Girdling Experiments on the Translocation of Topically Applied radioactive γBHC-1-C^{14} in certain Woody Plants with insect Galls

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(*With 3 Text-figures and 5 Plates*)

During recent years, not a few works have been published reporting that BHC and/or lindane may be absorbed and translocated from contaminated portions into plant tissues of various kinds of plants, when these insecticides are applied to the aerial parts of the plant, or added to soil in which plants are grown, and or dressed over seed before sowing. Yet relatively little is known at present of the mechanism of absorption and translocation of this kind of insecticide. From the standpoint of practical application of BHC and / or lindane (γBHC), this is the question to be solved urgently, and in fact, has continued to be a topic of discussion. In the previous report concerning the integrated control of chestnut gall wasp (vide Torii, '60, a, b, & '61), I inferred from the autoradiographic results obtained from the behavior of the radioactive γBHC-1-C^{14} topically applied to certain plants with insect galls that phloem transport seems to be solely responsible for penetration and translocation of the topically applied γBHC in plant tissues. The present paper is a continuation of the investigations previously reported by me. In the present study, girdling experiments were conducted using the same radioactive γBHC-1-C^{14} as before to determine whether or not phloem transport is the basic mechanism for translocation of γBHC into the plant tissues when γBHC is applied to the aerial parts of the plant. Generally, results presented here are sufficiently encouraging to make the use of aerial application of γBHC for the integrated control of chestnut gall wasp.

The present investigation owes much to the encouraging guidance given by Dr. S. Ishii, Chief of the the Ist Section of Insect Pests Control at National Institute of Agricultural Sciences, who kindly imparted the radioactive γBHC-1-C^{14} of his synthesis to me. To him, my heart-felt acknowledgement must be paid. This paper constitutes one of a series of reports concerning comprehensive studies on the integrated control of the chestnut gall wasp, which were initiated under the kind guidance of Dr. K. Yasumatsu, Professor of entomology at Kyushu University. His constant encouragement is heartily acknowledged by me.

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Materials and Methods

The experimental plants were wild chestnut tree and wild willow. Freshly-cut twigs of these trees with insect galls were used as the experimental material. Samples were collected at the scrub near our university campus, which was mixed with lots of wild chestnut trees infested by chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu. Twig samples were all flush and vigorous. Sampling and laboratory experiments were conducted on 11th July, 1960.

The radioactive γBHC emulsion used consisted of 3.2 mg. of γBHC-1-C^{14} (with specific activity of ca. 0.4 μc/mg.), 0.1 ml. of xylene, two drops of Triton X-100, and 125 ml. of water. The concentration of γBHC was originally estimated at about 25.6 ppm. (Ishii et al., '59). But the emulsion employed here seems to be somewhat weaker in its chemical nature than the original one, since it is the remnant left after Ishii's rice-plant root dip experiments.

Duplicate twigs were treated, each being about 40 cm. in length. For the counterpart, a ring about 2 or 3 cm. wide was made with a scalpel on the stem above or below the treated portion, to see if any effect of girdling the stem on the phloem transport exists. The other twig of each set was prepared for control use. The radioactive γBHC was applied topically to one fixed portion such as a foliage leaf, a flush terminal shoot, and a stem according to the experimental design. In an application of γBHC, a glass rod with one tip covered with absorbent cotten was used. After drying up the emulsion first applied, the second application was made on the same portion to cover its weak specific activity. Each treated twig with foliage leaves was transferred individually to a 500 cc. flask containing water and allowed to photosynthesize for 4 days under indoor conditions. To avoid contamination of water, the treated portion was designed to be at least 5 cm. or so apart from the flask-mouth which was covered with absorbent cotton.

All the macro-autoradiographs presented here were taken by "contact method". Four days after an application of γBHC, all the twig samples were pressed between blotters with a heavy electric iron and dried quickly. After ironing, dried specimens were placed on a sponge-like rubber mat which afterwards served as a cushion ; they then were kept inside a drying cage for one day to perfect their dryness. Some modifications were made of the technique of preparation of autoradiographs that was reported in the previous studies (vide Torii, '59 & '60). The main point of difference lies in the use of a 1 cm. thick sponge-like rubber mat for cushion use providing against a pressure fog due to bulky galls (see Fig. I). The exposure was continued untill 24th March, 1961, the autograph time being 252 days.

Previous to taking macro-autoradiographs, counts were taken of the disintegrations of the radioactive isotope by a thin-window Geiger-Müller tube held a few mm. from the cellophane cover over a treated portion. The reading of cpm. for the specimens ranged from 31.5 to 33.0, being substantially equal to those for natural background, although the radial rays might have been absorbed considerably by the cellophane cover as is usual with them. This is the reason why a fairly ample time of exposure, i.e.

252 days, was spent in taking autoradiographs. Together with experiments with γBHC-1-C^{14}, parallel experiments were carried out with the samples labeled with radiophosphorus P^{32}. Materials and the methods of experiment were essentially the same as those described above, excepting the chemical employed. As regards the formation of radiophosphorus P^{32}, refer to the explanation of Fig. III.

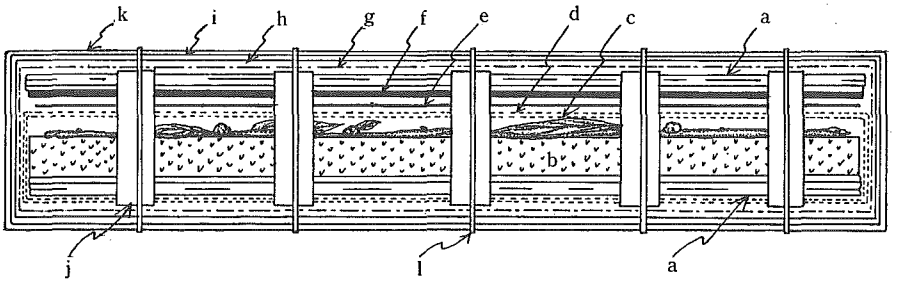

Fig. I. Illustrating the contact method for a macro-autoradiogram.
(Longitudinal section of a bundle.)

a : 3 sheets of board paper.
b : A 1 cm. thick sponge-like rubber mat for cushion use.
c : A dried specimen of a twig with insect galls.
d : 2 sheets of damp-proof cellophane paper.
e : Fuji X-ray film, non-screen type No. 200, highly sensitive to X-rays, γ-rays, and β-rays.
f : A thick, solid vinyl board.
g : 2 sheets of light-tight black paper.
h : 3 sheets of oiled paper.
i : 2 sheets of vinyl wrapper.
j : A rubber band.
k : 2 sheets of light-tight black paper.
l : A string; finally a bundle was bound tightly with this string.

Results

The autoradiographs are placed alongside of the corresponding plant photographs (phots. A-O). The distribution of the radioactive material is clearly revealed in them as the blackening of the plate, i. e. the precipitation of silver grains on the emulsion film. It should be pointed out in this connection that there can be seen a large spot of precipitation of silver grains (marked 'a') clearly outlined in a disc just at the portion one-third from the bottom of each of the autoradiographs excepting phots. A, C, and I. These spots have nothing in essence to do with the present experiments, because they are merely an outcome resulting from the accidental technical error in course of exposure as clearly judged from a substantially perfect coincidence in their position on the plate. In course of exposure inside an electric refrigerator, one set of specimens strongly labeled

with radioactive P^{32} were put by the side of another set of the present autograph preparations for a few days, although the two sets were shielded from each other with many thick glass plate septums set between the two. This must be the cause of the present accident ; no other cause cannot be considered. It may be said, however, to be "one consolation in misfortune" that these spots do not affect at all the degree of precision and accuracy in diagnosis of the present results.

In diagnosing the result, the picture of autoradiographs for experimental specimens needs to be compared carefully with that for control lest it should be confused with the blackening caused by various kinds of fogs. In the present experiments, the control autoradiograph shown on the lower half of the photograph J (marked "cont.") indicates a barely discernible picture of the whole control specimen excepting its foliage leaves, that is, of its stem, sprigs and galls. The control is a dried specimen of a chestnut twig with foliage leaves and chestnut galls. Presumably, this picture may be the outcome of chemical fogs caused by some sensitizing chemicals such as terpenes which are usually contained in woody plant tissues.

Series 1, ——Duplicate chestnut twigs with galls caused by the chestnut gall wasp were used. For the counterpart, a girdle was made on the stem about 2 cm. below the axil of a treated leaf (phot. A). The radioactive γBHC was applied topically to a fraction (in an oval shape with a major axis about 4 cm. long) of the top surface of one intact foliage leaf on each sample twig (phots. A & B). As can be seen on an autoradiograph in phot. A, the midrib and some ribs of the treated leaf gave a fairly clear picture on the film corresponding to the treated portion and its neighborhood, althogh the treated portion was not clearly outlined. As judged from the strongly sensitized tip of the treated leaf where the γBHC emulsion was accumulated in a dip, the above result may be ascribed in a measure to diffusion of γBHC over the surface of the treated leaf, since the γBHC used contained a surface active agent Triton X-100. But, appreciably spotted precipitation of silver grains seen at some portions such as an axillary bud on the flush terminal shoot, those of a leaf just above a girdle and a gall on the stem above the treated leaf may be an indication of physiological accumulation of radioactive carbon C^{14}. The flush terminal shoot, though faint, also gave a fairly appreciable picture on the film. But no perceptible image is visible at the positions of galls, foliage leaves, and axillary buds on the stem below a girdle. The sample specimen without a girdle (phot. B) produced as a whole a fairly clear autoradiograph as compared with the contrast specimen (phot. A). Especially, at one treated bud, the terminal shoots, the sprigs shooting from, and the galls formed on the stem above and below the treated leaf, moderate blackening was relatively clearly outlined on the film. This forms a noteworthy contrast to the results obtained from the twig sample with a girdle.

Series 2—— Duplicate chestnut samples similar to those in series 1 were used. The experimental technique was quite the same as that in series 1 except that topical application of γBHC was made to the underside of one intact foliage leaf. In this case, too, the aerial parts below a girdle are characterized by quite imperceptible precipitation of silver grains on the film (phot. C), whereas the intact specimen (phot. D) shows fairly strongly localized blackening all over the plant tissues, such as galls, flush terminal

shoots, sprigs, and some of the midribs, as compared with the girdled specimen. In both auotradiographs, the treated portion was clearly outlined on the film showing a comparatively fine venation thereat. Both images are far clearer than those in the case with a top surface application as judged from the comparison between the two results (cf. phots. A & B). The logical interpretation of this fact may be that γBHC enters more easily into mesophyl of the leaf past cuticular layer when applied to the underside of the leaf than when done so to the top surface of it. The same phenomenon was observed in the previous experiments reported by me (vide Torii, '60).

