THE PHYSIOLOGY OF GRAFTING

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The art of grafting two plants together is probably as old as civilization. Our ancestors undoubtedly learned it after observing natural grafts in the tops or roots of forest trees and vines. The basic reason for grafting is to propagate clones which do not readily produce adventitious roots on cuttings. It is also important as a means of changing plant form or to take advantage of special rootstocks and interstocks.

Stem Structure and Function

In order to intelligently discuss grafting physiology, some understanding of the structure of a stem and the function of some of its main parts is required. A summary of the essential functions, the locations and the cell types involved is given in Table 1 and illustrated in Figures 1 and 2.

Growth and Healing

The grafting operation mostly concerns those parts that make up the *vascular cylinder* — (a) the cambium layer and the functioning (b) xylem and (c) phloem, which immediately adjoin it. In the spring of the year, the cells in the cambium layer are activated by some stimulus from the opening buds. Side walls of the cells become thin and division occurs. As a result, the bark becomes loose and easily separated from the wood. It is said that the "bark is slipping." New cells developed from the dividing cambium gradually change into the specialized cells that make up the stem. Xylem cells are produced on the inside; phloem cells are produced on the outside. The activation of the cambium takes place in a wave from the top of the plant down and may not be complete until several weeks have passed. Once the cambium is activated, the cells will continue to divide until such time as the plant goes dormant in the fall or is inhibited from growing by adverse environmental conditions, such as lack of water or defoliation.

When a plant is wounded, there is a somewhat different chain of events in the healing of this wound. This is what occurs in the grafting process. The basic difference between healing a wound, such as a pruning cut, and graft healing is that a scion from another plant is inserted into the stock with the expectation that the two parts will heal together.

Phases of Graft Union Formation

The formation of a graft union can be discussed in terms of the separate problems that must be solved in order to have a successful graft combination.

I. The plant has to be able to heal the wound that was created when the graft was made.
   a. Exposed living cells (ray cells, cambium and its im-

Table 1. Important parts of stems of woody perennials of significance in grafting.

Function	Location	Cell types involved
Plant support and strength	Woody portion of the stem Includes the "old" xylem. Also "old" phloem in the bark	Elongated, narow cells (fibers) or shorter, rounded (*schlereids*); both dead; thick walls.
External protection from drying out and adverse environment factors	Outermost corky layer of bark (*periderm*)	*Cork cells* - dead, rounded cells with deposits of lignin and suberin in walls. *Cork cambium layer* - layer of living cells that produces the cork cells.
Water conduction	Outer layer of wood "New" *xylem*	Vessels. Long, barrel shaped, dead cells with thick walls and perforations in end and side walls *Tracheids* narrower, dead cells
Food conduction (translocation)	Inner layer of the bark "New" *phloem*	*Sieve cells* - special elongated cells, contain protoplasm with special openings on the end (sieve plates); associated with vertical distribution of organic materials. *Companion cells* - living cells adjacent to sieve cells; function uncertain. *Ray cells* - living cells that provide some horizontal distribution
Food storage and deposition of organic materials	Deposited in inner layer of bark (*phloem*) and outer layer of wood (*xylem*)	*Ray cells* - laterally distributed living cells of both phloem and xylem (see above). *Xylem and phloem parenchyma* - rounded, living cells that may show starch deposits.
Growth and production of new tissue	*Cambium* layer	*Cambium initials* - single layers of cells that divide to initiate growth *Immature* xylem and phloem cells and ray cells.

