

**Penetration and Translocation of  $\gamma$ -BHC 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.)\*\***

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*(With 1 Text-figure and 2 Plates)*

As evidenced minutely in my previous report (Torii, 1959), the early-summer-emerging females of the native parasitic wasps preying on the chestnut gall wasp oviposit again in the larvae or pupae (within the gall) of the same generation of the host-pest as host from which they themselves emerged, although they lay eggs onto the other various kinds of gall insects as well. From the angle of biological control, it is important to encourage positively and make the native natural enemies propagate as much as possible. For this purpose two control measures were recommended in the previous report. One is that applicable to the prevailing method that resorts to burning plucked galls. The other is that suitable for the application of  $\gamma$ -BHC. In both, special importance was attached to positive encouragement and utilization of such parasite progeny laid in a variety of galls. In the latter, therefore, the question needs to be answered of whether or not  $\gamma$ -BHC is capable of penetrating into plant tissues and being translocated to the parasite eggs inside the galls formed on a diversity of plants. Needless to say, proper timing of insecticidal application and plucking galls is also not less important than the action of  $\gamma$ -BHC. The difficulty involved in this problem has clearly been overcome by the establishment of what I call the "period fittest for possible biological control." As regards the former problem, some preliminary experiments were conducted with tolerably reliable evidence, as reported in the previous work. But with the object of securing more convincing evidence, a parallel experiment was being carried out by using radioactive  $\gamma$ -BHC labeled with  $C^{14}$ . The main portion of the present report concerns itself with the result obtained from this experiment. Together with this result, some general considerations have been given on the problems pertaining to biological control of the chestnut gall wasp studied. Both will serve as a supplement to the previous report.

As will be discussed in detail later on, recently increasing attention has been focused by many economic entomologists on the systemic nature of  $\gamma$ -BHC. Of late, it has been demonstrated by Ishii et al. (1959 et seq.) that " $\gamma$ -BHC does not easily penetrate the plant cuticle nor translocate within the plant tissues." Their conclusion is based on the result obtained from the detailed investigation by using emulsion of radioactive

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$\gamma$ -BHC-1-C<sup>14</sup> synthesized by Ishii. I have also found that, when applied to chestnut galls,  $\gamma$ -BHC dust or wettable powder is deposited within the layer at best 0.5mm. below the surface of the chestnut gall and does not easily penetrate to the depth of the gall cavity for each larva inside the gall. Originally, my parallel experiment on the systemic action of  $\gamma$ -BHC has its genesis in the investigation conducted by Ishii et al. mainly in 1958. Accordingly,  $\gamma$ -BHC used in my experiment is just the same material in chemical property as  $\gamma$ -BHC-1-C<sup>14</sup> that was synthesized by Ishii. In the present experiment, this radioactive  $\gamma$ -BHC was applied topically to some plants with insect galls, inclusive of herbaceous and woody plants, under laboratory conditions. Somewhat different aspect from that by Ishii et al. has been derived from the result. As regards the principal action of  $\gamma$ -BHC, however, my finding coincides in essence with that by Ishii et al. From the standpoint of integrated control, the systemic nature of  $\gamma$ -BHC seems to be promising, so far as the chestnut gall wasp and its parasitic wasps are concerned, as pointed out in the previous report.

Dr. S. Ishii, Chief of the 1st Section of Insect Pests Control at the National Institute of Agricultural Sciences, had the kindness to permit me to use his synthesized  $\gamma$ -BHC-1-C<sup>14</sup> and under his encouraging guidance this examination was carried out. For this my especial thanks are due him. This experiment was initiated as one part of more comprehensive studies on the biological control of the chestnut gall wasp. In this sense, my heart-felt acknowledgement must be paid to Dr. K. Yasumatsu, Professor of entomology at Kyushu University, who has been unstinting in encouragement, suggestions, and the loan of invaluable references, throughout the whole course of the studies. This report inclusive of the previous one, owes much to the unremitting encouragement shown by Dr. N. Yagi, ex-Professor of entomology at the Faculty of Textile and Sericulture, Shinshu University. His kindness is heartily acknowledged by me. Miss. M. Kamiya, ex-Member of our laboratory, has spared no efforts in preparing the manuscript. For this I am grateful to her.

## MATERIALS AND METHOD

A field collection of various plants with insect galls was made from the stands in the scrub forest of chestnut gall wasp infestation, near by our laboratory, on 23rd July, 1959. The plants collected were all flush and vigorous. They consist of 5 species of stems and twigs, inclusive of 1 species of herbaceous plant and 4 species of woody plants, and were brought into the laboratory for experiment immediately after the collection. Long freshly cut woody twigs and herbaceous stems, each with foliage leaves, were held individually in a 500 cc. flask containing water, each at the rate of one per flask. The water supply was replenished every day. The radioactive  $\gamma$ -BHC emulsion used consisted of 3.2mg. of  $\gamma$ -BHC-1-C<sup>14</sup> (with specific activity of 0.398 c/mg.), 0.1 ml. of xylene, two drops of Triton X-100, and 125 ml. of water. The concentration of  $\gamma$ -BHC was estimated to be equivalent to about 25.6 p. p. m. (Ishii et al., 1959). The emulsion actually employed by me seems to be somewhat weaker in its chemical property, since it is the remnant left after Ishii's root dip experiment. Presumably, this

fact seems to be responsible for the result secured in the present experiment, which differs in some measure from that obtained by Ishii et al. The method of topical application was very simple. Only the aerial parts of the freshly cut plants were treated. The  $\gamma$ -BHC emulsion was fully applied to two portions on the surface and/or the under surface of a foliage leaf with a brush to draw a disc about 1 cm. in diameter, respectively. The number of discs drawn was changed according to circumstances. In the case of galls, all the surface of each gall formed on a stem or a twig was coated. In the case with the stem, it was applied fully to a certain portion about 3 cm. long with a brush. For the preparation of dried plants, treated leaves, stems or twigs, and galls were marked with a white sewing thread, respectively. In each case, at least about 5 cm. buffer portion was left between the treated portion and the mouth of a flask to reduce danger of contamination by contact with water. Twelve separate lots of cut plants treated in this manner were kept under indoor conditions. The components of each series are shown in the radioautographs Series A-L. Topical applications were made on 23rd July. A week after applications, the sample specimens including treated portions were placed between filter and blotting paper in the botanical press and dried for 7 days in room temperature. Radioactivity of the treated portions and the other untreated portions was measured directly by use of a thin-window Geiger-Müller tube over a very thin sheet of cellophane paper at almost zero distance. The readings of cpm. for them turned out to be nearly equal to that for natural background, by somewhat greater in value than the latter in some cases. Radioautographs were made by placing a sheet of Fuji X-ray film, non-screen type No. 200, on the plants set on a thick cardboard and holding tightly in a new botanical press in the dark within the electric refrigerator kept below 5°C. for 162 days. The X-ray film used is characterized by its sharp sensitivity to X-ray. Plant specimens were held for comparatively longer period to compensate for radioactive decay (5568-year half-life for C<sup>14</sup>). By way of precaution against various sorts of background fogs, each cardboard on which plant specimens were set was covered with 2 sheets of damp-proof cellophane paper. A botanical press consisting of two sheets of thick cardboard was fastened tightly with 3 pieces of strong rubber bands. Each botanical press was wrapped up in a bundle with 2 sheets of damp-proof oil paper, and further with 3 sheets of light-tight black paper and a sheet of vinyl wrapper. Before developing the film, the wrapper of a botanical press taken out of the refrigerator was kept in the dark room for about half a day to be balanced with room temperature.

In order to distinguish the film blackening caused by radioactivity from that caused by a variety of background fogs, the following criteria\* were set up:

i) The blackening caused by radioactivity takes place in general on both sides of the film. Those caused by  $\beta$ -or  $\gamma$ -ray with high energy are the case in point. In a special case where  $\alpha$ -rays or electrons with low energy are concerned, blackening occurs

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\* These criteria were endorsed by the authorities of the Fuji photofilm company. For paragraph V and the effect of  $\alpha$ -rays and/or electrons with low energy, I am indebted to the company authorities. My appreciation is due them for their kindness.

at best on only one side of the film with which the radioactive material was in contact.

ii) The portion due to pressure fogs becomes less sensitive, being transparent when developed, and consequently becoming black when printed.

iii) Moisture fogs: The X-ray film has a tendency to become less sensitive when affected by moisture or drops of water. This adverse effect is observed on only one side of the film with which they were in touch. If some substances which produce chemical fogs such as terpenes are contained in water or moisture, blackening takes place on the affected side alone.

iv) When the film is in contact with some fog-producing ingredients such as terpenes which are often contained in some plant tissues, blackening is produced on the affected side alone, since they do not penetrate a celluloid base of the X-ray film.

v) In the case when scratches or streaks are produced by mechanical friction on the film, blackening is confined on the affected side of the film. When large dynamic pressure acted zonally on the film, the zone becomes less sensitive, being often characterized with many minute streaky blackening.

These criteria concern the appearances produced on a developed film. Accordingly, the word "blackening" and/or "transparency" mentioned above must be substituted for "whitening" and/or "blackening" on a print, respectively.

## RESULTS

Radioautographs are shown together with the corresponding plant photographs placed side by side in photos. A-L. Distribution of the radioactive material is clearly revealed in the radioautographs. An RI-free control plant specimen gave a comparatively clear picture on the X-ray film as is shown on its print, probably because of its imperfect drying. (The check plant, an oak twig, was dried up in room temperature for 2 weeks, but it was not pressed in a botanical press. In fact, the blackening of the film was judged as a chemical fog caused by some materials percolated from half-dried plant tissues). The results are grouped into 12 series according to photos. A-L.