Series 3——A set of stems of a chestnut twig were treated. One (phot. E) of the two was girdled at the portion about 1 cm. below the treated portion. The autoradiograph for this specimen has very little precipitation of silver grains as a whole. But blackening at the positions corresponding to the treated stem, flush terminal shoots, and galls on the stem above a girdle is easily traceable on the negative, though comparatively faint. A leaf on the stem just below the treated portion gave a fairly discernible picture of its midrib on the negative. Faint, but appreciable blackening can be seen at the position of a right side leaf on the lower stem below a girdle. Judging from the results of all the experiments, such blackening produced across a girdle seems to be attributed to the fact that a ring was made too near to the treated portion, resulting in the diffusion of the applied emulsion across the ring. On the other hand, another specimen without a ring (phot. F) showed more pronounced blackening on the whole. Especially so did a right side sprig with a flush young shoot. Precipitation of silver grains in galls, axillary buds, and flush terminal shoots also can be denied.

Series 4——Applications were made to a flush terminal shoot of a chestnut twig. The results are similar in outline to those obtained from the foregoing experiments. The darkest blackening was observed at the treated portion, and moderate one at various portions such as flush terminal shoots, sprigs, axillary buds, midribs, petiols, and some of the galls, roughly in this order in darkness. But, on the plant tissues below a girdle, inclusive of the stem, galls, axillary buds and foliage leaves (phot. G), no discernible blackening was observed at all. It is to be noticed that in the unringed sample the galls formed on a lateral sprig gave hardly any appreciable precipitation of silver grains on the film (phot. H)). This is one of the findings newly obtained in the present experiments.

Series 5——Chestnut galls formed on a chestnut twig were treated. In one sample, all the treated galls were picked off from the stem after a 4-day period of photosynthesis under indoor conditions. After then, each gall was cut in halves with a scalpel and pressed between blotters with a heayy electric iron and dried fully. The autoradiograph was taken of the section of these dissected galls (the upper half of phot. J, and cf. its magnified piece (phot. N)). In the other sample, the autoradiogram was taken of the treated galls as they were on the stem (phot. I). In this case, too, a tendency shows similarity in that blackening is darkest at the treated galls, comparatively pronounced at flush terminal shoots, young sprigs, midribs of the leaves on terminal shoots or sprigs, axillary buds and some of the node of insertion of the sprig. An artifact resulting from dripping of the applied emulsion can be seen at the middle and lower part of the film (phot. I).

The cross section of treated galls were appreciably defined on the film in a circular image, although blackening is not so dark. Undoubtedly, the central portion of each cross section is left unsensitized, its tint being the same in quality as that of the base of the film, and quite different from whitening resulting from pressure fog as shown in the control image. This may be considered evidence indicating that topically applied γBHC did not penetrate into the central tissues of the treated galls.

Series 6——Three twigs of a wild willow, *Salix integra* Thumb., with bowl-like leaf galls caused by *Pontania viminalis* were treated. A girdle was made on the stem of one twig sample (phot. K), and a leaf on the stem above the girdle was selected randomly to be applied with radioactive γBHC. In the other two intact twig samples, too, applications were made to a randomly selected leaf (phots. L & M). The underside of a leaf was treated in the two samples (phots. K & L), and the top surface of a leaf in the remaining one (phot. M). In all cases, applications were made to the whole surface of a leaf. The treated portion gave a fairly clearly outlined picture on the film in every case. Bowl-like leaf galls also showed some appreciable blackening. The images of the stem and / or the flush terminal shoot can only be discerned quite indistinctly. Precipitation of silver grains is on the whole relatively little as compared with that in the case with chestnut twigs. Probably for this reason, scarcely any effect of a girdle can be recognized. Comparatively quick wilting of this plant after cutting seems to be responsible for this phenomenon. The similar results were obtained from radiophosphorus P^{32}.

Summing up all the results obtained from the different experiments described above, the final outcome is presented diagramatically in the following figures (Fig. II, A & B). When freshly-cut chestnut twigs were treated topically with radioactive γBHC-1-C^{14} under indoor conditions under which the plants proceeded normally with photosynthesis for 4 days, acropetal translocation up to the upward tissues and persistence there as well as basipetal translocation down to the downward tissues and persistence there was plainly discernible (Figs. II, A & B). A girdle of the bark made on the stem below the treated portion clearly checked downward translocation of the isotope past the girdle (Fig. II, A). Evidence was obtained that hardly any downward and lateral translocation was observed to the galls formed on the lateral sprig (Fig. II B).

In the present experiments, autoradiograph preparations were made to determine whether or not a girdling of the bark made on the stem above the treated portion would really check upward translocation past the girdle. To my great regret, however, the result ended in failure on account of an accidental technical error in course of exposure inside an electric refrigerator. The cause is as follows: A set of preparations labeled with radiophosphorus P^{32}, parallel experiments for the present ones, were put by the side of one of the other set of preparations of girdling experiments with radioactive γBHC-1-C^{14}; radial rays emitted from the P^{32} were so strong that a majority of autoradiographs for the latter were so greatly spoiled together with some sets of preparations of P^{32} as to be blackened almost entirely. However, one of the concerning autoradiographs of parallel experiments with P^{32} was fortunately found safe. It is much suggestive of the effect of a girdle made on the stem above the treated portion on upward translocation

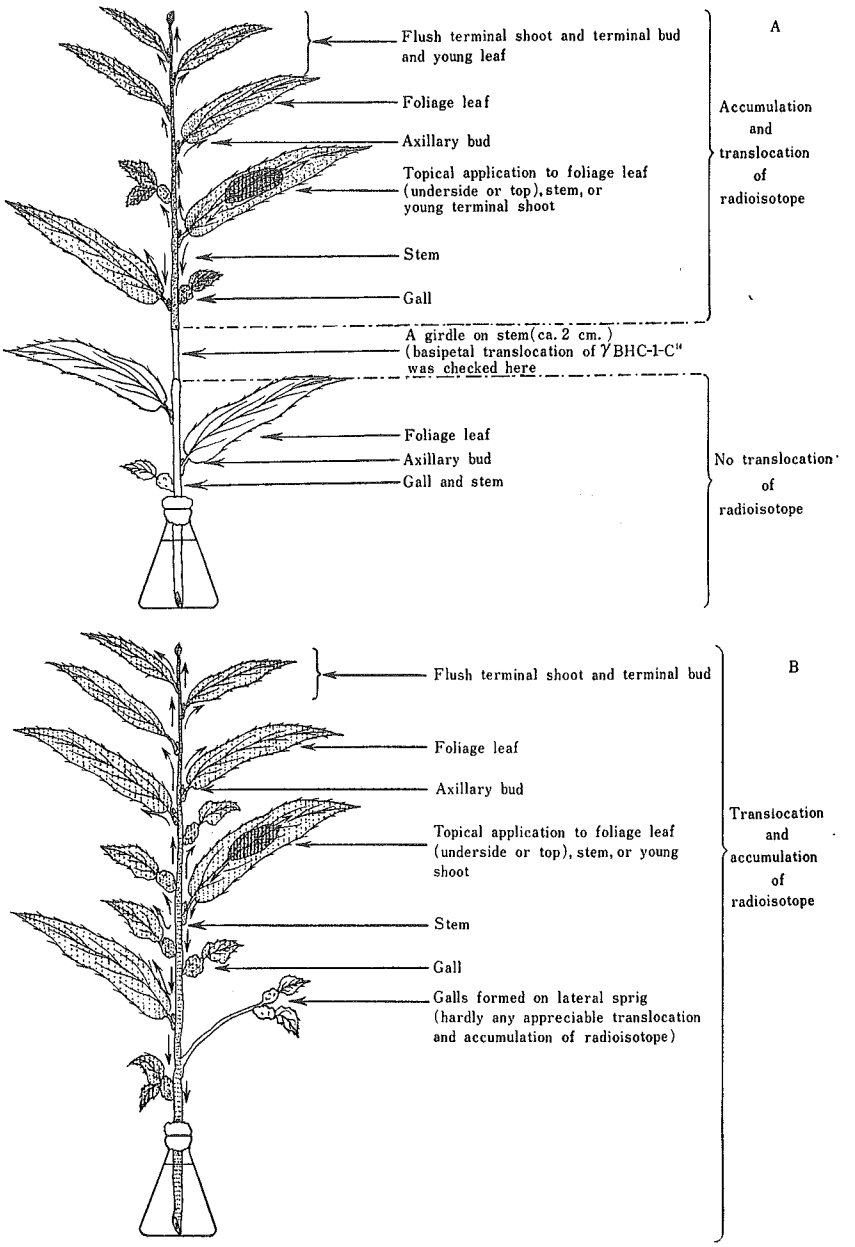

Fig. II. Schematic diagram of the distribution of topically applied γBHC-1-C¹⁴ in the plant tissues of a freshly-cut chestnut twig after a 4-day period of photosynthesis.——A. Translocation and accumulation in the case of stem girdling.——B. The same in the case with an intact twig without a girdle on the stem.

