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Modification of Vascular Tissue in Midvein of Quercus Alba Leaves Induced by Gall Development of Cynips Pezomachoides Erinacei

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MODIFICATION OF VASCULAR TISSUE IN MID-VEIN OF QUERCUS ALBA LEAVES INDUCED BY GALL DEVELOPMENT BY CYNIPS PEZOMACHOIDES ERINACEI

By E. FAY KENOYER

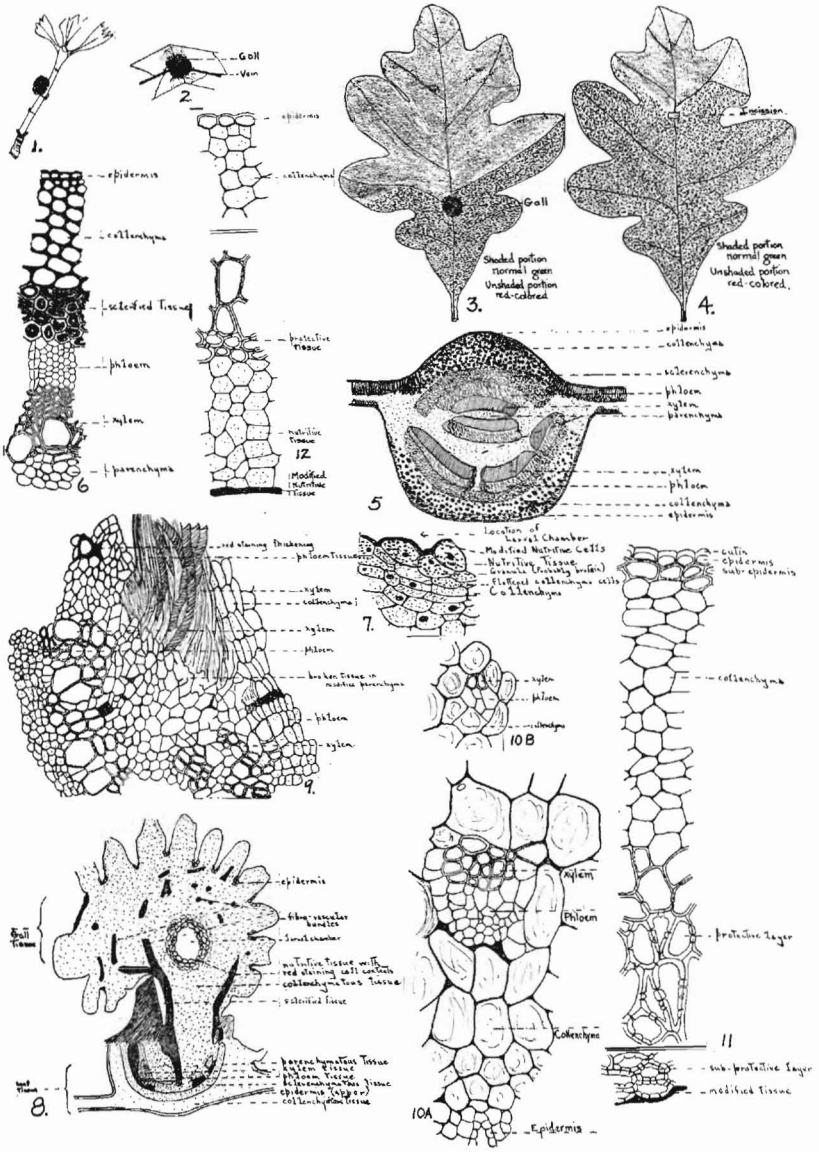
INTRODUCTION

In the autumn of 1933 Miss Agatha Griffin (3) studied the influence of interrupted translocation on loss of chlorophyll in leaves before autumn coloration. During the course of the observation, it was noted that a gall on the vein of leaves of *Quercus alba* produced strikingly similar results as cutting of a vein, *i. e.*, in both cases the area above the cut or gall growth lost its chlorophyll first and turned strikingly red several weeks before the portion below the cut or gall discolored. It was at that time assumed and substantiated hy superficial examination that the gall induced some sort of modification in the conducting tissue of the leaf, thus affecting translocation. To investigate the nature of this modification in the vascular tissues is the specific aim of the present study.

METHODS

The gall-infested leaves for this study were gathered at various intervals from the middle of June to the last of October. Specimens were obtained from trees on the Butler University campus, where galls were numerous. When taken to the laboratory, portions were cut from the leaves, each section bearing a gall. About 6-12 mm. of the vein were left on each side of the gall while about 3-6 mm. of tbe leaf blade were left on each side of the vein.