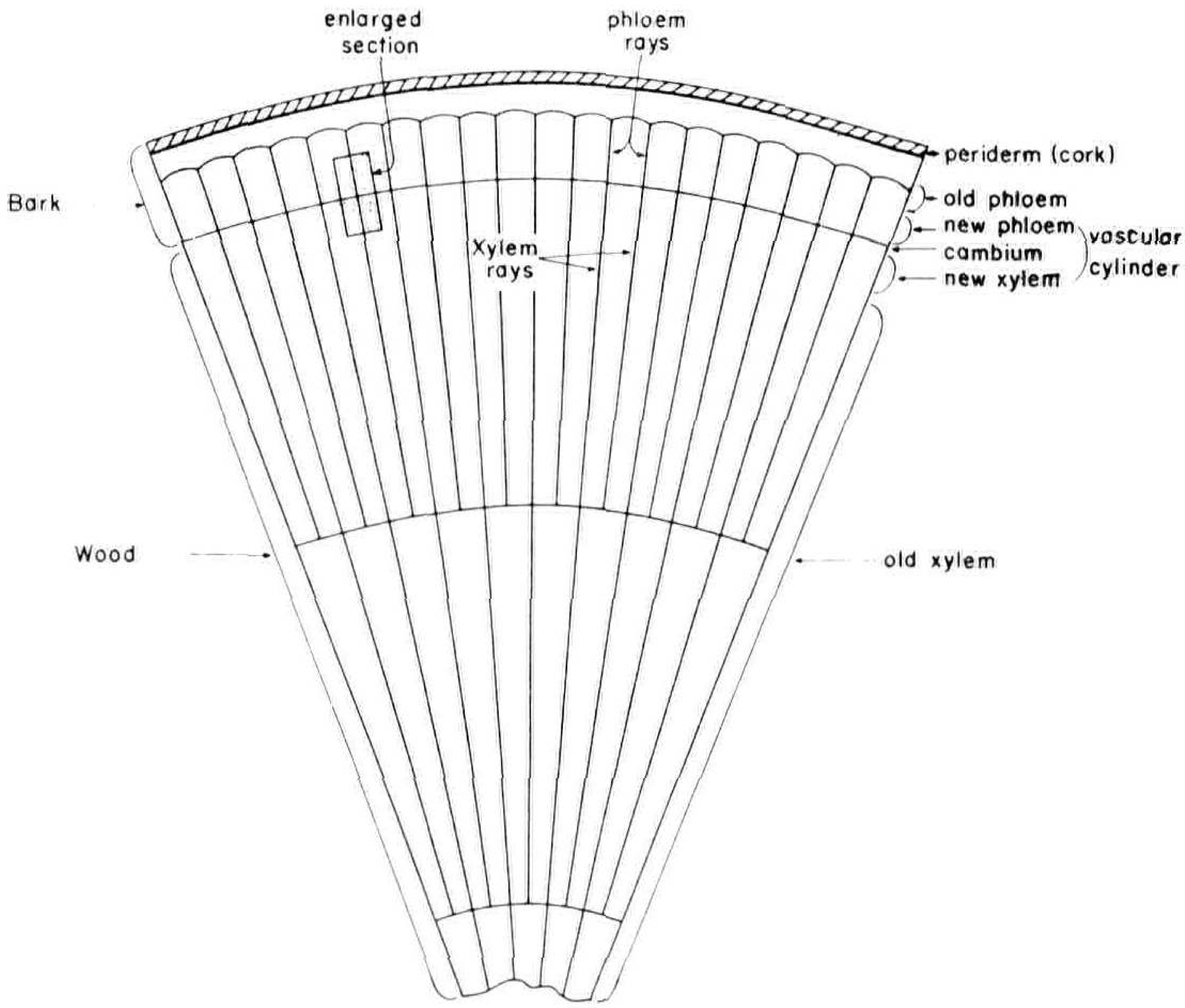

Fig. 1. Cross-section of woody stem shows parts important in grafting. Redrawn from Esau, K. Plant Anatomy. N.Y.: Wiley. 1953.

mediate derivatives) that are damaged turn brown and die (Fig. 3). This layer may be several cells deep, depending upon the length of exposure and particular drying factors. A plate of dry, necrotic tissue is produced over the cut surface (13).

b. Living cells, including ray cells and young xylem and phloem, below the necrotic surface begin to divide and to produce large, rounded parenchyma cells. The mass of white, solid tissue composed of these cells is known as *callus* tissue. It is produced on wounded stems, bases of cuttings and can be grown in an artificial media as tissue culture. The ability of the stock and scion to produce callus is the *first* prerequisite for a successful graft union.

c. Exposed dead cells (tracheids, vessels) are sealed off with a deposit of gum (1). The ability and speed of gum formation can be important. In one study, it was shown that apple (which is easily grafted) had the ability to plug and seal off the vessels quickly, whereas the more difficult-to-graft black walnut was much slower and desiccation was a problem (10).

d. Formation of a periderm or cork layer on the outside of the wounded area.

A. *Factors affecting callus formation*

a. *Genetic constitution.* Some varieties and some species are poor callus producers. In some cases this is evidently related to grafting difficulties. Slow callus producing plants can be sometimes combined by approach grafting, keeping both stock and scion on their own roots until the union heals.

b. *Supply of endogenous hormones and growth substances.* Callus formation is a function of at least two kinds of growth substances: auxins and kinins. Treatment of cuttings with growth regulators of the

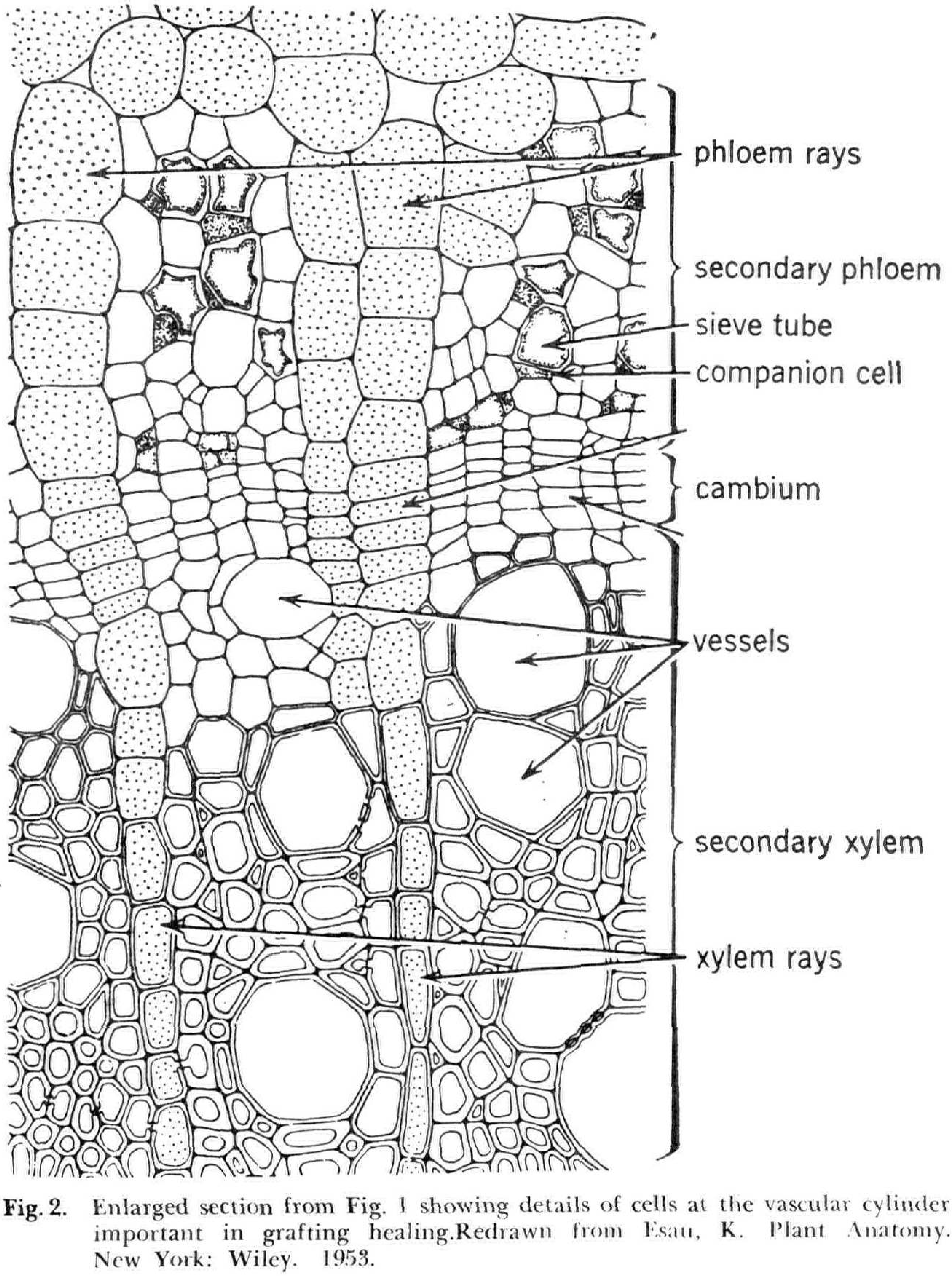

Fig. 2. Enlarged section from Fig. 1 showing details of cells at the vascular cylinder important in grafting healing.Redrawn from Esau, K. Plant Anatomy. New York: Wiley. 1953.