Series A.—A wild willow, *Salix integra* Thumb., was treated in this series. When a foliage leaf was treated topically, essentially no radioactivity was detected in that portion, irrespective of side. A treated stem gave a clear picture on the film corresponding to its position. A barely discernible picture is visible at both the upper portion of the twig near the treated stem and the lateral sprigs shooting from the treated stem. No appreciable picture can be seen at the flush terminal shoot, probably because of its hastened withering. Thus, evidence is clear that, when applied topically to the stem of a wild willow, *Salix integra*,  $\gamma$ -BHC is capable of being absorbed in its tissues and being translocated from the treated portion to some other untreated portions of the plant. Transparent portions caused by the pressure due to twigs were detected on the film, but they are hardly discernible on the print, as their black pictures have merged imperceptibly into its back.

Series B.—Freshly cut mugwort was dealt with in this series. Experimental results with the leaf and the stem revealed that  $\gamma$ -BHC was comparatively widely trans-

located into the whole plant tissues, although radioactivity was rather slightly appreciated. Judging from the fact that this herbaceous plant was most inferior in water-raising, hardly any effect due to moisture fogs combined with chemical ones on the film need be taken into consideration. A treated portion of a leaf, irrespective of side, was comparatively clearly sensitized. In the case when a stem was treated topically, radioactivity was clearly revealed throughout the whole plant, with a high concentration on the treated portion and a moderate, somewhat faint concentration on the other untreated portions such as foliage leaves and petioles. Bowl-like galls on mugwort stem, caused by a gall fly, *Rhopalomyia tubifex* Kieffer, were all characterized by a moderate concentration of radioactivity, but translocation to the other untreated portions of the plant, such as the stem and foliage leaves, was found to be very faint and scarcely discernible. Special attention should be paid to this fact. This seems to be connected with the special structural character of the herbaceous plants at large. It is characteristic of them that their aerial parts are poor in the xylem which serves as a water conducting tissue, as is reflected in the inferiority in water-raising, and further that the phloem exists so deeply in the inner parts of the stem that it can not be removed by girdling (Bonner and Galston, 1952). Judging from this structural feature, both the xylem and the phloem seems to share the responsibility for the absorption as well as the translocation of  $\gamma$ -BHC in the tissue of the mugwort under experiment. However, the phloem seems to be mainly responsible for this phenomenon. The reason for this lies in the inference that the nutrition for the then growing galls must have been supplied vigorously through the flow within the phloem whose function is to conduct nitrogenous food-substances from the leaves to the parts where growth is going on, and as a natural result the radioactive carbon absorbed in the phloem of the gall must have been prevented by such a flow from being translocated to the other parts of the plant. The wide-spread but weak translocation shown in the case when the stem and/or the foliage leaf was treated topically may be attributed to the hindrance of further accumulation of radioactive carbon in certain growing parts, which follows from the withering of the phloem hastened by the inferiority in water-raising of the plant, rather than to the direct hindrance of translocation ascribable to the paucity of xylem, because there is no vindicated evidence that  $\gamma$ -BHC is capable of penetrating into the xylem when it is applied to the plant by foliage or bark spray. This interpretation will be supported by the results shown in the following series, too.

Series C. —The treated oak foliage leaf showed a barely discernible concentration of radioactivity in the treated portions, with somewhat higher but faint concentration at the under side of the leaf. Probably, this may be ascribed to the fact that, when applied to the surface of the leaf,  $\gamma$ -BHC is easily volatilized without being absorbed in the leaf tissues on account of thick cuticle as well as paucity of the stomata thereon. Substantially no appreciable translocation was revealed in this series.

Series D. —Topical application to burry galls on the oak stem. A considerably high concentration of radioactivity was revealed at the treated galls alone. Barely appreciable translocation can be traced dimly in the foliage leaves. Distinct transparency on the film (blackening on the print), which is apparently the outcome resulting from pressure fogs,

was detected at the portion of contact with the stem.

Series E. —Oak knot galls, a foliage leaf of an oak twig with oak scale galls, and oak scale galls were treated topically, respectively. In every case, the highest concentration of radioactivity was distinctly revealed at the treated portion. When the oak knot gall was treated, moderate translocation took place in the foliage leaves adjacent to it. Foliar topical application produced wide-spread but faint translocation all over the treated leaf. When the under surface of a foliage leaf was treated, distinct, spot-like concentration of radioactivity was manifested at the treated portion, with moderate and noticeable radioactivity presented at the nearest scale galls, apparently indicating accumulative translocation to them. Furthermore, very weak radioactivity made the image of the leaf for its entire surface faintly visible on the film. When the scale galls were treated, moderate radioactivity was comparatively clearly outlined on the film, and translocation to the other untreated parts was observed to be rather less in degree, indicating persistence of radioactive carbon on the treated scale galls.

Series F. —Chestnut galls formed on the chestnut twig were treated topically. Clearly the highest concentration of radioactivity was indicated at all the treated galls themselves. Somewhat moderate intensity of radioactivity was also present on the stem. These findings are quite coincident with the case with the other insect galls treated topically, attesting persistence or accumulation of radioactive carbon on the insect galls. Absorption of  $\gamma$ -BHC by the treated plant is undeniable in this case, indicating that phloem must have played an important role in it. Pressure fogs are also visible at the portions of contact with large galls.

Series G. —A topical application to a flush terminal shoot of the chestnut twig with chestnut galls. The treated portion of the flush terminal shoot was clearly defined on the film. Again the galls on the stem far below the treated portion showed up as dark areas on the film (bright areas on the print), an indication of considerably remarkable downward translocation of radioactive carbon. The foliage leaves near them also indicated a much lower concentration of radioactivity.

Series H. —Young foliage leaves of the flush terminal shoot of the chestnut twigs with chestnut galls. The treated portion of a leaf, irrespective of side, gave barely discernible images on the film. The galls formed on the lower stem from the treated leaf showed up as much more distinct pictures on the film. Again evidence is clear that  $\gamma$ -BHC tends to be accumulated on the insect gall. In the sample at the bottom of the print, a black streak arising from the pressure fog due to contact with the stem is visible.

Series I. —A topical application to the stem of the chestnut twig with chestnut galls. Somewhat clear image can be observed at the treated portion, with the highest concentration of radioactivity at the callus formed on the stem just treated. The galls formed on the upper stem from the treated portion, especially those on the uppermost flush stem, are also clearly indicative of upward translocation of pretty amount of radioactive carbon and of its persistence in them. A moderate concentration of radioactivity was visible on the flush terminal shoots as well. A less appreciable concentration was present in the mature foliage leaves. Among the results concerning this series, what

attracts our attention is the highest concentration in the callus.

Series J. —(a) The stem of the *Quercus acutissima* twig with both knot galls caused by the Gelechiid moth, *Stenolechia querci* Shin. (Gelechiidae) and *Q. acutissima* galls. —The distribution of radioactivity quite resembles the case with the treated chestnut twig (Series I) in outline, excepting the image of the callus. In this case, too, persistence in the treated stem as well as upward translocation to the galls formed on the upper stem can not be denied. (b) The leaves issuing abnormally in clusters from the *Q. acutissima* gall. —Very weak and barely discernible radioactivity was detected on the treated portions, with an appreciably more concentration on the treated under surface of the leaf.

Series K. —The oak stem of the oak twig with oak knot galls caused by a kind of leaf roller moth, *Pelataea bicolor* Walsingham. In this case, too, the distribution of radioactivity quite resembles the preceding cases (Series I and Series J-(a)) in general outline, although somewhat higher radioactivity was revealed as a whole.

Series L. —The *Q. acutissima* gall with the leaves issuing rather abnormally in clusters from it. In one sample at the bottom of the radioautograph, the highest concentration of radioactivity in the treated galls was evidenced by a large and distinct blackening on the film (brightening on the print). Substantially no translocation was detected in the leaves. A much reduction in radioactive intensity was shown in the other two samples at the top of the radioautograph. Much stronger downward translocation was observed in the lower stem as well as in some leaves. For this the fact is responsible that some other treated galls formed on the lower stem had fallen off from the stem before the sample was set on the cardboard.

## DISCUSSION AND CONCLUSIONS OF THE EXPERIMENT WITH RADIOACTIVE $\gamma$ -BHC

It was revealed that, when the foliage leaf was treated topically with  $\gamma$ -BHC, the absorption of the chemical was evidenced to be somewhat stronger in its under side than in its upper side. The similar phenomena to this are also observed in the case with foliar spray of urea (Noguchi and Kuzuhara, 1954). In the latter case, it is believed that the thin cuticle as well as many stomata on the under surface of the leaf are in most cases responsible for such a phenomenon. It is also pointed out that, in the foliar spray of urea, use of a certain auxiliary substances such as Triton X-100 or Tween No. 80 results in the increase in percentage absorption by about 20–30%. In the present experiment, too, Triton X-100 was used as a surface activator. It is expected, therefore, that penetrating power of  $\gamma$ -BHC may be accelerated in a measure. Generally, chemicals soluble in lipoids are thought to be easily absorbed in the plant cell because of their having strong permeability into the cell wall, even if their molecular volumes are large. In the case with  $\gamma$ -BHC, its penetrating action into insect tissues is considered to depend on its solubility in lipoids. It is also known that  $\gamma$ -BHC is persistent in fatty materials. From these facts, it is not necessarily illogical to think that  $\gamma$ -BHC, though large in its molecular volume, may be absorbed in plant tissues. In the present experi-

