position of the mark relative to the hook implies that the hook is composed of a procession of tissue elements, each of which first curves and then straightens as it is displaced from the plant apex during growth. The steady form is produced by a parade of changing cells.

A root tip is another example of a steady form composed of changing tissue elements. Here, too, the form is observed to be steady only when distance is measured from the root tip. A region of cell division occupies perhaps 2 mm of the root tip. The elongation zone extends for about 10 mm behind the root tip. Phloem differentiation is first observed beginning at 3 mm from the tip, and functional xylem elements may be seen at about 12 mm from the tip. A marked cell near the tip will seem to flow first through the region of cell division, then through the elongation zone and into the region of xylem differentiation, and so on. This shifting implies that developing tissue elements first divide and elongate, and then differentiate.

In an analogous fashion, the shoot bears a succession of leaves of different developmental stages. During a period of 24 hours, a leaf may grow to the same size, shape, and biochemical composition that its neighbor had a day earlier. Thus, shoot form is also produced by a parade of changing elements that can be analyzed with kinematics. Such an analysis is not merely descriptive; it permits calculations of the growth and biosynthetic rates of individual tissue elements (cells) within a dynamic structure.

Tissue elements are displaced during expansion

As we have seen, growth in shoots and roots is localized in regions at the tips of these organs. Regions with expanding tissue are called **growth zones**. With time, the organ tips, bearing meristems, move away from the plant base by the growth of the cells in the growth zone.

If successive marks are placed on the stem or root, the distance between the marks will change depending on where they are within the growth zone. In addition, all of these marks will move away from the tip of the root or shoot, and their rate of movement will differ depending on their distance from the tip.

From another perspective, if you were to stand at the tip of a root that had marks placed at intervals along the axis, you would see that all marks would move farther away from you with time. The reason is that discrete tissue elements on the plant axis experience displacement as well as expansion during growth and development.

The growth trajectory is a cell-specific growth description that relates cell position to time

One way to characterize the growth pattern along an axis mathematically is to plot the distance of a tissue element

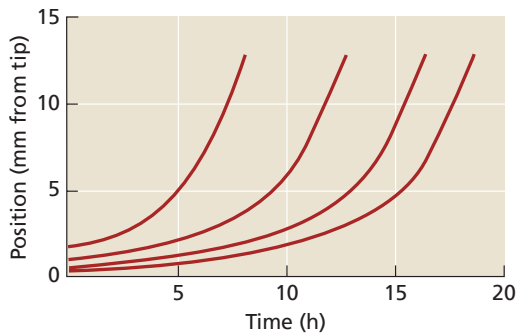


FIGURE A2.3 Growth trajectories of *Zea mays* (maize): Growth trajectories plot the positions of particles spaced along the root axis as a function of time. Note that distance is measured from the root tip. This is termed a material specification of growth, or a cell- particle- specific description, because real or material particles are followed.

from the apex versus time. The resulting curve is called the growth trajectory. With the apex as the reference point, the growth trajectory is concave. Marked tissue elements at different locations may be followed simultaneously to generate a family of growth trajectories (FIGURE A2.3).

The growth trajectory provides a way to convert spatial information into a developmental time course. For example, if the amount of suberin in the root increases with position, the growth trajectory will show us the time required for the observed accumulation of suberin in cells moving between any two spatial points.

As cells move away from the apex, their displacement rate increases

Looking at the growth trajectories of Figure A2.3, we see that as a cell of the plant axis moves away from the apex, its growth velocity increases (i.e., the cell accelerates) until a constant limiting velocity is reached equal to the overall organ extension rate. The reason for this increase in growth velocity is that with time, progressively more tissue is located between the moving particle and the apex, and progressively more cells are expanding, so the particle is displaced more and more rapidly. In a rapidly growing maize root, a tissue element takes about 8 hours to move from 2 mm (the end of the meristem) to 12 mm (the end of the elongation zone). The root tissue element accelerates through this region (see Figure A2.3 and FIGURE A2.4A).

Beyond the growth zone, elements do not separate; neighboring elements have the same velocity, and the rate at which particles are displaced from the tip is the same as the rate at which the tip is pushed away from the soil surface. The root tip of maize is pushed through the soil at 3 mm h^{-1} . This is also the rate at which the non-growing region recedes from the apex, and it is equal to the final slope of the growth trajectory.