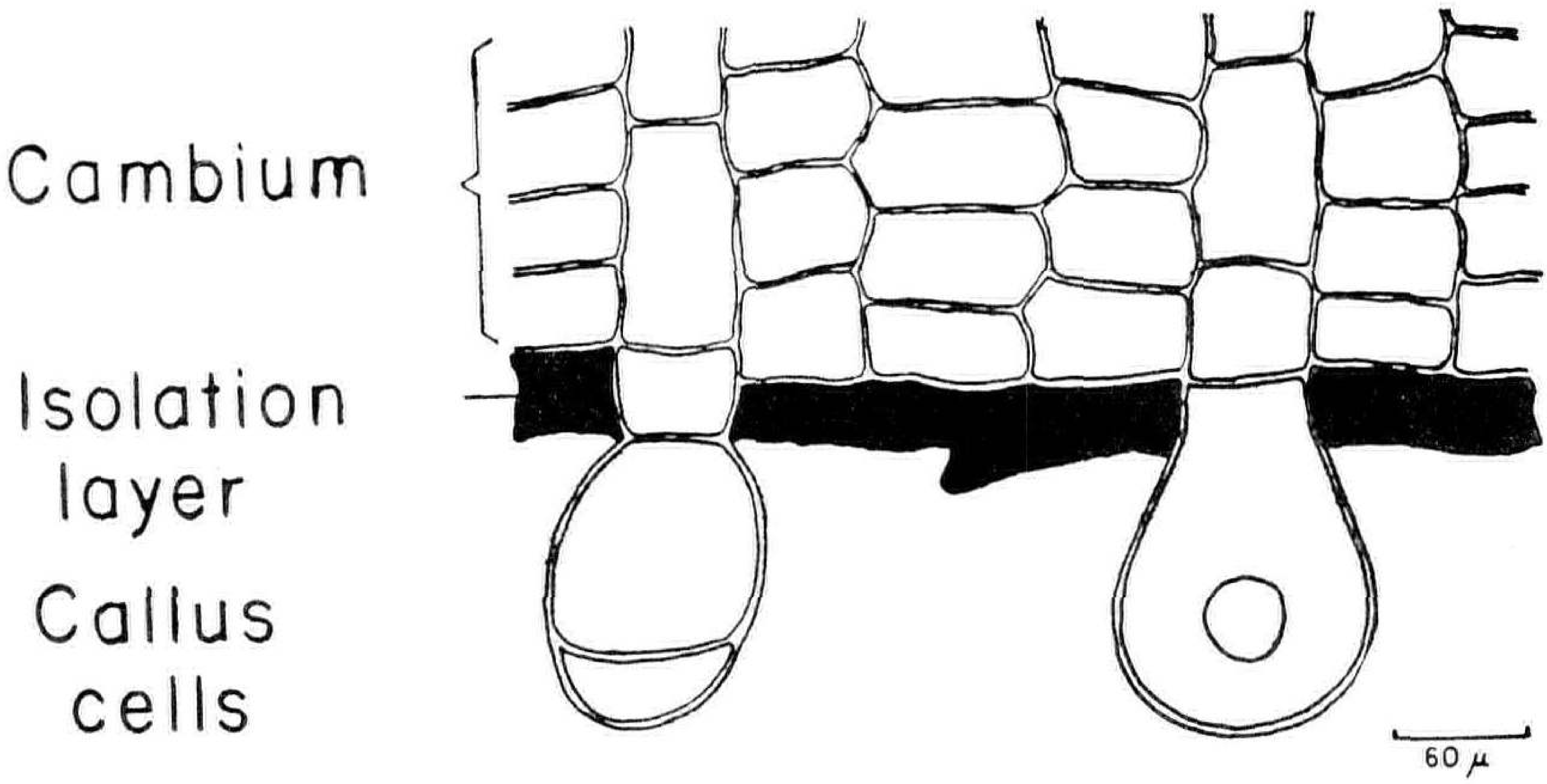

Fig. 3. Isolation layer from damaged cells at surface of wound and production of new callus cells from the rays. From Thiel (13).

auxin-type (IAA, NAA, IBA) stimulate callus formation. If one takes a piece of stem (without buds) or root or other plant organ and grows it without NAA or some other similar substance, some species will produce callus; others will not. If one takes a piece of callus and tries to keep it alive in artificial media by continual retransfers to new media, it will not survive without auxin (except in certain specific cases).

Kinin-like substances, such as kinetin, which are known as cell-division factors, also play a role in callus formation. For example, almond stems collected in winter respond to kinetin slightly but respond strongly to NAA (5). For continued maintenance of almond tissue in culture, kinetin is essential and certain other substances, e.g., thiamin (vitamin B_1) may be helpful.

A number of years ago Skoog (11) showed that relative amounts of IAA and adenine (a kinetin-related compound) would control the type of growth on tobacco pith culture.