Five stages were selected for study: (1) Veins showing only ruptures with few trichomes protruding. (2) Galls ranging in size from one to three millimeters. (3) Galls from three to five millimeters. (4) Galls from five millimeters to largest size of about one centimeter on veins showing no indication of brown coloration or modification. (5) Galls on veins which were hrown, discolored and displayed modification. The material was killed, some in medium chromo-acetic acid and some in formalin-acetic alcohol.


1.
Gall
Vein
2.
epidermis
collenchyma
epidermis
collenchyma
protective tissue
nutritive tissue
Modified Nutritive Tissue
12
Gall
Shaded portion normal green
Unshaded portion red-colored
3.
Incision
Shaded portion normal green
Unshaded portion red-colored.
4.
epidermis
collenchyma
sclerenchyma
phloem
xylem
parenchyma
5
xylem
phloem
collenchyma
epidermis
6
epidermis
collenchyma
protective tissue
phloem
xylem
parenchyma
red staining thickening
phloem tissue
xylem
collenchyma
xylem
phloem
broken tissue in nutritive parenchyma
phloem
xylem
9.
location of larval Chamber
Modified Nutritive Cells
Nutritive Tissue (containing protein)
Granula
Fluffiest collenchyma cells
Collenchyma
7.
xylem
phloem
collenchyma
10B
cutin
epidermis
sub-epidermis
collenchyma
protective layer
11
sub-protective layer
modified tissue
epidermis
fibro-vascular bundles
gall chamber
nutritive tissue with red staining cell contents
collenchymatous tissue
protective tissue
parenchymatous tissue
phloem tissue
sclerenchymatous tissue
epidermis (upper)
collenchymatous tissue
Gall Tissue
Leaf Tissue
8.
xylem
Phloem
Collenchyma
Epidermis
10A

EXPLANATION OF FIGURES

Figure 1. Gall of *Cynips pezomachoides erinacei* on a twig of *Quercus alba*.

Figure 2. Portion of the midvein of a white oak leaf showing the bent surface due to the growth of a gall.

Figure 3. Leaf of *Quercus alba* showing early red coloration in fall due to gall growth on midrib.

Figure 4. Leaf of *Quercus alba* showing early red coloration due to cut in midrib.

Figure 5. Outline drawing of a cross section of the normal vein of a white oak leaf showing arrangement of tissues.

Figure 6. Detailed drawing showing tissues found in the normal vein of a white oak leaf.

Figure 7. Detailed drawing of tissue around the larval chamber in stage 1. This tissue is similar in stages 2 and 3.

Figure 8. Outline drawing of stage 2 showing tissues of gall and vein. Note distribution of vascular tissue in gall, vein tissue pushed to one side of gall, and separation of groups of vascular tissue in vein due to proliferated parenchyma.

Figure 9. Detailed drawing showing vascular tissue running into gall from leaf vein, tissue of vein, and sclerified tissue pushed to one side of gall growth.

Figure 10. Cross section of vascular bundle and surrounding tissue in gall: A, large bundle; B, small bundle.

Figure 11. Detailed drawing showing tissues found in mature gall.

Figure 12. Tissues found in cross section of gall of *Cynips pezomachoides erinacei* on twig of *Quercus alba*.

When ready for use, dehydration and imbedding in paraffin were carried out according to standard methods. Sections were cut ten microns in thickness. Cross sections of the vein were made in all stages of gall development. The gall was left on the vein in the younger stages while in the older stages it was removed, because of its hardness and consequent difficulty in cutting. Cross sections of the gall itself or cuts longitudinal to the vein were also made. In arranging paraffin ribbons on the slides, serial sections were made. Due to the natural hardness and brittleness of the oak veins and the collapse of cells of the vein in stage 5, it was impossible to make complete serial sections in many cases. Thick cross and longitudinal sections forty to sixty microns in thickness were cut of the mature galls by hand and on the microtome. Twigs bearing galls were killed in formalin-acetic alcohol and imbedded in celloidon. Both cross and longitudinal sections of the twigs were cut on the sliding microtome, thirty microns in thickness. All sections were stained in safranin, counterstained in Delafield's hæmotoxylin, and mounted permanently in balsam.

Amount of vascular tissue in the vein was determined by measuring with the aid of an ocular micrometer around the ring of tissue or across isolated groups of tissue in the gall-infected veins.

