THE FORMATION AND DEVELOPMENT OF THE NORWAY SPRUCE GALL CAUSED BY *Adelges abietis* L.

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George H. Plumb[2]

The atypical growths known as galls and produced by plants in response to a foreign stimulus have long been a subject of interest to biologists. Much of the work done with them, however, has been of a systematic nature only, consisting of the classification of the galls themselves and of the organisms causing them. This has been particularly true of the galls caused by insects. Such a treatment disregards the fundamental problem involved, namely, that of the origin, nature, site of action, and effect of the causal stimulus.

In recent years a renewed interest has arisen, both here and abroad, in the synthesis of plant galls. This revival has been given impetus by the new lines of thought made possible by the accumulation of knowledge, and by the evolution of new tools and techniques. Among these are recent developments in the fields of genetics and plant physiology. The advances made in the studies of plant tissue culture are especially noteworthy, and will undoubtedly further contribute to a better understanding of the intricacies of atypical growth. And finally, the teleological philosophy which has all too frequently impeded a sound approach to the subject has been cast aside, and the investigations have been set on a truly experimental course.

It seems advisable to precede this work with a general account of the gall problem. The literature on this subject is scattered, and in no one place can be found a complete record of the many experiments performed and the various theories set forth in explanation of gall development. Yet even this compendium cannot pretend to be complete; rather it is selective. It is hoped, nevertheless, that the facts and theories here collated will aid in a broader understanding of atypical growth as a whole, than would a discussion of the immediate problem alone.

The Gall Problem in General

HISTORICAL

The etymology of the word "gall" indicates that the existence of these plant malformations has long been known. Connold (50) gives three possible sources, viz: from the Anglo-Saxon word "ge'lla", used in the sense of bitter; from the French "galer", in which the meaning is taken to be that of annoyance, teasing, and so forth; or the abrading of the skin, and from the Latin "galla", a term which denotes directly the abnormal growths on plants. The connotations of all of these word-sources collectively describe

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succinctly the gross properties of such proliferations. The term "cecidium", which is also applied to such growths, is from a Greek root meaning juice. It is also used to mean a gall-nut produced by the oozing of sap from punctures made by insects.

Galls were used in ancient times for medicinal purposes, for the making of ink, and later in dyeing processes. Thus, we find that Theophrastus (372-286 B.C.) spoke of gall-nuts as articles of trade, and of the superior quality of those obtained from Syria. Hippocrates (406-377 B.C.) wrote on the medicinal properties of galls, and on the application of divers preparations thereof. In addition, he described the appearance and form of numerous galls occurring on various host plants, and knew that at least one of them contained "animals". Pliny (23-79 A.D.) was aware that "flies" emerged from galls, but ascribed the growth of the latter to other causes.

During the Middle Ages there appeared the great herbals, bestiaries and other natural history cyclopaedias which culminated in the true botanical herbals of the sixteenth century. Perhaps the first mention of galls in the English language appeared in one of these, in 1398. This was the translation by John Trevisa of a nineteen-volume work entitled "De Proprietatibus Rerum" by Bartholomaeus Anglicus, an English Franciscan friar, and originally published in the early thirteenth century. This cyclopaedia has sometimes been credited in error to Bartholomaeus de Glanville who lived in the fourteenth century. The translation existed only in manuscript form (the Tollemache Ms.) for almost one hundred years; for it was not brought into print until about 1495. In it is the statement that "the mall (Mandragora) hath white leaves . . . and apples groweth on the leaves, as galles groweth on oaken leaves."

Various other similar English works contain information on galls. One Gervais Markham (153) edited in 1616 a revision of Richard Surflet's translation (made in 1600) of "Maison Rustique" or "The Country Farme," as it was entitled in English. This work was written by Charles Estienne, and was published in France in 1569. In this book galls and their uses are discussed in several places, particularly with regard as to their value in prognostication. Under the heading "Signs foretokening fruitfulnesse" is the following: "He shall know a fruitfull and fertile year, if he shall see in the Oke apples, commonly called Gals, a Flie engendered." This may be interpreted to mean that the naturalists of the period knew that insects were produced in these growths. No positive statement occurs, however, that they thought the galls were caused by the activity of the insects. In fact, it apparently was considered that galls were produced as a second sort of fruit by the tree, since the nature of the acorns was known and spoken of in connection with forest planting.

On another page of Markham one finds: ". . . the greater sort of gals or apples have this propertie in them, namely to presage and foretell three things, that is to say, war, dearth, pestilence; for if you open them which are whole, you shall find therein a little flie, or a little spider, or a little worm . . ." There appears to have been no realization that the "worm" and the "flie" might have been related, and might have had something to do with the occurrence of the galls.

The oldest illustration of a gall is thought to be that of *Cynips tinctoria.* It is to be found in the "Hortus Sanitatis," published in 1491 (Locy [144], Küster [128]). Many other natural history writers of the early centuries referred to galls on plants. It was not until the late eighteenth century, however, that any attempt was made to explain the connection between the galls and the insects found in them.

NATURE OF THE STIMULUS

Source

The great diversity in the known producers of vegetable malformations may be pointed out at this time. Galls can be caused by members of widely separated categories of living organisms. These include bacteria and fungi among plants; rotifers, copepods and nematodes, mites and insects among animals. Of the insects, gall-producing forms occur in different orders, including Diptera, Hymenoptera, Homoptera, Coleoptera, Lepidoptera, Orthoptera, Neuroptera, and Thysanoptera. Undoubtedly the majority of these gall formers belong to the families Itonididae and Cynipidae, respectively, of the first two orders named above. There is within these orders a trend toward specialization by definite groups. The galls formed by cynipids received the earliest attention and have rather dominated this field of investigation. This fact may be due to their prominence and conspicuousness on tree leaves, particularly those of oak, and to their importance as articles of trade since historical times. Then, too, the galls produced by cynipids are among the most diverse and complex known, and are for that reason perhaps more intriguing.

The true relation of insects to their galls does not appear to have been comprehended until about the middle of the last century. However, several scientists of the eighteenth century made shrewd guesses as to the origin of these plant malformations. Malpighi (1620-1694) was probably the first to render an opinion that the stimulus to gall formation was of animal origin. In his "Anatome plantarum" (Pt. II, De gallis), London, 1679, he expressed the opinion that the stinging of plant tissue by an insect gave rise to a gall. Malpighi stated that the liquid, which he called "ichor", injected by the ovipositor produced a swelling akin to that caused in animal tissue by the sting of a bee. Réaumur (1683-1759), on the other hand, held that the gall developed as a result of the physical irritation incited by the puncture, and by the presence of the egg and developing larva (176). Martin Lister (1630-1712) has been credited with the discovery of the association of insects with many galls. Thus, we have at an early date the basis of two different schools of thought as to the origin of the gall stimulus, one of which presumably has been disproved only in recent years.

It is not intended to include here a general review of all of the theories regarding the stimulus which brings about gall formation. However, it is necessary to call rather extensively on previous work in order to provide a logical background for the ever-narrowing (yet in a sense expanding) path of evidence leading toward the ultimate source. The workers of the latter part of the last century who expressed their views as to the biological nature of the stimulus, restricted themselves for the most part to galls

arising from tissues punctured by the ovipositor. In this case the stimulus was attributed variously to a fluid injected at the time of oviposition; or to the "irritating" presence of the ovum and of the larva after eclosion.

The idea that secretions of the insect salivary glands might be implicated in gall formation apparently came into being about the middle of the nineteenth century. Hofmeister (102), Adler (1, 2) and Beijerinck (14) all mention this possibility. Kerner (116) says: "Those who investigate galls consider that it is chiefly the acrid 'saliva' excreted by the larvae to liquify their food which acts on the cell tissue of the dwelling they have selected, but there is no doubt that other excretions may also take part."

There were, then, already in existence by the late nineteenth century, several hypotheses to account for atypical plant growths, namely: the injection of a stimulating fluid at the time of oviposition, the sheer physical irritation set up by the presence of a foreign body, the secretion of an active saliva, and the excretion of metabolic products. It now remains to develop these theories, some of which are still extant, with a view toward fitting them into an integrated concept of the problem.

Réaumur's original belief (since held by others) that the mere act of puncturing plant tissues produces atypical growth is no longer tenable; nor is that of the presence of an alien body. It is true that some galls begin to develop concurrently with oviposition. There are, however, many cases in which the egg lies in the tissue for a long period, and no gall develops until after eclosion.

There are insects which lay eggs in plant tissues without (as occurs in other instances) at the same time injecting the product of the so-called "poison" gland, e.g., certain sawflies. The response to this type of oviposition approximates that of the plant's response to a physical injury; that is, the swelling which ensues is due only to the growth and enlargement of the developing embryo. The belief that simultaneously with oviposition a "poison" is injected into the plant has some basis in fact. Adler (1) points out in the case of *Nematus valisnierii,* also a sawfly, galling willow, that before the larva has left the egg, the gall is well developed through the effect of a secretion coming from the mother insect, discharged into the wound at oviposition. It must be remembered, however, that only the initial impetus to gall production arises in this way. For complete development of the cecidium, a continued stimulus is necessary. As Beijerinck (14) said: "The gall-forming influence of the insect therefore cannot be a single simple impulse, but the same must continue acting during a longer time." This is, as will be shown, quite true.

The mere puncturing of a plant tissue by ovipositor or stylets, the physical pressure of an object in or on a tissue, and the chewing or ambulatory movements of an animal through the tissues may be considered as the possibilities involved in the mechanical stimulation theory. The first of these considerations has unquestionably been disproved. Were it so, the penetration of plant tissue with a needle, a nail, the stylets of any non-gall-forming insect or by any other sharp object would produce the same effect. Horsfall (104) sums up the situation in the following sentences: "Attention has been called to the fact that no species of aphid under consideration induces the

formation of zoocecidia. If galls develop in response to the mere mechanical presence of the piercing setae, as some conclude, it is difficult to understand why such growths do not arise on the infested plants since gall formations are induced in some of these plants by other species of aphids. On the other hand, the data of the writer would seem to indicate the presence of materials injected by the insect." Smith (197) failed to duplicate the injurious results of capsid bug feeding by piercing and scratching leaves by means of a needle. Rosen (183) inserted in very young grape leaves a number of fine, capillary glass tubes, and allowed them to remain there until the leaves had grown "to a fair size." Punctures were also made in small leaf areas and marked. Sections were then made of the wounded leaf areas, and while microscopic examination showed dead cells in the punctured regions, no depressions were found around the point of injury. These experiments add weight to the belief that the depression in the vine leaf below the insect is not due simply to a puncturing by the insect's proboscis. Annand (5), Parr (166) and the writer have used glass pipettes in a similar fashion for another purpose, and have observed a like effect.

The probability that the physical pressure of an object upon a plant tissue may lead to gall formation is also disproved by Rosen (*loc.cit.*). He made small glass knobs with a stem just long enough to pierce the grape leaf slightly, and hold the knob in place. After considerable leaf growth had taken place, "microscopic examination of the injured areas showed dead cells which the pins had pierced. A slight depression was noticeable where the head of the glass pin had pressed against the leaf surface, but nothing was obtained resembling the insect cavity, with its fringe of hairs."

Molliard (see Rosen, p. 348) observed that pressure on the surface of meristematic tissue caused a depression at the pressure-focus, and hyperplasia in the area adjoining the depression. The egg of a gall-producing insect may be laid on the surface of a plant tissue, e.g., a leaf. It is glued to the leaf and might be supposed to exert a pressure which would lead to malformation. But no gall tissue develops until the larva has emerged from the egg. Beijerinck (15) mentions such an instance with respect to the gall caused by *Cecidomyia poae* on *Poa nemoralis*. According to him, the egg is laid on the surface of the growing leaf, and after incubation and eclosion the larva migrates to the place of gall formation. The latter proceeds for the most part through abnormal enlargement of the covering epidermal cells, followed shortly by enlargement of the sub-epidermal cells, as a kind of callus growth.

Although the mechanical-stimulus theory scarcely is longer credible, it is always mentioned in any review of gall producers. Even in recent times a few investigators have held to this view. Barber (12), in his study of the goldenrod gall caused by *Gnorimoschema gallaesolidaginis* Riley, stated that "the gall results from mechanical injury to the plant parts caused by the feeding of the insect." Shively (189), in his paper on the gall of wild lettuce caused by the cynipid, *Aulacidea tumida,* states that: "Apparently, either the mechanical irritation of the eating process or a chemical irritation from a larval secretion or both are responsible for gall initiation." Cook (51, 53) has modified his earlier convictions, to allow for the possibility of chemical stimuli. Cook's admission of the part which chemical stimuli may

play in gall formation appears to have been overlooked by Leach (136), who has also imputed the mechanical theory to both Adler and Beijerinck. Kostoff and Kendall (124) likewise misinterpreted Adler. Yet Herbst (99) in 1895 in his classical treatise on morphogenic stimulus gives credit to Beijerinck and Adler for having established the chemical nature of the stimulus.

As stated at the beginning of this section, Malpighi was the first investigator to point to an animal secretion as the basic source of the stimulus to gall formation. In this case, a fluid injected by the ovipositor at the time of oviposition was involved. Since then, several other animal (insect) products, both metabolic and catabolic, have been implicated. Certain of these will be mentioned at this time. At the outset it is appropriate to quote from Laboulbéne (130) a passage which may well be borne in mind. This author states: "The same cause stimulates whether the liquid is secreted by the cells of a gland opening itself into the mouth with or without organs of suction, or it oozes through the very walls of the body of a larva, a helminth, or finally, it is provided by a galligenous bacterium. It is therefore neither a puncture, nor an incision, nor a foreign body which is able to produce a lasting plant outgrowth, a tree gall; it is the soluble material, elaborated by the animal or plant cells, and these liquid substances have a special action, necessary, indispensable."

One of the older theories implies that a liquid contained inside the insect egg (deposited in plant tissue) passes out through the integument, and so affects the cells about it, and even at some distance from it (Adler [1]; Laboulbéne [130]; Magnus [147]). In this connection, it was also thought that the stimulating fluid in the egg did not escape until eclosion, when it was released into the host-tissue (Magnus [147]). It has already been mentioned in the quotation from Laboulbéne that the idea has been entertained of a fluid escaping into the host from the body cavity of a larva, through its skin. These theories of fluids passing into the plant tissue from either egg or larval integument are of dubious validity. The theory based upon initiation of atypical growth concomitant with eclosion is based upon the fact that in many cases gall growth does not begin until the egg has hatched. Presumably the larva, upon leaving the egg, immediately begins to exert its influence upon the plastic cells surrounding it; and this effect was attributed to the fluid escaping from the egg shell. It is possible that such fluid might contribute to the initiation of atypical growth, since a prenatal influence is necessary in other cases.

Rössig (185) first advanced the idea that the stimulus originated in the Malpighian tubules. He did not believe that the tubules were the sole source of the energizing material, however; but thought that the oenocytes in some way preconditioned the blood for further treatment by the tubules. Triggerson (213) took up this theory, but discounted any connection between the oenocytes and the stimulating substance. The oenocytes are now considered to be in some way connected with the processes of growth and perhaps of reproduction, but the nature of their secretion is still unknown. Triggerson studied the development of the gall caused on the leaf of white oak by *Dryophanta erinacei* Mayr., a cynipid. He dissected out the Malpighian tubules, dried and ground them, dissolved the resultant powder in physio-

logical saline, and filtered the solution. The filtrate was then injected with a hypodermic needle into the midrib of white oak leaves, one drop to a puncture. Check injections were made with saline only. The solutions containing the filtrate penetrated one-fourth to one-half inch into the fibrovascular bundles and the midrib. As to the results: "The tissue was turned yellowish brown, and cracking appeared similar to that seen in many young leaves where gall formation has just started, but owing to the death of the larva, has ceased. While these experiments did not produce a gall, they give suggestions as to the work performed by the secretion of the tubules. Nothing of the above described appearance was to be seen in the checks." This theory is plausible, but it should have been compared with the effect of the injection of salivary gland extract, since this larva has mandibles and feeds on the plant tissue.

Laboulbéne (130) carried out some experiments on gall-producing insects. He made plant inoculations using the body fluids of galligenous larvae, collected after cutting off of the larval head. Injections of water in which the larva of *"Cecidomys"* had been washed or crushed were also made; and fragments of the integument of this same larva were introduced into a leaf or a bud. He claimed partial success from these trials. Whether or not all of these various observations were correct is problematical. Nevertheless, Needham (159) says of this work: "The discovery of Laboulbéne is meanwhile important that the grafting of little pieces of dead larvae succeeded in producing galls (cf. the persistence of the organizer effect after the death of the cells of the organizer region)."

Still another theory of the cause of gall formation must be touched upon. Both Küster (128) and Cosens (54), working with different species of the same genus of sawfly, concluded that the stimulus had its source in the larval excrement. Of this Cosens has to say: "Küster states that the excrement of *Pontania salicis* is capable of producing cell division. I have found this phenomenon occurring also in *Pontania pomum* Walsh and particularly good examples in the undescribed sawfly gall on *Salix serissima* (Bailey) Fernald (Fig. 72). While I have made no attempt to determine by experiment the cause of this unusual example of cell proliferation, yet it would seem highly probable that the enzymes, introduced into the protoplasm by the ovipositor of the producer and swallowed by the larva, have not entirely lost their power by passing through the digestive tract but are still able to excite cell division." Thus, Cosens indicates that the primary source of the stimulus is the mother animal, and does not imply that the larva in this case is capable of producing its own enzymes. Such a situation is most unlikely.

More recently LaRue (133, 134) investigated animal excrement as a source of the stimulus. He cites the observations of Smith (191), which are not in all cases valid, that "insects which lived with the mouthparts, whole head or fore part of the body buried in plant tissue never formed galls, but that the gall-forming larvae were always completely surrounded by plant tissue, from which facts he assumed that the excretions or secretions of the posterior end of the larva might act as a stimulus to gall formation" (from LaRue, 133). This author mentions the fact that auxin has been found to be present in the urine of various animals and hence it appeared likely that it would be present in the feces of insect larvae. He observed

that outgrowths appear around each pellet of feces in contact with the mesophyll in poplar leaves tunneled by leaf-mining larvae. Later he placed pellets of feces of a number of insect larvae, and of mice, in contact with the exposed mesophyll of leaves, and found that these induced the formation of cell outgrowths of this tissue. Injection of 0.00005 per cent heteroauxin in the mesophyll produced a mass of cell outgrowths twice as thick as the original leaf.

Some late work of Boysen Jensen (22) is reminiscent of that of both Laboulbéne and LaRue. The former investigator attempted to determine whether or not the larvae of *Mikiola fagi* secrete substances that cause leaf galls of beech. He placed larvae either directly on young leaves, or upon pads of lanolin paste which thereby separated the larvae from contact with the leaf surface. In addition, a similar paste upon which larvae had been kept for some time was put on leaves. As to the results of these experiments, Boysen Jensen says: "It appeared that one or more substances were secreted by the larvae causing the following effects: 1) an embryonic growth and cell divisions; 2) a cell elongation." He also treated decapitated *Nicotiana* hybrids with β-indolyl acetic acid, and compared the resultant callus formation with that of untreated plants. Boysen Jensen then concluded that the larvae secrete substances similar to growth hormones "in definite places of the beech leaves and in the gall, thereby making the latter adopt its definite form. Thus a special gall-forming substance does not exist." Unfortunately he did not attempt to localize the source of the stimulating material.

Uhler (215) removed young larvae of *Eurosta solidaginis* (Fitch) from the host plant (goldenrod), just as they were entering meristematic tissue, and galls were beginning to form. He macerated a number of these in saline solution over ice, centrifuged the solution, and injected it into immature plants with a hypodermic needle. No galls were induced by these injections.

Beck (13),[1] working with the same insect, injected into the plants the following solutions: 1) extracts of galls with organic solvents; 2) distilled water extracts of galls; 3) extracts of maggots made with both organic solvents and distilled water; 4) extracts of different media on which maggots had been growing, and 5) distilled water in which maggots had been kept for three or more days. Positive results were obtained only with distilled water extracts of maggots, and with water in which maggots had been kept.