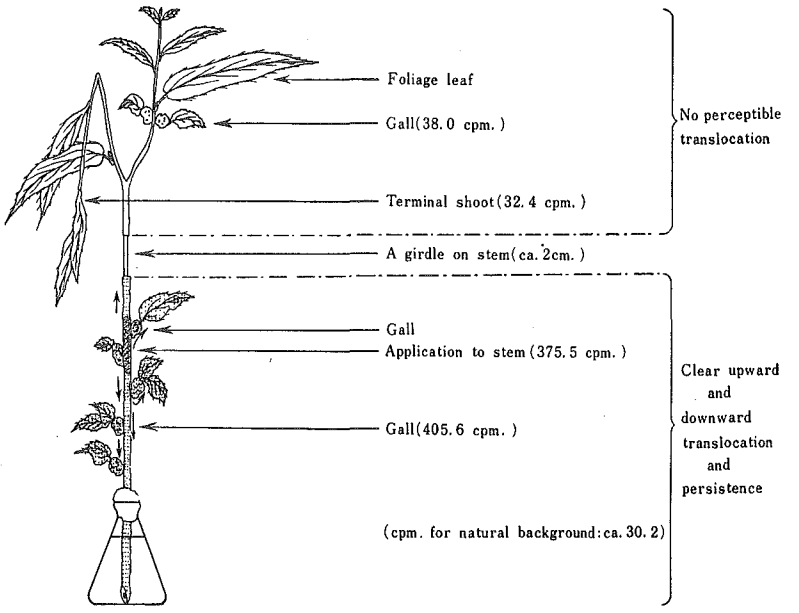

Fig. III. Schematic diagram of the distribution of topically applied radiophosphorus P^{32} in the plant tissues of a freshly-cut chestnut twig after a 5-day period of photosynthesis. Experimental: Preparation of a tracer P^{32}:——3μc. P^{32} in the form of orthophosphate in dilute hydrochloric acid + 25 cc. dist. water + one drop of Triton X-100. Autograph time: 3 days. The other techniques are quite the same as those in the case of topical application of γBHC-1-C^{14} (vide text).

past the girdle (phot. O). As can be seen on the autoradiograph (phot. O), very slight upward translocation up to terminal tissues past the portion of a girdle occurred. Blackening at untreated terminal tissues is by far the less in darkness as compared with a large-scale dense precipitation of silver grains on the treated portion. After covering the treated portion with a large lead block, counts of radial rays were taken a week after application at a distance of 15 cm. above this dried specimen by a Geiger-Müller tube. The reading of counts per minute for principal parts are indicated in Fig. III. The average values of cpm. for untreated portions above a girdle are substantially negligible. From both this result and that indicated on the autoradiograph, it may safely be said that in the case of topical application of P^{32} to a freshly-cut chestnut twig upward translocation past a girdle is substantially negligible. Then, is there any difference between the behavior of radiophosphorus P^{32} and that of radioactive γBHC-1-C^{14}? As clearly judged from 9 autoradiographic results obtained from the parallel experiments with P^{32} (phots. A′, B′, D′, F′, G′, H′, J′, L′, and K′), although it is feared that precipitation of silver grains is too dense to be traceable in detail and there can be seen more or less mutual contamination caused in course of exposure, hardly any substantial difference in

behavior can as a whole be recognized between the two. Namely, by dint of a girdle made on the stem below the treated portion, downward translocation was undoubtedly checked almost entirely ; upward as well as downward translocation via the stem onto growing tissues such as flush terminal shoots and galls was plainly evidenced. On the basis of these findings, it may not be illogical to draw an inference that in the case of γBHC-1-C^{14}, too, upward translocation past a girdle made on the stem may substantially be impossible. This implies that the xylem is not so much responsible for upward translocation of γBHC-1-C^{14} topically applied to aerial parts of the plants treated here; in other words, that the γBHC-1-C^{14} emulsion applied cannot penetrate so deeply into the inner part of the plant tissues as to reach the xylem. The result obtained from the autoradiographs of the cross section of chestnut galls (phot. J) warrants the propriety of the above inference, because scarcely any appreciable blackening was visible in the central portion of their pictures outlined on the film. It thus comes to this conclusion that radioactive γBHC-1-C^{14} applied topically to the aerial parts of the plants here treated moved basipetally as well as acropetally from the treated portions onto foliage leaves, terminal shoots, and galls via the phloem. In the previous studies (vide Torii, '60, a, b, & '61), I have drawn an inference that phloem transport is solely responsible for penetration and upward as well as downward translocation of γBHC-1-C^{14} applied topically to the aerial parts of certain woody plants, inclusive of some herbaceous ones, especially of those belonging to genus *Quercus*. It may be said that my previous statement thus has been demonstrated positively by the present girdling experiments, although some further supplementary experiments with γBHC-1-C^{14} may be necessary for drawing a final conclusion by a girdle made on the stem above the treated portion.

Discussion

On the problem of phloem transport

As one part of a series of studies on the integrated control of chestnut gall wasp, *Dryocosmus kuriphilus*, the present experiments were initiated. The prime object is, therefore, to ascertain whether or not γBHC applied to aerial parts of the infested chestnut trees penetrates deeply in and moves into the inner parts of plant tissues; that is, it lies in confirmation of such a phenomenon, if any, but not in the elucidation of concerning physiological mechanism. In fact, according to Crafts ('51) who discussed the mechanism of translocation in plants very minutely and intensively, very little was known up to that time of the possible association complexes possible between the common foods in plants and various materials such as virus, fluorescein, radioactive tracers, auxin, flower-inducing hormones, vitamins, and 2, 4-D, notwithstanding many papers reported association of these transported materials with food movement. Even the oldest problem of translocation of assimilates within plant tissues, comparable in importance with the circulation of blood in animals, was then a subject of much controversy in plant physiology, in spite of so many a theory of transport mechanics postulated by many plant physiologists until that time. In the famous book entitled "Principles of Plant Physiology" written by Bonner and Galston ('52), it is pointed out that lots of problems concerning

the mechanics remain unsolved. At present, too, our knowledge of translocation processes seems to be left in an unsatisfactory state. So far as the present experiments are concerned, I am also not in a position to go so deeply into such a difficult problem. The only thing inferable from all my experiments conducted to date is the fact that phloem seems to be mainly responsible for the penetration and translocation of radioactive γBHC-1-C^{14} and P^{32} topically applied to a certain portion of the twig of wild chestnut tree and willow. This founded on an indication that a girdle made on the stem distinctly hinders the isotopes from moving both upwards and downwards in plant tissues past the girdle under present experimental conditions. However, it may be of some service to the promotion of better understanding of the phenomena to deliberate on the subject concerned.

Not a few reports attesting to phloem transport studies have been published. As regards the effect of ringing a stem on the upward transfer of carbohydrates, there is the early work of Curtis ('20). He obtained evidence indicating the following fact in his phrase: namely; "these foods are carried up the stem chiefly through the phloem tissues even though much of the food to be transported is present as sugar or starch in the xylem tissues. Even if sugar presents in the vessel of the xylem at the time water is being carried in these tissues, it may not be carried past a region where a ring of phloem is removed." He thus concluded that "at least the upward transfer of sugar seems to be very clearly checked by the removal of a ring of phloem." In 1923, he further showed strong evidence offered from ringing experiments indicating that nitrogen or other nutrients are carried chiefly in the phloem, although he could not consider that his data are conclusively proving that such nutrients move upward primarily through the phloem and not through the xylem, with deliberation on the possibility that the treatments may have altered the xylem tissues. On the other hand, Stuot and Hoagland ('39) showed by isolating a strip of bark from the wood, that radioactive elements such as potassium, sodium, phosphorus, and bromine were rapidly translocated laterally from xylem to phloem in actively growing and transpiring willow and geranium plants. They concluded that "the evidence is consistent with the view that the xylem is the path of rapid upward movement of salt." According to Crafts ('51), the reverse movement found by Mason and Maskell ('28). As is aptly pointed out by Bonner and Galston ('52), there is nothing strange about these phenomena, because once inorganic elements enter the phloem, they will be carried by usual phloem transport. As is often observed, they are transported through phloem to younger leaves and growing terminal shoots where growing is active but transpiration is not so rapid. It thus is no doubt that phloem plays an important role in upward and downward translocation of inorganic elements, too. In their transport studies by using radioactive carbon C^{13}, Rabideau and Burr ('45) demonstrated that the carbohydrates photosynthesized by leaves exposed to radioactive $C^{13}O_2$ moved both upward and downward in plants studied. These workers thus concluded that this radioactive photosynthate moves in the phloem, since it would not pass killed portions of the stem. Their result and conclusion coincide with those in the present experiments in its outline. They obtained, however, another results quite different from the present ones; they observed that radiophosphorus applied to roots moved readily upward past

killed portions of the stem. According to the result obtained by Withner ('49) from the study on the distribution of radiophosphorus in maturing corn plants by applying the tracer as KH_2PO_4 through the culture solusion, this tracer apparently undergoes secondary movement in the phloem along with food materials, after its primary distribution th-rough the transpiration stream in the xylem. These results are quite different from those in the present experiments. Probably, they may be attributed to the difference in either the treated portion or plant species, or both. The behavior of these chemicals seems to be highly correlated with both dosage and intensity of the chemical applied. In the discussion of the mechanism of phloem transport, Crafts ('51) gives an instance of these phenomena. When applied in low dosage to the unifoliate leaf of a bean plant, 2, 4-D is "absorbed by the leaf cells, moved through the mesophyll and released into the phloem. Here, in association with food materials, it is rapidly translocated down the petiole and stem."·····"At higher dosage rates this chemical causes distortions, malformations of stems and leaves, and inhibition of roots. At still higher rates it kills the treated plants." This is very instructive, since 2, 4-D resembles γBHC in its nature in that both chemicals are originally toxic and cause injury to some plants. And his following final statement will furnish us with very useful information to the better understanding of the results obtained from the present experiments : "All experiments conducted to date indicate a correlation of 2, 4-D movement with translocation of foods in the plant."

In the studies cited above, the data are confined to the herbaceous plants with less developed xylem, some dealing with crop plants with more complicated bicollateral vascular bundle. According to Crafts ('51), with some herbaceous plants such as squash plants, "where a separation of phloem from xylem can not be made, steam ringing is the best known method for determining whether or not movement is restricted to the phloem, and the ringed plant being the control, one is still left in doubt as to whether the experimental plant is behaving in the same manner." With the woody plants, where phloem is distinctly separated from xylem, girdling the phloem from the stem will produce most reliable evidence of phloem transport. According to Bonner and Galston ('52), this kind of experiment was initiated by Malpighi (1675). No report has been published on the girdling experiment on the movement of γBHC applied to the aerial parts of the woody plants. An interesting instance was presented by Ball ('53). By a callus culture, he made histological studies of the accumulation of radioisotopes P^{32}, S^{35}, and C^{14} contained singly in the three kinds of culture media. Finding that C^{14} was uniformly distributed through the callus of *Sequoia* and highly concentrated in all living cells such as the marginal meristems and the cambia around the tracheid groups, all of which were considered to have had a higher metabolic rate than older cells, and correspondingly, have been utilizing sugar at a higher rate. On the basis of this finding, he concluded that the results are "interpreted to indicate a metabolic accumulation of this isotope over and above the physical process of diffusion," and it was "probably present in the living tissues as compounds of considerable complexity." Finding further that cell walls of tracheids (without protoplasts) showed pronounced accumulation of C^{14}, he assumed that the isotope there must have become a part of the cellulose or other constituents of the walls. To be sure, C^{14} contained in sugar may be accumulated in such a dead part,

as a result of metabolism of the sugar containing it. But, at the same time, it should be noticed that there may be some difference in both pattern and physiology between the peculiar metabolism in a callus in a tissue culture and metabolic processes of toxicants in normal plants. Nevertheless, this information offered by Ball needs to be paid some attention, since the tissues of insect galls belong to a kind of abnormal tissue just like the callus. He further pointed out an accumulation of C^{14} in tannin-containing and parenchymatous cells. This is very suggestive of an interpretation of my finding that topically applied radioactive γBHC-1-C^{14} was remarkably concentrated in the galls without exception. Recently a noteworthy report has been made public by Redske and Shea ('61). Using radioactive $C^{14}O_2$, they outlined the translocation patterns of radioactive photosynthate with the object of determining the feasibility of using a systemic selective herbicide for control of dwarfmistletoe, a parasitic plant on lodgepole pine. They found that "photosynthate produced in a terminal needle cluster of the pine was translocated basipetally to the roots, but also was accumulated in intercepting dwarfmistletoe plants," and pointed out "that the dwarfmistletoe acts as a biological girdle resulting in an accumulation of photosynthate above the site of infection," assuming that "presumably carbohydrates can be withheld from the roots in quantities sufficient to cause the characteristic decline of the tree that is associated with severe attack by dwarfmistletoe." These findings founded upon the results demonstrated by autoradiographic analysis and / or paper chromatography. In them the effect of girdling a stem on the transfer of photosynthate in plant tissues is clearly outlined. Putting the autoradiographic results of the presenet girding experiments together with the similar results cited above, it seems logical to assume that the translocation of γBHC-1-C^{14} topically applied to the aerial parts of the plants studied is mainly dependent upon the role of phloem.