- high or intermediate auxin plus high or intermediate adenine = callus
- high auxin plus low adenine = roots
- low auxin plus high adenine = shoots

The application of *various* externally-applied growth substances in grafting operations has not shown consistent practical benefit (4). Some experimenters have reported beneficial results; most do not. It seems that other factors, such as time of year, proper scionwood, correct technique, etc., are more important factors than supplying external growth regulating chemicals.

c. *Time of year and stage of development.* Callus proliferation varies with time of year. Sussex and Clutter (12) took pieces of stem every six weeks and determined callus formation at different times. It

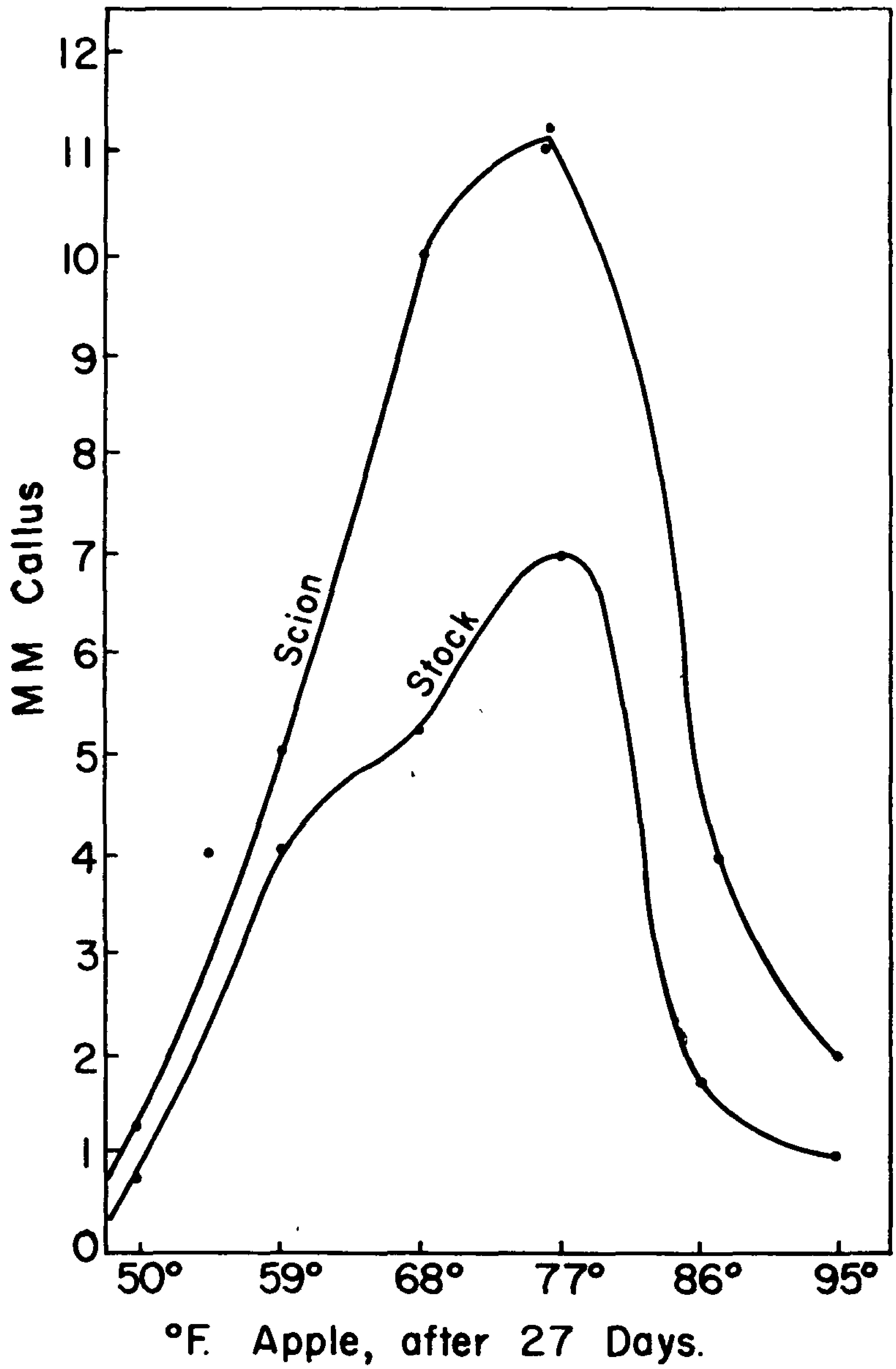

Fig. 4. Effect of storage temperature on callusing of apple scions. From Shippy (9).

