The Effect of Colony Size upon the Survival of Larvae of the Southern Green Stink Bug, *Nezara viridula*

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**INTRODUCTION**

Generally, pentatomids lay eggs in a batch and newly hatched larvae stay on their egg shells during the first instar. The young larvae have a strong tendency to form aggregation, though this habit diminishes as development advances. The southern green stink bug, *Nezara viridula*, provides a suitable material to study the meaning of its colonial life, because of its large size of an egg mass, e.g. 70–100 eggs per egg mass on the one hand. Its larval colony in the field, on the other hand, is easily noticeable with rapid determination of the size, for example, in terms of the ear of the rice plant. The colony of the larvae can be recognized up to the third instar inclusive, but it is not infrequent to see the larval colony composed of the fourth instar larvae when food is favourable in quality and undisturbed. The fifth instar or the final stage obviously has no tendency to form group and shows even an antagonistic behaviour when two individuals meet on a ear of the rice plant.

The larvae of the fourth and the fifth instars show a considerable degree of colour variation from green to entire black, while the abdomen of the black type turns to bright scarlet and the green type to light green.

Life tables of the southern green stink bug have shown that a heavy loss occurred in the early stages amounting to 80–90 per cent mortality up to the third instar inclusive (KIRITANI and HOKYO, 1962). Furthermore, HOKYO and KIRITANI (1963) claimed that mortality factors work upon the colony as a whole rather than upon each individual unit causing a total loss of the colony. In the present paper, only the results obtained from the laboratory experiments were dealt with in regard to the effects of aggregation upon the developmental rate and mortality. The ensuing experiment on the adult obtained from the present study will be reported in the future.

**MATERIAL AND METHOD**

Experiments were replicated seven times using the egg masses of the first and the third generations from April to December, in 1962. The details of the sources of the experimental materials and the rearing conditions are shown in Table 1. Each experiment was set up with the eggs taken from one batch of eggs within 24 hrs. after oviposition. One egg mass divided into the groups, 1, 2, 5, and 10 eggs with replications of 10, 5, 2, and 1, respectively. Rearing was carried out at 25°C under the 15 hrs. illumination. The larvae were reared up to the third instar in a glass container 8.5 cm in diametre and 1.5 cm high and from the fourth instar in a glass container of 11.5 cm in diametre and 7.5 cm high. The larval food was changed with intervals of two days and four days with the leaves of the potato plant and the pods of the common haricot beans, respectively. To keep the rearing number of individuals of each set constant throughout the experiment, dead individuals were

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Table 1. Design of the experiment and the number of adults emerged.

<table>
<thead>
<tr>
<th>Sources of parents</th>
<th>Generation of progeny</th>
<th>Starting date of experiment</th>
<th>Date of oviposition</th>
<th>No. of 1st instar</th>
<th>No. of emerged adults</th>
<th>Group size</th>
<th>Rearing condition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>at 25°C on potato plant until 3rd instar and on pods of common haricot beans thereafter.</td>
</tr>
<tr>
<td>Hibernal-cula</td>
<td>1st</td>
<td>23, iii</td>
<td>29, iv</td>
<td>40</td>
<td>M 2 7 3 1</td>
<td>F 3 2 3 1</td>
<td></td>
</tr>
<tr>
<td>Standing wheat</td>
<td>1st</td>
<td>2, iv</td>
<td>6, v</td>
<td>40</td>
<td>M — 2 3 5</td>
<td>F 1 2 2 4</td>
<td></td>
</tr>
<tr>
<td>Hibernal-cula</td>
<td>1st</td>
<td>23, iii</td>
<td>12, vi</td>
<td>40</td>
<td>M 2 2 3 3</td>
<td>F 1 2 2 3</td>
<td></td>
</tr>
<tr>
<td>Standing wheat</td>
<td>1st</td>
<td>2, iv</td>
<td>20, vi</td>
<td>40</td>
<td>M — 5 1 4</td>
<td>F 5 4 3 2</td>
<td></td>
</tr>
<tr>
<td>Field cage in paddy field</td>
<td>3rd</td>
<td>—</td>
<td>31, ix</td>
<td>40</td>
<td>M 1 2 3 6</td>
<td>F 2 5 2 4</td>
<td></td>
</tr>
<tr>
<td>Ditto</td>
<td>3rd</td>
<td>—</td>
<td>2, x</td>
<td>40</td>
<td>M 5 7 3 5</td>
<td>F 4 3 7 5</td>
<td></td>
</tr>
</tbody>
</table>