On the problems connected with BHC seed dressing and soil application

In recent years, increased attention has been given to the problem of the effectiveness of BHC presowing seed dressings and / or BHC soil applications. According to Lichtenstein ('59), a large percentage of the insecticide applied in spray or dust formulations finds its way into the soil and persists there over relatively long periods of time, depending upon the soil type and the chemical. Many works have been published reporting that BHC and/or γBHC belong to such a kind of insectcide (vide Torii, '60), proving effective in different measures according to circumstances. Then, the problem of effectiveness of γBHC, irrespective of the method of application, i. e. either aerial, soil application, or seed dressing, resolves itself down to translocation of γBHC into plant tissues. To the practical application of this BHC translocation phenomenon, close attention was paid by Weaver and Haynes early in 1955. From the same standpoint, Koshihara and Okamoto first applied BHC to the rice plant paddy field as pretransplanting soil application in 1957. On the concerning problem, I made a few comments in the previous report (Torii, '60). Since then, some works have been made public on the same problem. Ware and Gilmore ('59) observed that while initial deposits of granulated application of BHC on alphalfa were far lower than similar spray applications, the harvest residues were higher than those of sprays. They attributed this phenomenon to accumulation of BHC translocated

through the root system from granules deposited on the soil. Nikolova et al. ('59) showed that BHC introdued into the soil against certain insect pests, inclusive wireworms, at rates of 45, 72, and 108 lb. per acre did not cause a reduction in the yield of maize, except the case with the two higher rates, keeping the yield and quality of tobacco normal. Brass and Ware ('60) investigated the effect of soil type and concentration in the soil on the quantity of BHC taken up by forage plants in the green house by presowing application. They found that phytotoxic symptoms were proportional to the dosage of BHC and dependent upon the soil type. And they drew an inference that "adsorption on soil particles may conceivably make some of the BHC unavailable to the plant, or delay its availability," and "heavy BHC applications may exceed any adsorptive capacity of the soil, the excess providing nearly equal amounts of available toxicant in soils of different constituency."

What recently arrests our attention is that investigations concerning the effect of BHC predrilling dressing have remarkably increased in number. Their results are not always coincident with one another, some proving effective to control of the aimed-at insect pest and some other ineffective or rather phytotoxic. In the experiments conducted to control *Psila rosae*, an important pest of carrot, Ausland ('57) found that γBHC (lindane) seed dressing gave good control of the first generation of the fly, but not of the second, though sometimes reduced germination, affecting the flavour of the carrots. It was found by Buhl, K. ('59) that treatment of the cruciferous crop seed with γBHC (lindane) at 10 per cent. of its weight reduced the attack by the flea-beetle *Psylliodes chrysocephala* (L.) to economic levels, though it did not afford complete protection. In 1960, Buhl, C. ('61) published similar results obtained from the winter rape seed dressed with a powder containing 75–80 per cent. γBHC (lindane) at 5 per cent. by weight of the seed, with an adhesive. The dressing afforded excellent protection of winter rape against larvae of the same insect pest, but had no effect on *Phytomyza rufipes* Mg. mining the petioles. In field tests to protect flax seedlings from injuring by the flea-beetles, *Aphthona euphorbia* (Schr.) and *Longitarsus parvulus* (Payk.), Veenenbos ('57) suceeded in controlling the insect by treatment of the seed with γBHC (lindane) at 2.7–4.4 oz. 20 per cent. powder per bushel. In this case, the insect died in the greenhouse within two days of feeding on the flax seedlings, although seedling emergence was decreased by γBHC. Nolte ('59) also found that various seed treatments with preparation of γHBC protected rape against attack by *Psylliodes chrysocephala* (L.) and *Ceutorhynchus pleurostigma* (Marsham) and proved more effective than soil treatment at the time of sowing. Jameson ('60) used viscous aqueous seed dressings containing up to 16 per cent. γBHC for the control of turnip flea beetle attacking turnip and kale, and found that the dressing did not impair germination when applied at 50 ml. per lb. of seed. In field experiments, one of these dressing, containing only 4 per cent. γBHC, was highly effective in controlling the flea-beetle on kale seedlings, even against attack occurring up to three weeks after the seed was sown. Prior to this report, Jameson ('58) conducted laboratory expriments with the similar liquid seed dressing to discover in broad outline the mechanism of γBHC against turnip flea beetle attacking kale seedlings. The gist is as follows: The kale seedlings emerged freshly from seed coated with γBHC were

highly toxic to the flea beetle. This toxicity fell rapidly, nearly 90 per cent. of it being lost in 7–10 days, but the protective power of the seedling against material damage by flea beetle was maintained for another 2 or even 3 weeks. The initial protection appeared to be due to γBHC which penetrated the seed as a vapour or liquid and contaminated the cotyledons. The cotyledons may have received additional poison as they burst through the seed coat; they apparently carried a high concentration if γBHC on or near the surface when they emerged above the soil. When the plants are about a week old, active γBHC is translocated to the leaves through the roots and maintained toxicity at low level but preventing material damage by the insect, largely by fumigant action. This process outlined by Jameson seems to be very suggestive of the mechanism of controlling insect pests by BHC presowing dressing on some seeds.

Adverse effect of seed dressing on the plant that emerged from the dressed seeds is also reported. In field tests conducted to control *Oscinella frit* (L.) seriously attacking winter rye, Tiittanen ('59) reports that seed treatment with lindane (almost pure γBHC) gave no control and retarded the growth of the seedlings. According to Van Steyvoort ('58), a normal stand of a late variety of beet was obtained by presowing broadcast applications of 9 or 18 lb. per acre of commercial preparations containing 7.5 per cent. γBHC (lindane) which were made to control the millepedes injuring the germinating seeds. But this γBHC seed treatment was less effective, 5–10 per cent. of the plants being stunted. Nikolova ('59) also reports that treatment of seeds with 12 per cent. γBHC had no effect on the yield of maize at up to 2 per cent. by weight or of sunflower (Helianthus) at up to 4 per cent., but this last caused a slight reduction in the yield of sugar-beet. BHC preplanting seedling dip also produces a bad result, though it does not come within the category of seed dressing. To reduce damage by the pitch-eating weevil to loblolly pine seedlings, Thatcher ('60) studied the effectiveness of BHC preplanting dips, and found that its phytotoxic effects were evident, particularly in the heavier concentrations, where there was no weevil damage to mask chemical effects, the effects persisting through the second growing season. His findings agreed in many respects with the well-known fact that BHC soil applications often cause abnormal root development in Japanese larch seedling and red pine ones. All these results seem to be considered positive evidence indicating that BHC is easily taken up by some plants.

Most reports cited above are founded upon the practical observation of the degree of phytotoxicity, damage caused by insect pests, and change in yield, that is, upon the insecticidal effects manifested after γBHC applications. Some reports have been made public on the basis of bioassay and/or chemical analysis of the plant tissues. Such are the works published successively by Lichtenstein during recent years. Using seven species of crops as experimental materials, he ('59) studied the extent to which lindane may be absorbed and translocated from contaminated soils into plant tissues and the relationships among absorbance of insecticidal residues, soil types, and crops by colorimetric analysis and bioassay. And he found that lindane was absorbed into crops, and the degree was dependent on the crop, the soil type, and the concentration of lindane within the soil. From the practical standpoint, his above finding is of great importance. But, because of its extremely low solubility in water, the question may be raised of whether or not

γBHC might be translocated to the aerial parts of plants from the soil treated with γBHC. In this respect, too, Lichtenstein ('60) made extensive tests by resorting to very high concentration of γBHC and using a soil of minimum absorptivity and complexity. By colorimetric analyses and quantitative bioassay, he found that lindane is translocated via the root system into the aerial parts of the plant, no evidence being obtained of absorption through the leaf cuticle of lindane vapors from the soil surface.

Generally, little is known of the plant metabolism of hydrocarbon compounds containing chlorine. To pursue this question, San Antonio ('59) conducted instructive experiments with various plant species grown in soil treated with lindane by the method of 'reverse phase' paper chromatography. His method introduced the presence of an unknown oil-soluble chlorine-containing metabolite, in addition to unchanged lindane in the fibrous roots, edible root and leaf tissue of lindane-treated carrots which are well known to accumulate lindane. In view of his analytical data, he considered the unknown substance was not 1, 2, 4, -trichlorobezene, but may be pentachlorocyclohexene or a closely related compound, though the evidence is not conclusive. "Of unknown significance at present is the finding that the species (carrot) which had 'accumulated' the greatest quantity of lindnae was the only species in which the unknown substance was detected," is his opinion. It is well known that BHC or γBHC application results in the development of off-odors and off-flavors in the edible portions of some crop plants. As suggested by San Antonio, this fact seems to be much correlated with the presence of pentachlorocyclohexene in plants or a closely related compound, since it has a particularly disagreeable musty odor. At any rate, these results cited above furnish us with evidence indicating that BHC and γBHC are capable of being absorbed in and translocated to plant tissues when they are applied to some plants aerially, by soil application, or by seed dressing.

In Japan, too, increased attention has recently been focused to the effectiveness of pretransplanting application of BHC to the rice paddy field to control rice-stem borer larvae. As touched before, such a technique was initiated by Koshihara and Okamoto ('57). Of late, this problem has been discussed minutely by Ishii ('61). Attaching more emphasis to the mechanism of translocation of γBHC, he pointed out the following possible process of movement of the γBHC applied to paddy field soils.