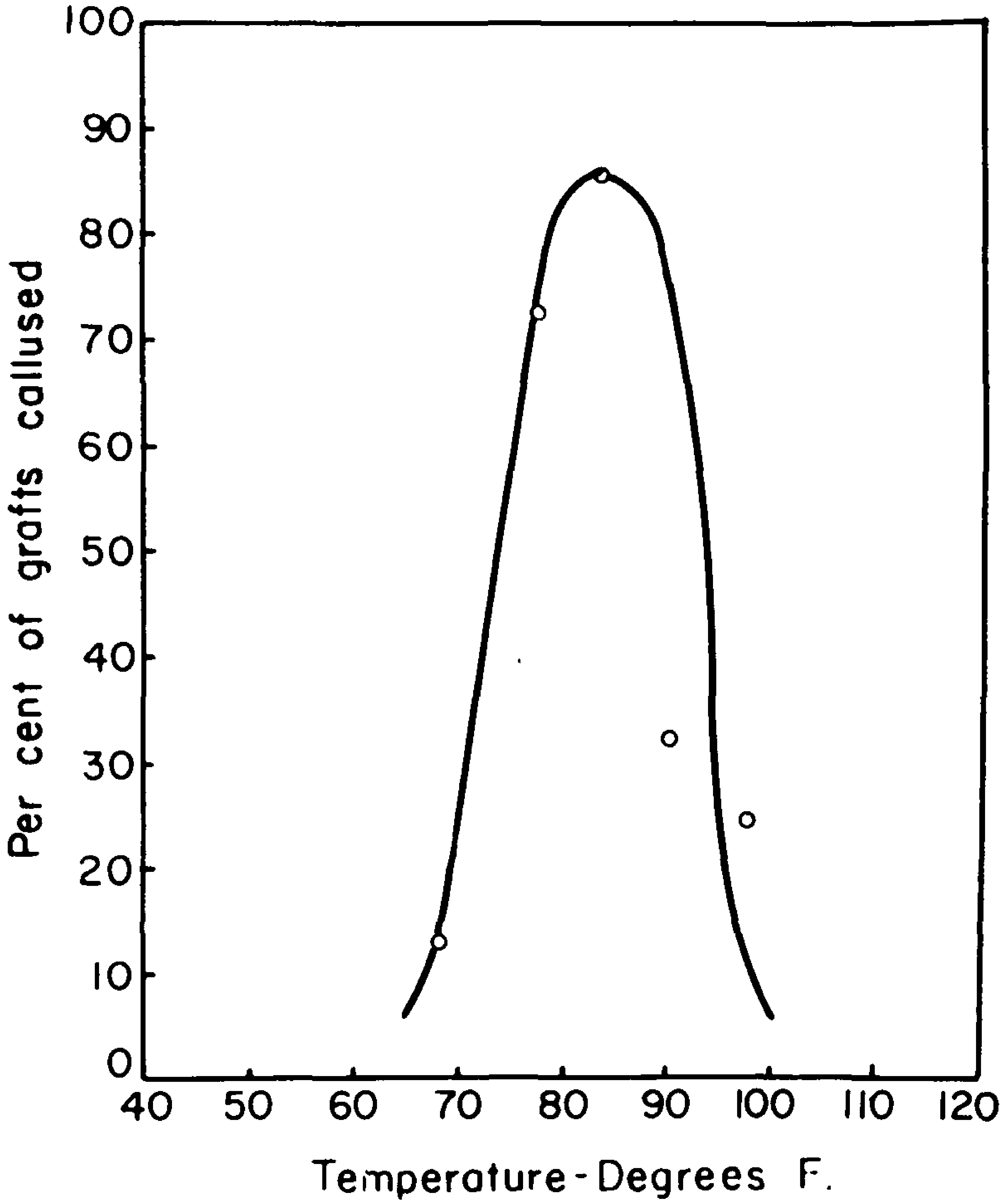

Fig. 5 Effect of storage temperature on callusing of black walnut grafts. From Sitton (10).

was greatest in spring when new growth occurred, decreased gradually into fall and winter. It increased again several months before bud break even though buds were dormant.

In some plants grafting must be done during the time of year the plants are in active growth. Budding operations and bark grafting require that bark be slipping. Side grafting of junipers is done when the plants are in active growth.

d. *Temperature.* Warm temperatures promote callus formation; cool temperatures reduce or inhibit it; high temperatures may be injurious or result in too much soft callus which is easily injured.

Figures 4 and 5 compare the effect of temperature on callusing of apple and black walnut. Apple is less sensitive to temperature than walnut. where the range of effective temperature is narrow.

e. *Moisture.* High humidity is essential for callus formation. Holding cut potato tubers, for instance, under somewhat dry conditions results in the formation of a periderm and deposits of suberin which seal over the wound; high moisture suppresses suberization and induces callus formation (2).

Probably the same thing is involved in a graft. High humidity of near saturation, and even free water deposit is conducive to high callus formation (Fig. 6). This is why it is essential to enclose the graft wound to keep it from drying out.

f. *Aeration* is important and different species vary in their response to the oxygen supply. Some reduction in aeration is permissible, at least in some species (Fig. 7).

g. *Polarity* is also important. Callus develops mostly at the base of cuttings or scions. This is related to the downward movements of auxins and other substances in the stem.

A scion placed upside down will usually fail to unite although a single inserted bud will reorient itself and grow to produce wide-angled branches.

An inverted bridge graft or bark strip may heal in place but does not grow normally. The latter has been used to some extent to produce dwarfing.