OBSERVATIONS

The gall of the agamic wasp, *Cynips pezomachoides erinacei*, is produced most frequently on the midvein of the lower surface of the leaf of *Quercus alba*. It occurs less frequently on the upper surface, and rarely on twigs (Figure 1). It is a spiny or slightly faceted, spherical to oval structure, about one centimeter in length when mature. The galls are a deep red when produced on the upper surface of the leaf, or range from a cream to light red when produced on the lower surface.

After the first galls were noticed on the trees early in July, it was observed that many ruptured places similar to those from which early stages of galls were protruding were present in the veins of leaves. To be sure that these ruptures were caused by *Cynips pezomachoides erinacei*, several leaves were marked for further observation. In all cases, galls of this wasp were later produced in these ruptured places. Veins showing similar ruptures were then collected and designated as developmental stage 1.

Galls developed from these first beginnings to a structure of about

one centimeter in diameter. When gall growth approached this size, it was found in many instances that a pinching of the tissues of the vein occurred beneath the gall (Figure 2). Later brown coloration and modification appeared in such pinched tissue. It was also found that before autumn leaf coloration occurred, the portion of the leaves above gall-growths turned red (Figure 3). A similar reaction was obtained when veins of normal leaves were cut. The portion of the leaf above the incision turned red first (Figure 4). This result is in agreement with those obtained by Griffin (3). It was further observed that frequently the veins of leaves showing this red coloration were brown and discolored with the appearance of disintegration. In many cases disintegration of tissues had gone so far in veins bearing mature galls that the galls fell easily from the leaf.

A histological study of normal as well as abnormal tissue influenced by gall growth is given below:

Normal Vein. The following arrangement of tissues appeared in cross section: Upper epidermis protected by a thick layer of cutin. Beneath this a collenchymatous tissue composed of comparatively thick-walled cells thickened at the corners. Directly below this was a thick-walled, red-staining tissue extending in a ring all around the vein, followed by a ring of phloem enclosing a ring of xylem. A thin-walled parenchymatous tissue lay in the center of the ring of xylem. At the middle of the vein beyond the ring of phloem was also a parenchymatous tissue. Beginning at the lower epidermis, a similar arrangement of tissues was found, except that the phloem and xylem did not form another ring, but extended up on each side of the vein just to the upper ring of vascular tissue (Figures 5, 6).

Stage 1. Cross sections of a ruptured vein on the lower surface of the leaf showed trichomes about four abreast just developing on the exposed surface of the newly forming gall; yet there had not been any gall tissue visible to the naked eye, nor could it be noticed under a hand lens. The trichomes were composed of slightly elongated cells similar to those composing the remainder of the gall. Epidermal tissue was present. The tissue directly surrounding the larva appeared to be broken down, forming an amorphous, red-staining substance. Outside of this layer were a few rows of large cells containing abundant protoplasm with red-staining granules. Just outside of this were a few rows of small flattened cells. The rest of the newly-formed gall tissue was composed of closely packed collenchyma cells (Figure 7). Orientation of the larval chamber

to the tissue of the vein was determined by comparison with tissue of the normal vein. It was found that the chamber lay chiefly in the xylem tissue, in close contact with the phloem. The central parenchymatous tissue of the vein had been proliferated and contained part of the larval chamber. Vascular tissue found normally in the lower part of the vein had been interrupted by the formation of gall tissue.

STAGE 2. Vascular bundles were present to supply the gall with food and water by the time it had become about 2 mm. in diameter. Figure 9 shows details of the vascular tissue running from the vein into the gall. Marked rearrangement of the tissue of the vein was noted by the time the gall had reached this stage. Vascular tissue was separated and rearranged into various isolated groups, due to proliferation of parenchymatous tissue between the vascular elements (Figure 8). This separation and rearrangement of vascular tissue was common in most of the infected veins studied.

Another source of rearrangement of the tissue of the vein was the proliferation of the tissue near the larval chamber. Parenchyma and vascular tissue proliferated, finally pushing up and out of the vein of the leaf to form the gall. In this process, tissue normally lying over the place in the vein where the egg is deposited, takes up a position to one side of the gall when the gall develops (Figure 9). In many cases a deposit of some brown substance was observed in this tissue pushed to the side of the gall as well as in the leaf blade close to the gall. This may be a tannin deposit; Winton (8) says that tannins are usually associated with brown coloring substances. In many cases this rearranged tissue also had become sclerified and had developed heavy red-staining walls and thickenings (Figure 9).