(1) Translocation through the root system; the root is capable of absorbing γBHC.

(2) The γBHC dissolved in paddy field water penetrates in the stem and the leaf sheath, being translocated from there to the other aerial parts of the rice plant.

(3) The γBHC dissolved in paddy field water moves upward by capillary attraction through the stem or the slit of the sheath part.

(4) γBHC is volatilized from the surface of the water of paddy field in which it is dissolved, and is deposited onto the aerial parts of the rice plant.

On this assumption, he interpreted the probable mechanism of the effectiveness of γBHC applied to the rice paddy field as follows: Among these possible processes, the last one cannot be considered to constitute so much an important part in the effectiveness of γBHC, in view of the result reported by Lichtenstein ('60). Then, it comes to this possibility : When applied aerially to the rice plants, the processes (2) and (3) may probably become the principal course in the effectiveness of γBHC. In the case of soil

application, the process (1) will further take part in in addition to the other two processes (2) and (3). In any case, γBHC will penetrate in the rice plant together with water, and be condensed as a result of evaporation of water, finally resulting in the ample concentration enough to cause high mortality of the larvae of a rice-stem borer. According to my opinion, the above processes all take part in the effectiveness of γBHC in more or less extent in a mutually combined state. Commenting on the results reported by foreign workers, he further points out aptly that it is particularly necessary for us to take into consideration the amount of γBHC applied in the interpretation as well as the practical application of such results.

Apart from the interpretaion of the mechanism of translocation, all the reports cited above seem to agree with one another in that almost undoubtedly γBHC is capable of being translocated in some plant tissues, irrespective of whether it is applied aerially, into soils, or as presowing dressing of seeds. Before interpreting these results or applying them to practice, due regard should be paid to the difference in the materials as well as the methods employed, such as the kind of insect pests and the host plants, beneficial parasites, the dose and the formula of the chemical, the weather conditions, the soil type, and, *inter a lia*, the season and timing of applications.

Biological meaning of phloem transport of γBHC as viewed from the integrated control of chestnut gall wasp

From the view-point of integrated control of chestnut gall wasp, the meaning of the phenomenon that γBHC is capable of being translocated mainly through the phloem of the chestnut tree needs to be explained. As clarified in the previous works reported by me (vide Torii, '59, '60), the key point of integrated control of this insect pest lies in the harmonious utilization of its resident parasitic wasps and γBHC by taking advantage of time gap between the emergence of this insect and that of the group of its parasitic wasps. It is a definite pattern that the latter emerge about 2 weeks earlier in early summer than the former does so, depositing eggs onto the larvae or pupae within the galls from which they themselves emerged, or the other insect galls then available for them. If timely applications of γBHC are made aerially to the aerial parts of the infested chestnut trees after the majority of the parasite wasps emerged, and just at the time when only a few host wasps started to emerge, the eggs laid by the parasite wasps inside the galls will be able to escape from the danger due to contact with γBHC, so long as γBHC does not penetrate so deeply in or is not translocated from the other portions to the inner part of the galls. The chestnut gall wasp, the host to be controlled, may surely live on the living tissues of the gall, or the phloem, for its supply of nutrients while it is in the younger larval stage. In proportion as it grows older, the lignification of the galls advances. When it is in the full-grown, prepupal, or pupal stage, i. e. the stage of breaking off absorption of nutrients, or in the more advanced stage, i. e. at the time just before emergence, its chamber within the chestnut gall will be far more lignified, resulting in the protection of its body against contacting with the γBHC transferred through the phloem. Such a time just hits on the time when the majority of its parasitic wasps emerged out and finished their ovipositing. Of most importance is therefore timing.

In fact, the results obtained from laboratory as well as field experiments clearly demonstrated the importance of timing of γBHC application. Now, it may be conceivable that some of the old pests happen to die by contact with γBHC after they were oviposited by their parasite wasps. In such a case, too, there may be some chance for the deposited eggs to evade from the toxicity of γBHC. According to Hassanien and Zaki ('58), when eggs and larvae of *Prodemia litura* (F.) were placed on filter paper in petri dishes, sprayed with 2 cc. of different concentrations of γBHC, embryonic development of the eggs was not inhibited at all for 24 hours, although the newly hatched larvae died after moving about on the treated filter paper. This is an encouraging finding for me, because all the parasitic wasps emerging early in summer never emerge until the end of August, i.e. until the time when far more time beyond the limit of the duration of residual effect of γBHC has passed. In the investigations conducted to test the toxicity of BHC to *Nepnatis serinopa* Meyr, a coconut pest, and to *Trichospilus pupivora* Ferrière, an important parasite of the pupae, Nirula et al. ('58) found that BHC spray (at 0.2 per cent., the minimum effective conc.) did not affect the immature stage of the parasite, which are passed whithin the host, and was harmless to adult parasites emerging six days after application. Their finding is also very encouraging for our case, although there is a wide difference in the species of insects and the host plant concerned between the two cases.

Summary

A girdle was made on the stem of a freshly-cut twig of chestnut tree with galls caused by chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, and/or that of wild willow with bowl-like leaf galls caused *by Pontania viminalis*. Radioactive γBHC-1-C^{14} was then applied topically to such plant tissues as a leaf, a flush terminal shoot, a stem, and galls located above or below the treated portion, respectively, and its penetration in as well as translocation within the plant tissues was traced by autoradiography. γBHC was found to penetrate and be translocated both upwards and downwards, but when applied to the upper part of the treated portion it was hindered in its entirety from moving downwards past the girdle. The experiments with a girdle made on the stem above the treated portion ended in failure by an accident occurred in course of exposure, but those conducted quite similarly in parallel with this by using radiophosphorus P^{32} showed that P^{32} was almost checked from upward translocation past the girdle made on the stem above the treated portion. In view of the other substantially equal results of the two sets of experiments, it was considered that radioactive γBHC might have behaved just like P^{32} under the same experimental conditions. Autoradiograph of the cross section of galls indicated undoubtedly that γBHC mainly persisted in the external surface layer of the gall without penetrating so deeply as to reach the inner part of it. On the above basis, it was concluded that when applied to the aerial parts of the plants examined the γBHC absorbed in the treated portion is capable of being translocated to several plant tissues acropetally as well as basipetally, especially being accumulated in flush terminal shoots and galls where growth is active, phloem being solely responsible for its translocation, under the present experimental conditions.

Founded upon this conclusion, the following discussion was made : First, some deliberations were made on the phenomenon pertaining to phloem transport from available foreign works rather historically. Secondly, on the problems of presowing or pretransplanting soil application or predrilling seed dressing or dipping of γBHC which now are one of the most prevailing topics of discussion in applied entomology, the mechanism of insecticidal effectiveness of BHC was discussed in detail, a special importance being attached to the fact of its phloem translocation observed. Finally, the role of γBHC in the "integrated control" of chestnut gall wasp was reaffirmed as follows: If γBHC is applied to aerial parts of infested chestnut trees once or at most twice in a week at the time when the greater part (ca. 80 per cent.) of the composite resident parasitic wasps which emerge about 2 weeks earlier than their host wasp, chestnut gall wasp, does so, finished emerging and a very few (ca. 10 per cent.) chestnut gall wasps completed their emergence, i.e. what I call the "period fittest for possible biological or integrated control", then, the eggs already laid by the composite resident parasitic wasps onto various insect galls, inclusive of chestnut galls, will be protected safely from the γBHC toxicity due to contact with it within the inner part of increasingly lignified galls. On the other hand, the remaining majority of chestnut gall wasps (ca. 90 per cent.) which enter gradually upon the period most active emergence after that time will easily killed in contact with

γBHC which persisted in the external surface layer of the gall when they emerge out of it. As a natural result of this process, the existing rate of parasitism for the composite resident parasitic wasps will be increased relatively to a great degree, accordingly a system of integrated biological and insecticidal control adjusted to meet chestnut gall wasp being established.

Literature Cited

Ausland, O (1959): *Gulrotflue* (*Psila rosae* Fabr.) *og gulrotsuger* (*Trioza apicalis* Fǿrst.) *bekjiempelse*. Meld. Plantev. 15 61 pp. (R.A.E., A 49: 145, 1961)

Ball, E. (1953): Studies of the accumulation of certain radioisotopes by a callus culture. Amer. J. Bot. 40 (5): 306-316.

Bonner, J., and A.W. Galston (1952): *Principles of plant physiology*. (Translated by Takamiya, A. and Y. Kokura): pp. 450

Brass, C. L., and G.W. Ware (1960): BHC translocation from treated soil and the effect on growth of red clover. J. Econ. Ent. 53 (1): 110-113

Buhl, C. (1960): Untersuchungen über die Wirkung hochprozentiger Lindansatgutpuder Zur Bekämpfung des Rapserdflohes (*Psylliodes chrysocephala* L.) Sund des Kohlgallenrüsslers (*Ceuthorrhynchus pleurstigma* Mrsh.) Z. PflKrankh. 67 (6): 321-326

Buhl, K. (1959): Beobachtungen und Untersuchungem über Biologie und Bekämpfung des Rapserdflohes (*Psylliodes chrysocephala* L.) in Schleswig-Holstein. Z. PflKrankh. 67 (6): 321-338

Crafts, A.S. (1951): Movement of assimilates, viruses, growth regurators, and chemical indicators. Bot. Rev. 17 (4): 203-284

Curtis, O.F. (1920): The upward translocation of foods in woody plants. I. Tissues concerned in translocation. Amer. J. Bot. 7: 101-124

———— (1923): The effect of ringing a stem on the upward transfer of nitrogen and ash constituents. Amer. J. Bot. 10: 361-382

Day, B.E. (1950): Absorption and translocation of 2,4-dichlophenoxyacetic acid by bean plants. Ph.D. Diss., Univ. Cal., Davis, Cal. (cited indirectly from Crafts' paper (1951).)

Hassanien, (Hassanein), M. H. and M.M. Zaki. (1958): Studies on the suscepitiblity of different stages of the cotton leaf-worm, *Prodenia litura* F., to certain chlorinated compounds. Bull. Sod. Ent. Egypte 42: 411-419 (R.A.E., A 48: 176, 1960)

Ishii, S. (1961): Problems connected with the mechanism of killing insects of BHC applied to soils. Noyaku 8 (3): 5-9 (in Japanese)

Jameson, H. R. (1958): The mechanism of the control of turnip flea beetle by benzene hexachloride dressing on brassicae seed. J. Sci. Fd. Agr. 9: 590-596

———— (1960): γBHC liquid seed dressing for the control of flea beetle. J. Sc. Fd. Agr. 11 (9): 528-534

Koshihara, T. and D. Okamoto (1957): Control of rice stem borer by the application of BHC dust in the paddy soil. Jap. J. Appl. Ent. Zool. 1 (1): 32-35 (in Japanese with a summary in English.)