ment, it has been revealed that a fair measure of variation in radioactivity was evidenced with plant species and/or treated portions. Probably, such may be attributed to the difference in thickness of the cuticular layer, the number of stomata on both sides of the foliage leaf, and the amount of lipoids in the plant tissues. Actually, it has been demonstrated that whether or not foliar absorption of urea is quick depends upon the plant species, environmental conditions under which the plants are placed, and the state of growth. Foliar topical application of radioactive  $\gamma$ -BHC to the leaf of woody plants showed substantially no, or barely discernible radioactivity at the treated portions. Wax on both sides of their foliage leaves also seems to have been responsible for this result, since it is chemically stable, hastening the volatilization of  $\gamma$ -BHC. Weak but noticeable radioactivity was clearly visible throughout the almost whole plant when the mugwort leaf and/or stem was treated topically with radioactive  $\gamma$ -BHC. It admits of almost no doubt that the systemic action of  $\gamma$ -BHC on the mugwort is undeniable. This is a fact to be noticed. As is well known, the cut mugwort withers soon after it was put in a vase, on account of its being relatively inferior in water-raising to some other herbaceous plants. Under these conditions,  $\gamma$ -BHC will be hampered greatly from being accumulated in a certain fixed portions such as various growing parts or other, no matter whether it be translocated through the xylem-vessels or the bast part. As already pointed out, however, the bast part or phloem seems to be responsible for the systemic translocation of  $\gamma$ -BHC in this case, from all the experimental results as well as the structural character of the plant of concern. Similarly, this interpretation seems to hold true in the case with the woody plants under investigation. It was evidenced that, when applied topically to the stem, irrespective of herbaceous or woody plants,  $\gamma$ -BHC persisted at that treated portion, and further was strongly translocated to the untreated other parts of the plant. And besides, what arrested our attention mostly was the fact that evidence was clear that  $\gamma$ -BHC persisted at the topically treated gall and/or callus, without showing hardly any signs of being translocated to the other untreated portion. The insect gall belongs to the tissue produced as a result of abnormal growth in thickness, and is usually characterized by the secondary tissues of the phloem as well as the xylem which were formed in, inside and outside of its cambium. The callus is referred to as the wound tissue, in the formation of which usually the xylem-vessels and the other parts of the xylem do not concern themselves, irrespective of herbaceous or woody plants (Ino, 1954). The sieve-plate and its pores of the phloem are coated with callus (Scott, 1927). Needless to say, nutritive substances are transported to these parts through the phloem. Putting all accounts together with the fact that the upward as well as downward translocation of  $\gamma$ -BHC to various growing parts was evidenced clearly, we may say that the phloem seems to be most responsible for the systemic translocation of  $\gamma$ -BHC topically applied to a certain part of the plant. In order to draw a final conclusion, however, more detailed examinations such as girdling experiment, chemical analysis, and bioassay seem to be necessary. At any rate, if the above-mentioned interpretation is tenable, there seems to be much possibility that a part of  $\gamma$ -BHC absorbed in the phloem may be easily broken down to intoxic substances such as trichlorobenzene or other, since it is very unstable to alkalis (Tasugi et al., 1955) and the cell sap of the phloem is alkaline (Ino,



1954). From this, too, the fact seems to be understood that, when taken up in the plant tissues from the treated soil,  $\gamma$ -BHC varies to a considerable extent in its toxic effect on the insects feeding on the plant according as the plant species concerned vary, even though the amount of  $\gamma$ -BHC applied may be more directly connected with the effect. This interpretation seems to be worthy of note.

Numerous reports have been published which attest to the systemic action of  $\gamma$ -BHC or lindane. Some of them are suggestive of the role of the phloem. After milling the wheat treated with radioactive  $\gamma$ -BHC, Bridges (1958) found that the residue in the bran was increased between two- and four-fold of the initial residue, while only 40-50 per cent. of it was present in the fine flour fraction. He found further that penetration of  $\gamma$ -BHC into cheese was low, and repeated applications caused a build-up of  $\gamma$ -BHC in the outer few millimeters of the cheese, having little effect on the amount penetrating more deeply. This seems to imply that, when absorbed in lipoids contained in the phloem,  $\gamma$ -BHC tends to persist in them, and unable to penetrate so much deeply. Such an action may be supported by the fact that  $\gamma$ -BHC is insoluble in water, because it is hardly thought that  $\gamma$ -BHC is capable of penetrating the inner part of the vascular tube and being transferred together with water to the other portions of the plant tissues. As reported previously in my report (1959), it was evidenced that sprayed or dusted  $\gamma$ -BHC barely penetrated the exterior few millimeters of the chestnut galls which were treated soon after the field collection made in early- and/or middle-summer. If we take into consideration the fact that nutritive substances must have considerably be hindered from flowing inside the phloem of the plucked galls, my results seem to be interpreted from Bridges' finding concerning the experiment with cheese. In his milling tests with wheat, Schesser (1958) also found that the highest residues were in the bran and shorts, and only 1.3-2.6 p. p. m. remained in the flour from wheat treated with lindane at 2.5-7.5 p. p. m. either 9-10 days after or after ageing for 18-24 months. Doane (1958) applied emulsion of lindane to American elms before leaf bud break, in the study of the control of European elm bark beetle. The residues were weathered under field conditions and were bioassayed at 4, 8, and 13 weeks after treatment. From the results, he concluded that the tenacity of lindane residues was remarkable in view of the volatile nature of  $\gamma$ -BHC and may possibly be explained by penetration into the bark.

Recently several reports have been published that various plants absorb and translocate  $\gamma$ -BHC and lindane. With special regard to possible fumigant action of  $\gamma$ -BHC, Koehler and Gyrisco (1957) demonstrated the systemic action of lindane in alfalfa upon the meadow spittlebug under conditions that eliminated the possibility of fumigant or contact action. According to them, the action was considerably more powerful within plants growing in treated soil than within those receiving foliar application of comparable concentrations. This was concluded by them to be due to systemic action of lindane, although they did not touch as to which part of the vascular bundle was responsible for such an action of lindane. They are of opinion that "if lindane is capable of acting as a systemic insecticide in the laboratory, it is feasible that such action also takes place to some extent in the field." Further they maintain that "frequent reports in the literature and among entomologists concerning off-flavors in crops which have

been treated with BHC have probably been the strongest evidence of the absorption of substances into treated plants." In fact, Gyrisco et al. (1959) reported that potatoes grown on the plots formerly treated with lindane were judged "probably-off" to "strongly-off," when tested for flavor and odor. In the experiments with mature cacao trees, Bowman and Casida (1958) demonstrated that a foliar spray of BHC resulted in definite off-flavors.

As regards the effect of soil applications, not a few reports have been made public. Howe (1950) found a remarkable reduction in the number of squash vine borer that attacked squash grown in soil treated with  $\gamma$ -BHC at the rate of 2 pounds per acre. Starnes (1950) gave the first positive evidence that  $\gamma$ -BHC was translocated in the reproductive tissues of lima beans and potatoes grown in the greenhouse in soil treated with lindane at several rates of comparatively high dosage. Many people other than the above two also have been known for long in having shown evidence that BHC is capable of being absorbed and translocated in various plants. For instance, Casida and Allen (1952), Terriere and Ingalsbe (1953), Ehrenhardt (1954), Gladenko and Fortushynyi (1954) and Wollerman (1955) are the case in point. Lilly and Haines (1956) also have offered evidences that lindane in the nutrient solution or  $\gamma$ -BHC in soil can be translocated to aerial parts of various plants tested. In Japan, too, Koshihara and Okamoto (1957 et seq.) have recently shown that lindane and  $\gamma$ -BHC were taken up by rice plant from soil treated just prior to transplanting of the plant and were toxic against rice-stem borer, indicating their systemic action. The report has been published by Linsley (1956) that analysis and bioassay demonstrated comparatively high residues of BHC or lindane in some root crops grown in soil treated with these substances.

On the other hand, negative results against the systemic action of  $\gamma$ -BHC has been offered by Anderson (1955). His finding is that, when applied to the soil of potted plants at the rate of 1 gm. per 6-inch pot, BHC 11.5 per cent. gamma isomer was "not found to be effective as a root-absorbed systemic poison" against first instar Mexican bean beetle larvae feeding on the potted plants. The conclusion drawn by Ishii et al. (1955), too, is rather negative. They conclude that " $\gamma$ -BHC does not easily penetrate the plant cuticle nor translocate within the plant tissues." However, they add that, in order to make the systemic action of  $\gamma$ -BHC clearer, radioactive  $\gamma$ -BHC with stronger specific activity should be used, since specific activity of the substance used was estimated at about 0.4 c/mg., which seemed to be too weak to trace its systemic action on the plants tested. According to Ishii's private communication from America (1960), he does by no means deny the systemic nature of  $\gamma$ -BHC, but is of opinion that it seems to depend upon either the intensity of specific activity, the dosage applied or both. In fact, they used  $\gamma$ -BHC of comparatively high concentration to cover its weakness in specific activity to such an extent as it sometimes caused plant injury. Judging from the results obtained from the present experiment, however, such an operation itself seems to have been responsible for the hindrance of absorption as well as wide-spread translocation of  $\gamma$ -BHC. The reason for this lies in the following two: One is that  $\gamma$ -BHC used in the present experiment seems to have been somewhat weaker in its specific activity than that used in the experiment conducted by Ishii et al., since it was

the remnant left after their root-dipping experiment. The other is that hardly any sign indicative of plant injury was observed at all the treated portions, while wide-spread translocation of radioactive carbon was clearly evidenced. Besides these reasons, attention should be paid to the difference in the plant species used as well as the method of application, too. As already discussed, it was clearly revealed by chemical analyses or bioassays conducted by many investigators that there was comparatively wide discrepancy in the amount of translocated  $\gamma$ -BHC or in the intensity of toxicity with the plant species, the dosages applied, and/or the method of application. And furthermore, as is generally known, the relative potency of  $\gamma$ -BHC varies greatly with insect species ((Lyon (1959); Rudinsky and Terriere (1959)). Similarly, the persistency of residue and/or residual effectiveness of  $\gamma$ -BHC is subject to fairly sharp fluctuations with the plant species (Decker et al. (1950); from Ozaki (1959)).