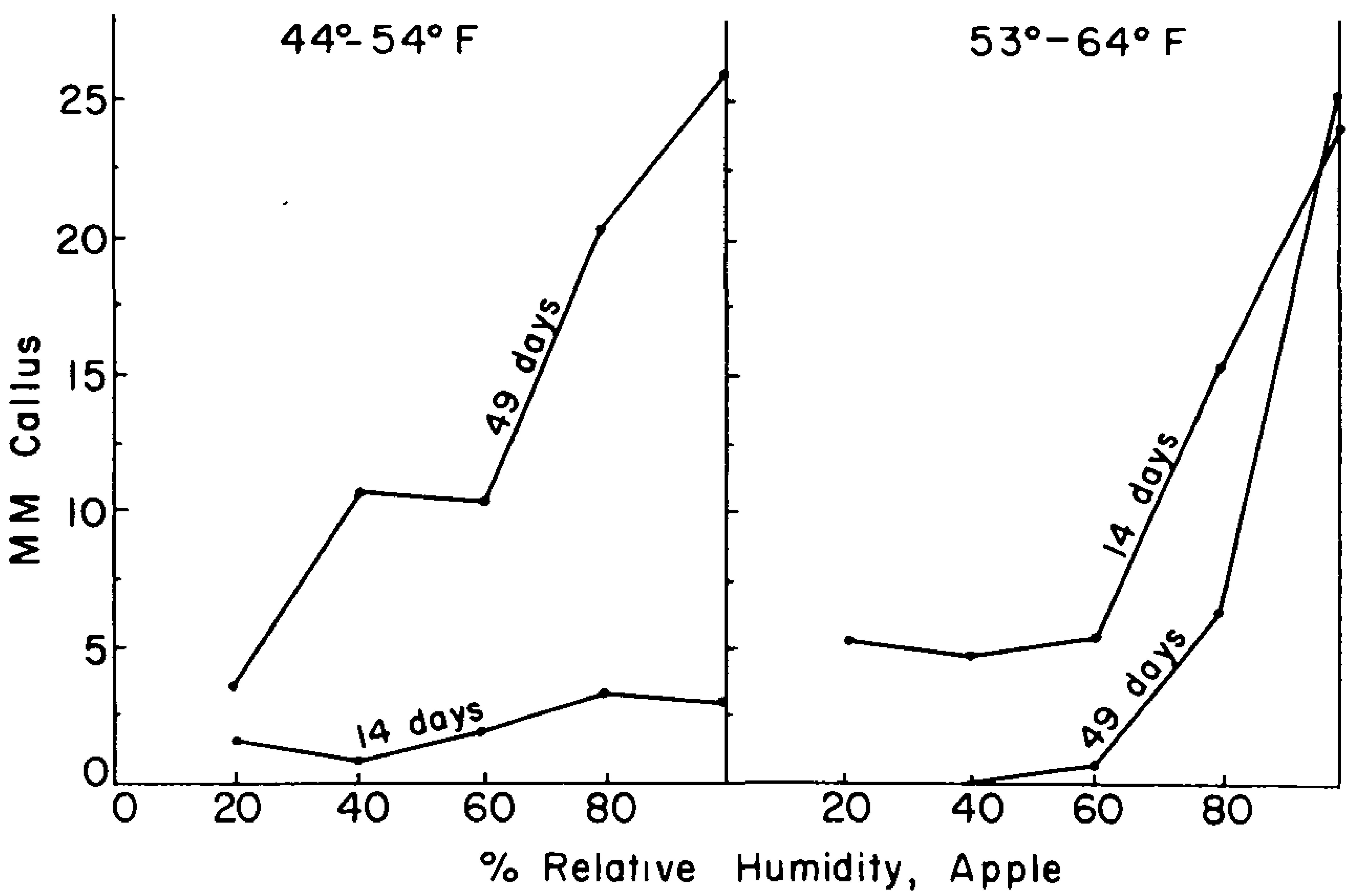

Fig. 6. Effect of moisture on callus formation of apple cuttings in relation to temperature. From Shippy (9).

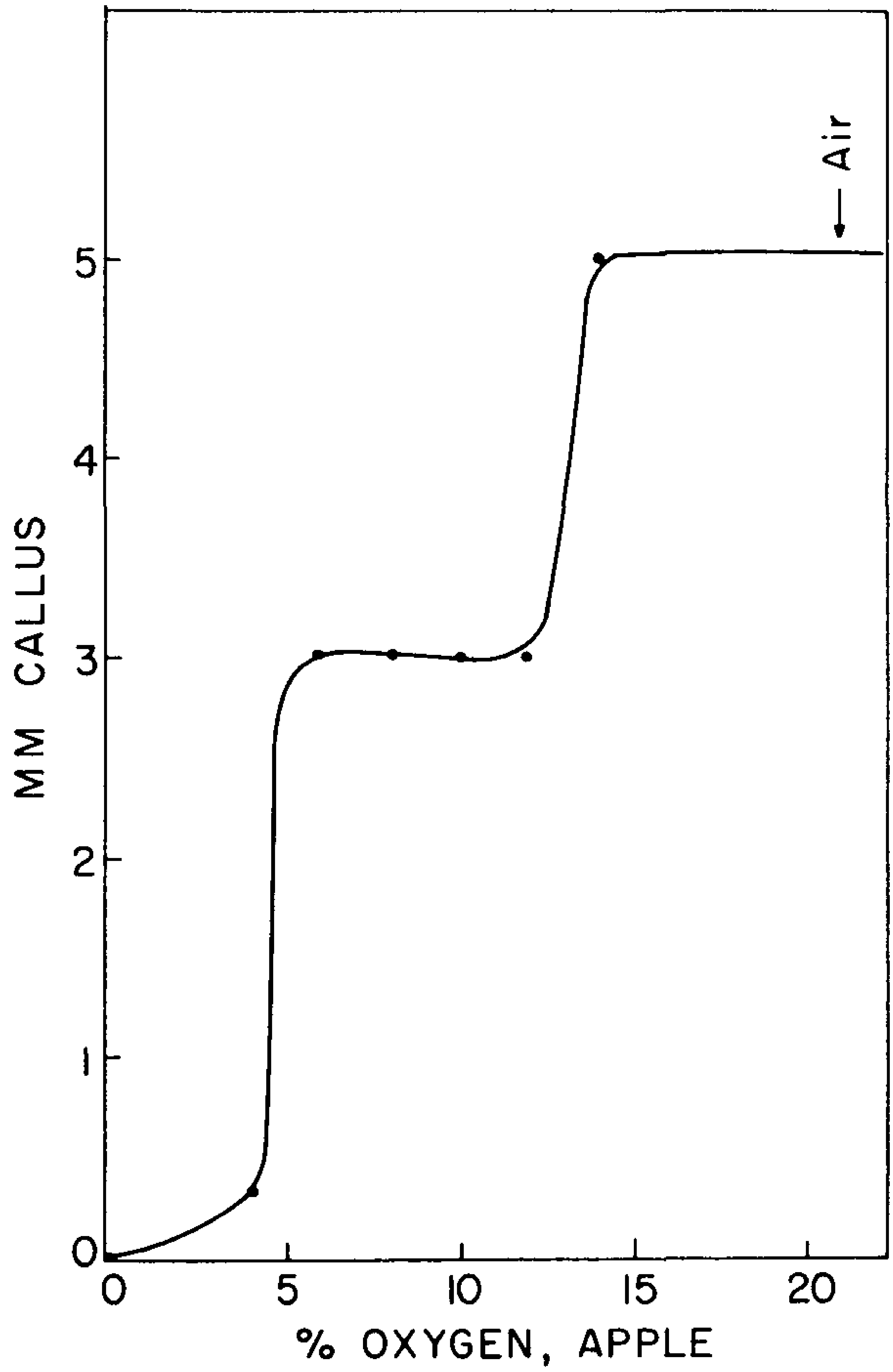

Fig. 7. Effect of oxygen supply on callus formation of apple cuttings. From Shippy (9).

If conditions are favorable, callus proliferates and fills a large part and possibly all of the cavity between stock and scion. Callus from stock and scion meet and intermingle and form a more or less continuous bridge. This may be completed within 5 - 7 days.

II. A connection must be established between water conducting elements (xylem) of the stock and scion. Water supply is the immediate problem of the scion.

Xylem formation is closely associated with new cambium formation and it is not always certain in the studies made whether one is prerequisite to the other. At any rate, new cambium and new xylem cells can be observed to have differentiated within one or two weeks from some of the callus cells. New xylem and cambium begin to appear at the vicinity of the end of existing vascular strands of both stock and scion. These may be parallel to the surface. As a result the appearance seems to be progression toward the center.