Lichtenstein, E. P. (1959): Absorption of some chlorinated hydrocarbon insecticides from soils into various crops. Agr. Fd. Chem. 7 (4): 430-433

——— (1960) : Translocation of some chlorinated hydrocarbon insecticides into the aerial parts of pea plants. ibid. 8 (6) :452-456

Mason, T.G., and E.J. Maskell (1928): Studies on the transport of carbohydrates in the cotton plant. I. A study of diurnal variation in the carbohydrate of leaf, bark, and wood, and of the effects of ringing. Amer. Bot. 42 : 189-253. (cited indirectly from Crafts' work ('51).)

Nirula, K.K., J. Antony, K.N. Sahasranaman, and K.P.V. Menon (1958) : Relative toxicity of the field weathered insecticide to the Eulophid parasite (*Trichospilus pupivora* Ferr.) associated with *Nephantis serinopa* Meyr. Indian. Cocon. J. 11 (4) : 124-132. (R.A.E., A 48 : 450, 1960)

Nikolova, V., K.Kirkov, A. Vartanyan, and D.Danova (1959) : The effect of BHC on maize, sunflower, beet and tobacco when introduced into the soil or used for seed dusting. Nauch. Trud. Minnist. Zemed. nauch. Inst. Zasht. Rast. 2 : 107-123 (R.A.E., A 7 : 341, 1961)

Nolte, H.W. (1959) : Die Bekämpfung des Rapserdflohes (*Psylliodes chrysocephala* L.) und des Kohlgallenrüsslers (*Ceuthorrhynchus pleurostigma* Marsh.) druch Sameninkrustierung. NachrBl. dtsch. PflSchDienst (N.F.) 13 (8) : 153-157

Rabideau, G.S., and G.O. Burr (1945) : The use of the C^{13} isotope as a tracer for transport studies in plants. Amer. J. Bot. 32: 349-356 (cited indirectly from Crafts' paper ('51).)

Rediske, J.H., and K.R. Shea (1961) : The production and translocation of photosynthate in dwarfmistletoe and lodgepole pine. Amer. J. Bot. 48 (6) : 447-452

San Antonio, J.P. (1959) : Demonstration of lindane and a lindane metabolite in plants by paper chromatography. Agr. Fd. Chem. 7 (5) : 322-325

Stut, P.R., and D.R. Hoagland (1939) : Upward and lateral movement of salt in certain plants as indicated by radioactive isotopes potassium, sodium, and phosphorus absorbed by roots. Amer. J. Bot. 26 : 320-324

Thatcher, R.C. (1960) : Toxicity of BHC to loblolly pine seedlings. J.Econ. Ent. 58 (1) : 175-176

Tiittanen, K. (1959) : Kahukärpasen torjuntakokeissa syysrukülla satuja tuloksia. Maatalos ja Koetoin. 13 : 110-116 (R.A.E., A : 209, 1961)

Torii, T. (1959) : Studies on the biological control of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu (Hymenoptera: Cynipidae), with particular reference to the utilization of its indigenous natural enemies. J.Fac. Agr. Shinshu Univ. 2 (2) : 73-149

——— (1960, a) : Penetration and translocation of γBHC topically applied to some plants with insect galls. (A supplement to and general considerations on studies on the biological control of the chestnut gall wasp.). ibid. 2 (3) : 161-190

——— (1960,b) : Conservation and utilization of resident natural enemies. Plant Protection 14 (11) : 19-24 (in Japanese)

——— (1961) : Studies on the biological control of the chestnut gall wasp, with special reference to the utilization of its resident parasitic wasps. The gist of the thesis for the prize of the Japan Soc. of Agr. in 1961 (Japan Soc. Agr.): 4-6 (in Japanese)

Van Steyvoort, L. (1958) : Le blaniule moucheté, possiblités de lutte. Publ. tech. Inst. belge Amélior. Better. 26 (2) : 73-84 (R.A.E., A 48: 202, 1960)

Veenenbos, J.A.J. (1957) : Zaadbehandeling met lindaan ter bestrijding van aardvlooien bij vlas. Landbouwvoorlichting 14 (3) : 142-146 (R.A.E., A 49:91, 1961)

Ware, G.W., and L.O. Gilmore (1959) : Excretion of BHC in milk from dairy cows fed

known amounts of BHC. Ohio Agr. Exp. St. Resear. Circ. 68 (cited indirectly from Brass and Ware's paper (1960))

Weaver, C.R., and J.O. Haynes (1955): Band placement of insecticides, for clover root borer control. J. Econ. Ent. 48: 190-191

Withner, C.L. (1949): Movement of P^{32} on maturing corn plants. Plant Physiol. 24: 527-529

ある虫瘿着生木本植物における局所塗布放射性 γ-BHC-I-C^{14} の移行に関する環状除皮実験

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クリタマバチ(*Dryocosmus kuriphilus*)の虫瘿をつけたクリとシバヤナギハバチ(*Pontania viminalis*)の葉身虫瘿をつけたヤナギとの新鮮切枝に環状除皮を行い，その上部及び下部にある葉，新梢，茎及び虫瘿にそれぞれ放射性 γ-BHC-I-C^{14} を局所塗布し，その植物体内浸透移行をオートラジオグラフィによつて追跡した。γ-BHC は塗布部から浸透し上下に移行するが，塗布部の下部環状除皮によつて下部移行は殆ど全く阻止された。塗布部の上部環状除皮結果は露出中の事故により失われたが，併行実験として同時に行つた放射性燐 P^{32} では，同じ実験条件下でその上部移行は殆ど阻止された。ラジオオートグラフィに表われた両者の略同様な成績にかんがみ，γ-BHC-I-C^{14} でも略同様の結果が期待された。また虫瘿断面のオートラジオグラフィにより，γ-BHC は供試虫瘿の表層に多く沈着し，内部への深達的浸透移行は殆どないことが明かになつた。

以上により，γ-BHC は供試植物の地上部局所塗布によつて，塗布部から吸収されてその上下の各植物組織に移行し，特に生長の盛んな新梢，虫瘿によく集積されること，その移行は主として師部の作用によることを結論した。

これにもとづき，次の諸項を論議した。まず，師部転流の現象を外国文献により史的に考察した。次に，現在内外で論議のまととなつている γ-BHC の播種または移植前土壌施用，及び播種前の種子浸漬または被覆の問題につき γ-BHC の殺虫機構を考察し，植物体の師部移行の事実を重視した。最後に，クリタマバチの統合的防除における γ-BHC の役割を次の様に再確認した。クリタマバチより約2週間早く羽化脱出する在来天敵蜂群が約80%羽化し終り，宿主たるクリタマバチが僅か10%内外しか羽化せぬ頃（筆者のいう生物的または統合的防除最適期）を適期として γ-BHC を週内精々1，2回被害樹の地上部に撒布すれば，各種虫瘿に産下ずみの既羽化の天敵蜂群の卵は木化の進んだ虫瘿内で γ-BHC の接触から逃れて安全に保護される。一方つづいて大量羽化期（約90%）にはいるクリタマバチは脱出時虫瘿の表層に残留沈着する γ-BHC に触れて皆斃死する。こうして在来天敵蜂群の従前の寄生率は相対的に著しく高められ，クリタマバチ防除に適した「天敵と薬剤の調和的併用法」が確立される。

EXPLANATION OF PLATES

EXPLANATION OF PLATE I

A–C : Autoradiographs and photographs of the plants labeled with radioactive γBHC–1–C^{14}. Autograph time : 252 days. The left side : dried plant specimens; the right side : its autographs.

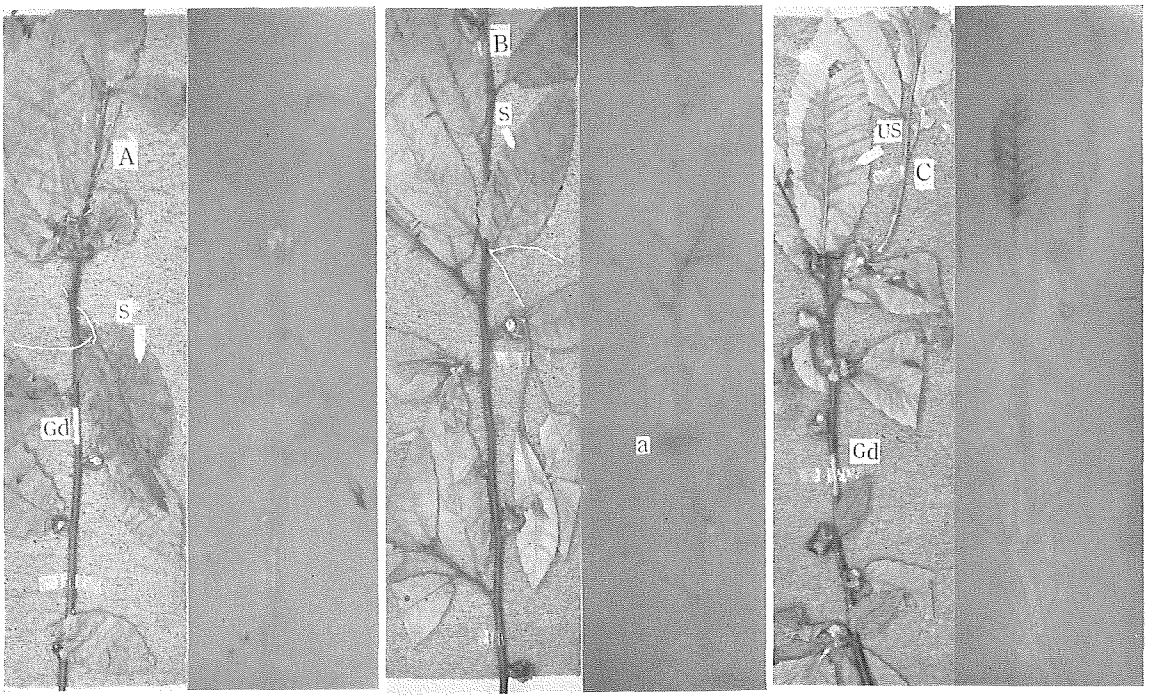
A' & B' : Autoradiographs and photographs of the plants labeled with radiophosphorus P^{32}, comparable with those in the upper row, respectively. The central part of a large blackened patch on each autoradiograph appears to be rather less blackened on account of its having precipitation of very dense silver grains which assume a brownish black color on the negative.