Rosen (183) attempted to reproduce the effects of *Phylloxera vastatrix* on grape leaves. He had taken nymphs from galls, placed them in starch solution and obtained tests for sugar, while checks were negative. He qualified the results, nevertheless, by saying: "I am not sure, however, that I did not introduce wild yeast or other micro-organisms along with the nymphs." Then he placed nymphs in water and sprayed young vine leaves with this fluid, and injected the same into leaves with fine glass tubes. All gave negative results. Apparently he did not macerate the nymphs, but depended on diffusion of the enzymes from them into the water.

Martin (154) induced stem galls of sugar cane by artificial injections with insect extracts. Adult leafhoppers (*Draculacephala mollipes* and

1 Thesis seen by courtesy of Dr. C. D. LaRue.

Peregrinus maidis) and a mealybug (*Trionymus sacchari*) were macerated in water. The extract was injected into sugar cane with a hypodermic needle, and gall growths resulted.

Keller (113) was "disposed to accept the initial influence of a secretion on the delicate plant tissue" in his study of *Chermes* galls on *Picea*. He knew that this insect possesses salivary glands; but maintained that their secreting capacity was so small that they could not be the source of the stimulus. Instead, he held that the latter stemmed from the epidermal glands, which actually produce the white, waxy flock that covers mature females.

The work of Gurwitch and Franck (93), and Magrou and Magrou (148) may be mentioned briefly. The first writers conceived that the morphogenic stimulus for cell division is due to "mitogenic rays." The latter men followed this conception in their investigations of *Bacterium tumefaciens,* and concluded that the galls were produced as a result of the mitogenic rays stemming from the bacteria. In the light of the more recent work on the chemical nature of the products of gall-producing organisms, there can be no place for such an approach.

Many of the older concepts of the source of the gall-forming stimulus have now been reviewed. Some of these have been discarded, others have been retained. The facts pertaining to one of the most probable and important sources of the stimulus, namely, the products of insect salivary glands, will be taken up at this point.

Considerable work has been done which establishes the salivary glands of insects as the producers of digestive enzymes. With the discovery that insects may carry and transmit plant diseases, there was initiated a great interest in the feeding habits of insects (particularly sucking insects) and their oral secretions. Thus, much of the research has involved insects which are not gall producers, but which either directly or indirectly do cause the death of plants or at least of plant cells.

Smith (197) tried to reproduce the harmful effects of capsid bug feeding by scratching and puncturing leaves with needles. These experiments yielded negative results, so he then made use of insect salivary glands and saliva. The capsids which he studied cause the death of cells in the region of the feeding puncture, leading to distortions and russeting of the apples on which they feed. More than one species of capsid is involved, however. Some species cause the above harmful effects while others do not. Smith made histological studies of the salivary glands of both types, and could detect no differences between the two. Neither did he find bacteria present in the glands. Salivary glands were taken from both kinds of bugs and pricked into leaf tissue. Only those from the harmful species (*Plesiocoris rugicollis*) killed cells under the glands, while those of the non-injurious species (*Psallus ambiguus*) gave no effect. A large droplet of saliva exudes from the beak of the latter species just before it punctures the tissue. This droplet remains on the leaf surface and eventually dries up without injury to the underlying cells. Such droplets drawn up into a fine capillary and injected into plant tissue likewise fail to cause injury. On the other hand, the droplets exuding from the puncture of *P. rugicollis* do kill the underlying cells.

This work supports the observations made previously by Busgen (32), Busse (34), Gruner (92), Plateau (171) and others. Plateau and Gruner excised the salivary glands of insects, triturated them, and tested the solution on starch, a much sounder procedure than using the insect entire or macerated. In neither of these methods can one isolate the precise organ from which the active material originates. Both of the above investigators found that starch was changed to sugar by enzymes in the glands. This was an important discovery, for it had been believed that starch granules were drawn up through the mouthparts of sucking insects, and then ground up by little toothlets in the pharynx. This is, of course, an impossibility because the channel of such a mouthpart could not receive an object of such size.

There exists an extensive bibliography on the injury caused in this manner, or by actual tissue damage caused by the physical effects of puncturing (or by a combination of both) during feeding by insects of this type (see Brown [27]; Carter [36]; Granovsky[89]; Horsfall [104]; Weber [221]; Zweigelt [239]). But there is a distinct difference between cell injuries of this sort, and tissue aberrations of galls. In the first instance, cells are killed at the time of feeding, leaving necrotic areas. In the latter case, plastic tissues are molded in a pattern which frequently is repeated from generation to generation. Death of the gall tissues may occur with the cessation of feeding, but on the other hand, galled tissue may resume nearly normal growth after emergence of the insect (Stewart [201]). Although there is not an exact parallel between the two types of feeding, the former does point to the salivary glands as the source of a highly potent secretion, capable of affecting certain cell constituents.

Despite the fact that many investigators have postulated that the salivary glands are the source of the gall-stimulus, they have not proved the point by using salivary glands or their products, disassociated from the insect body. Keller (113) interpreted the statements of two previous workers to mean that the salivary glands were the source of the stimulus, although he refused to accept this thesis. Keller says: "Although Ratzeburg and Frank neither expressed it definitely, their explanation obviously meant that from the puncture-wound of the stem-mother there goes forth an effective agent, which causes the degeneration of the plant tissues. This agent must stem from the mouth parts of the insects and can naturally only be a gland secretion. Now salivary glands occur, of course, in the anterior portion of the intestinal tube of plant lice."

Cosens (54), employing larvae of *Amphibolips confluens* Harris, concluded that the larvae were able to secrete an enzyme capable of converting starch to sugar. By means of sections he discovered salivary glands and their openings below the mouth. Unfortunately, however, he placed entire larvae in the starch solution rather than excised glands only. Thus, absolute proof that the glands alone are the source of the enzyme is lacking, although Cosens states that they are.

An exception is the work of Parr (166, 167) who, by his work on two different species of gall-forming scale insects, has demonstrated that the salivary glands are the source of the stimulus. He dissected the glands from *Matsucoccus gallicolus* Morrison and macerated them in glycerine solution. The solution was drawn up into tiny micro-injection bulbs, the open ends of which were inserted into twigs of pitch pine, the host of this scale insect. A

swelling and injury similar to that caused by the insect resulted. Glycerine solution checks showed no such effect, but only a very narrow ring of dead tissue immediately around some of the bulbs. The same technique was used in his study of the golden oak scale, *Asterolecanium variolosum* Ratz., with the same results.

So far, the source of the stimulus in the case of sucking insects has been traced to the salivary glands. It has been supposed that the enzymes (or other materials) formed in these glands modify the cell behavior so that it reacts in a manner different from normal. Lewis and Walton (140) have found such a modifying substance in the cells of a gall formed on witch hazel by an aphid, *Hormaphis hamamelidis* Fitch. But according to these investigators, the material is distinct from the salivary secretion. It is "secreted by glands opening into the stylar canal," and is injected into a young leaf by a "stinging" process which is different from that of feeding. This material shows staining and refractory characteristics which are not the same as those of saliva. It breaks up into globules which eventually find their way into the nucleolus, and are redistributed during mitosis. Repeated injections of the material are necessary, however, for continuation of gall formation. Only the stem mother is capable of initiating gall growth. These findings are most unusual and interesting; particularly as regards a stimulating material separate from the normal salivary secretion.

There is still another unique theory as to the source of the stimulus. This involves the thought that although the "enzymes" originate in the salivary glands, the latter are not the actual producers of them in a metabolic sense. Rather, they are the products of symbionts.

It has long been known that many species of insects harbor microorganisms within their bodies, both intra- and intercellularly. An excellent account of this fascinating relationship may be found in Steinhaus (199). The microorganisms found in insects include bacteria, protozoa, yeasts and molds. They may be found in the lumen of the intestinal tract, in what are apparently special outfoldings of the intestine, in cells of the intestinal wall itself, in modified Malpighian tubules, and in mycetocytes which may lie scattered singly or be gathered into a more definite mass called the mycetome. The identity, and the role, of these organisms has been a subject of intense speculation. The exact part that they play in the life of the insect is not easily determined. It has been thought that those found in the gut were used as food by the insect; it has also been considered that they are merely incidental and are in no way implicated in the economy of the insect. However, there is definite evidence that in some cases, at least, these organisms are important to the existence of their host.

Since a complete account of what is known concerning the symbionts found in the intestinal tract of insects is to be found in Buchner (29), Steinhaus (199) and Wigglesworth (231), this type will be mentioned but briefly. These symbionts are presumed to function in nutrition and metabolism, or by providing accessory factors which supplement an incomplete diet. Certain insects (termites) appear not to be able to digest wood if the flagellates normally found in the gut have been killed (Cleveland [48]). On the other hand, many of the other wood-feeding insects do not possess symbionts, and thus are not dependent on them for digestive function.

When the presence of symbionts intracellularly and in organs separate from the intestinal tract, or only indirectly associated with it, is considered, the relationship with the life of the insect becomes even more obscure. Leydig (142) observed in aphids a peculiar organ which has been variously termed since that time. This organ contained bacteria-like organisms. In 1910 Sülc (205) proposed the names of "mycetome" for the organ and of "mycetocyte" for the individual cells composing it, and in which the symbionts occur. The latter he described as being *Saccharomycetes.* It has been shown that symbiosis is hereditary, that is, the organisms are transmitted from generation to generation from the mother insect to the egg (Gier [86], Uichanco [216]). The symbionts may reach the female by way of the sperm (Mansour [151]). Thus, the symbionts are present in each of the developmental stages of the insect. Further, the symbionts may well be specific for the host-species. Mahdihasson (149) was able to separate species of coccids by means of blood smears which contained symbionts characteristic of the specific insect. Tóth (210), as a result of his extensive work on the symbionts of certain aphids, suggested that in view of the constancy of their occurrence, the aphid genera might need revision. Tóth (208) found that aphids which feed on branches, stems or roots had larger salivary glands than those which feed on leaves. He also observed that all aphids which are symbiont-free have no salivary glands. Such aphids were short-lived, and had rudimentary mouthparts. Oviparous females with this type of mouthpart also lacked glands, but had a reduced symbiont-quantity for egg infection.

The work of Haracsi (94) has the widest implications, from both a morphological and a biological standpoint. Haracsi studied the embryology of certain aphids, including *Prociphilus bumeliae* Schrk., *Lachnus roboris* L., and *Pemphigus bursarius* L. and *P. spirothecae* Pass. He maintains that the salivary glands are derived from the mycetome-anlage, rather than from the ectoderm. An anterior portion of the anlage gradually separates from the basic mass, divides, and further develops into the glands. The duct is formed by an invagination of the fore-gut ectoderm. This is an entirely new concept of the origin of the salivary glands; and at the moment it is difficult to say whether or not Haracsi is correct. However, it may be borne in mind that the insect intestine is a tripartite structure, the segments of which are not all derived from the same basic germ layer.

Thus, the salivary glands contain the same symbionts which are found in the mycetome. Regarding the purpose of the symbionts thus situated Haracsi has this to say: "The function of the salivary gland microorganisms consists therein, to deliver to the host enzymes for the decomposition of difficultly soluble plant materials (cellulose, hemicellulose, starch, proteins, etc.), in this way the animal easily reaches fluid nourishment, consequently the food-stuff-content of the plant tissue can be utilized completely . . . Likewise, one can perceive, that the symbionts also play a role in the case of the origin of frequently-occurring gall formations after the effect of the sucking of beaked-insects." The author's summary contains no direct statement as to whether he attempted to isolate enzymes from the glands. According to Michel (156), however, "Haracsi has been able to separate these substances in prepared artificial symbiont cultures."

Other workers have tentatively proposed somewhat similar theories as to the relation between insect symbionts and the host plant, but have not carried their ideas as far as did Haracsi. Lutz and Brown (146) described a new bacterium from a gall-forming insect, and from the gall tissue. "The senior author has been wondering for some time if all plant galls, including those supposed to be directly caused by insects, are not fundamentally due to either bacteria or fungi." They injected witch hazel leaf buds with the bacteria, but results in all cases were negative. (See also Carter and Cotner [40], Herford [100]).

Carter (36) found a distinct association between the symbiont flora of a mealybug and its ability to cause green spots on the host plant. A non-spotting strain of the same insect possessed a different type of symbiont in its mycetome than did the spotting strain. By feeding the mealybugs on a grass rather than on pineapple, the normal host, he found that the green-spotting capacity was lost upon transfer back to pineapple (Carter [39]). Coincident with the loss of the spotting capacity was the disappearance of the symbionts from the mycetome.

This new theory of the source of the stimulus and of gall formation is most interesting, and appears to be supported by some indirect evidence. If it should prove to be true, it would supply an even closer linkage between plant galls presumed to be caused directly by insect products, and those malformations produced by bacteria, for example. It is known that different species of the same insect genus produce different galls on the same host plant tissue. In fact, some of these gall-producers are more easily separated on the basis of the form of their galls than on their own intrinsic morphological characters. Tóth and others have shown that the occurrence of at least some insect symbionts is remarkably constant, and different for each species. If the galls are produced by the effect of symbiont metabolic or catabolic products, it would be more easy to account for the quite diverse forms of the galls.

Composition of Salivary Secretion

To recapitulate briefly, Malpighi thought that the material injected into the plant by an insect at the time of oviposition was a poison or venom akin to that of a bee. This poison affected the cell sap, and also caused the movement thereof to be concentrated in other than normal channels; thereby altering the growth potentials and resulting in the development of abnormal tissue. Triggerson, who proposed that the Malpighian tubules were the source of the stimulus, suggested "that something of an enzymic nature might be present." Cosens' opinion that the stimulating excrement of one gall-former contained enzymes introduced at oviposition, and which were passed unchanged through the larval intestine, has already been quoted, as has the work of LaRue. Hofmeister thought that a fluid secreted by the salivary glands, even before eclosion of the larva, possessed "amylolytic and proteolytic enzymes." Most of the subsequent work on the nature of the stimulus, moreover, has been done with insect salivary glands, which undoubtedly are the major source, directly or indirectly, of the stimulating substances.

The earliest information on the composition of arthropod saliva was obtained chiefly from that of the various blood-sucking bugs. An excellent

review of this work is to be found in Zweigelt (239). Burmeister (in 1832) ascribed a poisonous nature to the saliva. Geise (in 1883) stated that the salivary fluid was strongly alkaline and corrosive, and that these properties increased lymph flow toward the locality of the puncture (cf. Malpighi). He also pointed out that the mere physical puncture could not produce the effect observed. Weddle (in 1885) not only worked on the effect of bedbug bites (saliva alkaline), but also conjectured that in the case of aphids the saliva had the power of increasing sap flow. Blath (in 1899), without, it appears, having made independent investigations, accredits such increased sap flow as the basis for the swelling of plants attacked by aphids. Busse (in 1905) discounted this theory, stating that in this belief the botanists were not intelligible, but considered the alkalinity to be of importance for the activity of ferments (cf. Beck [13]).

Plateau (in 1874) made some actual experiments on the properties of water scorpion salivary glands. He found that the glands, macerated with starch paste, could transform the starch to glucose. Gruner (in 1901) detected a similar reaction, using larvae of *Aphrophoris salicis*. Büsgen (32), in referring to the change of starch to sugar by enzymes, states: "A similar material must indeed be of great advantage to the aphids. The solution of material in a cell under continuous slow suction of the formed sugars induces an osmotic stream thither toward the pierced cell, which always supplies nourishment to the animal." More recently Davidson (61) and Herford (100) have confirmed this conversion capacity of insect saliva.

The results of many investigations into the cause of the death or atypical growth (or both) of plant cells following the feeding of non-gall-forming insects have been published. The authors point out that cell injury may be due to actual laceration, to a substance secreted by the insect, or to a combination of both of these. Yet some sucking insects can feed without, apparently, injuring the host tissue. For example, Medler (155) summarizes his investigation thusly: "A histological study of the feeding punctures of *Aceratagella sanguinolenta* (Prov.) and *Macrosteles divisus* (Uhl.) shows that these leafhoppers cause no apparent internal injurious effect in alfalfa leaflets. . . . On the other hand, the nature of plant injury by *Empoasca fabae* (Harris) is unique, and a combination of the insect's feeding habit in vascular tissue and the action of a specific compound injected during its feeding process. This secretion causes hypertrophy in affected cells, and its effect is first characterized by nuclear enlargement and prominent safranin-stained nucleoli."

The actual cases in which some attempt has been made to determine the chemistry of isolated salivary secretion in the case of gall-forming insects are only too few. Parr (167) excised the salivary glands of *Matsucoccus gallicolus* Morrison, macerated them in glycerine solution, injected them into pitch pine twigs, and found that swelling and injury similar to those caused by the insect were produced. In his work with *Asterolecanium variolosum* Ratz. (166), the same technique was employed with like results on chestnut oak. Checks were negative, although Annand (5) found that 1 per cent glycerine solution injected into kale caused the development of small tumors in the midrib. In addition, Parr made micro-chemical analyses (both qualitative and quantitative) of the constituents of excised

glands, and found that amylase, invertase, and a protease were present. Tests for cellulase, oxidase and peroxidase were negative.

Martin (154) in 1939 induced the formation of stem galls of sugar cane by injection of insect extracts. Adult leafhoppers (*Braculacephala mollipes*) were macerated in water and the resultant extract was injected with a hypodermic needle into young plants, below the growing point. Control plants remained normal. According to Parr (166): "The stimulus for gall production has been shown to be chemical in nature and has been artificially produced by injections of salivary extract from the insects. Failure of the salivary extract heated to 60° C. to produce galls points to an enzyme or enzyme-like substance or groups of substances as the cause of gall formation." Yet Martin says: "In recent studies (1941) galls have been produced with an extract prepared, as described above, from adults of the corn leafhopper, *Peregrinus maidis,* and the pink sugar-cane mealybug, *Trionymus sacchari.* It was also shown that stem galls developed when extracts prepared from the corn and green leafhoppers were sterilized at 15 pounds steam pressure for 20 minutes, thus indicating that the stimulus to gall formation is chemical rather than biological in nature. It is possible that insects carry certain growth-promoting substances which when injected into plants during their feeding result in gall formation."

It is rather difficult to reconcile these contrasting findings. Martin, of course, used entire insects rather than excised organs, so that no one organ was isolated as being the source of the stimulus. Parr's work indicated that enzymes were involved, for sterilized gland extracts failed to produce galls. Even though Martin employed whole insects, surely any enzymes present would have been destroyed by such treatment. On the other hand, it is possible that growth-promoting substances as mentioned by Martin, would not be inactivated by the sterilization method which he used. If auxin were involved, it would not be so destroyed.

In addition, Beck (13) recently found that washings of larvae of *Eurosta solidaginis* Fitch contained invertase and maltase; and a proteolytic enzyme capable of digesting only simple proteins. The water in which maggots were macerated contained amylase in addition to the above enzymes, and also a proteolytic enzyme which could digest more complex proteins. He also caused cell enlargement of the pith region in young stems of goldenrod by injecting extracts of the maggots. Similar results were obtained by injections of trypsin, bacto-peptone, bacto-tryptone, tryptophane, 3-indole acetic acid and naphthalene acetic acid.

The situation may be further complicated by the presence in the plant tissues of the so-called "stylet sheath." This sheath, a two-layered tube, may be found in the tissues of plants wherever certain sucking insects have fed. It remains in the tissues, retains stains, and is an excellent source of information as to what specific cells the stylets have touched upon. It has been considered in the past that (1) the sheath is entirely of plant origin and is formed in protective response to injury; (2) the sheath is partly of plant, partly of animal origin; (3) the sheath is entirely of animal origin. The work of Smith (195), Storey (202), and Carter (37) appear to have proved that the third possibility is correct. Carter fed a mealybug on agar blocks, and found that when the stylets were withdrawn, a feeding track or

sheath is left behind. These sheaths could be dissected out entire, from the blocks.