It has been demonstrated adequately that this is an "induction phenomenon", brought about by a stimulus from the existing vascular region. This can be illustrated by some experiments on grafting buds onto pieces of root tissue in culture (Fig. 8). A new vascular stand is initiated. The physiological basis for initiation is not understood. except there is evidence that auxin is involved.

The practical application of this information is the well known need to have a reasonably close match of the cambial region of the stock and scion. A large area of contact is not usually necessary, however, since once a continuous connection of the vascular systems is achieved at any one point, it will continue to develop rapidly.

III. The next step is that a continuous connection between the food connecting elements of stock and scion must be

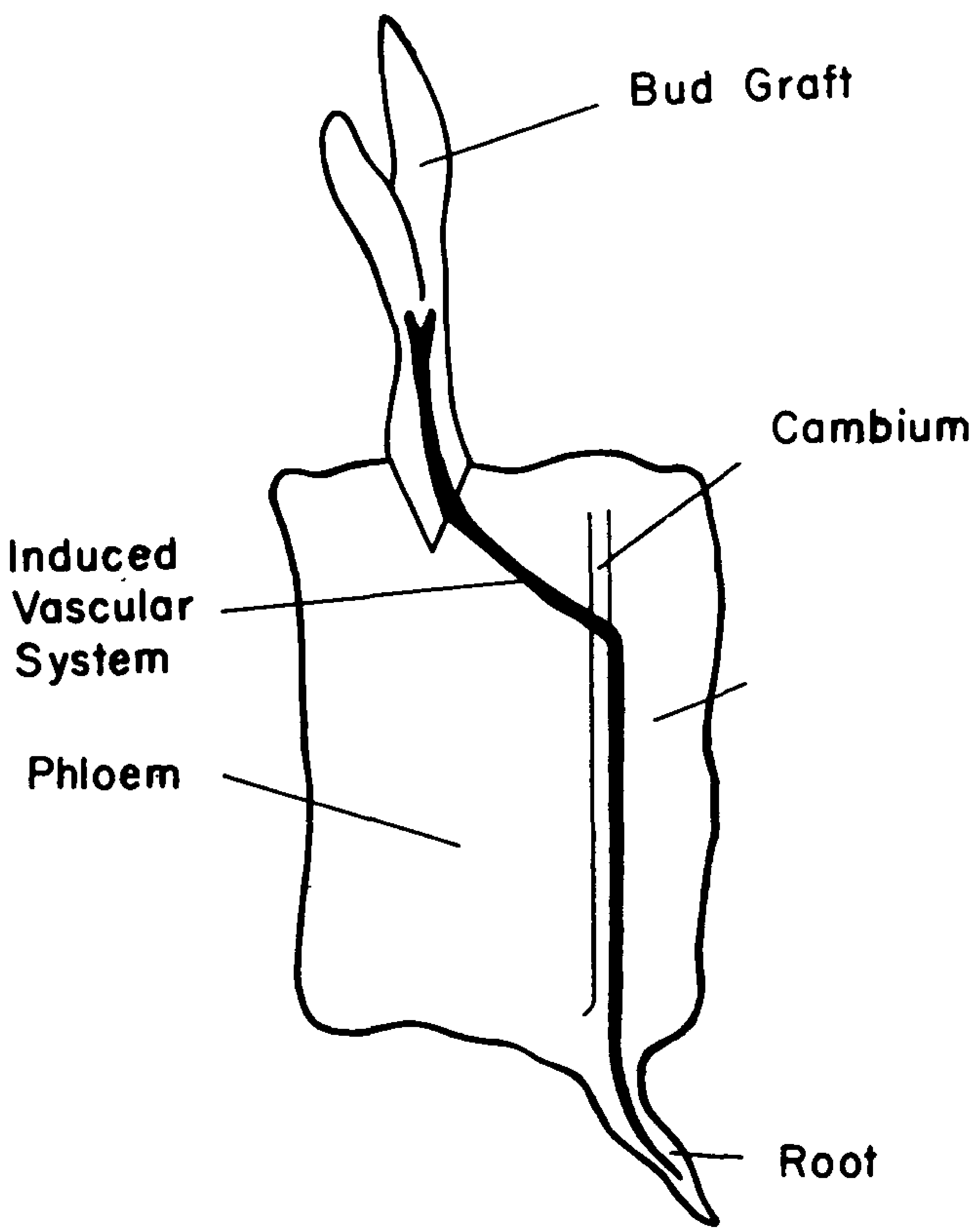

Fig. 8. Induction of a vascular cylinder by grafting a bud on a piece of root tissue From Gautheret (3).

achieved. Timing is less critical since the scion usually has considerable stored food. Once a continuous cambium is present, it will begin to form phloem cells.

Studies with movement of viruses through graft unions indicate that this connection occurs somewhat later than the *xylem connection* (2).

V. A strong supporting framework within the branch must be established between stock and scion.

Once a vascular system is established, cambium will produce fibers and other strengthening elements at the union just as above and below it. The union should be just as strong as any other place in the plant. However, it may require some time after a graft is made to develop full strength and support.

In some graft combinations a continuous permanent connection between stock and scion is never achieved. The plant grows well for a time, has phloem and xylem connections evidently, but has no strength.

Some time after the graft is made, the top can be broken off at the union to leave a clean break (7. 8). This may occur in one-year-old nursery trees of some combinations, e.g., apricot/almond (5). Clean breaks have been known to occur after 20 years of seemingly normal growth in other cases — for example, peach/almond.

V. The stock and scion parts must be able to grow together and exist in relative harmony for a long period of time. If this occurs, we say they are *compatible*. If for any reason the combination fails or one part has an adverse effect on the other, we say they are *incompatible* (7, 8).

Incompatibility takes a number of forms and much more understanding of the physiological basis for most kinds is needed for reliable methods to make an early forecast of incompatibility.

Fig. 9 shows growth rate during a seven-year-period of a series of varieties of almond grafted to Marianna 2624 plum. Some (Texas and Peerless) are perfectly compatible and grow at constant rate. Other varieties are gradually inhibited in growth, become unhealthy and (sometimes) die. In other cases, as in "blackline" in walnuts, trees may be normal for 15-20 years and then breakdown at the union.