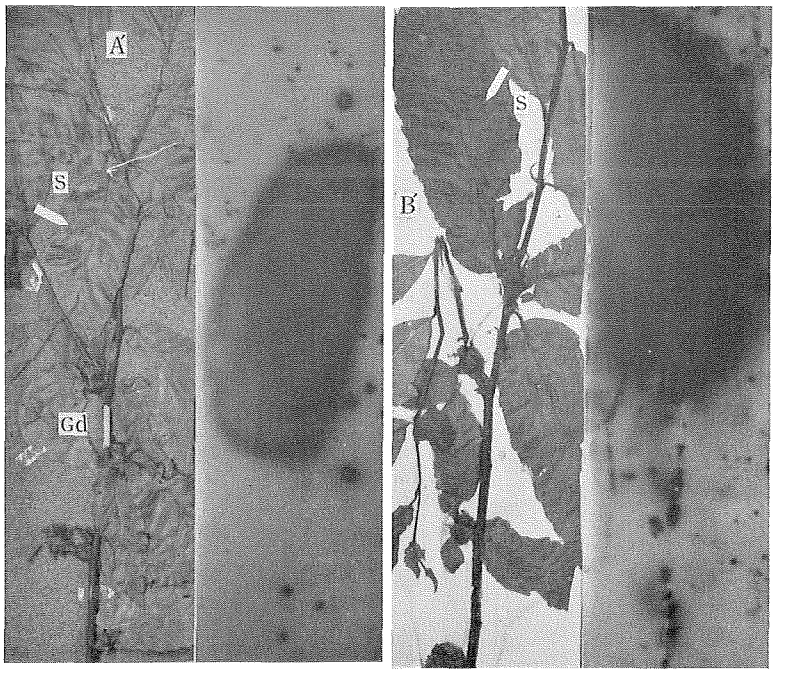
A : Chestnut twig with chestnut galls, with a foliage leaf, a fraction of whose top surface was labeled; a girdle was made on the stem located below the labeled leaf.

B : Parallel sample placed under the same experimental conditions as A excepting that no girdle was made.

C : Chestnut twig with chestnut galls, with foliage leaf, a fraction of whose underside was labeled; a girdle was made on the stem located below the labeled leaf.

Notation : 1) S : Top surface of a foliage leaf.
2) US : Underside of a foliage leaf.
3) Gd : Girdle.
4) a:Spot–like artifact blackening accidentally caused in course of exporsure.


A
S
Gd
B
S
a
US
C
Gd


A'
S
Gd
S
B'

EXPLANATION OF PLATE II

D–F : Cf. the explanation of Plate I.

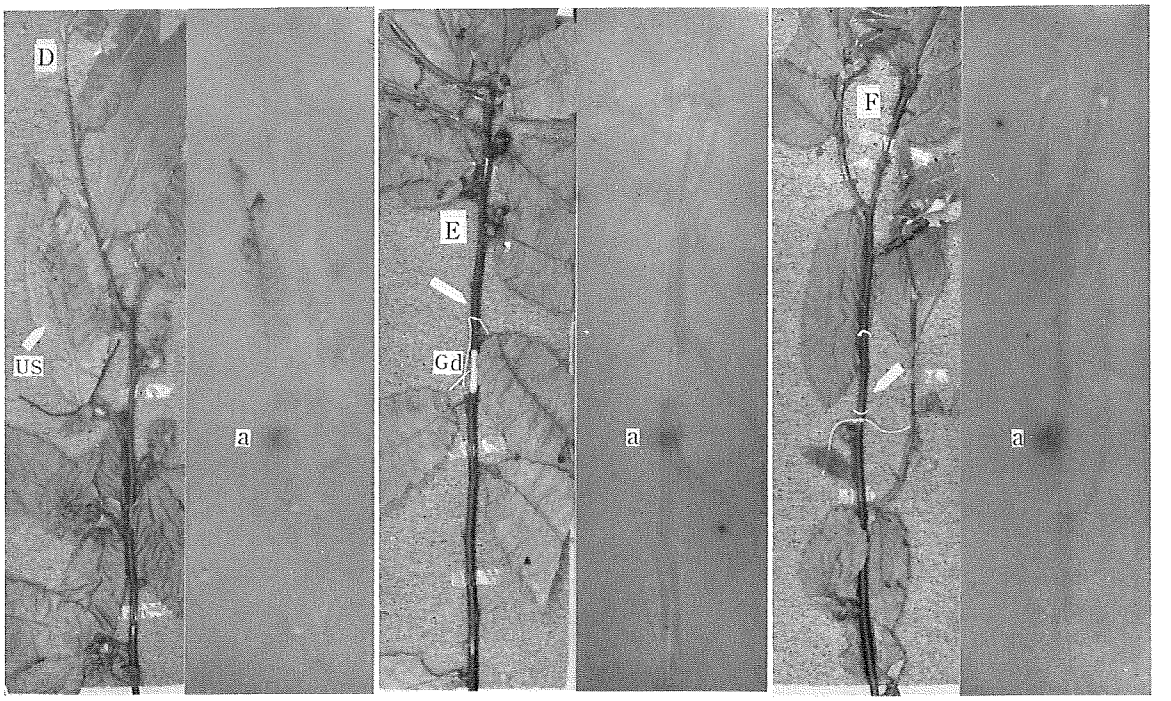
D' & F' : Ditto.

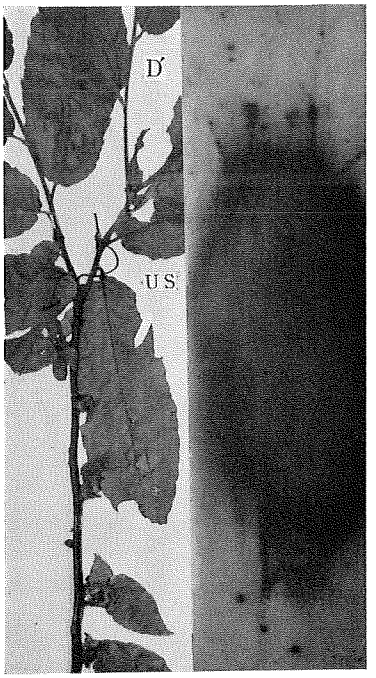
D : Chestnut twig with chestnut galls, with a foliage leaf, a fraction of whose underside was labelled.

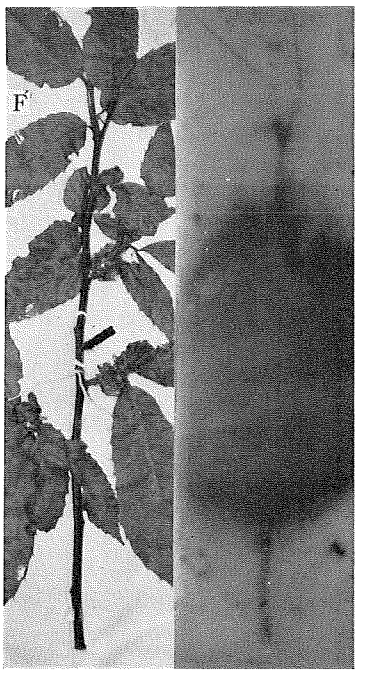
E : Chestnut twig with chestnut galls, a fraction of whose stem was labeled at the upper part of a girdle.

F : Parallel sample placed under the same experimental conditions as E excepting no girdle was made.

Notation : Cf. notation in Plate I.


D
US
a
E
Gd
a
F
a


D'
US


F'

EXPLANATION OF PLATE III

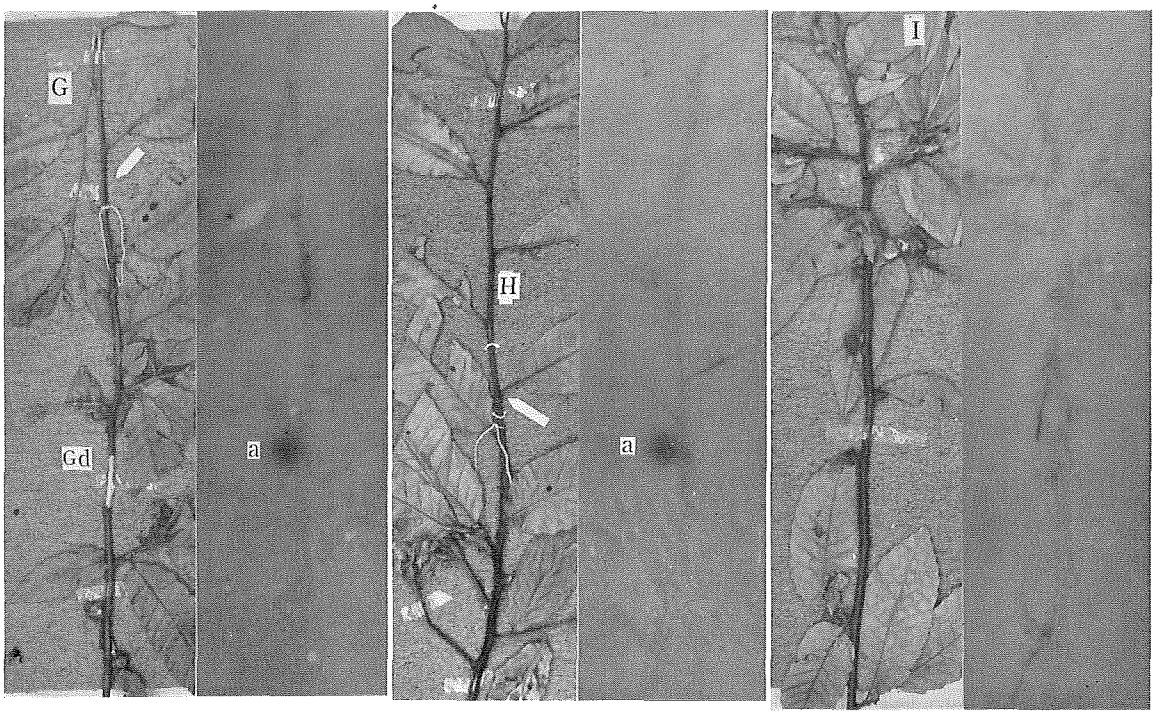
G–I : Cf. the explanation of Plate I.