If the sheath is wholly of animal origin, its source presumably is the salivary glands. These glands, then, must be of a most complex nature. The sheath itself, once it has become set from a fluid state, may be completely inert, or on the other hand, certain components of it may remain capable of action. Storey (202) has suggested that an "electrolyte" issues from the insect with the fluid, independent of the sheath-forming saliva, or as a soluble component of it at the time of its ejection. It is also possible that the sheath itself, even after gelling, contains an active principle which is slowly soluble and is given off gradually. In any event, it is evident that the constitution of the saliva in the case of non-gall-forming insects is quite different from that of gall formers, even if both produce a stylet sheath.

Sukhov (204) found that the stylets of *Myzus persicae* Sulz., including the points, were completely enveloped in, and moved inside, the stylet sheath. Thus, the fluids taken up by the aphid are filtered through the sheath, and permeability tests showed that bacteria cannot pass through it. This fact, of course, eliminates the proposal of Lutz and Brown (146) that bacteria transmitted by plant-feeding insects are involved in the production of galls; that is, aside from the possibility of sheer mechanical contamination. It does not, however, completely exclude the theory that symbionts in the glands produce enzymes which may pass into the host at the time of feeding.

Not all gall insects (nor indeed, all plant-sucking insects) form a stylet sheath. The sheath, then, may or may not play a part in gall formation. In some instances one or more of its constituents may be involved. In other cases, the saliva may serve only for extra-intestinal digestion concomitant with the gall stimulus, and no sheath is formed. The actual connection of the stylet sheath with gall formation thus is quite uncertain, whereas the sheath of non-gall-forming insects frequently occludes the vascular tissues.

All the possible sources of the stimulus have now been mentioned, and in a few cases the composition, in part, of animal secretions which might be implicated in gall formation has been given. Unfortunately, the qualitative micro-chemical methods available at present are inadequate, and furnish only an indication of what may be present. The various enzymes detected in gall-forming insects are common also to insects that do not form galls.

A recent work of White (229) is very interesting in this connection: "Tissue cultures were isolated from crown-galls on *V. rosea* rendered bacteria-free by heat therapy. These showed a very rapid discoördinate growth in vitro and when grafted back into healthy host plants produce typical tumors which regularly exceed in size those generally produced by direct multiple needle puncture inoculations of tumefacient bacteria." White points out that a *real increase* in virulence of the tumor tissues involved may have taken place *in vitro*. Here, we have the effect of the causative organism persisting to such an extent, in the absence of the organism, that the affected cells are capable of autonomous tumor growth. And Gautheret (82) says: "This virological hypothesis of plant cancer is evidently only tentative. It is not altogether arbitrary, however, because some viruses are capable of breeding tumours in plants (Black)." And further: "It thus appears that

the cancerization of plant cells is not a genetic phenomenon, but that it is similar to the enzymatic adaptation so frequent in micro-organisms."

As matters stand, then, the composition or even the nature of the stimulus cannot be set forth. Further, a study of the galled tissues gives no clue as to what has affected the cells. In a pertinent statement Wells (223) says: "I cannot find a single structural fact produced by any one in the study of the initial stages of Hemiptera galls, that throws any light whatever upon the profound problem of the nature of the highly specific stimulus applied by the insect to the embryonic plant tissue."

Site of Action

One is probably quite correct in stating that the prime requisite for gall formation, aside from the stimulant, is the presence of plastic, immature plant tissue. This tissue does not necessarily have to be cambium or another of the more or less well-defined meristematic strata. It need only be in such a state that an outside stimulus can alter its normal growth pattern. There appear to be no known cases in which insect galls have been formed on tissues that have become completely mature. This fact rather limits the initiation of gall formation to a time when the cells of the various plant organs are at least in an early stage of development. That such a situation prevails may be shown by a study of the life history of a gall insect and the atypical growth which it produces. The insects themselves may lie dormant during the time when the plant also is dormant, and only become active with the renaissance of new growth. Indeed, it may well be that the plant, the physiological system of which is so different at this time, furnishes some material which the insect can use only then, to synthesize the stimulant.

The entire problem of precisely what cells and what tissues may enter into gall formation, and whether their entrance into abnormal growth is in any way limited, is rather obscure. Galls occur on roots, stems, buds and leaves of plants. Thus, it appears that almost any organ of a plant may be affected and able to respond to the stimulus. There are instances in which the cambium or a definite meristem are involved, but many others in which galls occur on tissues that are plastic but that contain no restricted meristem. As an example, many diverse insect galls are to be found on the leaves of deciduous trees. The leaf is preformed in miniature before the bud opens. When the latter has opened, the leaf grows in size chiefly by increase in cell size, although new cells may be formed gradually throughout the leaf (Eames and McDaniels). The insect attacks the leaf while it is still growing, and perhaps not until it has attained a fair size. The leaf, as mentioned, contains no well-delimited meristems, but the cells of the leaf as a whole are in a labile condition. In this connection, many detailed descriptions of the anatomy of galls are to be found, particularly in the works of Cook, Wells, Cosens and Felt. These authors do not, however, solve the problem outlined above. According to Shively (188), the type of gall produced on two species of *Latuca* by three different species of *Aulacidea* is dependent on the site of oviposition.

The only suggestions as to the specificity of the elements entering into galls are to be found in Butler (35) and in Cook (53). The latter says: "Wells's studies reaffirm the idea expressed by the writer that all galls originate with the excessive development of parenchyma tissue." Cook prob-

ably is correct in this statement. Butler lists various galls of both plant and animal origin, by host tissues—pith, epidermis, cortex, vascular cylinder, leaf—but in all of these, cells of a parenchymatous nature are the ones involved.

Effect of the Stimulus

Growths which are similar in appearance to galls have been formed on plants following treatment with various materials, such as simple inorganic chemicals and complex organic compounds, including carcinogens, alkaloids and growth hormones. Some of these cause abnormal mitoses to occur, as well as other irregularities in cell behavior and make-up. How these agents bring about these atypical reactions, that is, their actual effect on the cell, is not known. There must be a close parallel between the action of these synthetic agents and that of the gall-producing stimulus. However, the composition of the latter still is unsolved, as is the mechanism of its action upon the plant cell. Parr (166) has traced some of the chemical changes in the tissues galled by *Asterolecanium variolosum* Ratz. (on oak), but has not thoroughly explained the meaning of these changes. Beck (13), on the other hand, has given a logical explanation of the effect of the stimulus. He "concluded that the secretion of the proteolytic enzyme by the maggot and the elevated pH tended to keep conditions in the maggot infected region of the stem similar to conditions in the growing point of the stem and thus continue active growth in this region for periods much longer than normal. It was also concluded that the hydrolysis of tryptophane, to the growth-promoting substance 3-indole acetic acid is a second important factor in this case of gall formation."

The stimulus which leads to formation of galls affects not single cells, but cell tissues (Fisher and Holloman [74], LaRue [135], Needham [158]). The effect of the stimulus may lead to hyperplasia or hypertrophy (or both) of the cells involved. But this effect is conditioned by several factors, namely, the state of the cell, the nature of the tissue of which the cell is a component, and the location of this tissue in regard to the plant as a whole. The cells need not necessarily be part of a true cambial layer. They must, however, as previously stated, be in a plastic, labile condition. The state of the cell at the time of gall initiation may be of the greatest importance. Haracsi (94) believes "that the gall formation depends primarily on the plant, especially its physiological and development condition, and that insects play only a secondary role. . . . The Fundatrix which emerges from the fertile egg in the spring is, chiefly owing to its favorable situation, alone gall-forming, the other generations are, however, equally capable thereto." Burdon (30) held that the stimulus is non-specific from one species to another of the spruce gall aphids of the genus *Chermes*. Boysen Jensen (22) even stated that "a special gall-forming substance does not exist." It is known that plant cells, which are by all ordinary criteria mature, may change their form and function and play an entirely different role in the plant economy (see Eames and McDaniels [65]). Insects, however, cannot so affect mature plant cells.

Once gall formation has begun, involving hyperplasia and hypertrophy, gall development may take one of two courses. The resultant gall may be a kataplasma or a prosoplasma (Beijerinck [14], Küster [128]). The first

category includes the galls of indefinite form in which differentiation is not too divergent from normal, and is not consistent. In contrast, a prosoplasma is a growth in which differentiation is specifically and widely divergent from normal. Such galls are quite constant in form and structure from one to another, and from generation to generation.

This leads to the question as to whether or not any unique tissues actually are present in galls. Cosens (54), Cosens and Sinclair (57), and Küstenmacher (126) assert that nothing structurally new is to be found in gall tissue. Wells (223, 224) holds a contrary opinion. The evidence, however, appears to favor the former view. Other workers (Butler [35], Küster [127], Needham [159], Went [227]) consider that abnormal growth is not a direct and abrupt transition of the cell into something new. Rather it is an escape of the cell from the inhibitions imposed on it by normal growth factors and patterns (Smith [192]). When this occurs, its hitherto suppressed potentialities are disclosed. The stimulus itself may play an important part, however. If it is in the form of an evocator, then which of the latent possibilities is to be expressed is dependent upon its nature. But if a particular tissue is stimulated to hypertrophy and hyperplasia, by whatever cause, its ultimate external form (and internal arrangement) may be determined by the stresses fixed by its position in the plant (Thompson [207], Weisse [222]).

To summarize, the effect of the stimulus and the reaction of the plant are interdependent upon location and condition of the cells affected, and upon the nature of the stimulus itself.

The Norway Spruce Gall

This gall is commonly known as the "pineapple" gall of spruce, because of its resemblance to that fruit (Figure 1). It is a polythalamous prosoplasma (Figure 2), and is borne only at the base of the one-year or current shoot. In the past, most investigators have assumed that the gall tissue is made up of cells of the needle-leaves. None of them, however, in studying the gall, have traced at the same time the development of the normal shoot from the bud. This work includes such a study as a necessary prerequisite to an understanding of what tissues go to form this gall. Concurrently, the development of the gall from the differentiating bud tissues has been investigated. An attempt has also been made to isolate the origin of the gall stimulus, and to investigate its nature.

MATERIALS AND METHODS

Infested and uninfested twigs of Norway spruce were collected and fixed in a series from dormancy through the growing season to completion of development. The twigs were brought into the laboratory where the desired specimens were finally selected. Since the bud is covered tightly by closely appressed bud scales, the latter were removed with forceps to insure satisfactory fixation. This was done carefully under the dissecting microscope to avoid injuring the bud tissues. The buds were then clipped from the twigs close to the base.

The fixative used was a modified Bouins' fluid, containing 70 per cent ethyl alcohol, 85 cc.; formalin (40 per cent), 10 cc.; glacial acetic acid, 5 cc.; picric acid, 2 gr. Fixation was carried out in partial vacuum generated by a water pump. The tissues were kept in vacuum until all air bubbles had ceased to come from them. Air was then admitted gradually to the container, thereby assuring complete infiltration of the tissues with the fixative. The tissues remained in the fixative for from 24 to 40 hours, depending upon their size. Then they were washed in 70 per cent alcohol until the picric acid was completely removed.

The tissues were dehydrated with tertiary butyl alcohol after the method of Johansen (108). Following dehydration, infiltration was accomplished through a butyl alcohol-paraffin oil-paraffin mixture (see Johansen). The paraffin used has a melting point of 56-58 degrees C. It was found that the sooner the material was infiltrated and embedded, the easier it was to section.

Considerable difficulty was experienced in embedding the larger pieces, such as large buds and whole galls, without air bays. Finally a technique was evolved that was more satisfactory than any tried heretofore. Metal "L" embedding boxes were employed, and were slightly chilled before using. The box was arranged according to the size of the tissue, and filled with molten paraffin, which hardened rapidly at the periphery. The tissue was quickly added and oriented, and sides of the box removed. Gentle blowing of the breath caused the top surface to skim over. It was found that if the block were plunged directly into cold water at this point, as usually recommended, air bays inevitably resulted. This is due to the fact that the stronger bottom and side-walls of paraffin, on contracting, force the upper layer of paraffin away from the object. A prominent ridge with an air bay beneath it was always found running across the block.

Instead of plunging the block into water as soon as the surface could bear the weight, the breath was blown continually across it until the entire upper surface ceased to shrink downwards. The congealing paraffin had now shrunk so as to be in close contact with the object. The block, still adhering to the base plate, was then inverted and immersed to a depth of only a few millimeters. It was held in this position for about a minute, then totally immersed, base down. If the block is placed in water too soon, air bays develop. If immersion is delayed too long, the paraffin crystallizes and the inner portion surrounding the object is crumbly. The lapse of time after embedding and before immersion varies with the size of the object, and can be determined only by experience. Small air bays which occasionally occur can be pricked out and filled in with a hot needle, but such treatment should always be followed quickly by chilling.

Some material was double-embedded satisfactorily in pyroxylin and paraffin. It was found, however, that unless the methyl benzoate was completely removed (in benzene), the oily surface made embedding difficult. The adhesive used was that recommended by Johansen for the purpose, and the sections were further protected by dipping in a 1 per cent ether-alcohol solution of pyroxylin following xylene. The slides were clearer if this coating was removed after staining, but the stains used were partially taken out by this procedure. Further, some sections or parts of sections came loose and dropped from the slides in the alcohol-ether solvent. When the material was fresh enough to section easily, plain paraffin embedding proved to be the best.

The sections were cut at 12 micra on a Spencer rotary microtome. Thinner sections were not advisable because of the loss of depth. The sections were stained with Sharman's modification (187) of Foster's schedule (77) for meristematic tissue, making use of tannic acid, iron alum, safranin O and orange G. The staining procedure is very rapid, for it takes only about 15 minutes to run the slides through, in contrast to 48 hours by Foster's method. It was found that iron chloride substituted for iron alum gave better results, as the latter stained too rapidly and left a muddy deposit in the cells. Dehydrating and mounting were carried out by the usual methods.

THE NORMAL SHOOT OF *Picea excelsa* (Lam.)

The Mature Shoot and Dormant Bud

This description includes the upper part of the mature stem of *Picea* and the bud that it bears. The accounts of the morphology of the dormant bud are taken from Busse (33), Koch (120), Korody (121), and Warren (219). Lewis and Dowding (139) have described the growing point and bud of *Picea canadensis* (Mill.); while Crafts (58), Cross (60), and Sterling (200) have described these structures in *Sequoia sempervirens* (Lamb.) Endl., and Foster (75) those of *Abies venusta*. Other details have been taken from Chamberlain (41) and Eames and McDaniels (65).

Below the bud is found the stem formed during the previous growing season (Figures 3 and 4). The stem is bounded externally by the epidermis, a uni-cellular layer in which the cells have thickened walls. Medially to the epidermis is the cortex, which is made up of several tissue layers. Proceed-

ing from the outside inward, these are the hypodermis, the phellem, the phellogen, the phelloderm and the cortex proper. The hypodermis is composed of thick-walled stone cells or sclerenchyma; and is found concentrated in the angles between the bark-platelets or pulvini at the upper ends of which the needles are borne. The phellem is made up of cork cells, thin-walled, large, irregularly rectangular or polygonal, and devoid of cell contents. This layer is the external derivative of the phellogen or cork cambium which lies just internally to it. The internal derivative of the phellogen is the phelloderm, a tissue composed of rather loosely arranged cells very similar to those of the adjacent cortex proper; but arranged in more or less definite rows. Medial to the phelloderm is a cortical tissue composed chiefly of typical parenchymatous cells, indefinitely arranged. In this layer the resin ducts are found.

The woody cylinder, an endarch siphonostele, is composed of primary phloem, secondary phloem, the cambium, and the xylem. Primary and secondary xylems are not easily distinguishable in this case. The central part of the cylinder is occupied by the loose spongy aggregation of pith or medullary cells. Crossing the woody cylinder are wood rays composed of parenchymatous elements.

At the upper end of the twig, and just below the bud proper, is to be found a layer of thick-walled cells (Figure 5) which separates, in effect, the bud from the twig. This is the collenchyma plate of Korody (121). As she points out, this plate was first described by Schroeder in 1869 in his study of *Acer platanoides;* and termed by him the "Markzwischenstück." Later Busse (33) in 1893 described this structure in *Abies alba,* and it was called by him the "Knospenscheide." Lewis and Dowding (139) picture in *Abies, Picea* and *Larix* the same structure to which they give the name of "crown." Apparently they were unaware of the previous works. Extensions of the plate pass upward into the mass of overlapping protective bud scales; and downward for a short distance to bound the inner surface of the vascular elements. The plate is pierced marginally by the procambial strands which pass up and into the bud. During the late growing season or early dormancy the medullary cells just below the plate begin to break down (Figures 3 and 5), thereby leaving a gap as far down as the lower extension of the plate. This cavity can be observed at the node in old branches, as well as the plate itself.

Immediately above the plate is a region of flattened parenchymatous cells filled with tiny droplets (Figure 6). These gradually pass distally into a cone of large thin-walled medullary cells arranged in columns and filled with gummy masses of tannin (Figure 7). The latter seem to be formed as the result of the aggregation and coalescence of the droplets found in the flattened cells.

Laterally to the medullary cone run the procambial strands (Figure 8) which will form the vascular elements. The protophloem stains more densely than the proxylem. Externally to the procambial strands is a tissue composed of elongate cells (Figure 9) from which the cortex and cortical derivatives will be formed. The needle rudiments arise from this layer. Basally the needle rudiments contain prevascular elements which are continuous with the procambial strands (Figures 9 and 10). The leaf-proxylem elements arise approximately in the region of the leaf axil (Crafts [58],

Esau [69]). Differentiation proceeds both acropetally and basipetally, in the latter case until connection is made with the older xylem elements in the shoot; this process is intermittent and periodic. Leaf-protophloem elements originate only in contact with older differentiated phloem. Thus, the differentiation of this vascular tissue proceeds in an acropetal direction only, and is continuous. Procambial differentiation is also continuous and acropetal, so that a connection is always found between it and the leaf elements (Figure 9).

The growing point at the apex of the shoot is not a single unit of discrete cells. Rather it is composed of several distinguishable cell-types (Figure 11). At the uppermost tip of the point is a single layer of apical initials. Below this is a group of polygonal cells, variously termed subapical initials (Cross [60]) or subterminal "mother cells" (Foster [75]). Proximad to these is found the rib meristem or pith mother cells. Laterad to these three groups is a layer of peripheral meristem, which may merge indefinitely into the rib meristem. The peripheral meristem gives rise to the cortex, epidermis, provascular tissue, and to the leaf primordia. The rib meristem is the precursor of the pith. Both the peripheral meristem and the rib meristem are derivatives of the subapical initials. The latter, in turn, originate from inner derivatives of the apical initials.

Development of the Shoot

The dormant bud already contains the precursors of all of the elements that are found in the mature shoot. Many of these can readily be recognized. This type of bud, then, amounts to a telescoping of the shoot that is to come (see Figure 3) and is typical of the *Abietaceae*. Some of the tissues that are present in the mature shoot and its derivatives cannot be distinguished in the juvenile shoot, however, even at the time when it is emerging from the bud. Since we are not concerned with the ontogeny of the bud itself, this account will be confined to the development of the shoot proper, and the differentiation of the leaf tissues.

The development of the juvenile shoot is characterized by extreme linear growth as opposed to a lesser proportional diameter increase. It is, in effect, the unfolding or extension of the telescoped organs. During this time the provascular elements differentiate further and elongate with the shoot. As the shoot increases in length, the leaves, which have been packed closely together in the bud, become spaced farther and farther apart (Figure 12). This is due, of course, to the elongation of the shoot between the needle insertions. Since the stem-vascular system is continuous with that in the leaf, the branches from the former also elongate. Thus, the point at which the vascular branch of a particular leaf is joined to the stem vascular elements eventually comes to lie at a considerable distance below that leaf.