Putting all the above results together, I arrive at the following conclusion: When applied topically at a proper dose to certain aerial portions of herbaceous or woody plants used,  $\gamma$ -BHC seems to be capable of penetrating at best to the phloem under favorable conditions, but incapable of penetrating so deeply to the depth of the xylem in most cases. The phloem or the bast part seems to be responsible for the translocation of  $\gamma$ -BHC which was absorbed in it. If these are tenable, it implies that the previous statement made by me (1959) has been vindicated that the eggs deposited onto various kinds of galls by the early-summer-emerging wasps parasitic on the chestnut gall wasp (host pest) can survive the toxic action of  $\gamma$ -BHC, only if it is applied to around what I call the "period fittest for possible biological control" at a proper dosage. I found that there are some reports suggestive of the propriety of this statement. In the investigation of the action of lindane on the immature stages of a bean weevil infesting stored haricot beans, Schvester (1958) found that, when already infested beans were treated with  $\gamma$ -BHC at 3 and 5 p.p.m. and stored at 25°-26°C., development of the weevils had proceeded normally up to the time of adult emergence, so that damage to the beans was not reduced, although  $\gamma$ -BHC at such dosages progressively reduced the numbers of the adults that emerged from the treated beans. This clearly suggests that  $\gamma$ -BHC has no deeply penetrating effect on stored beans. From the results of trunk implantation of a systemic insecticide Am. Cyanamid 12880 applied to balsam firs, Giese et al. (1958) found that larvae of *Tetrastichus whitmani* (Gir.) and *T. macrovitchi* (Crawf.) continued to develop in the galls on larvae killed by 12880 which was used about the end of the growing season when a lower rate of translocation would be expected. Their finding is very suggestive, although the chemicals, insect pests, and the plants employed are entirely different from those in my case. Let us suppose that the early-summer-emerging parasitic wasps should have deposited their eggs onto the gall insect (host pest) which lived in the cavity lying very near the surface of the gall, and further the galls were treated with  $\gamma$ -BHC at a proper dosage. Then, it is feasible that such eggs may continue to develop in the gall on the host pest, even if the host pest should have died of contact with  $\gamma$ -BHC which penetrated to the inner part of the cavity for the host. It is still more possible, if the host pest be the larvae of the chestnut gall wasp in midsummer, since the larvae at that time

must be in the resting pupal stage, being unaffected by the cell sap containing  $\gamma$ -BHC.

By the way, from the angle of integrated control of the chestnut gall wasp, the duration of the residual effect of  $\gamma$ -BHC needs to be noted. There are at least two conditions for it. Firstly, in order to effect complete kill of the emerging chestnut gall wasps, it is necessary for its residual effect to persist for at least two weeks. Secondly, for the purpose of conserving the native parasitic wasps as many as possible, it is to be hoped that its residual effect does not last so long a time as to last until their eggs ( $F_1$ ) deposited in the chestnut gall emerge out as adults. Is it really possible to satisfy these conditions? It is said that the residual effect of  $\gamma$ -BHC is of rather short duration, since  $\gamma$ -BHC is comparatively high in volatility. In the test of the effect of chlorinated terphenyl on the persistence of residual action of  $\gamma$ -BHC, Ishii and Matsuda (1959) found that the addition of chlorinated terphenyl to  $\gamma$ -BHC was ineffective in restraining  $\gamma$ -BHC from evaporating in the greenhouse. From this they concluded that, when applied to crops at dosage of common use under weather conditions,  $\gamma$ -BHC with or without chlorinated terphenyl will probably become ineffective in its toxic action on insect pests in a few days, since it remains attached very little on the crops treated. In the investigation of some insecticidal residues on various vegetables, Brett and Bowery (1958) reported that, when dusted at the rate of 30 pounds per acre when the vegetables were ready for harvest, lindane (1%) residues were detectable up to the 3rd day on tomatoes and up to the 4th day on snap beans, and only as a trace on collards by the 13th day. They further report that "rainfall effected a greater reduction of residues during the early period after application than during later periods." Mistic and Martin (1956) also pointed out the importance of various climatic factors which affect insecticidal toxicity. According to them, BHC was completely ineffective in controlling the boll weevil following a 24-hour exposure of treated plants to 0.87 inch of natural rainfall. The residual effectiveness of BHC was greatly reduced by a 24-hour exposure of treated plants in outdoor shade at high temperature. When treated plants were exposed to outdoor weather conditions for 48 hours, BHC was virtually ineffective. Repeated applications of BHC at 5-day intervals did not result in accumulated toxic residues which could be measured in terms of either initial or residual control of the boll weevil. Nirula (1956) has also reported that, when wettable powder spray of BHC at 0.1 and 0.2 per cent. was applied to leaflets from fronds of young coconut palms, BHC lost all toxicity after a maximum of 20 days and, in general, mortality fell to less than 40 per cent. in a week. According to Hassanein and Zaki (1957),  $\gamma$ -BHC gave complete mortality of adults of a beetle of stored food products, when they were confined for 20 hours on filter paper that had been impregnated with solution of 0.3mg.  $\gamma$ -BHC per sq. cm. and allowed to dry for an hour, but lost effectiveness, when they were confined on the filter paper similarly impregnated but left in the light and air for 2-12 days, the activity of  $\gamma$ -BHC decreasing by up to 66 per cent. in 12 days. Allen and Rudinsky (1959) found that sprays of 25% lindane wettable powders in water suspension completely protected the bark of freshly cut Douglas-fir trees against the Douglas-fir beetle attack for at least 8 weeks after applications, but after 19 weeks no successful attacks

of the beetle had occurred on the bark treated with lindane at 3 pounds active ingredient to 100 gallons of water. Dow and Willis (1959) also showed that the continued effectiveness against a gnat, *Hippelates pusio*, in soil was demonstrated with BHC 40 days after the initial application at the rate of 2.5 pounds of gamma isomer per acre.

All the results cited above clearly indicate that the residual effectiveness of  $\gamma$ -BHC varies to a considerable extent with treated plants, insects to be controlled, and the dosage employed. Such may be ascribable to the difference in degree of absorption and/or persistence in the plant tissues and of breakdown of  $\gamma$ -BHC or lindane, and further to weather conditions under which investigations were carried out. Nevertheless, it may safely be expected that the residues of  $\gamma$ -BHC will remain insecticidally toxic for one week at least or about one month at the maximum under outdoor weather conditions. If that is the case, it is quite possible to satisfy the above two conditions necessary for the integrated control of the chestnut gall wasp under study. However, due regard should be paid to the fact that there are many investigations reporting strong and durable persistence of  $\gamma$ -BHC in soil in the case of high-dosage soil application. Shorey et al. (1958), for instance, demonstrated the very long residual life of  $\gamma$ -BHC in soil. According to them, when applied as dusts in a permanent pasture sod, BHC at 8 pounds of gamma isomer per acre was giving excellent control of the larvae of European chafer in pasture sod after 3 years, but had lost its effectiveness by 6 years after application. In applying  $\gamma$ -BHC to chestnut groves, therefore, special attention should be paid to the method of its application not to render it accumulated in soil.

Summing up the present experiment with radioactive  $\gamma$ -BHC-1-C<sup>14</sup> topically applied to some freshly cut plants with insect galls, the following conclusions have been drawn:

Weak but noticeable wide-spread translocation of  $\gamma$ -BHC was revealed more clearly in treated mugwort than in the other treated woody plants. In view of both the experimental conditions and the structural characters of the plants examined, the phloem into which  $\gamma$ -BHC penetrated seems to be mostly responsible for such systemic translocation.

The degree of foliar absorption of  $\gamma$ -BHC in the woody plants was found to be stronger in the under surface of a leaf than in the upper side of a leaf, when  $\gamma$ -BHC was topically applied to the foliage leaf. This seems to depend upon either thickness of the cuticle and/or the wax layer of a leaf, numbers of the stomata on the side of a leaf, or all of them.

When topically applied to a certain portion of the stem,  $\gamma$ -BHC persisted most strongly in the treated portion, showing fairly spread translocation over the untreated parts. This translocation varied in degree with plant species, being most strong to galls, comparatively remarkable to the upper flush terminal shoot in every case. When applied to a certain part of flush terminal shoot,  $\gamma$ -BHC was translocated to the lower untreated region of the plant, and especially strongly to the galls formed on the lower stem, indicating its capability of downward translocation. Persistence of  $\gamma$ -BHC in the callus was remarkably strong in its degree. When topically applied to insect galls,  $\gamma$ -BHC persisted strongly in treated galls, with barely or scarcely discernible translocation to the other untreated portions. Judging from the time of plant collection as well as its nature

of a kind of abnormal growing tissue, it was concluded that the flow of materials converging to galls from all nutrient-producing organs must have hampered applied  $\gamma$ -BHC from translocating to the other untreated portions, since the treated galls in a growing state at that time must have been concentratively supplied with nutrient substances by foliage leaves.

On the basis of all the present results obtained from topical stem and/or gall application, and further many people's results demonstrating translocation of  $\gamma$ -BHC to reproductive parts of various plants, it was concluded that, when topically applied to a certain aerial parts of some plants, inclusive of herbaceous and woody ones,  $\gamma$ -BHC shows a strong tendency to accumulate in growing parts, inclusive of abnormally growing tissues, such as the callus or insect galls, of the treated plants.

From every angle, the phloem seems to be mainly responsible for absorption and translocation of topically applied  $\gamma$ -BHC. Informations corroborating this interpretation were given in detail.

It is to be noted in this connection that, in drawing the above conclusions, careful investigation was carried out to the full of the radioautographic results obtained on the basis of 5 diagnostic criteria for such an investigation, which have been clarified by me.