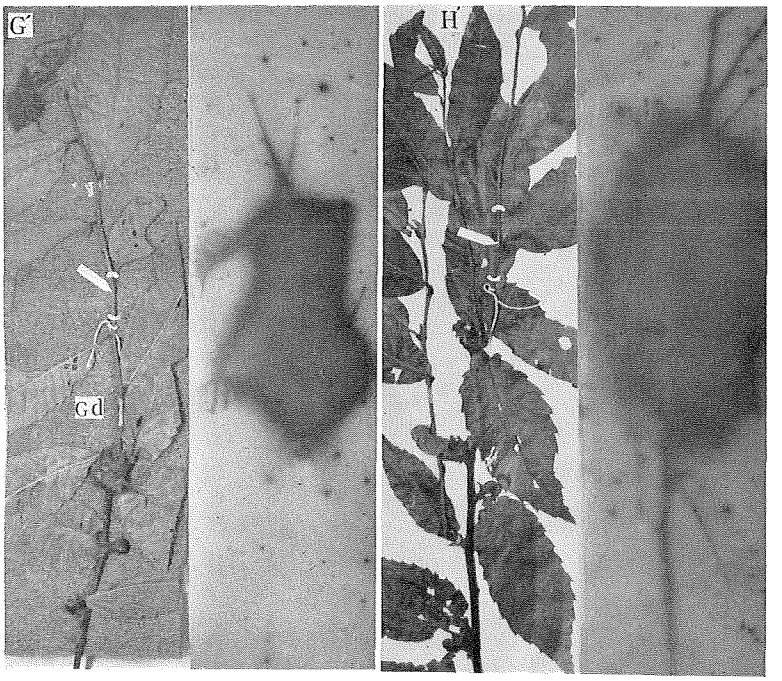
G' & H' : Ditto.

G : Chestnut twig with chestnut galls, a fraction of whose flush terminal shoot was labeled at the upper portion of a girdle.

H : Parallel sample placed under the same experimental conditions as G excepting that no girdle was made.

I : Chestnut twig with chestnut galls, all of whose galls were labeled. Notation: Cf. notation in Plate I.


G
Gd
a
H
a
I


G'
Gd
H'

EXPLANATION OF PLATE IV

J–L : Cf. the explanation of Plate I.

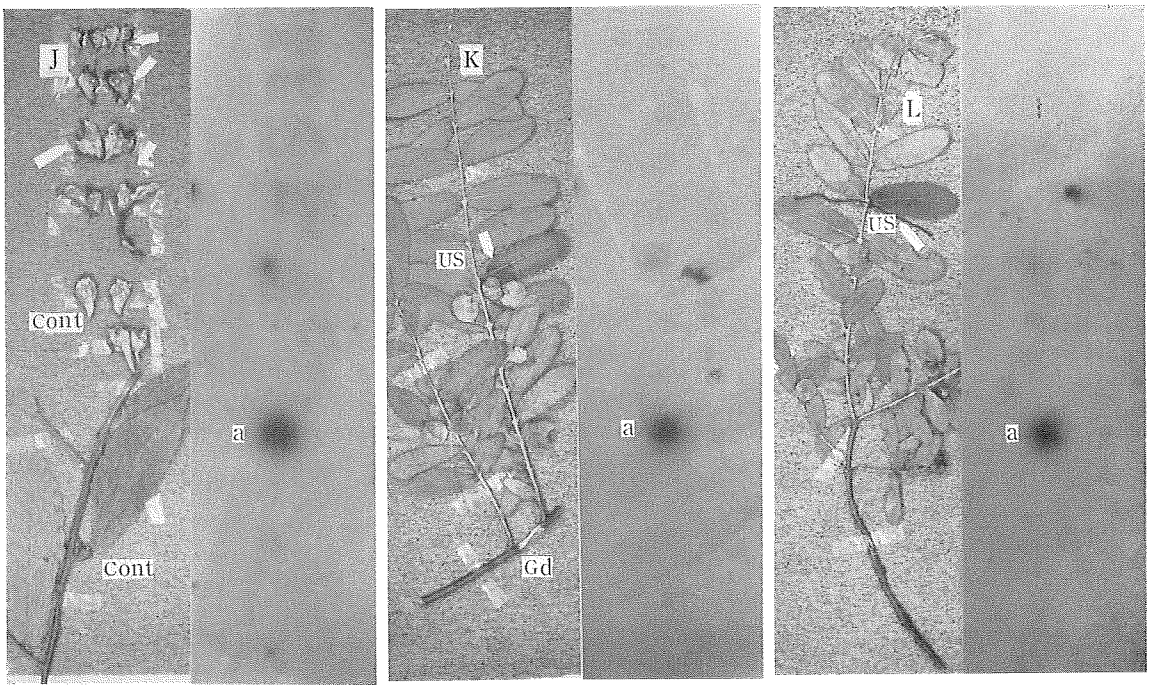
J'–L' : Ditto.

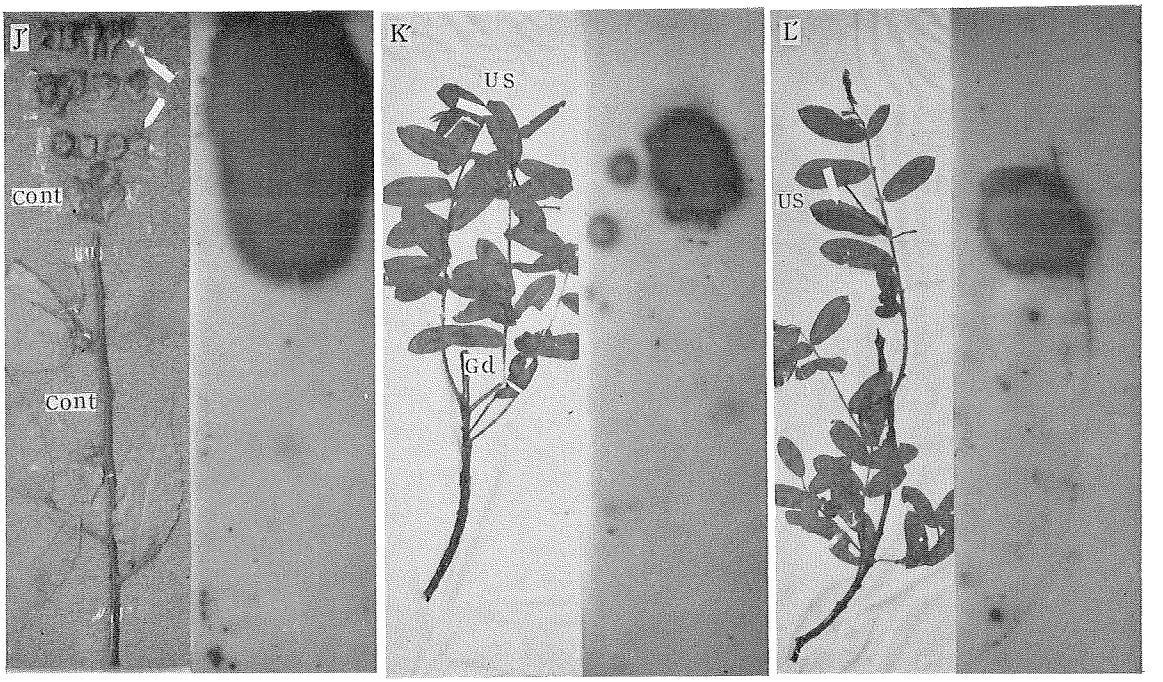
J : The upper row : Chestnut galls, each of which was dissected in halves.
The lower row : Control chestnut twig with a chestnut gall.

K : Willow twig with bowl–like leaf galls caused by a willow sawfly, *Pontania viminalis*; the under side of a foliage leaf located above a girdle made on a stem was labeled.

L : Parallel sample placed under the same experimental conditions as K excepting that no girdle was made.

Notation: Cont. : Control. The others are the same as those in Plate I.


J
Cont
Cont
a
K
US
Gd
a
L
US
a


J'
Cont
Cont
K'
US
Gd
L'
US

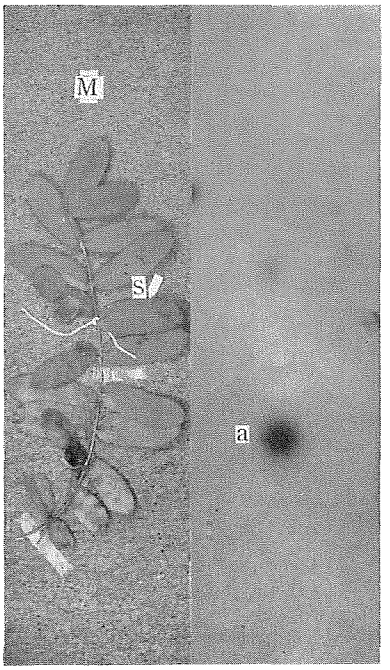
EXPLANATION OF PLATE V

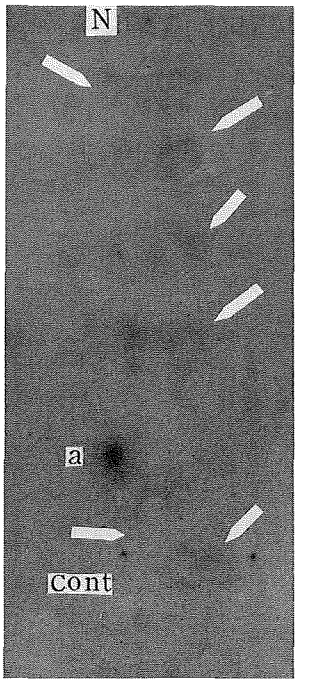
M : Willow twig with bowl-like leaf galls caused by *Pontania viminalis*. The top surface of a foliage leaf was labeled with radioactive γBHC-1-C^{14}.

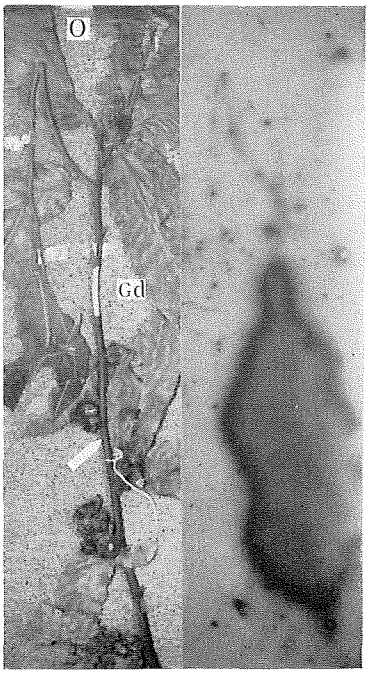
N : Magnified piece of autoradiographs of the cross sections of chestnut galls (Cf. phot. J).

O : Chestnut twig with chestnut galls, a fraction of whose stem was labeled with radiophosphorus P^{32}. A girdle was made on the stem located above the labeled portion.

Notation : Cf. that in Plates I & IV.


M
S
a


N
a
Cont


O
Gd