The growth velocity and the relative elemental growth rate profiles are spatial descriptions of growth

The slope of the growth trajectory (see Figure A2.3) at any given point is equivalent to the growth velocity at that region of the axis. The velocities of different tissue elements are plotted against their distance from the apex to give the spatial pattern of growth velocity, or **growth velocity profile** (see Figure A2.4A). Figure A2.4A confirms that growth velocity increases with position in the growth zone. A constant value is obtained at the base of the growth zone. The final growth velocity is the final, constant slope of the growth trajectory equal to the elongation rate of the organ, as discussed in the previous section. In the rapidly growing maize root, the growth velocity is 1 mm h^{-1} at 4 mm, and it reaches its final value of nearly 3 mm h^{-1} at 12 mm.

To characterize the local expansion rate, we must consider the growth velocities at the apical and basal ends of a small segment of tissue. If the apical end of the segment is moving faster than the basal end, the segment is elongating. The velocity difference, divided by the segment length, gives the relative growth rate of the segment. Now, imagine that the segment shrinks to a point at location x . To find the relative growth rate, r , at point x , we can use calculus: The value of r is given by the velocity gradient, dv/dx , where v is the growth velocity. This measure of the local growth rate is called the relative elemental growth rate (Erickson and Sax 1956) or REGR. It represents the fractional change in length per unit time and has units of h^{-1} . If the growth velocity is known, the relative elemental growth rate can be evaluated by differentiation of the growth velocity with respect to position (FIGURE A2.4B). Or we can approximate r by measuring the relative growth rates of closely spaced segments of initial length L . For each segment, $r = (1/L)(dL/dt)$. The relative elemental growth rate shows the location and magnitude of the extension rate and can be used to quantify the effects of environmental variation on the growth pattern.

The relative elemental growth rate profile is similar to growth localizations obtained from discrete marking experiments used to find percent size change per hour over the surface of an expanding organ. However, because marked tissue elements are constantly moving, care must be used to find appropriate time and space intervals for a marking experiment to localize growth. In contemporary research computer-assisted methods, including automated particle tracking, are the basis for growth analyses with high resolution in space and time. Recent work includes quantitative descriptions of effects of environmental variation on growth rate patterns, and molecular studies to find the genes and proteins responsible for the responses to environmental stress (Zhu et al. 2007; Walter et al. 2009).

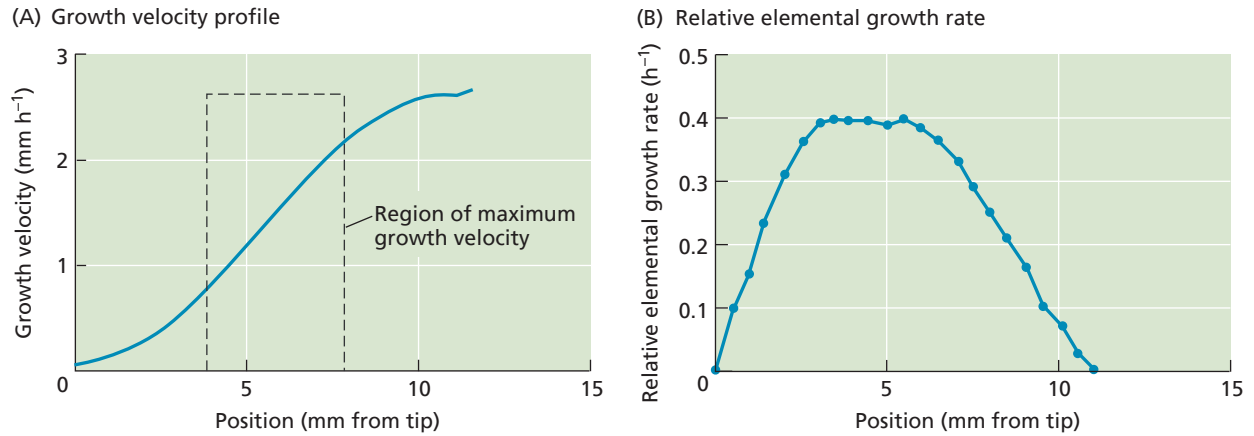


FIGURE A2.4 The growth of the primary root of *Zea mays* (maize) can be represented kinematically by two related growth curves. (A) The growth velocity profile plots the velocity of movement away from the tip of points at different distances from the tip. This tells us that growth velocity increases with distance from the tip until it reaches

a uniform velocity equal to the rate of elongation of the root. (B) The relative elemental growth rate tells us the rate of expansion of any particular point on the root. It is the most useful measure for the physiologist because it tells us where the most rapidly expanding regions are located. (Data from W. Silk.)

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