A study of the gall tissue (Figure 9) showed typical large masses of xylem and phloem running up into the gall. It was found that these vascular elements branched out into the collenchyma when they reached the gall tissue, so that small vascular bundles were obtained, each oriented so that the xylem was toward the center of the gall and the phloem was toward the outside. In size, the bundles ranged from small, containing only one to few xylem elements, to large, containing several xylem elements (Figure 10).

Epidermis, collenchyma and nutritive tissue were similar to that found in stage 1, except that in the pedicel-like structure supporting the gall a centrally-located area composed of cells elongated with their long axis vertical to the leaf blade was noticed. The ends of some of the cells

were at right angles to the sides, while others were oblique or tapering. The walls were somewhat thickened and simple pores were noticeable in the walls.

STAGE 3. Tissues of the vein and gall were similar for the most part to those found in stage 2. Beneath the epidermis was a thick layer of collenchymatous tissue well supplied with vascular strands. The cells enclosed a crystalline substance and prominent large nuclei. Adjoining on the inner side was a layer, a few cells in thickness, which contained large nuclei, as found in the collenchyma, and other smaller irregular bodies staining red with safranin. Inside of this tissue was a broken-down amorphous tissue staining uniformly red throughout.

STAGE 4. A large amount of vascular tissue leading into the gall structure was present. In some instances, even the lower row of vascular tissue in the vein was tapped to supply the gall. The collenchymatous cells below the epidermis of the vein showed signs of collapse in this stage, especially at each side of the vein. This had been noticed in a few of the younger stages after gall-tissue had pushed out of the vein and forced the vein-tissue to each side. Some of the parenchymatous tissue of the vein also showed evidence of disintegration.

STAGE 5. The most striking feature was that most tissue of the vein seemed to be in a state of collapse. In fact, it was very difficult to obtain sections of these stages, because of the brittleness and degeneration of the tissue. A longitudinal section of a mature gall (Figure 11) showed epidermal tissue present. Directly below this was a thin layer of compact red-staining cells having sclerified walls. When material was observed macroscopically, it appeared to be composed of a glistening crystalline layer. Beneath this was a layer of collenchyma which also appeared crystalline, macroscopically. This tissue made up about one-half of the gall growth, and contained only remnants of protoplasm. Beneath this and extending to the larval chamber, was a layer of red-staining sclerenchymatous tissue. This consisted of enormous, very thick-walled, elongated cells containing numerous simple and branching pores extending partially or all the way through the cell wall. There were no large thin-walled cells surrounding the larval chamber as observed in younger stages, but a compact layer of smaller, somewhat flattened yet nearly isodiametric, six-sided sclerenchymatous cells was present.

GALL BORNE ON TWIG. A cross section of a gall formed on a twig showed development similar to that found on the leaf (Figure 12). Proliferating parenchymatous tissue of the pith had pushed some of the

vascular tissue to each side of the gall growth. The cells of the pith had undergone a peculiar change. Those opposite the gall had become sclerified, while in the center of the twig was a deposit of a dark heavily-staining substance. Then toward the gall from this deposit were the ordinary, only slightly larger, pith elements.

VASCULAR TISSUE OF VEIN INTERRUPTED BY GALL GROWTH

It was found that the amount of vascular tissue present in a section through a gall-infected vein did not equal the amount found in the normal vein. It was also seen that this was caused by interruption of the vascular tissue by gall-growth. Reference to Table I gives some idea of the amount of such interrupted vascular tissue.

TABLE I

SHOWING INTERRUPTED VASCULAR TISSUE DUE TO GALL GROWTH IN VEIN

Surface of leaf bearing gall	Vascular tissue in normal leaf. Lineal microns	Vascular tissue left in gall-infected leaf. Lineal microns	Amount of vascular tissue interrupted. Lineal microns	Percentage of vascular tissue interrupted
Upper	1696	1431	265	15.6
Upper	2014	1484	530	26.3
Upper	1802	1590	212	11.7
Upper	1908	1484	424	22.2
Upper	1908	1484	424	22.2
Lower	1696	636	1060	62.5
Lower	1908	1272	636	33.3

The average loss of vascular tissue due to interruption by gall growth in the vein when galls were produced on the upper surface of the leaf was 27.7 per cent. In most cases, only the upper part of the ring of vascular tissue found normally in the upper part of the vein was affected. When galls were produced on the lower surface, however, it was found that more tissue had been interrupted. Not only had part of the row of vascular tissue extending around the lower part of the vein been affected, but also the lower portion of the ring of vascular tissne found in the upper part of the leaf had been interrupted. In one case, only the phloem of the lower portion of the ring was affected, leaving the xylem unaffected.