On these conclusions, the following inference was drawn: When applied to the aerial parts of the infested chestnut tree at proper dosage at what I call "the period fittest for possible biological control",  $\gamma$ -BHC presumably would not produce any direct adverse effect upon the insects living inside the lignified gall, inclusive of the chestnut gall wasp (host pest) and its parasitic wasps (natural enemies), until they emerge out as adults. In view of the relatively short duration of residual toxicity of  $\gamma$ -BHC under natural weather conditions,  $\gamma$ -BHC thus applied will not prevent the eggs deposited onto the lignified\* chestnut and/or other insect galls by the early-summer-emerging natural enemies from continuing to develop in the lignified galls, but will effect complete kill of the adult wasps emerging out of the galls, without distinction of host-pest and natural enemies, for about one week or so, and probably partial kill for at least one month. Accordingly,  $\gamma$ -BHC applied in this manner at the chestnut grove at least will satisfy the purpose of controlling the chestnut gall wasp and at the same time of positive conservation of natural enemies parasitic on the chestnut gall wasp. The integrated control of the chestnut gall wasp, viz. biological control combined with chemical one, which was recommended in the previous report (1959), has thus come to stand on a more reliable foundation which has been vindicated by the results obtained from the laboratory work.

It was pointed out that, in the case of application of  $\gamma$ -BHC to the chestnut trees in the chestnut grove or orchard, due caution should be exercised not to make  $\gamma$ -BHC accumulated in soil, since it is believed to be a root-absorbed poison persistent in soil

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\* The majority of the chestnut and/or other woody galls become browned to withering at the middle of August in Ina districts. This is evidence of the fact that they were already in a considerably advanced stage of lignification, i.e. death of cells, at the beginning of July.

for so long a time as to be almost incredible.

## GENERAL CONSIDERATIONS WITH SPECIAL REFERENCE TO BIOLOGICAL CONTROL

(Ecological Problems on the Interrelation between Host and Parasites)

### i) Host Parasite Relation of Emergence Period

As clarified in the previous report (1959), the parasitic wasps (resident natural enemies) preying on the chestnut gall wasp (host pest) emerge out of the host galls about 2 weeks earlier and complete their emergence about as many days earlier than the host pests do so, the first appearance of the former occurring regularly about 20th June. This host-parasite relation was kept substantially constant every year in Ina district for 5 years during which my investigations were carried out. From the ecological standpoint, it is a very interesting phenomenon worthy of note. To make the biological meaning of this phenomenon clearer is an important problem that underlies success or otherwise in the establishment of a proper time of biological control of this host pest by making the best use of this host-parasite interrelationship. This phenomenon seems to arise from the difference in various characters inherent in both wasps such as the number of generation, a mode of development of the overwintering larvae during autumn and winter, and a state of diapause during winter. As already clarified, most of the female parasitic wasps studied deposit their eggs, viz. their second generation progeny, on the same generation of host from which they themselves emerged, and probably are able to complete two generations, at least, on a single generation of the host. Consequently, the majority of the early-summer-emerging adult parasitic wasps must be their progeny which emerged from the eggs laid by their parent autumn-emerging adult females on the subsequent generation host (vide Fig. 1). Judging from such a mode of life cycle of the two, therefore, the host wasps may be taken to have been in a state of more or less slowed-down development or of somewhat deeper diapause than the wasps parasitic on them. If that is the case, the latter will continue further development more quickly than the former in the following spring when diapause is broken. As pointed out in the previous report, such a mode of host-parasite interrelationship may surely be of much benefit to the parasitic wasps which make twice separate attacks on the same host species. The host-parasite relationship somewhat similar to this was pointed out by Simmonds (1948) in the interrelation between the sugar beet webworm, *Loxostege sticticalis* L., and its ectoparasite, *Cryptus inornatus* Pratt, observed in North America. According to him, similar multi-attach on a generation of overwintering hosts occurs with the Ichneumonids, *Cryptus sexannulatus* Grav. and *Ephialtes caudatus* Ratz., parasites of the codling moth, *Cydia pomonella* L., in the South of France. At any rate, it is thought that probably such host-parasite relationship may be of stereotyped relation peculiar to a certain climate. To find out such host-parasite relation, if any, and trace its genesis to its origin will surely open the new way to positive encouragement as well as utilization of native natural enemies, and further to "integrated control" in the sense of complementary use of chemical and biological control, on which

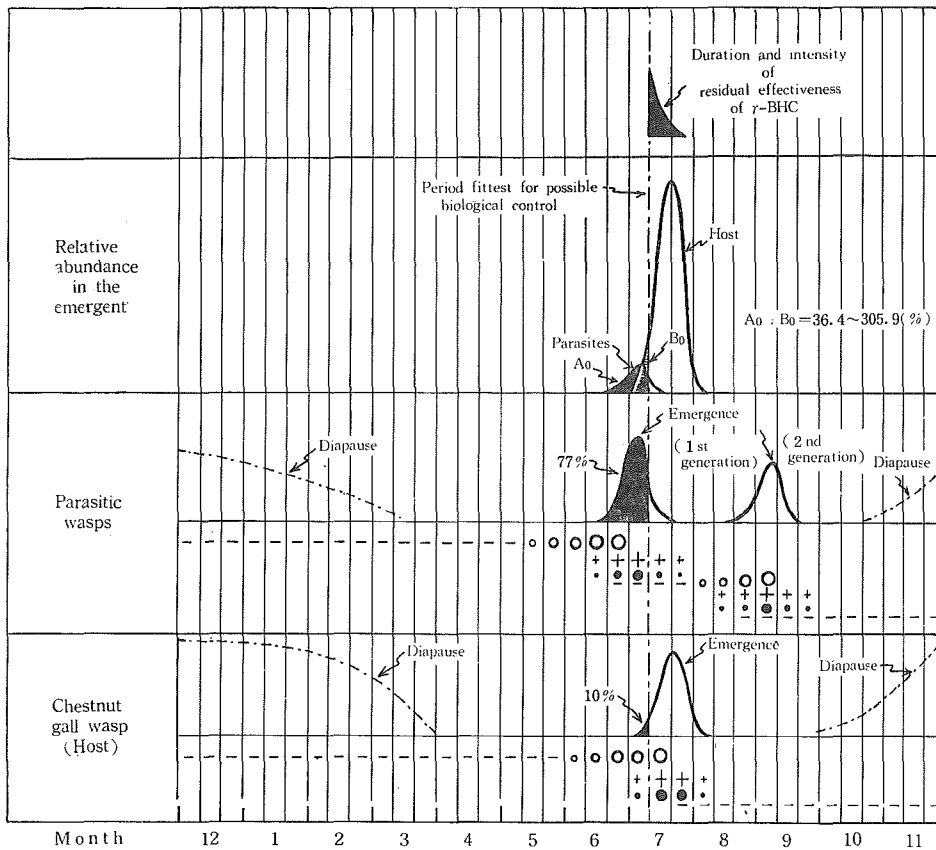


Figure I.

Schematic representation of the host-parasite relation between the chestnut gall wasp and its parasitic wasps. Diapause curves were drawn on the basis of both the state of defoliation and/or budding of the chestnut trees, and the emergence relation between host and parasites in Ina district.

Note:    { - : Larvae.    + : Imagoes.  
           { O : Pupae.    ● : Eggs.

leading ecologists' attention is now focused.

#### ii) Ecological Grounds for the recommended Control Measure

The essential point of the control measure of the chestnut gall wasp, which was recommended by me, lies in the following two: (1) To find out such a proper time as about 75% or so of the early-summer-emerging parasitic wasps emerge out and barely 10% or so of the host wasp do so by that time, viz. what I call the "period fittest for possible biological control." (2) To find out such a proper measure as the early-summer-emerging parasites or their eggs laid as their second generation can survive the measure as much as possible, and nearly all the host pests emerging subsequently, viz.



about 90% or so of all the emergent host pests, can be killed by the measure; in other words, the most rational control measure practicable in the "period fittest for possible biological control." As an emergency, or stop-gap means to suffice the second requisite, a single application of  $\gamma$ -BHC in that period has been recommended in addition to the method of plucking galls to be carried out simultaneously at that time. Hardly any people but Steiner (1938), a pioneer in this line, have hitherto reported the control measures like this. According to Dr. Yasumatsu (1955, 1956), Steiner's work runs as follows: In the control of the white apple leafhopper, *Typhlocyba pomaria* McAtee, he investigated fully the period of emergence and/or of oviposition of both the host pest and its natural enemies. On the basis of this result, he conducted insecticidal spray by the most effectual method, and further constructed a control schedule with the best care not to kill its natural enemies. By this method, he first effected considerable numbers of kill of the first-generation apple leafhoppers, and then left the control of the subsequent three generations of the host pest in the activity of the gradually increasing natural parasitic wasps, such as *Anagrus armatus* and *Aphelopus typhlocybae*, thus succeeding in 80-90 per cent. destroy of the host pest population. In this way, he established a precedence of success in the combined use of insecticide and natural enemies. Although quite resembles in its principle the control measure recommended by me, Steiner's method differs in the main point of operation from my one, as a natural consequence of the difference in the insect species dealt with.