Examination of the union in the almond/Marianna 2624 case shows a failure in the region of the phloem (bark) resulting in gradual girdling of the tree, although the wood portion appears normal and strong. Inserting a compatible interstock between the two incompatible parts does not overcome the bark breakdown in this kind of incompatibility. Instead, whatever it is that exists in the incompatible top that causes the bark breakdown moves across the interstock and causes breakdown at the union below it.

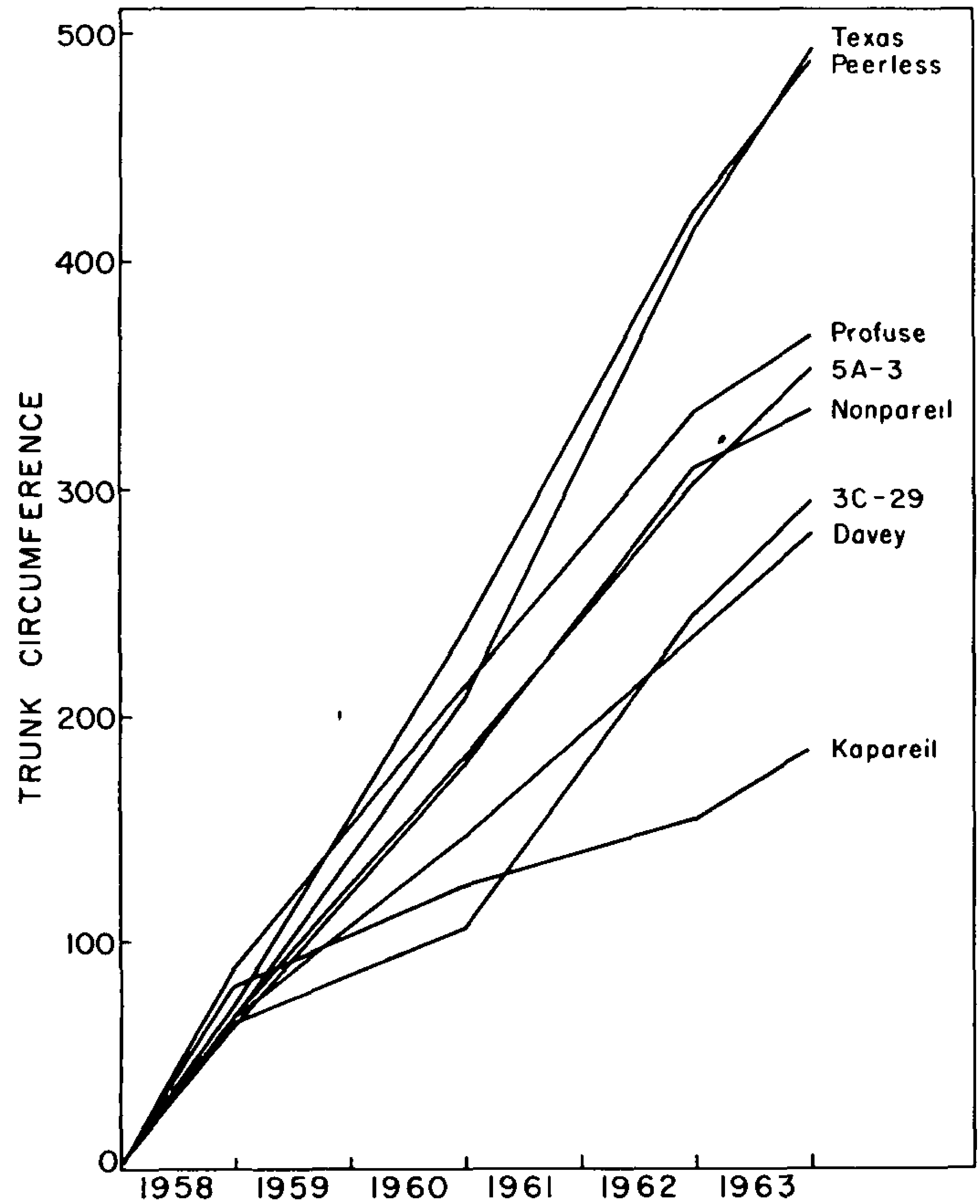

Fig 9. Effect of different degrees of incompatibility of different almond varieties grafted to Marianna 2624 plum rootstock. From Kester, *et al.* (6).

In other kinds of graft combinations, for example pear/quince, obvious breakdown occurs at the graft union throughout the xylem (wood) area. In this case an interstock of a compatible variety will overcome the incompatibility, although some reports suggest that environmental conditions, notably temperature, can influence this effect. A very small piece of interstock, inserted by double budding, is known to be sufficient to correct this kind of incompatibility. Recently Gur in Israel has given evidence that incompatibility of pear/quince is due to accumulation of hydrocyanic acid resulting from breakdown of amygdalin. Rootstocks differed in their ability to produce this compound.

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MODERATOR BRIGGS: Thank you, Dale. To continue on this morning we are going to hear about teaching college students some of the methods of grafting. We have with us Mr. O. A. "Jolly" Batcheller. He is the Chairman of the Department of Ornamental Horticulture, California State Polytechnic College, Pomona, California — Jolly:

METHODS OF TEACHING GRAFTING

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The theory of grafting and budding is easily learned. The conditions and after-care also present no problem, but to teach individuals the actual skill and manipulative practice of budding and grafting is more difficult. It is to this matter I am going to direct my presentation.

Our senses which help us learn: sight, hearing, and touch, are perhaps the most important in this experience because the actual material we are working with is so small that class demonstration does not have the desired effect and can actually detract from the presentation, unless accompanied with larger models. If the students cannot see what is actually being done, they may get the wrong impression or lose interest and be distracted.

The use of the blackboard is helpful, but not always do our drawings appear to others as they do to us. The use of colored chalk improves this, but still this is a two-dimensional presentation while the actual material is three-dimensional.

I have found that after a preliminary presentation by lecture of the reasons for grafting, the limitations, the conditions, and the after-care, that an actual demonstration with living ma-