Even before the shoot elongates from the bud, the xylem elements can be distinguished from those of the phloem. The spiral thickenings of the secondary wall characteristic of scalariform protoxylem cells appear (Figure 13). Resin ducts may also be distinguished (Figure 12). From this point on, stem development is largely a matter of further differentiation and elongation. The cells of the medulla also divide chiefly in a linear direction (Figure 13). No further details of the normal development of stem vascular elements or medulla will be given here, as these tissues are not involved in

the gall process. The remaining part of this section will be devoted to the differentiation of the leaf structure and of the cortex.

At the time when the bud is still dormant, the cells of the leaf rudiments (aside from those of the provascular elements) do not differ too greatly in appearance from those of the cortex (Figures 9 and 14). The cytoplasm and the nuclei of the leaf cells stain more densely than do those of the latter. Further, the leaf cells are more regularly rectangular or square, are somewhat smaller, and are arranged in definite rows. Otherwise there are no salient features that distinguish one cell type from the other, and there is no sharp line of demarcation. The leaves or needles of spruce are borne on short, upwardly-twisted needle-stalklets (Figure 1), the cells of which are cortical in nature. No trace of the future needle-stalklet can be seen at this time.

When the bud has burst and the shoot is growing out, there is a noticeable change in the appearance of the cells of the needle proper. Xylem and phloem cells of the vascular system can be distinguished, as well as those of the transfusion tissues and endodermis (Figures 15 and 16). It may be seen that the xylem is uppermost (Figure 15). This situation is due to the connection of the leaf vascular elements with those of the stem, in which the xylem is mediad to the phloem. Laterad to the vascular strands are the cells of the leaf mesophyll (Figure 15). These cells are quite uniformly rectangular, about cubical in shape, and are arranged in regular linear and transverse rows. They are filled, like the cells of the medulla, presumably with tannin, and are indeed very similar in appearance and arrangement to medullary cells. External to the mesophyll are the differentiating cells of the hypodermis (Figure 15), which will be libriform fibers when mature. The outermost tissue is composed of cells of the leaf epidermis—elongate rectangular cells with prominent, large, round or oval nuclei (Figure 15).

Toward the proximal end of the needle there is now a decided transition in cell type. Near the junction of the needle with the stem there is a bilateral, inward tapering of the base. The change in the cells appears at about the plane of diminishing diameter (Figure 17). Below this point the cells obviously are cortical in nature and merge with the cells of the stem cortex from which they are indistinguishable (Figure 17). The epidermal cells of this basal region also are different from those of the leaf. They are more nearly square, and the nuclei and cytoplasm stain more densely than in the cells of the leaf epidermis. This differentiating basal region is to be the needle-stalklet which bears the needle-leaf. It is in effect an outward extension of the shoot, containing all shoot tissues excepting pith.

During this early stage of leaf development, growth takes place by enlargement and division of the cells. As the shoot elongates from the bud, however, and basal leaf cell prolifieration begins, the latter takes place from an intercalary meristem. This meristem is located just distal to the terminus of the needle-stalklet, and just proximal to the first mesophyll cells (Figure 19). The demarcation line becomes more apparent as the shoot continues to elongate. The leaf epidermal cells develop their peculiar type of interlocking dovetailed connections. The mesophyll cells pull apart on the line of their transverse rows, leaving air spaces between each row of cells (Figure 18). The inward shrinking of the lateral cell walls causes the individual cells to assume a double-concave form.

This shrinking process of the mesophyll cells apparently begins in the distal, older section of the leaf and proceeds progressively toward the base. The proximal first few transverse rows of mesophyll cells retain their original shape as they have been but recently formed. Cells continue to be formed by the intercalary meristem during linear growth; and those older cells distal to them are pushed outward. For a detailed account of the morphology of the adult spruce needle, see Marco (152). An account of the aging process in the growing needle of conifers is given by Alexandrov and Djaparidze (3).

By the time needle elongation is about complete, the cells forming the abscission layer may be found (Figure 19). These comprise a transverse layer two to three cells deep. They are situated at the distal end of the needle-stalklet and are a part of it, not of the leaf proper (Figure 19). These cells are narrow, irregular rectangles, frequently trapezoidal in shape, with thick walls and narrow lumens. They apparently are proximal derivatives of the intercalary meristem, but may be redifferentiated cortical cells of the needle-stalklet.

The cells of the true cortex, that is, the layer of cells between the bark elements and the vascular elements, change but little during their course of development. Cell proliferation is chiefly anticlinal—at least all of the mitoses observed were taking place in a linear direction. These cells retain their parenchymatous nature from the dormant bud stage through maturing of the stem. They are, when mature, comparatively large with thin walls, varying in shape from almost round through polygonal to irregularly rectangular. The most noticeable change is in the relation of the nucleus to cell size. In the dormant bud the nucleus occupies a relatively large proportion of the cell lumen, while in the mature cell it occupies but a minor volume (Figure 9).

The nucleus of these cortical cells does not appear to increase much in size during the maturation of cells, while the over-all size of the cell increases greatly.

The bark elements, aside from the epidermal cells, cannot yet be distinguished at the end of the period of stem elongation. Trichomes are present, however, on the shoot epidermis (Figure 20), and occasionally on the basal epidermis of the needle-stalklets. Completion of bark differentiation takes place during the so-called "hardening off" period that follows.

The relationships of the various tissues of the growing shoot as described above should be borne in mind as the development of the gall is taken up. Particularly should it be remembered that the needle is not borne directly upon the shoot itself, but upon a needle-stalklet; and that the cells of this stalklet are cortical in nature.

THE GALL

Life History of *A. abietis*

Adelges abietis Linn. belongs to a genus whose members are chiefly gall makers on conifers. Detailed accounts of the morphology and life history of this insect may be found in Annand (4), Börner (18, 19, 20), Cholodkovsky (43), Mordwilko (157), and Wilford (233, 234). Many of the

other species of this genus have exceedingly complex life histories, involving several different alternate host trees. The life cycle of *A. abietis,* however, is comparatively simple, as it is a one-host species confined to Norway spruce. This species apparently is totally parthenogenetic, since males have never been found.

When the gall matures in mid-summer, the cavities of the gall open on the suture lines already present and quite readily visible (Figure 40). The mature, winged forms, which are called alatae non-migrantes, emerge from the cavities. The term "non-migrantes" simply means that these forms do not migrate to a host tree of a different species. They may fly to another host tree of the same species, Norway spruce (*Picea abies* (L.) Karst., syn. *P. excelsa* Link) ; but most of them remain upon the same tree that bore the gall. They go to the needles of the host, and upon them deposit egg masses that are concealed beneath the wings. The female dies at the close of oviposition, her body remaining over the eggs.

The wingless forms that emerge from these eggs are nymphs of the fundatrices. Upon eclosion they migrate to the twigs and, after selecting a suitable site, insert their stylets into the twig and never leave this place again during life. During the winter, nymphs may be found hibernating at almost any point on the current shoot, usually in the crevices between the pulvini, as well as upon the base of a bud. Many of the nymphs which are fixed elsewhere than on the base of a bud die during the winter, as well as some of those which are on the buds. Some, however, are able to live through the winter on the twig. These never molt to produce fundatrices, but die in the spring. This phenomenon may be connected with nutritional differences in the twig-bud level, as will be shown later.

The nymphs resume feeding activity in the spring, and molt to become fundatrices or stem mothers at about the time when the buds are beginning to swell. After becoming mature they begin to lay eggs, each of which is covered with fine, waxy threads, by means of which they adhere together. The eggs hatch at a time after the bud has burst and the new shoot has begun to elongate from the bud. These nymphs or gallicolae that emerge from the eggs then crawl into the open bud.

Chronology

After the work on this problem had been started, it was found that the bibliography pertaining to this subject is more extensive than had been supposed. During the past 200 years many European scientists have investigated this gall, but most of them have neglected the results and theories of others. A few English and American workers have also dealt with spruce galls, but they, in turn, have almost completely overlooked the European literature. Therefore, it has seemed expedient first to set forth briefly what has already been done. Further reference will be made to some of these previous works as the present findings are taken up.

An illustration of a gall presumed to be that of *A. abietis,* on "red fir" occurs in the "Commentarii" of Mattioli, first published in 1544, and in a finely illustrated edition in 1565. Clusius, in 1576, published a good description of the gall (Küster [128], Löw [145]).

Linnaeus mentioned this spruce gall in the "Flora Lapponica", 1737, and stated that it is not a male flower but is produced by insects. Later, in his "Fauna Suecica", 1745, he named the insect and gave a good description of the gall. He stated therein that the cells of the galls contain "little animals", and also noted the waxy wool covering the mother "which causes the strawberry (gall)."

Geoffroy (85) in 1762 again described the "psyllid" of spruce and the gall: "It produces at the end of the branches of this tree a particular monstrosity. The tip of the branch pricked by the insect mother which has deposited her eggs, expands itself and forms a squamous tuberosity, like a little pineapple. Beneath the scales of this knob, are the cells, in which are found the little insects which are destined to produce the animal complete and winged."

DeGeer (84), 1773, made a most excellent study of the development of this gall, and of the life history of the insect that produces it. His work is illustrated with a plate of 29 figures, including longitudinal and cross sections of the gall. His account of the life history of *A. abietis* is correct in all details. DeGeer stated that the gall is composed of the needles of the spruce, the basal portions of which have enlarged owing to the feeding of the fundatrix in the bud, in the spring. He thought that the stylets punctured the young and tender leaves, which are still confined in the bud. Thus, the gall is preformed by the fundatrix for the reception of the gallicolae, and according to DeGeer, the feeding of the latter is responsible for the formation of the cells and further development of the gall. He also noted the presence of unaltered portions of the leaves protruding from the gall, and that some of these are almost as long as normal leaves.

Kaltenbach (112), 1843, reaffirmed what DeGeer had already observed. He explained the formation of lateral twig galls, however, as owing to the fact that the fundatrix had pierced only one side of the bud.

Ratzeburg (173), 1844, added little that was new. He did note that the stylets of the fundatrix could not directly touch all of the needle rudiments which go to make up the gall. Those rudiments not so touched are affected by an abnormal flow of sap from the vascular bundles serving them, and which have been punctured by the aphid.

Leuckart (137), 1859, denied that the stylets could pierce the individual needle "as Kaltenbach and also DeGeer asserted . . . " He observed that before the opening of the bud, "the axis of the young shoot with the needles attached to it begins to swell; there begins therewith the first anlage of that remarkable pineapple-like gall, . . . " and that the stylets are "sunk deep into the axia of the growing spruce-bud."

Kaiser (111), 1865, stated that the fundatrix never begins to lay eggs until the cover scales on the bud begin to raise themselves, for if the eggs hatched before the bud had opened, the young gallicolae would starve. He also noticed that the basal, swollen portions of the needles were not green but whitish or yellowish white, whereas the points thereof remained green. Kaiser further stated that the gallicolae cannot feed in normal needles, but only in those previously affected by the fundatrix.

Winkler (236), 1878, gave a histological description of the gall, apparently made from sections, but published no illustrations. He noted that stomata were lacking in the epidermis of the gall proper, but were present in the unaltered portion of the needle. An account of the numerous trichomes along the suture lines of the cells is given. Winkler observed the presence of starch granules in the gall cells, and the absence of chlorophyll, which he thought to be due to the fact that the gall develops "in the dark of the forest." He also found lacking the vascular bundle sheath, which surrounds those elements in the normal needle. The high tannin content of the gall was detected by him.

Frank (80), 1880 (2nd Ed., 1896), observed the thickening of the shoot axis and of the lower part of the needles before gallicolae were in the gall. He found the cells of that part of the needle to be full of starch grains, and to contain no chlorophyll or stomata. Frank says further: "As soon as the little aphids have gathered at the base of the needles, the formation of the gall chambers commences. Through further growth, the lower part of the needle remaining in the meristematic condition, raises the pad-like broadening over the base still further, . . . " He also detected the disappearance of starch grains from the gall toward its maturation, and the onset of lignification at that time.

K. (110), 1884, who remains anonymous, added little that was original. He, too, observed the lack of chlorophyll in the swollen, basal part of the needle, and its presence in the upper, normal portion. K. did mention finding some needles which were whitish only, from base to tip.

Keller (113), 1885, said: "I maintain that the influence of the stem-mother on gall production is entirely subordinate and must regard the larvae as the limiting factor in the transformation of the shoot." He then listed various reasons why he thought this to be so. Keller admitted "the initial influence of a secretion on the delicate plant tissue," but considered the aphid salivary glands too small and the quantity of secretion too scanty to be of any importance. Instead, he held that the secretions of the aphid epidermal glands were absorbed by the spruce twig. These secretions maintained the twig axis and needles in a meristematic condition, so that they became abnormal in their first growth. With the tissues thus prepared, the larvae then accomplished the formation of the real gall by transforming the needles into cellular chambers, and the stem-mother takes no part in this. Keller found: "that after the removal of the stem-mother and the eggs deposited by her the gall-formation always ceases, even if the needles of the young shoot already are swollen."

Dreyfus (64), 1889, thought that the diversity in form between the gall of *Chermes* (*Adelges*) *abietis* and that of *Ch. strobilobius* was due to the difference in the place of feeding. The former species pierces the bud from its base, the latter species at the center of the bud. He also considered that the stage of development of the plant parts concerned as well as the location of the place of attack, influences the gall formation. Dreyfus wondered whether each species of gall-forming insect injected a different "toxin."

Kerner (116), 1891, pointed out that this spruce gall differs from certain other types of gall in that it is "produced not from a *single* organ or some part of it, but from a *whole group* of adjoining plant-members." He said

All photomicrographs, with the exception of Figure 4, are of longitudinal sections.

FIGURE 1. Gall of *A. abietis*, showing needle-stalklets on normal shoots and normal needles borne on gall. 1.5 x natural size.

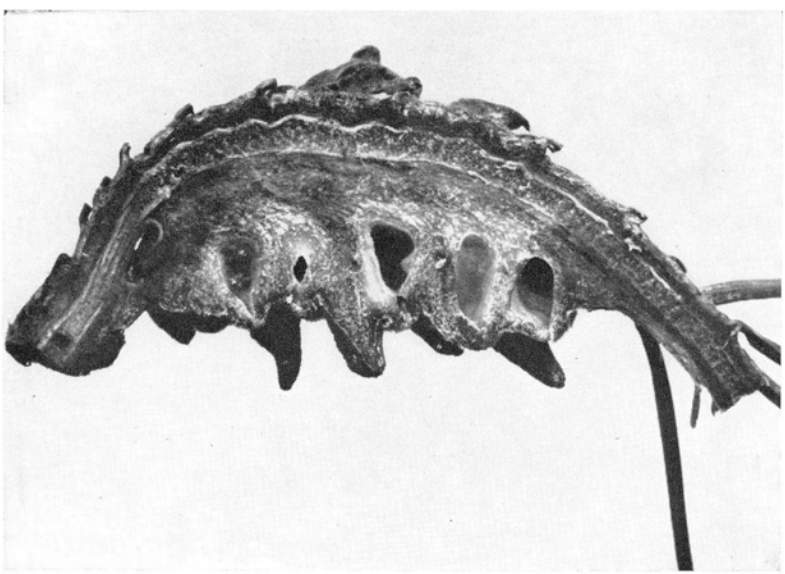

FIGURE 2. Longitudinal section of lateral gall, showing normal cortical tissue on one side of the shoot, and hypertrophied tissue with chambers, on the other side. 3.0 x natural size.

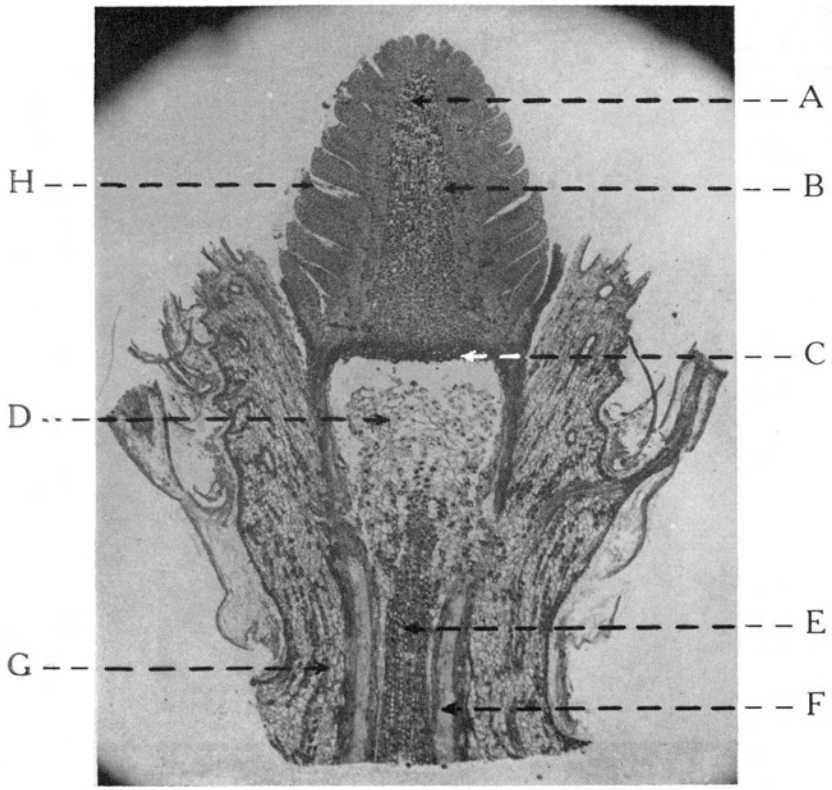

FIGURE 3. Dormant bud and one-year stem. Mag., 19.0 x.

A. Pith or medulla
B. Procambial strands
C. Collenchyma plate
D. Disintegrating cells of pith
E. Pith
F. Vascular elements
G. Cortex
H. Needle rudiments

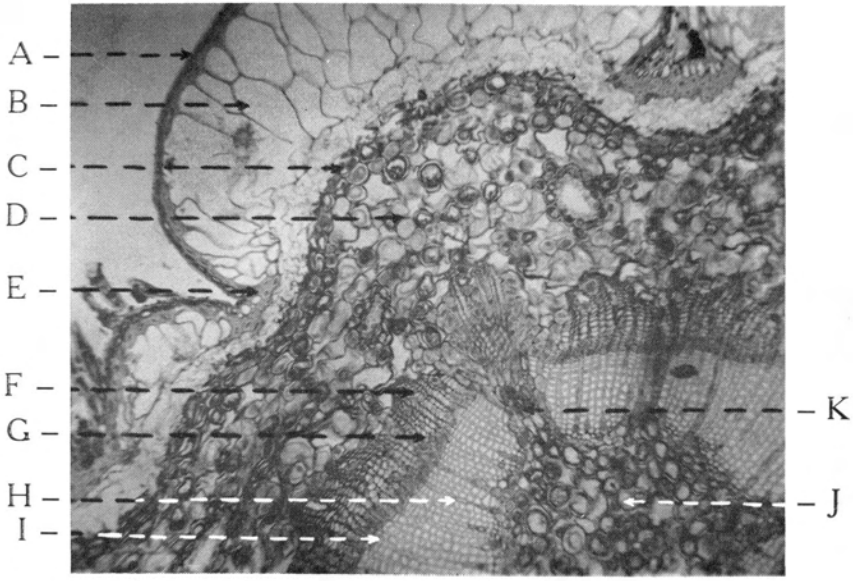

FIGURE 4. Cross section of two-year stem. Mag., 99.0 x.

A. Epidermis
B. Phellem
C. Phellogen-phelloderm
D. Cortex
E. Hypodermis
F. Primary phloem
G. Secondary phloem
H. Secondary xylem
I. Cambium zone
J. Pith
K. Wood ray

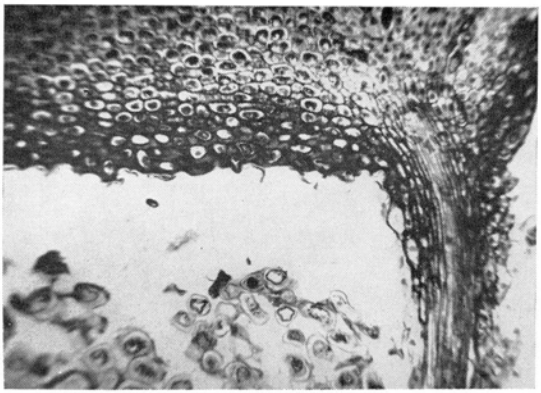

FIGURE 5. Collenchyma plate and vascular strands. Mag., 104.0 x.