Now, the essential feature of the control measure presented by me lies in to destroy the natural balance formerly existed between the population of the host and that of the parasites, and then to construct a new balance so as to hold the host population at a subeconomic level. For this purpose, by taking advantage of the host-parasite relation now existing, it was attempted to encourage the increase of the parasite population as much as possible and at the same time to repress the host population as much as possible. Success or otherwise in this attempt depends upon whether or not the increase in the parasite population really produces the large-scale reduction in the host population. According to the present investigation (vide Table X in the previous report in 1959), it was found that the relative increase in the host population did never result in the same-rate relative increase in the parasite population, but the relation between the rates of relative increase for the two fluctuates to a considerable extent. This means that the host population is simply related to the parasite populations in such a manner as what Milne (1957, 1959) calls "imperfect density dependence". In fact, it is almost impossible to believe that the density of the former depends perfectly upon that of the latter, if the term "perfect density dependence" means such an "exact linear (or curvilinear) relationship" between the two as is maintained by Milne. If so, is it impossible to expect successful control by my method? The answer is "No." As is well known for long, Salt (1936; from Allee et al., 1950) observed that, in the experimental study on the host-parasite interaction concerning the host moth, *Sitotroga cerealella*, and its parasitic Chalcid hymenopteran, *Trichogramma evanescens*, "as the density of parasites in a fixed host population is increased, the number of hosts that escape steadily decreases, that of hosts that die steadily increasing,

although even at high densities of parasites some hosts occasionally escape." Surely, Salt's result was obtained under known controlled experimental conditions. But, probably, nearly similar interaction to this will be expected in Nature; for, it is believed that, in the words after Thompson's expression (1939; from Allee et al., 1950), the greater the relative abundance of the parasite becomes, the more likely is the attack by the parasite to increase, since the essential character of the parasite is that it increases at the expense of its own host. Actually, in his study on the natural control of Florida red scale on citrus, Muma (1959) has recently reported that evaluation studies on a statewide sample basis have demonstrated a decided negative relationship between relative abundance of a certain parasite and/or rates of parasitism, and intensity of scale infestations. Pickett (1959) also points out that there is evidence that several general predators are effective against the eye-spotted bud moth, the most serious pest to apples in Nova Scotia, "especially at low-prey densities." Surely, these findings must be the foundation of so-called "integrated control" which covers control by means of "the artificial multiplication of native or established parasites and predators for mass liberation at a time when they are at a low population level in the field," although "more detailed investigation may be needed" (Simmonds, 1959). In like manner, such a host-parasite relation would also be accountable in part for Dr. Yasumatsu's spectacular success in the natural control of a famous scale pest on citrus, *Ceroplastes rubens*, by means of the interareal transfer of its most effective control agent, *Anicetus beneficus*, from areas of declining pest population to those of increasing pest population (Yasumatsu, 1956). Another question may be raised as to the intraspecific competition within parasite population, which supervenes upon both increase in its own population and paucity of host population. In fact, Salt (1936) observed that, under experimental conditions, "the number of parasite progeny reaches a maximum and then decreases, and that of progeny of the individual parasites steadily decreases" (Allee et al., 1950), which is indicative of intraspecific competition within its own population for food and space. Similarly, Ullyett (1953) also experimented with *Ephestia* population under controlled laboratory conditions, and found that "the spatio-temporal increase in the larval population automatically produces a compensatory reduction in the number of progeny in the succeeding generation," and that "the reduction in progeny becomes progressively greater as the population grows." Such natural regulation or compensatory action will surely occur to more or less extent in Nature. But it, if any, will hold true equally both parts of parasite and host population, and sufficient time will be necessary for it to be effectual. Even if the parasite population should increase to such a level as to attack the entire members of its host population, its members will surely scatter as a natural result of continual dispersion or migration which normally supervenes on host-searching activities in Nature. Especially so they do, since the parasitic wasps under study are originally native in this district, formerly parasitizing on various gall insects inhabiting this district. If the parasite population should reach its maximum level, if any, by virtue of its positive encouragement, one need no more worry about large-scale quick reduction in its population caused by such natural regulation than one cannot expect too much the similar result in host population in Nature. Paucity of host population may be rather a question in

our case; for, barely 10 per cent. or so of the host population can survive the control operation to be taken at what I call the "period fittest for possible biological control," only if it is proper, and further the host survivors are a little larger or far smaller in number than those of its parasitic wasps, so far as they are estimated from samples secured for the past five years (vide Table IX-a, Torii, 1959). As Ullyett (1953) says, the presence of an adequate host (prey) population is obviously an essential prerequisite for the developing parasites in the environment. The word "adequate" may be applied not only to "species" but also to "number." On the other hand, in Thompson's words (1929), the parasitic wasps are also thought to "require for their continued existence and propagation, not merely the presence of certain hosts, but also a certain definite complex of environmental conditions quite independent of the host." In other words, parasites in general, being an imperfectly density dependent control agent, may originally be "not [only] a damping [of the upward component] but [also] an enhancing of the downward component of fluctuation" in their biotic environment, since they "must always remove some individuals from the host (prey) population in order to permit the continued existence of their own population" (Milne, 1957). If so, in our case, too, there is almost no danger of the parasite population becoming extinct as a result of eradication of the host population, however severely the parasite members may attack comparatively small number of the host members outliving the control measure. Especially so, since there are usually some omissions in the operation of control measure, however rationally the control measure may be contrived. Probably, the host pests barely survive the control operation as well as the attack by its natural enemies, but nevertheless will be held far below economic levels for long. Truly, complete extinction of pest population is not an ultimate goal of natural control but rather an antithesis of control.

In the previous report (Torii, 1959), I concluded, on the basis of hypothetical host-parasite situation applied to empirical data, that an adequate control measure combined with positive encouragement of parasitic wasps concerned would result in speedy extinction of the host (pest) population. This is, of course, an inference on the hypothesis that the control measure was effected with favorable success. In Nature, however, it will be inevitable that the measure would fail to cover all the sources of the pest population in its fullest, such as the chestnut galls on the trees grown scatteredly in fields, valleys, and untrodden mountains, no matter how carefully it may be carried out. Consequently, evaluation of such an ideal result as mentioned is only a reasoning on semi-empirical hypothesis to show the effectiveness to be brought about by it. Needless to say, what is aimed at is not literal extinction of the pest population but natural or biological control. "Control" here meant is not complete extinctive control of the pest population but control occurring at subeconomic levels, or to restrict its increase below economic levels. In the phrase after Milne's (1957), the prime object of the present studies is to add successfully lethal factors, acting in concert, to an existing lethal or hindering factor as much as possible. Milne (1957) maintains that "in no case has it yet been proved that several different kinds of enemy, acting in concert, can control an insect species at economic levels (or any other levels),"

and further adds that there are many evidences of occurrence of "devastating outbreaks despite formidable arrays of parasites, predators, and pathogen—sometimes amounting to dozens—attacking a single species." To be sure, such may happen sometimes in Nature. But nevertheless, I can not necessarily agree with his opinion in its entirety; for, such may be the case when no effort is made at all to upset the natural balance formerly existed to the augmentation of the restrictive action of any one or all of the natural enemies upon the increase of the pest population. As is generally accepted, natural enemies show a strong tendency to density dependence, apart from the question of "perfect" or "imperfect." What is most essential, therefore, is to find out the way to utilize natural enemies, native or introduced, most effectively. Of course, it does not necessarily mean the utilization of natural enemies alone, but of every possible means. Now, the fact that the density of pest population is higher than that of parasite population may be attributed partly to higher degree in biotic potential on the part of the pest and partly to optimum environmental factors favorable for the increase of the pest population. It is usually very difficult, however, to alter the nature of biotic potential of any insect. Herein lies the importance of utilization of native natural enemies by means of their positive encouragement; to make in this manner the existing environmental factors departed in different direction from the optimum suited for the increase of the pest population is the method that stands to providence of Nature.

*On the Mathematical Prediction of the "Period fittest for possible biological Control"*

In the previous report, it was shown that, on the basis of the trend of cumulative percentage emergence of the composite parasitic wasps, the "period fittest for possible biological control" can be predicted statistically from logit 75 per cent. date. Evidence is clear that this is quite accurate and precise to answer the purpose of our practical use. But there is some apprehension of its practical use being avoided from some prejudice against mathematical methods involved. Indeed, we, economic entomologists, should always bear in mind the fact that "we all know of good control methods which have been ineffective in reducing the problems because they were unacceptable to the farmer" (Smith and Hagen, 1959). The following method of prediction, though somewhat rough in its nature, may be more manageable and "biological" in the opposite sense to "mathematical": Firstly, field collections are made of the chestnut galls amounting to about 500 g. or so on 15-20th June (in Ina district), and then the collected galls are kept in an indoor emergence trap. The indoor emergence trap is a proper-sized, well-ventilated box with one side fitted with a pane, or otherwise five or so paper bags will be substituted for it, instead. Care should be exercised not to rot the galls. Secondly, counts are taken of the numbers of the emergents, inclusive of the chestnut gall wasp and its parasites, every day or at intervals of one day at least. Then, the date of the first appearance of both the parasite and the chestnut gall wasp is recorded. In this case, the term "first" in the "first appearance" does not necessarily signify the literal "first," but a proper initial time in the period when the continued, not-intermittent, emergence of the wasps is observed. Finally, either the date about 20 days after the first appearance of the parasite or the date about 10 days after the first appearance of the host wasp is deter-

mined to be the "period fittest for possible biological control." The above steps are all necessary for our purpose. It is noted in this connection that, if the records of the emergence relation between the host and the parasites are kept every year through the above process, this prediction will be all the more precise. To make this prediction more reliable, we should determine the area to which this is applied, because such a host-parasite relation is usually apt to be subject to various environmental factors peculiar to each locality. Furthermore, attention should be paid to the fluctuations of the weather conditions during the period from the first appearance to the date predicted; for, as is usual with the other predictions, this predictive method is also founded upon the premise that all the factors concerned are kept normally without fluctuating so widely from the fluctuations formerly shown.