DISCUSSION

In a description by Kinsey (4) of the tissues found in galls belonging to Cynips, the following tissues are recognized: *Epidermal layer:* outer covering of gall with peculiar faceted surface in many species. *Spongy parenchyma:* occupying the central portion and constituting the major portion of material in all spongy and more hollow oak apples of this genus. Poorly developed in the subgenus Antron and absent, as far as he could see, from the galls of the subgenus Acraspis. *Protective zone:* apparently absent in Acraspis—sclerified tissue. *Collenchyma:* lying directly beneath the epidermis. A second layer in which the cells have thickened walls and usually crystalline contents—bulk of material in Acraspis. *Nutritive layer:* innermost tissue of the gall, lining the larval cell. A distinct layer in young galls of many species, soon becoming reduced by the feeding of the larval insect (and probably by absorption by other plant tissues) to a thin, broken layer of partially empty cells. Poorly developed in any but the very youngest gall of Acraspis. Possibly directly descended from phloem.

In the present investigation the following tissues were recognized: *Epidermis:* tissue covering the outside of the gall and becoming heavily cutinized in older stages (Figure 11). *Subepidermal layer:* present only in mature gall. Consists of a layer only a few cells deep, sclerified, having a thick wall and occasional pores (Figure 11). *Collenchyma:* found under above layers. Comprises the major portion of gall. It consists of nearly isodiametric to elongated cells, having deeply-staining walls thickened a trifle more at the angles than elsewhere. In young stages it contains abnndant protoplasm with large nuclei; later, crystals develop and finally only remnants of cell contents are left (Figures 7, 11). *Nutritive zone:* lining larval chamber in young galls; composed of large, loosely associated cells of parenchymatous character, and containing abundant protoplasm with red-staining granules, prohably protein (Figure 7). *Protective zone:* In mature galls, this tissue is found inside the collenchyma. It is a very heavy-walled, sclerified tissue, having enormous simple or branched pores extending partially or all the way through the cell wall (Figure 11). *Subprotective zone:* In the mature galls there is a thin layer a few cells in thickness instead of a large-celled area surrounding the larval chamber. This consists also of sclerified cells with wall pores, but they are not radially elongated. These are more nearly isodiametric.

Since *Cynips pezomachoides erinacei* belongs to the subgenus Acras-

pis, it may be seen that the present data differ from those given by Kinsey in two essential places, (1) the presence of a subepidermal layer of sclerenchyma and (2) the presence of an inner sclerified layer below the collenchyma, consisting of radially elongated cells of a protective zone, and more nearly isodiametric cells of a subprotective zone. There are two explanations for such a difference. Either younger stages of the gall were used for observations in the former report or there are variations in the structure of this gall and the structure of other galls of this species, so that the wasp, *Cynips pezomachoides erinacei*, may produce galls either with or without the subepidermal and protective zones.

The occurreuce of the gall of *Cynips pezomachoides erinacei* on the twig as well as on the leaf is "rare, although not unknown," according to Kinsey. Several such galls were noticed during collection of material.

Insertion of the egg into the central portion of the vein in contact with xylem and phloem tissue is in accordance with the description by Beyerinck. Orientation of the vascular bundles in the gall tissue (Figure 10) with the xylem inside and the phloem outside was found to correspond with that described by Küster (6) for the normal gall. Exceptions are numerous, however, according to him. Distribution of the vascular tissue through the gall seemed to indicate that some of the strands might form a closed network, for in some sections it was found that the vascular tissue was continuous up each side of the gall and almost met in the center. The presence of very small vascular bundles, seen in a cross section of the gall, would indicate a system similar to that found in the leaf in which the bundles finally end in minute branches consisting of only one xylem vessel and few phloem cells. Few vascular elements were also found extending out into the trichomes (Figure 8).

According to several investigators, the enlargement of the cells of the nutritive zone is due to the storage of abundant food substances. It was noted that cells surrounding the larval chamber contained large red-staining granules. This has been noted by Kostoff and Kendall (4), who point out the accumulation of food material in various types of tissues where foreign substances are introduced iuto the living tissue.