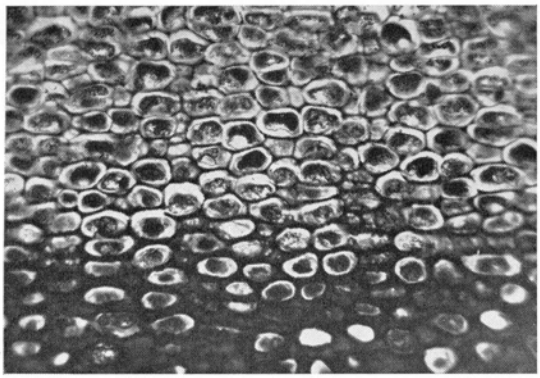

FIGURE 6. Flattened cells above collenchyma plate. Mag., 214.0 x.

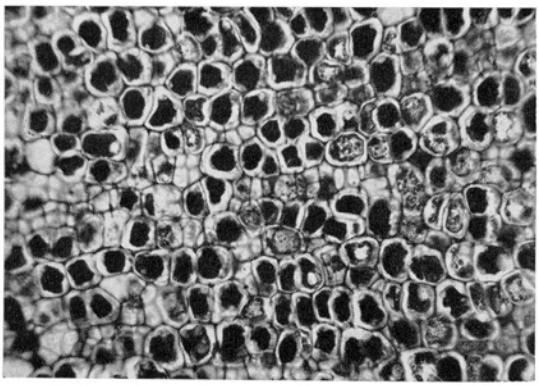

FIGURE 7. Pith or medullary cells above plate. Mag., 214.0 x.

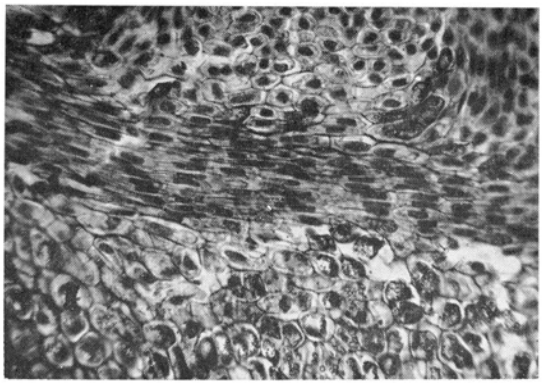

FIGURE 8. Procambial strands in dormant bud. Mag., 214.0 x.

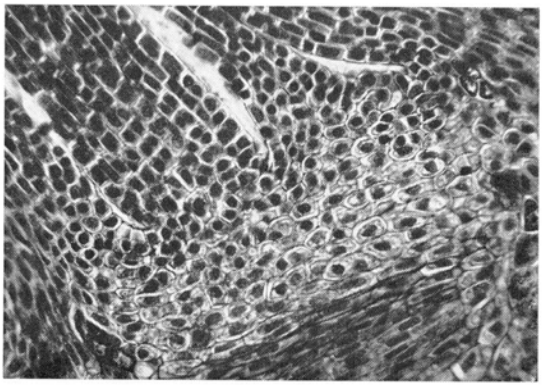

FIGURE 9. Needle rudiments, bud cortex and procambial strands. Mag., 214.0 x.

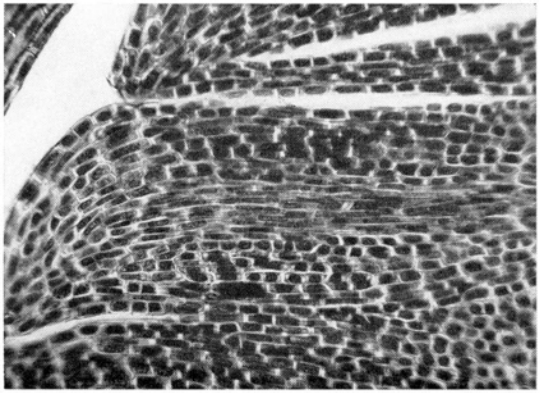

FIGURE 10. Procambial strands in needle rudiment. Mag., 214.0 x.

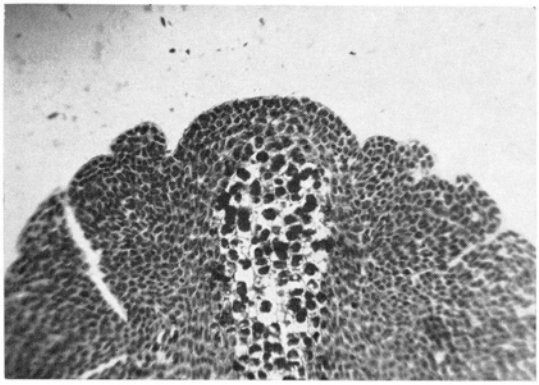

FIGURE 11. Growing point of bud. Mag., 104.0 x.

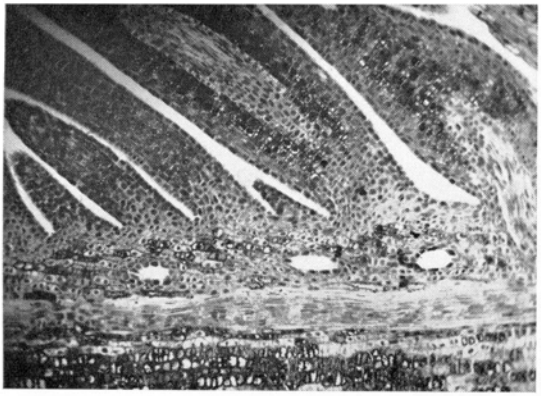

FIGURE 12. Beginning of shoot elongation. Mag., 77.5 x.

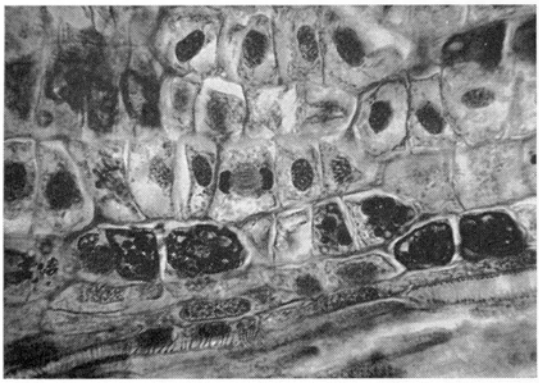

FIGURE 13. Linear division in cells of medulla; mitotic figure. Mag., 508.0 x.

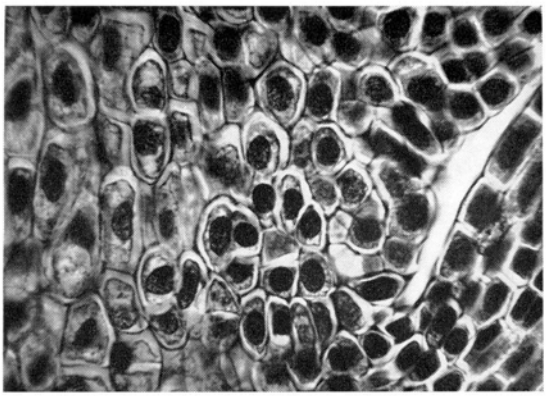

FIGURE 14. Cells of cortex and leaf rudiment. Mag., 508.0 x.

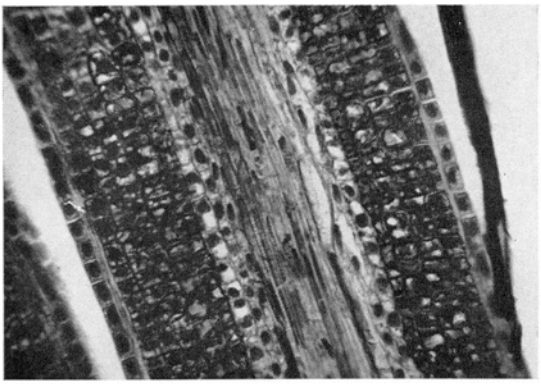

FIGURE 15. Growing needle showing differentiating vascular elements, endodermis, mesophyll, hypodermis and epidermis. Mag., 214.0 x.

FIGURE 16. Differentiating vascular elements in growing leaf, showing xylem with spiral rings. Mag., 508.0 x.

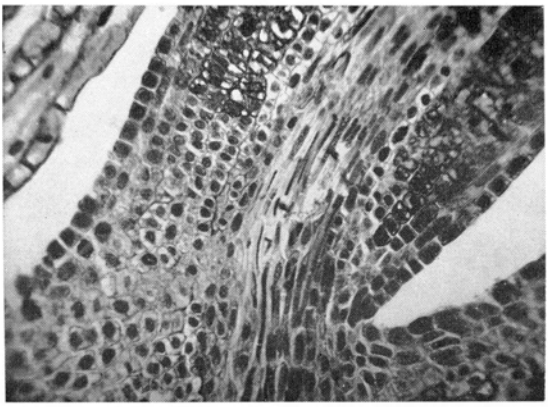

FIGURE 17. Contrast between mesophyll cells of needle and cortical cells of needle-stalklet, early growth stage. Mag., 214.0 x.

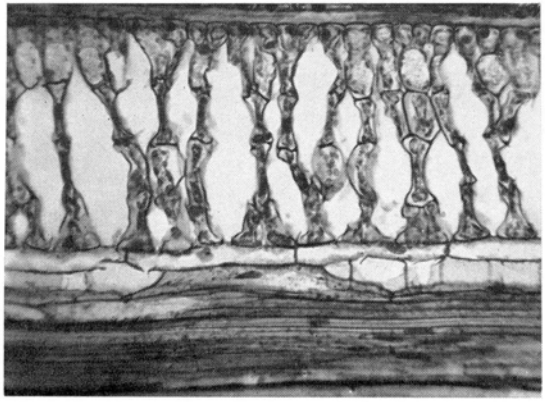

FIGURE 18. Later needle development, showing shrinking of mesophyll cells. Mag., 214.0 x.

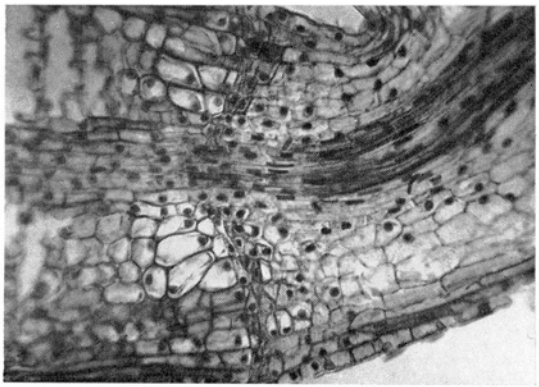

FIGURE 19. Base of needle and needle-stalklet, showing abscission layer and zone of intercalary meristem. Mag., 214.0 x.

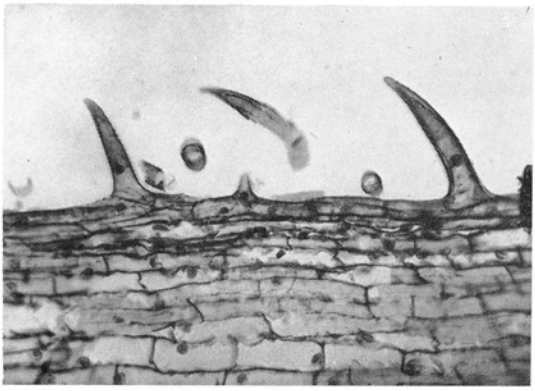

FIGURE 20. Developing shoot, showing trichomes. Mag., 214.0 x.

FIGURE 21. Base of bud, showing stylets and stylet sheaths of fundatrix, and evacuated cortical cells. Mag., 214.0 x.

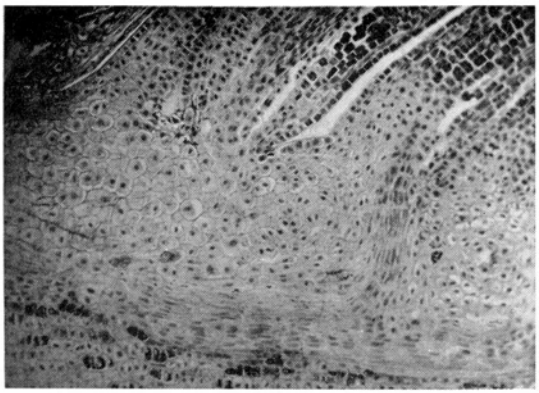

FIGURE 22. Base of elongating bud, showing hypertrophied cortical cells of shoot and needle-stalklet, and stylet sheath. Mag., 77.5 x.

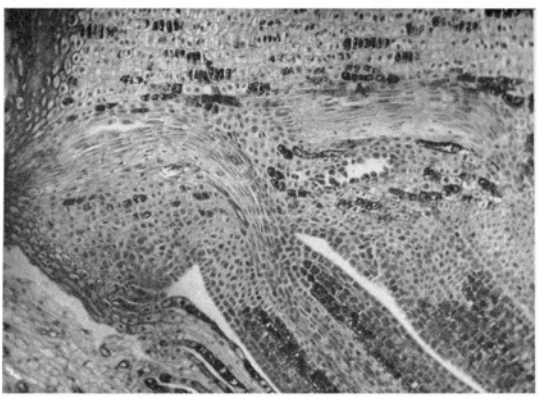

FIGURE 23. Normal cortical cells of shoot and needle-stalklet, opposite side of bud shown in Fig. 22. Mag., 77.5 x.

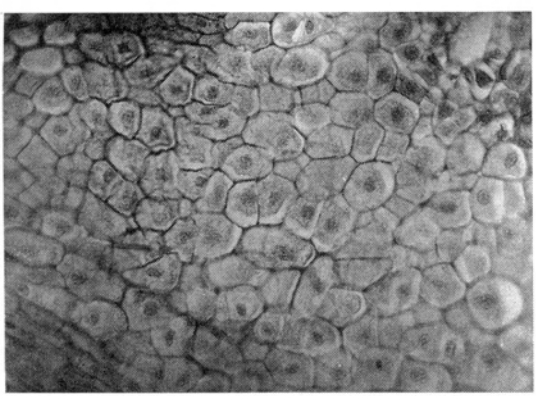

FIGURE 24. Hypertrophied cortical cells of shoot and base of needle-stalklet; note stylet sheaths and clumped chromosomes. Same section as Fig. 22. Mag., 214.0 x.

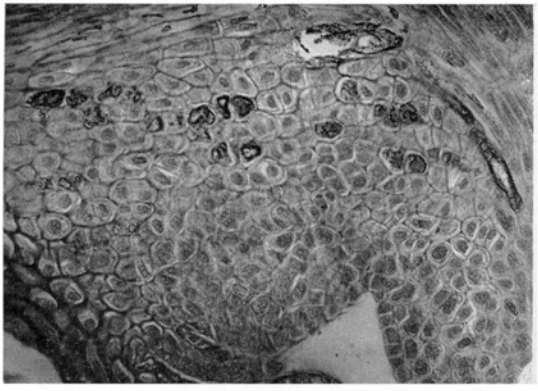

FIGURE 25. Normal cortical cells of shoot and base of needle-stalklet, same side of bud as Fig. 23. Mag., 214.0 x.

FIGURE 26. Hypertrophied cells of shoot cortex and needle-stalklet, later stage of development. Note early accumulation of starch grains. Mag., 77.5 x.

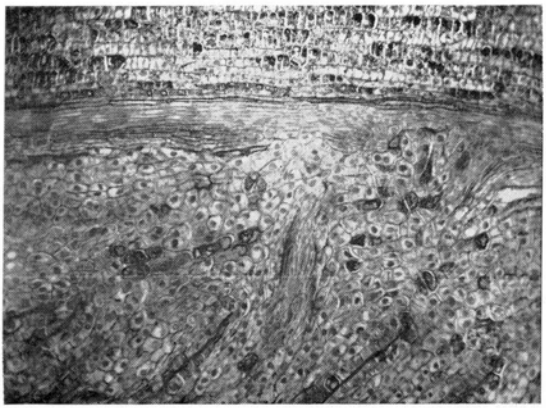

FIGURE 27. Normal cortical cells of shoot and needle-stalklet, later stage of development; note absence of starch grains. Same section as Fig. 26, opposite side. Mag., 77.5 x.

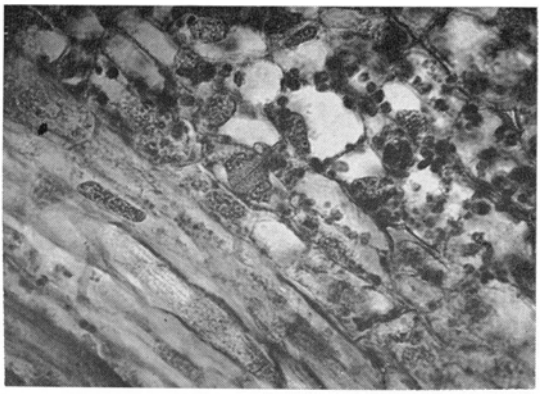

FIGURE 28. Mitosis in hypertrophied cell, plate formed; note starch grains. Mag., 508.0 x.

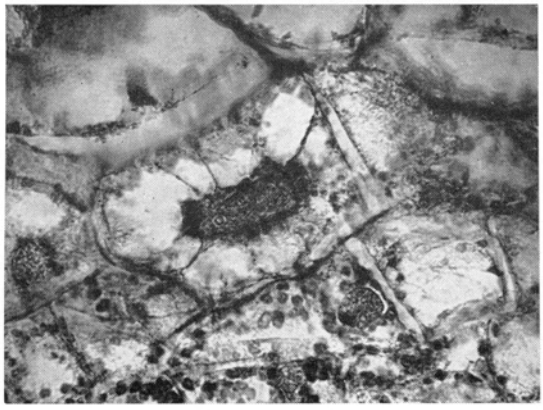

FIGURE 29. Hypertrophied cell, showing disintegrating nucleus and filamentous cytoplasmic strands. Mag., 508.0 x.

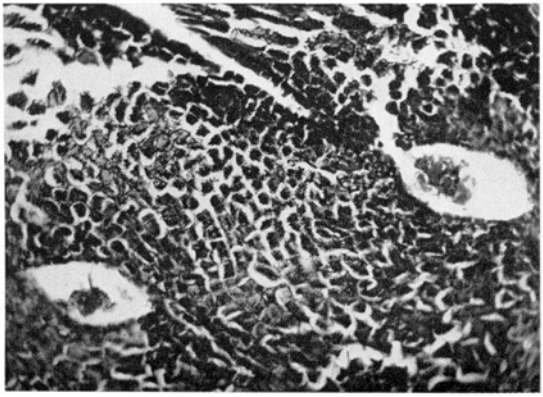

FIGURE 30. Young gallicolae in initial chambers or pockets. Mag., 77.5 x.

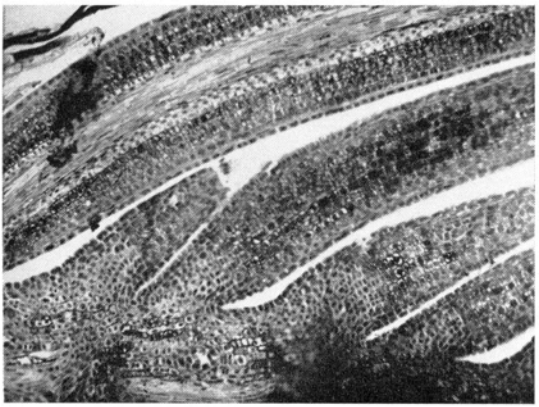

FIGURE 31. "Chambers" or "pockets" in normal shoot. Mag., 77.5 x.