#### *On the Weather Regulation of an Introduced Key Parasite*

As already discussed in detail in the previous report (1959), severe frost damage which is a frequent occurrence in late spring in Ina district seems to be greatly responsible for the propagation and permanent establishment of an introduced key natural enemy, *Torymus beneficus* Yasumatsu, parasitic on the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu. Further discussion needs to be made on this subject from some newly acquired references. As regards the importance of weather regulation in the domain of biological control, many reports have been made public since Howard and Fiske (1911; from Allee et al., 1950) pointed it out. According to them, climatic or weather regulation is referred to as "catastrophic" factors, one of the two large categories into which all the natural causes of mortality in insect population are divided. It also belongs to what Smith (1935) calls "density independent" mortality factors. It comprises "largely the physico-chemical aspects of the environment" and those "intrinsic defects in adaptation characteristic of the species" (Thompson, 1939; from Allee et al., 1950). In the present time, too, not a few reports have been published which attach importance to the role of climatic or weather factors. In the biological control of the spotted alfalfa aphid, *Therioaphis maculata*, in three distinct climatic areas in southern California, Bosch et al. (1959) utilized three different imported parasites, and found that these three parasites showed a tendency to varying climatic adaptation. Their conclusion rests on the following findings: The first species was found to be dominant at 2 different areas A and B where winters are considerably colder than at an area C and the species overwintered in diapause. The second species without an ability to pass winter in diapause was found to be dominant at another area C where is under the mild winter conditions. And the third species was found to be fairly abundant at only an area A. Further they add that there appear to be two important situations that adversely influenced the potential effectiveness of the introduced parasites, the first of which, the periodic mowing of the alfalfa, apparently having an extremely destructive effect on the parasite populations. This viewpoint may be compared to my one that propagation as well as establishment of the introduced key parasitic wasp, *T. beneficus* must have been affected severely by the large-scale felling down of the chestnut trees grown in the grove where this species was liberated (vide Torii, 1959). They attach importance to the role of the alfalfa as a shelter for the imported parasites, while I do so

to the chestnut gall as the very object of their oviposition. Concerning the parasites attacking Florida red scale, Muma (1959) points out that, among factors influencing the rate of parasitism, low temperature has a striking effect on it as a climatic factor. According to him, the confinement of adult parasites at 40° F. for 12 hours resulted in nearly 100 % mortality; following cold defoliating winds, adult parasites were strikingly reduced in the grove under observation. From the field data secured during the winter 1957-58 of freezing temperature, he further found that parasite numbers were strikingly lower in the postfreeze samples, and 15 to 40 % of the parasite mortality can probably be attributed directly to the freezing temperatures. Thus he concluded that low temperatures and insecticides all will reduce parasitism, appearing to be most critical, with low temperature seeming to have the greatest influence. Pickett (1959) also pointed out that, "contrary to popular belief, exceptionally low temperatures or other abnormal weather fluctuations may favor the pest species by destroying or retarding the development of beneficial species." In the case of *T. beneficus* introduction into Ina district, too, there are much apprehensions that, due to "catastrophically" mortal factors such as heavy frost late in spring, its population may be subject to drastic restrictions which result in wholesale inhibition of a population increase. This inference may be understood still more, if it is taken into consideration the fact that heavy frost late in spring occurs just at the time when the introduced adult parasites are active in host-seeking and oviposition, as previously pointed out.

In the meanwhile, there is no doubt that such "catastrophic" or climatic factors would likewise inflict adverse effect on the host (pest) population. Nevertheless, it would be expecting too much to rely on such factors alone in the control of the pest population, as already pointed out by me (1956). The reason for this is as follows: As aptly pointed out by Nicholson and Baily (1935; from Ullyett, 1953), such catastrophic factors, being density independent, however severe they may be, normally destroy only a particular fraction of the pest population and do so independently of its density, no matter how large it may be in number. And further it is quite impossible for us to utilize such factors at our disposal.

#### *On the Importance of Integrated Control Program*

Recently economic entomologists' attention has been focused on the problem of combined use of insecticides and natural enemies. The statement that "the development of integrated control programs opens new horizons for the utilization of natural and biological controls" is the one concluded by Smith and Hagen (1959) in their recent thesis "Integrated Control Programs in the Future of Biological Control." "Integrated control" refers to "the complementary use of chemical and biological control," where the best use is to be made of "naturally occurring biological control as well as biological control effected by manipulated or introduced biotic agents." They emphasize that "to insure success in the battle with the insects, an ecological or comprehensive approach to control is necessary, which does not depend entirely on biological or cultural controls nor does it exclude chemical control." Surely, either of them alone can never be a panacea for all problems on insect control; for, "our agricultural field and orchards are not works of Mother Nature but are highly artificial man-made "factories" which

bring about the drastic alteration of the natural balances that once existed." Probably, it would not always bring us successful results to rely upon either of them alone, since the pests to be controlled are not always one species alone. There are, indeed, some exceptions to this. Control of forest insects is for the most part the case in point. In the forest which is relatively less affected by artificial factors, maintaining greatly its original primitive conditions, the most use may be made of natural control to establish new balances in our favor. Now, viewed in this manner, the present study, inclusive of the previous studies (1959), may surely be said to be an approach belonging to the category, "integrated control." Herein lies another importance of the present study, although evidence for effective control in the field needs to be secured.

According to Smith and Hagen, integrated control does not simply mean to make full use of biological control but also to utilize chemical control where necessary and in a manner that is least destructive to the biological control. And further, its program required for the control of permanent pests must be contrived not to disrupt natural control of potential and intermittent pests. As is well known by economic entomologists, potential pests usually tend to increase only if conditions are favorable, and fluctuate in numbers, becoming high at a certain time, or low at other times as the activities of the existing natural control agents fluctuate. Therefore, it would be better for us to leave the suppression of such potential pests in the action of existing natural control agents. Chemical controls are often more harmful against such natural control agents than are so against the permanent pests, resulting in the production of potential pests to very significant pests. Non-selectively toxic chemicals should be used only at the times and where natural or biological control is inadequate, if any natural or biological control is present. As has been increasingly realized of late, the continuous use of chemical methods entails a number of deleterious side effects (Kato, 1953, 1955; Hueck, 1953, Glick and Lattimore, 1954; Kobayashi and Yoshimeki, 1955; Yasumatsu, 1955 et seq.; Goodarzy and Davis, 1958; Fenton, 1959; Fukushima, 1959 et seq.; Klostermeyer, 1959; Oatman, 1959; Pickett, 1959; Simmonds, 1959; Smith and Hagen, 1959). Among very complicated side effects, the following three, *inter alia*, are the major problems to which our special attention should be paid: The first is that non-selectively toxic pesticides would exert a very harmful influence upon the natural or biological control existing in an individual area. The second is that the routine repetitive applications of widely toxic pesticides against arthropod pests frequently create the development of pesticide-resistant strains, as a natural consequence of a long-term influence of pesticides upon the pest population. The third one which is liable to be overlooked but nevertheless very important and highly economic is that the other pests that formerly were insignificant are apt to be staged anew as serious pests, as has been emphasized repeatedly by Dr. Yasumatsu and some others in Japan, too (Yasumatsu, 1955 et seq.). On the other hand, natural or biological control, too, can not always be a panacea for all the pest-controls. For instance, as already clarified by Morris et al. (1956) in their very intensive and long-term studies on the population dynamics of spruce budworm in eastern Canada, sometimes any parasites suffer some unknown limitation and appear to be incapable of overtaking their host in the case where environmental conditions are

favorable for the host to rapid population development, although they respond to host density, in a delayed manner, and are capable of exerting control in the case where the increase in host population is not too rapid or sustained. And according to Varley and Edwards (1957), the number of hosts attacked by each individual of their parasitic Chalcid wasp, *Mormoniella vitripennis*, was independent of host density at high host population density. These findings clearly indicate that, in case of a large-scale outbreak of host, it will be a fairly long time before any parasite has a noticeable effect on it. This situation will often be a menace to farmers and growers. From economic standpoint, the above-mentioned defects inherent in both chemical and biological control need to be covered up by any means. It is just integrated chemical and biological control that makes us cope with these situations. Needless to say, to make chemical control compatible with biological one, it is necessary to have precise knowledge of both pests, their natural enemies, and the influence or potential influence of the pests on the plants. In the studies conducted by me, the prerequisite to integrated control may be said to be satisfied to a considerable extent, so far as laboratory experiments are concerned. The success, if any, lies in the following two: The first is that the period of emergence for the host wasp was revealed to be separated temporally from that for its parasitic wasps. The second is that the insecticide used,  $\gamma$ -BHC, already in use among some growers, was evidenced luckily to suffice our purpose by our laboratory experiments; namely, that  $\gamma$ -BHC, though its systemic action undeniable, appears to be almost incapable of penetrating into the cavities inside the chestnut gall which are occupied by both the larvae (or pupae) of the chestnut gall wasp and the larvae (or eggs) of its parasitic wasps, at least at the "period fittest for possible biological control." Stern et al. (1958; from van den Bosch et al., 1959) found that cocooned parasites can survive insecticidal treatment with even the most toxic phosphate material. From this, van den Bosch et al. (1959) discussed as follows: "Thus, if materials were to be applied when the parasites were largely in the cocoon stage and residues did not exceed several days' duration, chemical treatment might actually shift the parasite-host ratio greatly in favor of the former. Under this condition aphids surviving insecticidal treatment would be subject to heavy parasite attack and therefore greatly hindered in their ability to cause rapid reinfestation of the treated fields." This inference is just to the point and just in accord with what I have laid special emphasis.

One of the prime requirements of integrated control is, of course, the presence of natural control agents capable of checking the pests at subeconomic levels all or at least part of the time. Natural control agents, however, do not necessarily mean so-called effective or key natural enemies alone. From the angle of integrated control, importance should be attached to inconspicuous native parasites rather than the former. The latter play an important role in checking potential and/or intermittent pests below economic levels. In Smith and Hagen's phrase, "it is with such types of pests that integrated control is specially valuable." Needless to say, even with permanent pests, the control aided by natural control agents, inclusive of inconspicuous ones, may be significant in reducing the degree of damage, as is pointed out by Smith and Hagen, too. In this sense, it may be said, so to speak, to have adopted a prudent policy that, in the



previous studies, importance was attached to all the detected native parasitic wasps of ten species, inclusive of dominant and inconspicuous species, *in toto*, as an object of positive encouragement and utilization for the control of the pest wasp.