Cosens (1) attributes the accumulation of food at the source of irritation to another cause: "Summing briefly, the larva secretes an enzyme capable of changing starch to sugar, which acts on the starchy constituents of the nutritive zone and accelerates the rate of their change to sugar. The material thus prepared supplies nourishment for both the larva aud gall. The protoplasm of the latter is thus rendered unusually

active, since it receives an abnormal quantity of available food material in a limited area. The hypertrophy and cell proliferation and probably also the appearance of vestigial tissue or other primary characters are the response of the protoplasm of the host to the additional food supply." Weidel (7) postulates that chewing by the larva causes development of the protective tissue from the starch parenchyma, and development of the nutritive tissue from the protective tissue in *Andricus globuli*.

Esau (2), in working with the curly top virus in sugar beets, found interesting stages during the infection. First, the process begins with the development of giant cells. These arise from phloem parenchyma or pericycle cells adjacent to the first normal sieve tubes. Primary hypertrophy usually accompanies the hyperplasia manifesting itself in the phloem or pericycle cells farther removed from the sieve tubes. This may, however, fail to take place. There is then primary necrosis or death of giant cells. In the second stage, secondary necrosis, the giant cells collapse. These cells die, probably because they have developed abnormally and do not function as healthy cells. In secondary hypertrophy and hyperplasia, phloem parenchyma cells are proliferated around the collapsed cells. They commonly occur in plants when cells are injured or dead. "Resembling wound repair reactions, they do not require the presence of a specific infection to explain their occurrence."

These conceptions of the accumulation of nutritive material around the larval chamber offer a partial explanation of data recorded. Due to an irritation, either by the chewing of the larva, a secretion of an enzyme, or both, the parenchyma of the vein is stimulated to produce large cells closely surrounding the source of irritation, the egg and later the larva. Cells farther removed from the source of irritation, or vascular tissue, are also stimulated to proliferation and a gall-growth results. By enzymatic action or the chewing of the larva, cells of the nutritive tissue are degenerated and are seen as amorphous, broken-down tissue lining the larval cavity. In older stages, the entire disappearance of the nutritive zone and the production of the protective tissue, seen first as a row of heavy-walled cells and then as a thick layer of tissue surrounding the larval chamber, may be, as suggested by other investigators, a natural reaction of the plant toward a source of irritation.

The abnormal rearrangement of the tissue of the vein by parenchymatous proliferation as well as the pushing of the vein tissue to the side as the gall pushes through the epidermis of the vein, offer evidence for an explanation of the disintegration of the tissue as noted in veins bear-

ing galls of late stages. The general straining of tissue caused by such development would naturally stretch some cells and crush others, so that, under such abnormal conditions, cells would tend to die and disintegrate. Thus, the vein beneath the mature gall is found to be brittle and disintegrated, in many cases resulting in interrupted translocation.

Although there was interrnption by gall growth in approximately 20 per cent of the vascular tissue of the vein in galls borne on the upper surface of the leaf, and from 33 to 62 per cent in veins bearing galls on the lower surface, it is not thought that this interruption is sufficient to be the cause for early red coloration in gall-infected leaves. Interruption of the vascular tissue of the vein is seen in early stages of gall development. However, it is not until later in the summer that red coloration in the leaf is apparent. Degeneration and collapse of cells in the vein caused by the death of the strained tissue increases as late summer approaches. Thus, it is thought that the collapse of the strained tissue is evidently the chief cause of the brown coloration of the veins and results in early red coloration in the leaf.

CONCLUSIONS

1. Early red coloration in the autumn leaves of *Quercus alba* above the growth of a gall of *Cynips pezomachoides erinacei* is evidently due to interference in the translocation channels in such leaves.

2. Partial interference is apparently caused by: (a) Deflection into the gall of about 20 per cent of tbe vascular tissue of the vein on leaves bearing galls on the upper surface, and 33 to 62 per cent in veins of leaves bearing galls on the lower surface. (b) Deflection of vascular tissue of the vein into the gall where it branches in a partially open network prevents the normal translocation of food, water and minerals to the tip of the leaf.

3. The chief interference is caused, however, by the collapse of cells in the vein, due to the strain on these tissues as a result of hyperplasia, hypertrophy and consequent gall growth.

4. The young gall of *Cynips pezomachoides erinacei* is differentiated into epidermis, collenchyma and nutritive tissue.

5. The mature gall is differentiated into epidermis, subepidermis, collenchyma, protective zone and subprotective zone.

6. Galls of this species are found on the stem of *Quercus alba* as well as on the leaves.

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