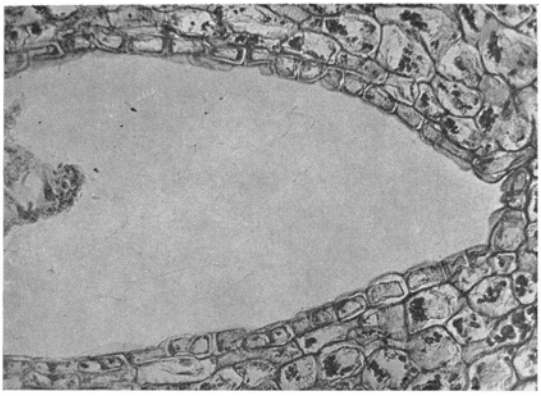

FIGURE 32. Portion of gall chamber, showing gallicola, unaltered epidermal cells, and hypertrophied cortical cells. Mag., 160.0 x.

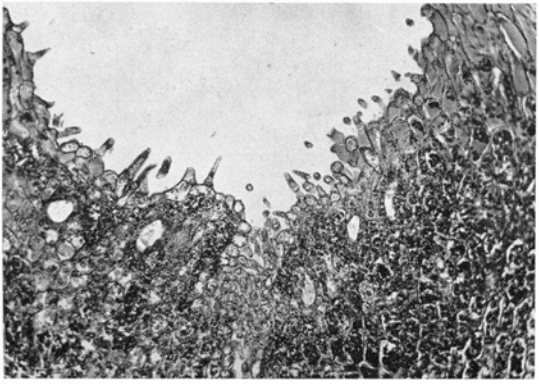

FIGURE 33. Mouth of chamber and exterior surface of gall, showing epidermal cells of cavity, and trichomes closing mouth of cavity and on external epidermal cells. Mag., 77.5 x.

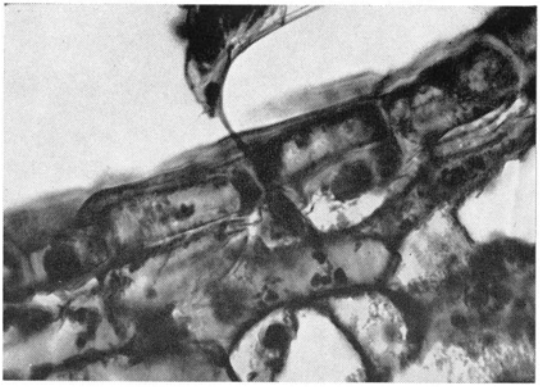

FIGURE 34. Stylets of older gallicola piercing cortical calls. Mag., 517.0 x.

FIGURE 35. "Giant" cortical cells in body of gall. Mag., 104.0 x.

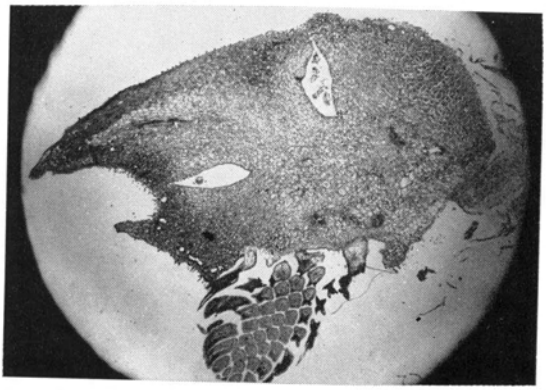

FIGURE 36. Section of entire small gall, showing two chambers with gallicolae, and vascular elements running out into "cone." Mag., 20.0 x.

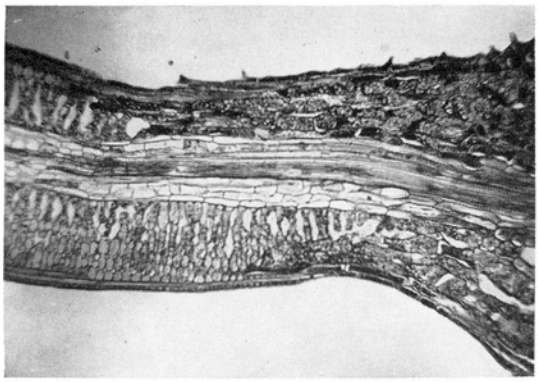

FIGURE 37. Base of needle and upper part of "cone," showing hypertrophied cortical cells and trichomes, absence of abscission layer and intercalary meristem, involvement of needle cells and vascular elements. Mag., 58.0 x.

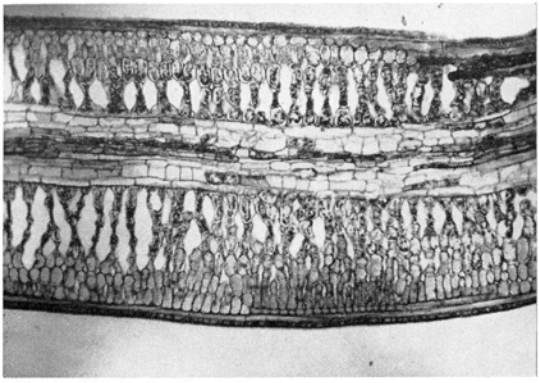

FIGURE 38. Normal needle structure distal to affected basal region. Mag., 77.5 x.

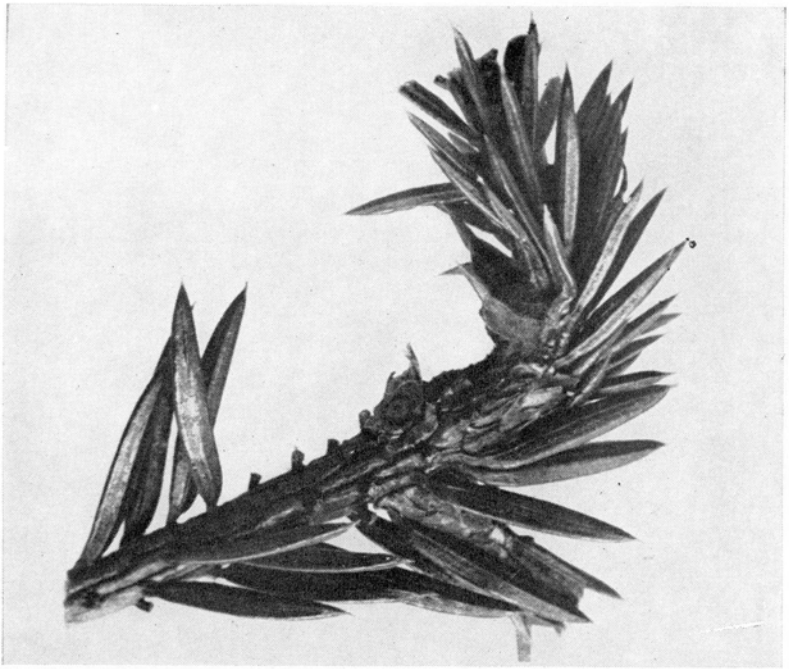

FIGURE 39. Gall formed after injection of fundatrix salivary extract and transfer of eggs. Mag., about 2.5 x.

FIGURE 40. Mature gall, cavities opening and alatae non-migrantes emerging. Note "cones," short needles and suture lines with trichomes. Mag., about 2.0 x.

further that the aphid "can directly influence only a few cells of the young shoot hidden in the bud. Nevertheless thousands of cells on the shoot soon begin to assume an altered form, a proceeding which reminds us strongly of the action of a ferment . . . "

Juduch and Nitsche (109), 1895, added nothing original. They did affirm that the gall in all its essential parts is to be found before the eggs have hatched, upholding Frank in this, and denying the thesis of Keller since he removed not only the eggs but also the fundatrix, from the bud. They did admit that the gallicolae contribute to the enlargement of the gall chambers, however.

von Tubeuf (214), 1896, studied this and other spruce galls especially from the standpoint of trichome development. He was the first, it is believed, to state that the gall is formed, not from the leaves themselves, but from proliferating needle-stalks. von Tubeuf held that since this is the case, each cell is a separate gall, but that many such "standing together" form the gall as a whole.

Cholodkovsky (43), 1897-98, presented his excellent work on the life cycles of many of the species of gall-forming *Chermes*. He also reviewed, to date, much of the previous literature on this subject. In addition, Cholodkovsky went into the manner of formation of the various spruce galls. He observed the effect on the resultant gall of the distance at which the fundatrix was fixed, from the bud. In 1905, he published on the biology and anatomy of the salivary glands of *Chermes* spp.

Fernald (72), 1897, gave the first good account of this species in the United States, although he gives credit to R. A. Cooley for having done the actual work. He admits that no reference was made to the previous work done abroad on the problem, but material was sent to Cholodkovsky, and Buckton and Ratzeburg are quoted. The initiation of the gall is credited to the fundatrix, and the gallicolae are presumed to have some influence in its further development. They noted that only those fundatrix nymphs hibernating at the base of a bud survived the winter, and that even some of these died. The origin of the gall was attributed solely to the physical stimulation of piercing of cells by the setae. The unusual mobilization of starch in the gall tissue was observed, and it was suggested that the starch is the food of the gallicolae.

Weisse (222), 1902, studied the phyllotaxy of many galls, including those on spruce. He noted, in a longitudinal section of one of the latter, that the stem and especially the "leaf-bases" were swollen. The cells or cavities he interpreted as being the "armpits (Achselhöhlen) of the metamorphosed needles." For the asymmetrical galls of *A. strobilobius,* Weisse stated the principle "that on them the needles show almost the same divergence which one observes in the normal vegetative bud. Therefore the slight torsion which has taken place in the normal twigs with their pushing out, has almost entirely ceased with the galls." To account for cell formation, he says: "Also in the galls has indeed the axis, compared with the bud, undergone a considerable stretching, which is scarcely inferior to that of the normal shoot. However, there occurs a considerable swelling of the leaf bases, so that in the gall the contact between leaf bases does not cease as in the normal twig, but coalesces still further." The same applies to the galls of *A. abietis.*

Börner (19), 1908, brought forth his monumental work on the genus *Chermes* and closely related genera. He went to considerable length to show that the gall is not made up of the spruce needles, *per se,* but of the stem cortex and the "cortical-stalklets" on which the needles are borne, and which belong to the cortex. Apparently he overlooked the work of von Tubeuf. Börner pointed out that galls of this type could not be produced on *Abies* spp. because the true firs lack these cortical extensions or needle-stalklets. He considered that each gall-forming species possessed a specific salivary secretion, to account for the variation in gall type from one species to another. But he also maintained that it depended "less on the intensity of the louse puncture than on the susceptibility of the plant tissue, whether those proliferations form or not." Börner removed the eggs of the fundatrix and observed the failure of the gall to develop in the absence of gallicolae. He also transferred eggs to opening, normal spruce buds, and found that the gallicolae which emerged from them would not feed on normal needles, and died shortly. Later, Börner (20) in 1909 replied to Nüsslin's denial of his assertion that the gall was solely of cortical origin. He pointed out that the tissues of coniferous leaves are not capable of gall formation through the action of *Chermes* spp.

Nüsslin (163), 1908, as just mentioned, contended that Börner was in error as to the tissue composition of the gall, and held it to be a mixed cortex and needle gall. In support of this, he used Börner's findings that the abscission zone between needle and cortex-stalk becomes "degenerate" through proliferation of the latter.

Burdon (31), 1906, found that as soon as the fundatrix begins to suck, in the spring, the cells about the apex of the stylets begin to increase rapidly in size, and to divide. The mitotic figures seemed to be normal, but numerous nucleoli appeared. Chlorophyll and tannin disappeared from the proliferating cells, and the tannin, he believed, was really a glucoside which was transformed into the starch that accumulates in these cells. He removed fundatrices from the buds about a week after they had begun spring feeding, and found that the shoots emerging later from the buds still bore galls. Thus, he considered "that the poison injected during that week of the insect's life continues to act on the tissues of the plant after the death of the mother." In 1908 Burdon (30) wrote concerning the relationship between the insect and its salivary secretion, and the nature of the host bud. "Since, however, we find that different species give rise to the same type of gall, it seems clear that the character of the gall is not due to any specific difference in the composition of the injection. On the contrary, I think that the composition of the injection is probably the same in all species of *Chermes,* and that the characters of the gall depend almost entirely on the ratio between the strength of the injection and the power of the embryonic tissue of the bud to resist it."

Cosens (54), 1912, stated that: "The gall in this case is a joint production of the cortex of the stem and the bases of the leaves of the host." He noted that whereas a normal leaf contains not more than two resin ducts, "a cross section near the base of these leaves (abnormal) cuts from four to six resin ducts." Cosens was not aware of the work of von Tubeuf, nor of the controversy between Börner and Nüsslin on the origin of the gall tissues.

Küster (129), 1952, pointed out that the affected tissue "undergoes thus another development-fate than the other part of the needle, and herein we see the occurrence of a 'manifoldness,' which in the corresponding normal part does not come to expression." This compares with E. F. Smith's (192) opinion that the plant cells possess latent qualities which are brought to expression by the stimulus as "the remover of an inhibition."

Herrick and Tanaka (101), 1926, studied the life history of *A. abietis* and the relationship of the various stages thereof to formation of the gall. They added nothing that was not already known.

Weber (220), 1930, stated that although the galls caused by various species of *Chermes* "are constructed very differently, they are, however, all similar according to their origin-cause. The gall formation always depends on a scale-like broadening of the basal part of the needle-anlage . . . " Weber quotes Zweigelt concerning the "struggle" of the affected plant tissues to assert their normality against the effect of the stimulus (cf. Burdon [30]). Then he says that in the case of *Chermes* galls, the opening of the gall is due to a reassertion of the "primitive nature of the component parts" after "relaxation of the foreign stimulus." The latter, of course, means cessation of feeding by the gallicolae.

Zweigelt (240), 1931, quoted chiefly from the works of Börner, Nüsslin, Nitsche and others, concerning this gall. He observed, as had others, that if the gallicolae failed to colonize the incipient gall, no further development of it occurred.

Ross (184), 1932, in addition to what was just mentioned under Zweigelt, stated that: "The tissue altered by the stem-mother cannot, however, revert to the normal condition, if the gall has attained a certain age and the mother animal has begun egg deposition." He also said that: "The lower part of the young spruce needle remains for a long time in a meristematic condition." (cf. Beck). The affected tissue of the shoot axis remains likewise. Ross found that stomata were lacking on the swollen part of the needles or are abnormally formed, that the hypodermis does not develop in that part of the needle, and that the vascular bundle sheath is wanting.

Warren (219), 1933, studied the relation of the hibernating nymph to the host tissues. He found that the stylets of the nymph seek out the lateral elements in the base of the bud and above the collenchyma plate. These elements are the anlage of what will be the future stem cortex. Warren also noted the morphological differences in the distal, normal part of the needle, and the basal, hypertrophied portion, but failed to see the significance thereof. He, too, was unaware of previous work on this gall, and believed that it was formed of actual needle tissue.

Friend (81), 1935, observed that if either fundatrix or eggs were removed from the bud, the abnormal needles fail to develop further and eventually atrophy and fall away (cf. DeGeer, Keller). Friend further found, as had Kaiser and Börner previously, that the gallicolae can feed only upon swollen needles, and hence cannot, of themselves, cause a gall to develop.

Origin and Development of the Gall

The gall that is caused to develop by *A. abietis* is commonly called the "pineapple" gall of spruce (especially in the European literature) because

of its external appearance (Figure 1). Outwardly, the gall seems to be made up of a number of rhomboidal "scales" or "flaps," each surmounted by a cone-shaped prolongation which bears a needle (partly or completely formed) at its upper end. The distal margin of each flap is bounded by an indistinct impressed line. This line marks the suture upon which the flap will open when the gall is mature (Figures 1 and 40). Beneath each flap is the cavity or chamber in which the gallicolae are found, and from which the alatae emerge when the flaps open (Figure 40).

Most of the galls are lateral; that is, only one side of the new shoot is involved in the abnormal growth. These are caused by the feeding of but one fundatrix. The unaffected side of the shoot develops normally and does not die. If more than one fundatrix feeds on the bud, however, the complete shoot is involved, and a perfect "pineapple" is formed. In this instance, the shoot usually develops but little above the gall, and dies when the gall matures.

If a bud at the base of which a fundatrix nymph is hibernating is opened and examined at any time during the dormant season, no difference may be detected between it and one that is uninfested. The over-wintering nymph, then, apparently does not affect the tissues of the bud in any obvious way. If an infested bud is examined in the spring, however, at the time when it has just begun to swell, a palpable change from normal may be seen. The basal part of the needles on the side of the bud just above the fundatrix is swollen, while the other needles appear quite normal (DeGeer [84], Kaltenbach [112]). If either the fundatrix or the egg mass is removed from the bud at this time, so that no gallicolae are produced, these abnormal needles fail to develop and eventually atrophy and fall away. Further, if the abnormal needles are removed and the fundatrix is left, no gall develops because the gallicolae, upon eclosion, crawl into the open bud and fix themselves only upon those needles the basal part of which is swollen. They are unable even to survive upon normal tissues, nor can they of themselves cause a gall to develop (DeGeer [84], Kaiser [111], Keller [113], Börner [18, 19], Friend [81]).

It is evident, then, that neither the fundatrix nor the gallicolae independently can cause the formation of the gall. The fundatrix must first "precondition" the tissues involved, and this occurs only in the spring (cf. Kennedy [115]). No complete gall develops, however, unless this affected tissue is further stimulated by the gallicolae. The fundatrix must effect some nutritional change in these abnormal tissues—a change without which the gallicolae cannot survive. This phenomenon is somewhat akin to certain of the crown-gall tumor relationships. Braun and Laskaris (26), working with attenuated bacteria, found that massive tumors were produced following a combination of needle prick inoculations and application of lanolin paste containing indole acetic or naphthalene acetic acids, a centimeter back of a cut surface. Neither treatment alone was able to produce significant overgrowths. Black (16) obtained tumor development by puncturing bacterium-infected stems. The younger the tissue punctured, the greater the proportion of wounds from which tumors developed. Tumor development usually did not take place after five weeks following wounding.

The stylets of the fundatrix nymph seek out the lateral tissue in the base of the bud, and above the collenchyma plate (see also Warren [219]). They

pierce the epidermis and periderm, pass upward laterad to the plate, and terminate in the tissues of the vegetative cone. Here may be found numerous ramifying stylet tracks (Figure 21) where the insect has partially withdrawn the stylets and again thrust them forward. The cells in which the majority of these stylet tracks occur are true cortical cells, although the stylets occasionally enter the bud scale bases. No tracks have been found in the medulla, nor does the aphid appear to seek out the phloem cells of the procambial strands, as do so many other plant-sucking insects.

The feeding of the fundatrix causes actual damage to only a restricted number of cells. Passage of the stylets is both intra- and intercellularly. In the immediate vicinity of the stylet sheath or stylets, there is a small group of cells which apparently has been killed. These cells are frequently, although not necessarily, first stimulated to hypertrophy before being killed. The cells that have been fed upon do not have torn walls, such as results from capsid bug feeding, nor do the walls collapse (Figure 21). Plasmolysis of the cells does occur, and the contents are withdrawn (Figure 21). The feeding effect appears to be similar to that caused by true aphids and by coccids, which have long, slender, comparatively weak stylets, in contrast to those sucking insects which have a stout rostrum and strong stylets (see Brown [27], Davidson [61], Painter [165], Parr [166], Zweigelt [239]).

The overwintering fundatrix nymph draws nourishment from the cells of this cortical tissue in the fall before hibernation, and in the spring before maturing. It is possible that feeding may occur during the winter on warm, sunny days, but this is rather unlikely. It is during the spring feeding that the fundatrix must inject into the bud the material which causes the initiation of gall formation.