Those which satisfy the other requirements of integrated control are timing of pesticide applications to avoid susceptible stages of natural enemies, elucidation of the nature of insecticides such as selective or otherwise, and establishment of the minimum dosages required to control pests. However, "the insecticide itself may be selective in its toxicological action." [A particular insecticide] "may be selective at low dosages, but not at high dosages." And further "proper timing of insecticides can produce a selective action on the pest-parasite complex" (Smith and Hagen). From the standpoint of integrated control, therefore, any insecticide, even being a wide-range, non-selective toxicant, may in some cases satisfy these three requirements at one time, only if it is properly used and it has no, or little, residual action. Viewed in this light, the application of  $\gamma$ -BHC in a manner that prevents the native parasites from being killed on a large scale as recommended by me in the biological control of the chestnut gall wasp may surely be said to have sufficed these requirements of integrated control at a time. At any rate, chemical control should be utilized in a selective manner or as an emergency or stop-gap means as much as possible, as emphasized in the previous report (Torii, 1959). It should be used only when the pest population became economic or threatened to be economic, and according to an integrated control program; when the pest population became subeconomic, it should be withheld as soon as possible.

In the previous studies (1959), it was revealed from laboratory experiments that various native parasitic wasps preying on the chestnut gall wasp lay their eggs as their second generation onto various insect galls formed on various scrubs or herbaceous plants such as mugwort, oak and *Quercus acutissima* seedling. The systemic nature of  $\gamma$ -BHC against these insect galls were also found to be substantially insignificant. From the angle of integrated control, therefore, scrubby groves, shrubs, hedges, and headlands grown with herbaceous plants such as mugwort at the bordered area of the chestnut orchard or grove had better be preserved as much as possible; for, such vegetations act as preserves and provide these parasitic wasps with the objects of oviposition. On the importance of the careful investigation of ecosystem at the bordered areas, recently stress has been laid by Yasumatsu (1959) and Pickett (1959) from their unique standpoint, respectively.

## SUMMARY

As a supplement to the previous studies on the biological control of the chestnut gall wasp, *Dryocosmus kuriphilus* Yasumatsu, laboratory experiments were conducted on penetration and translocation of radioactive  $\gamma$ -BHC-1-C<sup>14</sup> topically applied to various freshly cut plant species of five, inclusive of herbaceous and woody plants with insect galls, which were held individually in flasks containing water. All the plants employed in this experiment were those with insect galls onto which the early-summer-emerging parasitic wasps preying on the chestnut gall wasp deposited their eggs as their second generation.

Macro-radioautographs (autograph time: 162 days) revealed that the radioactive carbon C<sup>14</sup> in  $\gamma$ -BHC-1-C<sup>14</sup> was translocated from the treated portion through the plant examined almost systemically, with the highest concentration at the treated portion. A herbaceous plant, mugwort, showed faint but clearly noticeable distribution of radioactive C<sup>14</sup> in almost entire part of the body. The degree of translocation of radioactive carbon in the woody plants varied in a measure with the plant species as well as the treated portions. Hardly any discernible concentration of radioactivity at the treated portion nor any appreciable translocation through the entire body was revealed when the foliage leaf of the woody plant was treated. A little stronger tenacity of radioactivity was recognized at the under surface of a leaf. When applied to a certain portion of the stem, without distinction of herbaceous or woody plants, radioactivity was highest in concentration at the treated portion, with moderate translocation to the other untreated portions, especially with considerably strong accumulation at the galls formed on both the upper and lower portion of the treated stem, indicating upward and downward translocation of radioactive carbon. The maximum radioactivity was revealed at the callus on the treated woody stem. A treated flush terminal shoot gave a weak but clearly outlined picture on the X-ray film, showing a moderate concentration of radioactivity at the galls formed on the lower stem. Topical application to galls resulted in the highest concentration of radioactivity at those portions, with substantially no translocation to the other untreated portions. On the basis of both the difference in the structure of the vascular bundle of the stem between herbaceous plant and woody one, and a high concentration of radioactivity at abnormally growing tissues, viz. galls and the callus, indicative of downward as well as upward translocation, it was concluded that the bast part (the phloem) seems to be mainly responsible for absorption and translocation of  $\gamma$ -BHC in the plant tissues used. Putting this interpretation as well as the structural character of insect galls together with the previous finding (1959) that, when applied to the chestnut galls at dosages of common use,  $\gamma$ -BHC penetrated barely into the depth of about 0.5 mm. from the surface of the gall, we may say that  $\gamma$ -BHC application at dosages of common use seems to exert hardly any harmful influences upon the insects (eggs, larvae, or pupae of both host and parasites) living inside the lignified cavity of the gall, in so far as it is used at the "period fittest for possible biological control." On the basis of this interpretation as well as the relatively short duration of its residual effectiveness, it

was concluded that, when applied to at the "period fittest for possible biological control" at proper dosages,  $\gamma$ -BHC will be capable of killing the greater part of the chestnut gall wasps emerging in its almost entirety within the subsequent about 2 weeks, and besides exert hardly any adverse effect on the eggs deposited by the early-summer-emerging parasitic wasps as their second generation onto various kinds of insect galls. Accordingly, even  $\gamma$ -BHC allows compatibility of its own non-selective action with the positive encouragement and utilization of natural enemies, only if its application is proper. This implies that even  $\gamma$ -BHC, a powerful and comparatively wide-range toxicant, can be bestowed with, so to speak, temporally selective nature by taking advantage of the time discrepancy in the emergence and or oviposition period between host and parasites. Such is just an answer to one of the requirements of "integrated control."

As a supplement to the previous studies on the biological control of the chestnut gall wasp, detailed discussion was made from the ecological standpoint.

Temporal discrepancy in the emergence period between host and parasitic wasps may be ascribed to the difference in depth of diapause and/or growth rate during autumn and winter. The fact that the parasites emerge by about 2 weeks earlier than the host may surely favor their host-searching activity, because the former have at least 2 generations a year, while the latter is a single-brooded species.

Native parasitic wasps, inclusive of comparatively dominant one and otherwise, were considered to be what Milne calls "imperfectly density dependent"; for, the fluctuations in their yearly relative abundance were not "perfectly" proportional to those of the host, showing a more or less wavy trend. Consequently, even in case when the parasite population increases greatly in density and at the same time the host population is suppressed to such an extent as to be incomparable with the former, there will be almost no risk of the former being affected practically by the paucity of the latter, and the rate of parasitism will be increased all the more in Nature. This is the reason why the pest population may be prevented from breaking out on a large scale and can be checked at subeconomic levels almost continuously by the positive encouragement and utilization of native natural enemies. Otherwise, the increase in population density of the native parasitic wasps investigated will probably be almost incapable of overtaking that on the part of the permanent host (pest) population under natural conditions. The period most suited for performing such an operation as to make the native parasite populations capable of surpassing the host population is what I call the "period fittest for possible biological control."

In view of the fact that the statistical prediction of the "period fittest for possible biological control" requires somewhat mathematical training, the more manageable method has been recommended. The period in question will fall on the date about 20 days after the first appearance of the early-summer-emerging parasitic wasps, or otherwise that about 10 days after the first appearance of the host, the chestnut gall wasp. In Ina district, the "first appearance" will be determined by the state of continuous, not intermittent, emergence of the insects out of the chestnut galls collected at the grove or orchard on 15-20th June.

After due consideration of the newly acquired literature, the previous inference has

been reaffirmed that whether or not the key parasitic wasp, *Torymus beneficus*, introduced into Ina district may propagate vigorously and be established permanently depends upon the weather conditions in late spring, especially upon the severity of heavy frost that happens to fall late in spring when the wasp is just active in host-seeking and oviposition at the liberated site.

With the elucidation of the "period fittest for possible biological control" as well as making the action of  $\gamma$ -BHC temporally selective by taking advantage of the temporal discrepancy in the period of emergence and/or oviposition between host and native parasitic wasps, a new horizon has been opened for the "integrated control" of the chestnut gall wasp in the sense of the complementary use of chemical and biological control.

On the basis of the ecological distribution of the plants with various insect galls onto which the native parasitic wasps lay their eggs as their second generation, the importance has been pointed out of the preservation of such plant species at the bordered areas near the chestnut grove or orchard.

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## **EXPLANATION OF PLATES**

#### EXPLANATION OF PLATE I

- A: Wild willow twig. Figures 1, 2, and 3 indicate the three dissected portions of one intact twig; namely, the lowermost, the middle, and the uppermost portion in this order, respectively.
- B: Mugwort. Each specimen represents a different sample treated in a different manner, respectively.
- C: Foliage leaves of oak twig.
- D: Burry gall on oak twig.
- E: From the top to the bottom: Oak knot galls; Foliage leaves of oak twig; Oak scale galls.
- F: Chestnut twig with chestnut galls.
- Note: 1) A white circle and an arrow indicate the portion to which  $\gamma$ -BHC was applied topically.
- 2) S: Surface of a leaf; U: Under surface of a leaf.
- 3) Radioautograph time: 162 days.





EXPLANATION OF PLATE II.

- G: Chestnut flush terminal shoot with chestnut galls. Con: RI-free control sample specimen.
- H: Chestnut flush foliage leaf.
- I: Chestnut twig with chestnut galls.  
Ca: Callus on the stem.
- J: *Quercus acutissima* twig with knot galls (n) caused by the Gelechiid moth, *Stenolechia querci* Shin, (Upper); Abnormal leaves issuing in clusters from the *Q. acutissima* gall (g) (Lower).
- K: Oak twig with knot galls caused by a kind of leaf roller moth, *Pelataea bicolor* Walsingham (n).
- L: *Q. acutissima* gall with abnormal leaves issuing in clusters.
- Note: As regards the marks, see Note in Plate I.