If the basal region of an infested bud is examined at the time when the bud is swollen, but has not yet opened, a difference will be found within the tissues of the bud itself. In most cases, but one side of the bud will be affected; the other side will not (Figures 22-27). On the unaffected side, the picture will be the same as that described in the section dealing with the normal development of the shoot (Figures 23, 25, 27). On the affected side above the fundatrix, however, a different situation obtains. Here, the cortical cells between the medullary cone and the bases of the needle rudiments are in a state of hypertrophy (Figures 22, 24, 26). These cells are much larger than those on the normal side of the incipient shoot. Many of these cells appear quite normal otherwise, and are dividing, for mitotic figures may be found (Figure 28). Burdon (31), too, noted that in this gall "the mitotic figures seem to be of the usual somatic type, and I have found no indication of heterotypical mitoses, such as one would expect to find were the growth of a cancerous nature." Nêmec (160), with regard to *Heterodera* galls, found that division of giant-cell nuclei took place by mitosis. He also pointed out that other organisms may remove substances from plant tissues without stimulating the production of giant cells or the development of galls.

Some of the cells, on the other hand, present an abnormal appearance (Figure 29). The nuclei no longer are discrete bodies, but seem to be disintegrating. The cytoplasm of such cells is distributed in long, filamentous strands, radiating from the nucleus to the periphery of the cell. Whereas the normal cell nucleus contains one or two nucleoli, many of the abnormal cell nuclei contain four to six nucleoli. Burdon (31) found numerous

nucleoli in such cells. Kostoff and Kendall (122) stated that there is an increase in number and size of the nucleoli in the giant cells that occur in nematode galls on *Nicotiana* roots (see also Christie [45], and Nêmec [160]). Because of this enlargement of the cells, the cortical stratum on this side of the young shoot is deeper than the same tissue on the normal side (Figures 22 and 23). It will be noticed that starch accumulation has already begun in the cortical cells (Figure 26).

This cell hypertrophy extends upward into the leaf rudiment for a variable distance, depending upon the position of the rudiment in the bud, so that the former presents a swollen appearance. It usually encompasses a greater total length of those rudiments that are nearest the base of the bud, hence nearest the terminus of the stylets. In these affected tissues the cells stain less densely than do the cells of normal tissues. It is noticeable that in some of the swollen needle rudiments there is yet no transition from typical cortical cells to those of the leaf-mesophyll type. The latter may be distinguished in normal needles which are distal to the abnormal ones. If there is such a transition, it occurs only towards the upper end of the needle, and the proximal cells of mesophyll type are in the same disorganized condition that has just been described for the cortical cells.

The eggs deposited by the fundatrix hatch shortly after the bud has burst and the new shoot is elongating from it. The wingless nymphs or gallicolae that emerge from the eggs crawl into the bud and go to the basal region. Here, they enter into the acute angles formed by the vegetative cone and the leaf rudiments, which are still closely appressed to each other at the base of the bud. The stem in the distal, normal portion of the bud (or young shoot) has begun elongation, so that the needle rudiments here are separated from one another. As has been stated previously, the gallicolae go only to the needle rudiments already affected by the fundatrix. The initial "pocket" in which the gallicolae are found at this time (Figure 30) has its counterpart in the normal shoot, owing to the diminished diameter of the needle stalklet as it approaches the shoot (Figure 31).

Unlike the fundatrix, the gallicolae do not insert their stylets permanently in the host tissue and remain thereafter in that place. Their stylets are relatively short and may be withdrawn easily. Hence, the gallicolae are free to move about, feeding here and there. It is apparent now that the surface cells over which they move and which come to line the cavity that later develops are epidermal cells of the shoot (Figures 32 and 33). Thus, the epidermis is still continuous over the gall, within the cavities and without.

The short stylets of the young gallicolae are unable to penetrate beyond the second or third layer of cortical cells bounding the region in which they are feeding, but those of the older gallicolae can penetrate farther (Figure 34) to about the sixth row. The first few rows of cells surrounding the pocket are not stimulated to hypertrophy to the extent of the cortical cells forming the bulk of the gall. The cells immediately around the pocket attain a size slightly larger than normal (Figure 32), but much smaller than the outlying cells. The latter grow eventually to a size several times that of normal cells (Figure 35).

Further reference to Figures 4 and 32 shows that the epidermis is the only bark element which comes to expression in the gall structure. Cells of

the hypodermis, the phellem, and the phellogen-phelloderm all are lacking. The cells lining the cavity, as seen in Figure 32, are rectangular in outline and are rather thick-walled. It then might be questioned whether they are not of hypodermal origin. However, Figure 33 shows the trichomes which serve to occlude the cell openings, and which also occur to a lesser extent on the outer gall surface. Occasionally they are found within the cavity itself. Figure 20, on the other hand, illustrates the trichomes which are to be found on the developing normal shoot. Therefore, the cells in question must be epidermal cells. Burdon (30) noted that "The net result of these various changes is that almost all previous differentiation of the stem has been obliterated, and in its place a parenchymatous tissue, consisting of abnormally swollen cells with extremely thin walls, has been formed." In this statement Burdon erred. The missing elements are not obliterated in his sense, because differentiation of these elements does not occur, but rather is suppressed.

There is greatly increased radial growth by the cortical cells of the shoot and needle-stalklet. This increment is brought about both by hypertrophy and hyperplasia of the cells. Because of this diameter increase in the cortex beneath the pockets, they are pushed gradually farther from the central cylinder. Lateral or tangential increment parallel to the circumference of the shoot causes formation of the "scales" or "flaps" that enclose the chambers containing the gallicolae. Contrary to Wilford (233), the cells are not in actual communication with each other. This would be possible if the chamber walls were formed by the meeting of swollen needles, as previously thought by most investigators. Since the gall is formed by the outward proliferation (and hypertrophy) of cortical cells, however, there is a discrete partition between the chambers (see Figures 2 and 40).

The form of the flaps enclosing the chambers is occasioned by the position of the needle-stalklets on the shoot. In spruce, the latter are arranged in a rising clockwise or counter-clockwise spiral. Thus, any particular chamber is bounded laterally above and below by two other chambers (Figure 40). It is doubtless the growth-pressure due to this arrangement that gives the flaps their characteristic external rhomboid shape. The "ceiling" of a chamber is formed by the "floors" of the two chambers bounding it above and laterally, each of these two chambers contributing equally. The "floor" of this particular chamber is formed in like manner by the "ceilings" of the lateral chambers below it (Figures 2, 36 and 40).

Just how the chambers maintain and increase their internal dimensions is not clear. It would seem that growing pressure would cause complete coalescence of the sides of the chamber. But the original cavities formed by the narrowing of the needle bases in the normal shoot maintain themselves in the gall, with greater lateral expanse. It is possible that once the initiation of the gall is begun, growth is more or less uniform throughout it, and the cavity is enabled thus to maintain itself. On the other hand, as previously pointed out, the cells of the first few rows bounding the cavity do not attain the size reached by cells elsewhere in the gall (Figures 32, 35 and 36). This would seem to indicate that the growth of these cells is inhibited, or at least approximates normal, and that the stimulus is directed to the outlying cells. Unfortunately, and in contrast to the fundatrices, the gallicolae do not form stylet sheaths, so their exact feeding pattern cannot be traced.

They do, however, feed upon all surfaces of the cavity, but perhaps not in the cells immediately lining it. The stimulus must diffuse outward from cell to cell, or else promote a chain reaction. In the latter event it would catalyze a change in one cell, the effect of which is passed on to cause a similar change in the next cell.

Weisse (222), previously cited, noted that contact between leaf bases does not cease in the gall as in the normal shoot, owing to linear growth of the latter. This would not account for maintenance of the chambers, however. The chambers may persist because the tissues are to some extent following normal growth patterns. Figure 2 shows the angle maintained with the stem by the recurved normal cortical-stalklet. The space thus created below the latter, between it and the stem, perseveres despite abnormal growth of the surrounding cells. These spaces are the "Achselhöhlen" of Weisse.

The vascular elements pass uninterrupted from the stem vascular system through the gall tissue, and out into the needle which may be borne upon it (Figures 1 and 37). The distal, unaltered part of the leaf is quite normal in appearance (Figure 38). Winkler (236) observed the presence of stomata in the unaltered portion of the leaf, and their absence in the epidermis of the gall. He also noted that the vascular bundle sheath found in the needle was lacking in the gall. Warren (219) likewise noted the absence of these elements in the gall, and in addition that "In a normal young needle the extreme basal part lacks a complete endodermis. . . . " Frank (80) and Ross (184) made similar observations. These authors failed to see the significance of their findings. The elements which they could not find in the gall do not occur in the tissues which are stimulated to abnormal growth. The gall is made up of stem cortical cells, and of the needle-stalklets which are simply extensions of the stem cortex.

On some galls, or on part of a gall, needles may be found of approximately the same length as normal needles borne directly upon the shoot (Figures 1 and 40, also Börner [19]). These needles have continued linear growth despite the fact that they are borne on the flaps enclosing the gall chambers. This situation arises when the insect stimulus fails to reach up far enough to inhibit differentiation of the intercalary meristem. The needle-stalklet from which they rise may still be altered into the larger cone protruding from the scale covering the chamber (Figures 1, 2 and 40). However, the needle meristem just beyond the distal end of the cone has been able to differentiate and function normally. The cells of the cone and of the main body of the gall (Figures 2 and 36) are obviously of similar origin, and respond in the same manner to the stimulus.

There is a fine relationship between the breadth of the sphere of the insect stimulus and the future development of the needle. If the stimulus affects a needle rudiment before differentiation of the intercalary meristem, the needle does not grow linearly, but retains its initial length. Stunted needles of this type are common on the galls (Figures 1 and 40). An examination of such a needle and the cone-shaped tissue bearing it shows a complete lack of a basal meristem and an abscission layer (compare Figure 37 and Figures 17 and 19). This cone, the modified needle-stalklet, bears trichomes (Figure 37) which are not normally present on the stalklet except occasionally at its very base.

Needles may also be found that are in various stages of development between the two extremes. Their development is arrested by the infiltration of giant cells from the cone into the needle proper. An actual growth of such cells into the needle, rather than an alteration of leaf cells takes place, as can be seen in Figure 37. This infiltration of giant cells destroys the site of the abscission layer, and the intercalary meristem as well, thereby halting further linear expansion of the needle.

Burdon (30) thought that the extent to which the gall developed was an expression of the relation between "strength" of the stimulus and the "power" of resistance to it of the embryonic bud. It is, rather, an expression of the state of development of the bud at the time when the stimulus reaches the cells, or whether it does not reach them at all. E. F. Smith *et al.* (194) reported "secondary" crown gall tumors on inoculated plants, arising at a distance from the site of inoculation. These were bacteria-free, and were evidently not the result of *direct* diffusion of metabolic products, either of bacteria or of tumor tissues. Braun (23) rediscovered these secondary galls, and considered that they depended for their initiation, not exclusively on properties of the bacteria, but also on characteristics of the host plant.

It has been shown that the gall proper is composed only of parenchyma cells of the stem cortex and needle-stalklets. Cells of the leaf, *per se,* do not form a part of the gall. The stimulus may affect the basal meristem of the leaf not at all, so that such needles can attain approximately normal length, or it may inhibit differentiation of the meristem and these needles do not grow but simply mature. On the other hand, needle expansion may be halted by destruction of the meristem through infiltration by giant cells of the stalklet. In any event, formation of the abscission zone is repressed, so that needles can never drop from the gall.

All of the previous investigators of this gall, with the exception of Börner, von Tubeuf, and Cosens, (see also Francke-Grosmann [79]) have been in error in maintaining that it is composed of altered leaf tissue. Nüsslin held that it is a mixed cortex and needle gall. This viewpoint may be acceptable, depending on the definition of what constitutes a gall tissue. However, it is apparent that leaf tissues are not altered by the stimulus in the manner that the cortical cells are affected. Abnormal leaves should, perhaps, be thought of rather as "appendages" of the gall proper.

There do not appear to be any "new" structures in this gall. Trichomes, outgrowths of epidermal cells, occur in excessive numbers on the exterior of the gall, and occasionally in the interior of the chambers. They play no essential part in the gall aside from forming a closure for the lips of the cavities, and appear approximately the same as those found on the normal shoot. The cortical cells which make up the body of the gall differ from those of the normal shoot chiefly in size, for in shape they closely resemble those found in the two-year stem. No differentiation of bark elements or abscission layer occurs in the gall. In the early stages of development at least, there is regular mitotic division. Shortly, however, disintegration of the nuclei takes place, and thereafter no mitotic figures have been found in the gall cells. Early gall growth may be due partly to hyperplasia, but the greater part of the abnormal growth appears to be due to cell hypertrophy.

It is possible that any stimulus that similarly affected the cortical cells of stem and needle-stalklet would cause the development of a gall of the

same shape and form. The galls arising from the feeding of other species of *Adelges* on other species of spruce are essentially the same, although this general statement must be qualified. The alteration of a tissue is modified, moreover, by its position in the plant, and its resultant relationship to other tissues (Dreyfus [64], Thompson [207], Weisse [222]). Galls of a definite and reproduceable shape are not formed on species of the genus *Abies,* the true fir, as a result of feeding by the balsam wooly aphid, *Adelges piceae* Ratz. The tips of the twigs are transformed thereby into gouty clubs (Balch [8], Claus [47]; see also Francke-Grosmann [78, 79]). The needles of *Abies* are borne directly on the twig, on flat pads, rather than on cortical-stalklets as in the case of *Picea.* The fact that such stalklets occur on spruce may account for the constancy of the gall form.

SALIVARY GLAND ENZYMES

In view of the many diverse, and at times conflicting, theories regarding the source of the gall stimulus, it was deemed advisable to attempt to isolate this source in *A. abietis.* It is apparent that initiation of gall formation by the fundatrix must be connected with feeding by this form. The most obvious insect organs that might be suspected of involvement in the process would be the salivary glands.

It has been pointed out previously that a few investigators have succeeded in causing gall-like growths on plants by means of injection of insect extracts (Martin [154], Parr [166, 167]); and Smith (197) reproduced the injury caused by the feeding of capsid bugs, by pricking the salivary glands into leaf tissue. Parr, however, has been the only worker who isolated and used the glands only; Martin used extracts of macerated whole insects.

The methods of Parr (166) were used in obtaining salivary gland extracts of *A. abietis.* Fundatrices were placed, ventral side down, on a small droplet of rubber cement in a hollow-ground slide. The cement was allowed to harden for a few minutes before dissection. The insects were covered first with 70 per cent alcohol, which removed most of the filamentous wax covering; and helped to reduce surface tension. The alcohol was pipetted off and replaced with a modified Ringer's solution. The dorsum of the insect was cut away at the lateral margins by means of micro-scalpels made from finely drawn glass rods. By breaking off the tip of such a rod on a bias with forceps under the microscope, the rods could be given a sharp cutting edge at any desired angle.

When the dorsum has been removed, the salivary glands may be seen at the anterior end of the body cavity. These glands are comparatively small in the fundatrix nymph, but once the latter has moulted and the fundatrix begins to feed and to lay eggs, they increase in size rapidly. Eventually they come to occupy practically the entire anterior third of the body cavity. The paired salivary glands are arranged symmetrically, one gland on each side of the pharyngeal framework. Each gland consists of three major lobes, and a short distance down the lateral branch of the salivary duct is located a much smaller accessory gland. An excellent account of the morphology and histology of these glands is to be found in Cholodkovsky (44); (see also Tóth [208], and Weber [220, 221]).

The glands are translucent, and it is rather difficult to see them and to locate the main salivary duct. In order to obviate premature puncturing,

they were stained with neutral red, a vital stain which should have no effect on the cell contents. The Ringer's solution was pipetted off and replaced with the same solution with a small amount of neutral red dissolved in it. The glands took up the stain rapidly, and after a few minutes, the stain-solution was removed and replaced two or three times with Ringer's until all excess stain was removed. The glands then stood out in strong contrast to the underlying body wall, as they absorbed the stain more rapidly than the other body tissues. The alimentary tract, and the ovarioles, which contained eggs in various stages of development, were removed with fine forceps. The main salivary duct could now be seen and was severed with fine-pointed iridectomy shears. The glands could then be drawn up into the tip of a fine pipette and transferred to a 30 per cent glycerine solution. Twelve pairs of glands were used, and the amount of glycerine solution approximated seven cubic millimeters. After the glands were placed in the glycerine solution, they were crushed with a fine glass rod. The solution was allowed to stand overnight before use.

The glycerine solution was injected into the bases of the spruce buds by means of micro-bulbs made from lengths of melting point tubes. A short length of tubing was inserted into a hole in a rubber plug held in the mouth of a small rubber tube. The end of the glass tubing was rotated vertically in a micro-flame held horizontally and emanating from a glass capillary. When the end of the tubing had been closed, a gentle air pressure exerted by the mouth through the rubber tube expanded the glass tubing to form a small bulb. The other end of the glass tubing was double drawn in a tiny flame to form a fine-pointed pipette, which was closed.

Spruce buds that had no fundatrices attached were carefully selected. Then a micro-bulb was taken from the storage box, and the tip of the neck was broken off with a pair of forceps. The bulb was then held in the fingers for a few moments. This procedure warmed up the air within the thin-walled bulb and partly expelled it. Then the bulb was placed in a holder consisting of a pair of specially prepared forceps. After the bulb was placed in the holder, the open tip of the stem was inserted into the droplet of glycerine-salivary gland extract. The cooling of the bulb, aided by capillarity, drew a quantity of the extract up into the stem.

The epidermis of the stem just below the bud selected was then pierced by a fine needle point, and the tip of the stem of the bulb was inserted into the opening. The fine tip of the stem could then be driven up into approximately the position in which the fundatrix stylets are found. This procedure was very difficult owing to the danger of breaking the delicate bulbs; and the location of the end of the tip could not be fixed with certainty. Check bulbs were inserted into similar uninfested buds. Some of these check bulbs contained plain 30 per cent glycerine solution, and others contained glycerine solution plus neutral red dissolved in it. A few bulbs containing a similar extract of the salivary glands of *A. cooley* were also inserted in buds of Norway spruce. In all, 44 bulbs were used in this experiment, carried out at the experimental forest of The Connecticut Agricultural Experiment Station at Rainbow, Connecticut.

A similar experiment was set up in the greenhouse at New Haven. During the winter, twigs were cut and rooted in sand in flats. These developed nicely in the spring, with but few losses. Forty-three bulbs were used here.

This experiment was a failure, however, due to the entrance of an opossum which trampled over the cheesecloth-covered flats, thereby breaking off all of the bulbs.

The injections were made at Rainbow on April 18 and 19, 1945. By May 5, some of the shoots had elongated sufficiently from the open buds that an examination of the base could be made. In order to do this, some of the loosened bud scales had to be removed, but this could be done without injury. On this date, of the shoots examined with a lens, three showed positive results, that is, needles swollen in the basal region, and one doubtful. When examined again on May 8, all four were positive, and an additional one showed swelling, making five in all. The frequent subsequent examinations failed to yield positive results on any of the other shoots.

On May 8, twigs bearing fundatrices and egg masses were clipped from suitable trees. The buds (or shoots) above the egg mass on each twig were pinched out. These twigs were wired snugly to the above five positive twigs, so arranged that the egg mass touched the injected twig just below the expanding shoot. When next examined on May 14, these eggs had hatched and young crawlers or gallicolae could be seen entering the shoots.

Of these five injected twigs, four failed to develop further, although additional nearly mature eggs were transferred with a brush to all of them. The affected needle-stalklets and stem cortex on the fifth injected twig continued to develop as in a naturally infested shoot; and it could be seen that a small but perfect gall was forming. During the summer this gall continued to grow, and appeared no different from the "normal" galls. It was finally clipped from the tree just before maturing and preserved. This gall is shown in Figure 39.

None of the check shoots exhibited any effect from the injections. A few of the shoots died, doubtless from mechanical injuries received during the insertion process. Most of the bulbs remained in place and could be found in the twigs even a year later. The fine tips of the glass stems did not seem to interfere in any way with growth, and all of the shoots formed above them were quite normal.

This experiment appears to prove conclusively that the seat of the gall stimulus in *A. abietis* is in the salivary glands. It is believed that this is the first instance in which a complete gall of this species has been formed in the absence of the initiating insect. The gall formed on oak by Parr in this manner is an open pit-gall, with raised margins only, which does not close. A perfect gall would not have been formed in this case, it is true, without the presence of the gallicolae. Owing to the very small size of the latter, it would be most difficult manually to dissect out the salivary glands intact. Maceration-extracts might have to be used, and such a procedure would be open to the criticism that the true source of the stimulus cannot be known by this means. Further, due to the exact position of the gallicolae in the gall tissues, it is rather dubious whether such injection methods could simulate their effect.

It has been reported that folic acid represses, temporarily at least, growth of mammalian cancer cells. A supply of this material was obtained through the courtesy of Dr. E. L. R. Stokstad of the Lederle Laboratories, Inc. It was in the amount of 10 mg., 95 per cent pure, in 20 per cent ethanol solu-

tion. The alcoholic solution was very difficult to handle. Because of its low surface tension, it was apt to run out of the bulb stem before the tip could be inserted in the twig. In order to overcome this difficulty (despite which some injections were made), about an equal quantity of the folic acid-ethanol solution was placed in approximately 0.005 ml. of 30 per cent glycerine. This mixture could be injected more easily, but of course reduced the already low concentration of folic acid. A total of 34 injections was made at Rainbow and at New Haven in early June, 1945, in young, developing galls. No inhibition of gall growth was detected in any of the galls so treated.

TABLE 1. QUALITATIVE TESTS FOR ENZYMES IN FUNDATRIX SALIVARY GLANDS

Test for	Test	Aphid No.	Reaction	Check	Remarks
Amylase	Starch, iodine-potassium iodide	1	Negative	Negative	Female had about completed oviposition
		2	"	"	"
		3	"	"	Female had just started oviposition
		4	"	"	"
		5	"	"	"
Invertase	Sucrose, Benedict's soln.	1	Negative	Negative	Cuprous oxide crystals could not be detected with certainty in any of the tests.
		2	"	"	
		3	"	"	
		4	"	"	
		5	"	"	
Protease	Glycyl-L-tryptophane + bromine water	1	Positive	Negative	Strong reaction
		2	"	"	" "
		3	"	"	Weak reaction
		4	"	"	" "
		5	"	"	" "
Oxidase	Benzidine	1	Positive	Negative	Strong reaction
		2	"	"	" "
		3	"	"	Weak reaction
	p-Phenylene diamine hydrochloride	4	"	"	" "
		5	"	"	" "
Peroxidase	Benzidine + hydrogen peroxide	1	Positive	Negative	Color intensified
		2	"	"	" "
		3		"	Not certain
		4	Positive	"	Color intensified
		5	"	"	" "

Tests were made for certain enzymes in the salivary glands of fundatrices. These were qualitative only, and it must be remembered that the tests used are not absolutely reliable. The glands were crushed in each case, before testing. The results are presented in tabular form (see Table 1). The tests indicated that an amylase, a protease, an oxidase, and a peroxidase were present in the glands. Surprisingly enough, a positive reaction for invertase could not be obtained, although the reagent gave satisfactory results when tested on a known sugar. Despite the fact that these enzymes

were identified in the salivary glands of *A. abietis*, it is not believed that they are the substances that cause gall formation. All of these enzymes have been found in plant-feeding insects that do not produce galls. Of course the exact composition of, say, an oxidase in the insect may not be that of an oxidase in another insect; but such differences cannot be determined by the methods available.

It was thought that growth-stimulating hormones might be secreted by the salivary glands. An experiment was set up whereby the presence of auxin might be detected, if present, after the methods of Bonner (21), and Overbeek and Went (164). Peas were planted in sand, in March, 1946, in a dark chamber placed in a darkened room. When the etiolated pea stems had attained a suitable length, fundatrices were obtained, and the salivary glands were removed by the method already described. But few fundatrices were available at this time, from rooted infested cuttings in the greenhouse, so that only eight pairs of glands could be used. These were macerated in a drop of 30 per cent glycerine, which was then washed into a small Petri dish, using a total of 15 cc. of potassium diphthalate buffer solution (pH 3.95), in successive small quantities. Three split pea stems were placed on the surface of the fluid, and the dish was covered.

At the same time, split pea stems were placed in dishes containing, respectively, 25 cc. of the buffer solution used above; 25 cc. of indole acetic acid solution (3 mg. per liter); 20 cc. of indole acetic acid solution of the same concentration, plus 5 cc. of the diphthalate buffer solution; 25 cc. of tap water. Typical curvature was obtained only in the plain water. A slight positive reaction was obtained in the dish containing only indole acetic acid solution; and a slight acid curvature resulted in the dish containing both indole acetic acid and diphthalate buffer. All of the other tests were negative.

It is quite possible that the failure to find auxin present was due to the fact that so few pairs of salivary glands were used. A comparatively large amount of fluid is necessary in order to float the pea stems. On the other hand, this test is exceedingly sensitive, and perhaps should have indicated auxin had it been present. The tests were confounded by the growth of molds in the cultures. This would indicate that a phosphate buffer would be preferable since it has no carbon source.

At the same time, 30 rooted cuttings in the greenhouse were treated with both indole acetic and indole butyric acids. The former was used at a concentration of 10 mg., the latter at a concentration of 15 mg., per 1 g. of lanolin (anhydrous, U.S.P. XII). A small quantity of this paste was smeared onto the base of the buds (which were just beginning to swell) with a small glass rod. In some cases the paste was smeared directly onto the epidermis; in others, the epidermis was first peeled away, and in still others, needles were removed, leaving the open needle-stalklet which was then covered. No swellings were obtained similar to those caused by the fundatrix. In some of the twigs treated, a more vigorous growth of the new shoot was noted. This growth was asymmetrical, that is, confined to the side of the shoot on which the paste was applied. The eccentric elongation caused a curvature in the shoot. It had been planned to place eggs on these twigs if the needle-stalklets and cortex appeared to be affected.

The writer doubts that auxin plays any part in gall formation. This hormone must be present in such quantity in a growing shoot that the

amount added by an insect would make very little difference. Nor would this material as a source of gall stimulation account for differently formed galls caused on the same tissue by different insects.

However, Link *et al.* (143) obtained curvatures of *Avena* coleoptiles with ether extracts of several species of aphids and of the host plants. They comment on this finding as follows: " . . . No answer can be given to the question whether an aphid whose extract is effective in the coleoptile test derives its effective substances from the host leaf cells from which it sucks its food or whether at least in part, it itself (and/or some organism within its digestive tract) makes some or all of these substances from the materials obtained from the host. Hence, no answer can be formulated to the question whether an aphid incites auxone disturbances and whether these play roles in the growth disturbances associated with insect parasitism."

In this connection, the theory that symbionts may play a part in gall formation may be recalled. The work of Haracsi (94) points to the mycetome anlage as the embryonic tissue from which the salivary glands are derived, at least in the species studied by him. If this is true, the widest possibilities are presented for the investigation of gall formation. Sülcs' studies (205) of the mycetome provide additional indirect evidence that symbionts may be involved in this process. He described the symbionts in the mycetome of a number of species of Homoptera, as yeasts. *Chermes* (*Adelges*) *abietis* was one of the insects studied, and the symbiont from its mycetome was named *Schizosaccharomycetes Chermetis abietis* Sülc. It is known that yeasts may elaborate materials which may be used as nutrients by plant cells. This additional food might lead to increased growth or hypertrophy of the cells to which it was available.

CHEMICAL COMPOSITION OF THE NORMAL BUD AND SHOOT

The chemical composition of the normal bud and shoot, and of the gall tissues was investigated. It was thought that such an investigation might be of assistance in explaining the formation of the gall. Sections were cut both free-hand and on the freezing microtome. In each case, the blade was washed and wiped between successive cuts. The following reagents were used in these tests:

Starch — Iodine-potassium iodide
Proteins — Millon's reagent
Sugars — 1. Phenylhydrazine hydrochloride plus sodium acetate
2. Copper tartrate plus potassium hydroxide
Lignin — Phloroglucinol plus hydrochloric acid (25 per cent)
Aldehydes — Diphenylamine (1 per cent) plus sulfuric acid (concentrated)
Fats — Sudan III

It is realized that many substances other than those tested for may be present, but at least some indication is given of the composition of the various tissues. The results are presented in Table 2.

In the bud, starch is most highly concentrated in the medulla. In the two-year shoot, it is most abundant in the cortical tissue immediately below the lateral extensions of the collenchyma plate into the bud scales; and lateral

to the cavity below it, to the point of taper of the cavity. Proteins were most highly concentrated in the needle rudiments and in the cortical cells bounding them medially. Sugars, in the form of fructose and sucrose, were present in the bud cortical cells and in the provascular strands. These were also found in the lateral extensions of the collenchyma plate. The vascular elements bounding the cavity just below the plate contained sugars, but lower in the stem they were absent. Lignin was most concentrated in the secondary xylem of the stem.

TABLE 2. DISTRIBUTION OF SUBSTANCES IN SPRUCE TWIG, OCTOBER, 1945

Tissue	Starch	Proteins	Sugars	Lignin	Aldehydes	Fats
Bud						
Bud scales	−+[1]	−+	—	++	—	—
Needle rudiments	+	+++	—	—	—	—
Medulla	+++	—	—	—	—	—
Cortex	++	++	+	—	—	—
Procambial strands	—	+	+	+	—	—
Collenchyma plate	—	−+	—	—	—	—
Two-year stem						
Pith	++	—	—	−+	—	++
Secondary xylem	—	+	—	+++	—	—
Cambium zone	—	—	—	—	—	—
Secondary phloem	—	—	−+	—	—	—
Primary phloem	—	—	—	—	—	—
Cortex	++	+	—	—	—	—
Phelloderm	—	—	—	—	—	++
Phellogen	—	—	—	—	—	++
Phellem	—	—	—	+	—	+
Hypodermis	—	−+	—	++	—	—
Epidermis	—	—	—	—	—	—

1 — Not found
−+ Trace
+ Present
++ Concentrated
+++ Highly concentrated

These findings may explain why the overwintering fundatrix nymph can live only when fixed at the base of a bud. Sugars, proteins and starch occur in abundance only in the bud itself, with the exception that starch is quite concentrated in stem-cortex. It is probable that the nymph cannot find nourishment enough in the two-year stem, when fixed thereto, to enable it to survive.

In a young gall, starch is highly concentrated in the cortical cells which compose the gall, as are proteins. Sugars are present, but not concentrated. Starch is not found in the epidermal cells that line the gall chambers. It is present in the cortical cells that immediately surround the chambers, but becomes more abundant in the cells farther from the cavities. Starch is particularly concentrated in the cells nearer the outside periphery of the gall; and especially in the cells near the lips that close the chambers.

The chemical composition of the stem, bud and gall, as far as investigated, throws little light on the process of gall formation. It may be that

these insects require some substances for their nourishment other than sugars and proteins. This fact appears to be borne out by other investigations on certain plant-feeding insects. The gallicolae of *A. abietis* secrete honeydew, which may be found as discrete globules within the chamber. Rawitscher (175) believes that the excretion of honeydew represents the removal of superfluous carbohydrate, particularly by those insects that feed in the sieve-tubes. However, Rawitscher points out that honeydew is also excreted by insects that do not feed in the sieve-tubes.

Wigglesworth (231) cites Weber (220) on the constituents of manna, a similar metabolic product excreted by the coccid, *Trabutina mannipara,* that feeds on tamarisk. It contains 55 per cent of sucrose, 25 per cent of invert sugar, and 19.3 per cent of dextrin. Wigglesworth points out that the significance of this excessive feeding is alleged to be due to the deficiency of protein in the cell contents. Therefore, the insect must ingest large quantities of material in order to get the required amount of protein. He states, however, that there is little support for this theory. Wigglesworth draws upon the works of Davidson (61) and Tóth (209) for contrary evidence. The carbohydrate content is about 90 per cent and the protein content is about 5 per cent of the solid matter in the sieve-tube sap of plants. The honeydew of *Aphis rumicis* contains 85 per cent of carbohydrates (invert sugar, 24.5 per cent; sucrose, 16.7 per cent; dextrin, 39.4 per cent), and 3 per cent of proteins. The excrement of *Trialeurodes* feeding on the same plant as the aphids, contains neither carbohydrate nor protein (Weber [220]). Wigglesworth concludes that excessive quantities of material are absorbed to enable the insects to obtain some substance other than protein.

Michel (156) gives an analysis of the sieve-tube sap of oak and of the honeydew of an aphid, *Lachnus roboris* L., that feeds on oak. His table is here reproduced:

	Sieve-tube sap per cent[1]	*Honeydew per cent*
Invert sugar	17.5	19.1
Cane sugar	44.7	25.9
Melezitose	...	46.3
Dextrine	37.5	6.7
Nitrogen	0.3	0.6

Michel comments that it is evident that protein sources other than the plant sap are available to the aphid, and hypothesizes that proteins may be delivered to the aphids by their symbionts contained in the mycetome. (See also Peklo [168], Peklo and Satava [169]).

MATURING OF THE GALL

Toward the close of summer, in late August and early September, the gall chambers open on the preformed sutures that mark the "lips" of the cavities (Figure 40). The gallicolae, which previously have molted twice, molt for a third time to produce the alatae non-migrantes. The last molt may take place within the cavity, at its mouth, or the nymph may crawl to the twig or a needle before molting. As the time for this event to take place approaches, the gall gradually begins to lose its green coloration and takes

[1] Per cent of dry weight.

on a yellowish-brown appearance. At the time when the cavities open, there are still traces of green, however. Following the opening of the cavities, the entire gall rapidly dries and hardens, and assumes a deep blackish-brown color.

This process raises the question of what causes the gall to ripen, and particularly just at the time when the gallicolae are ready to emerge. Various theories have been advanced to account for the maturing and opening of the gall. DeGeer (84) detached a closed gall from the tree and placed it on his desk. After a few hours he noted that it was becoming brown, and that the cells had opened. He stated that the latter opened because of desiccation of the gall tissue, and that this is the reason why natural opening occurs. Frank (80) said that "The opening occurs through desiccation and is a result of tissue-tension, since opened galls lying in water close themselves after a time." K. (110) believed that the gall dried and discolored owing to sap withdrawal by the gallicolae. Küstenmacher (126) thought that the opening was brought about by pitting and lignification of the "parenchyma-complex," and the "heterogeneous thickening of the parenchyma walls." In the desiccated zone, the vascular bundle was stretched and torn. Börner (19) stated: "As soon as the sucking of the nearly matured gall nymphs ceases, then expires the stimulus for the sap stream which has nourished the gall, and the desiccation and opening of the gall commences necessarily as a result thereof."

It was thought that maturing of the nymphs and consequent cessation of feeding by them might be the cause. This view rests on the possibility that the gallicolae pump into the gall tissues some substance that keeps the cells alive; and that when the cells no longer receive it, they die.

A number of galls in the early stage of development (completely formed and closed, however) were fumigated so as to kill the gallicolae within. Ethylene dibromide was selected as the only fumigant easy to handle and least harmful to plant tissues. A short length of large-diameter glass tubing was fitted with a sleeve at one end, consisting of a thin rubber membrane. The other end could be closed by means of a cork stopper, to the inner side of which a small pad of paper toweling was affixed. Needles on the stem next to the gall selected were pinched off with forceps, and the rubber sleeve was slipped over the gall and the twig bearing it. The sleeve was closed from below with a pinch-cock. The ethylene dibromide was applied to the paper pad on the stopper in counted drops from a dropping-bottle. In this way the dosage could be varied. Exposures were varied as well as dosages, for it was not known what combinations of dosage and exposure the gall tissues could stand without being killed. Thirteen galls were treated in this manner.

Five of the galls were killed by the treatment. Of these five, the cavities opened eventually in two, while no cavities of the other three galls opened at all. One gall was removed from the tree the day after fumigation and all nymphs were found to be dead. The remaining seven galls exhibited no subsequent signs of injury, except that in some instances needles were killed on the twigs bearing them or on the galls themselves. On only two galls did any cavities open prematurely, and these did not open until 35 days after fumigation (June 4). When finally examined in early September, it was found that six of the galls had live nymphs in at least one cavity.

This experiment must be regarded as inconclusive, although it points to the fact that maturing of the gallicolae (and consequent cessation of feeding) does not affect opening of the cavities. It sometimes happens that the cavities of galls fail to open, and the insects are trapped within them. It scarcely seems only fortuitous, however, that the maturing of the insects and the opening of the cavities coincide nicely. On the other hand, there is nothing that indicates why the gall tissue dies and the cavities open. Once the cavities have opened, desiccation seems to be hastened. The vascular strands running outward from the stem-vascular elements into the gall appear to remain intact. It cannot, therefore, be an interruption in the water supply which at first causes drying of the gall and opening of the cavities.

There is, however, an interesting phenomenon connected with maturing of the gall. The younger galls have an abundance of starch in the cells. As the gall matures, this starch accumulation disappears. Hartwich (97) has stated that the abundant starch found in the nutritive layer of *Infectoria* galls is not used directly for the nutrition of the larvae. Rather it is transformed into other substances (Francke-Grosmann [78], Kraemer [125], Küstenmacher [126]). Two of these substances were found to be tannin and lignin. If a gall of *A. abietis* is taken from the tree when almost mature, lignin (phloroglucinol — HCl test) is found to be abundant. A completely mature gall, that is, one that has dried, when sectioned and tested, gives a strong, positive reaction over the entire surface. If the theory of Hartwich is correct, it would account for the disappearance of starch from the cells of this gall when the latter is maturing.

It is possible that the opening of the chambers may be at least partly due to stresses that result from the composition and position of the galled tissue. The cover flaps are formed from modified needle-stalklets. By reference to Figure 40, it may be seen that the normal stalklets tend to straighten out more or less at right angles to the twig. If the galled stalklets follow this same trend, which is inherent to them, then the flaps would be pulled open (see Thompson [207], Weisse [222]).

SUMMARY

The morphology of the dormant bud and developing shoot of Norway spruce has been studied; and the derivation of certain tissues from their precursors has been established. This work has also shown that the gall caused on this spruce by *A. abietis* is formed from cortical cells of the stem and leaf-stalklet, not from the cells of the leaf itself as generally supposed. Differentiation of the intercalary meristem and abscission layer in the needle is either completely inhibited, or is affected not at all. The epidermal cells that line the gall chambers are unaltered. The external form of the gall may be due to the stresses enforced by the position of the tissues affected.

The successful overwintering of the fundatrix nymph at the base of the bud may be due to a higher content of possible food materials in the bud, since the two-year stem contains comparatively little of these. The fact that starch accumulates in the gall cells during its growth may indicate that it and its derivatives are not primary nutrients of the gallicolae. The disappearance of starch on maturing of the gall may be due to its transformation into lignin, since the desiccated gall is completely lignified. No discrete

nutritive zone such as is found in many cynipid galls occurs. The gallicolae feed in the first few rows of cells surrounding the gall chambers.

Extracts of the salivary glands, injected into normal buds of spruce in the spring just at the time of swelling, caused the initiation of gall formation in five buds. The transfer of gallicolae-producing eggs to the injected buds carried gall formation to completion in one case.

Certain enzymes were detected in the salivary glands. Enzymes of the same type are present in the saliva of plant-feeding insects that do not form galls, however. The failure to find an amylase in the salivary glands of the fundatrix may have been due to faulty technique. But the absence of this enzyme, if actual, may carry over into the gallicolae; and this would aid in explaining starch accumulation in the gall.

A test for auxin, by means of the etiolated pea-stem technique, was negative. This may have been due to the use of an insufficient number of glands. It is not believed, however, that auxins play any part in the formation of this gall. Indole acetic and indole butyric acids failed to cause a growth stimulus in the bud similar to that caused by the fundatrix.

Folic acid injected into young galls failed to inhibit further development at the concentration used.

Fumigation experiments failed to show that maturing of the gallicolae, hence cessation of feeding, is necessarily correlated with maturing of the gall and opening of the pockets.

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