| Soy beans field    | 3rd                   | —                          | 22, x               | 40               | M 5 5 4 6            | F 5 3 6 4  |                  |

replaced by the same number of larvae obtained from the spare sets of the same size group. The fresh weight of adults was weighed at their emergence. The synchronism of hatching in different egg groups was observed at intervals of 2 hrs. until completion of hatching.

In order to know the actual size of the larval colony under natural conditions, censuses were carried out for the second generation in 1962 in the paddy field of the early planting rice. As mentioned above, the larvae of the first instar remain on the egg shells forming dense aggregation, and the onset of the feeding behaviour of the second instar eventually leads more or less to the disintegration of the original colony by individual movement and by extrinsic factors such as wind. Then the feeding groups of larvae are established individually on each ear of the rice plant. Since each rice plant was composed of about 20 ears, the number of larvae was determined in terms of the number per ear for the second and the third instars. Thereafter, only the number of larvae per rice stubble was examined.

Colour variation of the larvae was divided into eight types according to KOBAYASHI (1959) as type A to H in the order of decreasing darkness. In order to simplify the interpretation of the data, an index of degree of colouration (I.C.) was calculated by giving numerals 1 to 8 for the types of A to H, respectively. Then,

$$I.C. = \frac{1n_1 + 2n_2 + \cdots + 8n_8}{N}$$

where $N=n_1+n_2+\cdots+n_8$, and $n_1$, $n_2$, ..., and $n_8$ indicate respectively the number of individuals of each colour type.

**THE SIZE OF A LARVAL COLONY UNDER NATURAL CONDITIONS**

The results of the rearing experiment obtained from the laboratory with regard to the population density are often misleading without the knowledge of the actual colony size under natural conditions. The first instar larvae when hatched remain on the egg shells forming a dense aggregation throughout the stage without feeding. After the first moulting to the second instar, larvae begin to feed on the limited number of the ears of the rice plant. The results obtained from the paddy field with regard to the colony size for the second generation of *Nezara* were represented in Table 2. The mean size
Table 2. Colony size of the larvae in the paddy field in terms of per ear (A) and per stubble (B). The second generation in 1962.

<table>
<thead>
<tr>
<th>Instar and stage</th>
<th>No. of colony observed</th>
<th>Colony size</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Larvae per ear*</td>
<td>More than two larvae per ear</td>
</tr>
<tr>
<td>2nd early</td>
<td>140</td>
<td>9.65</td>
<td>14.46</td>
</tr>
<tr>
<td>2nd late</td>
<td>172</td>
<td>9.87</td>
<td>12.62</td>
</tr>
<tr>
<td>3rd early</td>
<td>39</td>
<td>4.87</td>
<td>6.03</td>
</tr>
<tr>
<td>3rd late</td>
<td>167</td>
<td>4.75</td>
<td>6.86</td>
</tr>
</tbody>
</table>

* One larva per ear inclusive.

<table>
<thead>
<tr>
<th>Instar</th>
<th>No. of stubbles observed**</th>
<th>Larvae per stubble</th>
<th>% stubbles which supported more than two larvae</th>
<th>Maximum no. of larvae per stubble</th>
</tr>
</thead>
<tbody>
<tr>
<td>2nd</td>
<td>461</td>
<td>14.96</td>
<td>69.2</td>
<td>86</td>
</tr>
<tr>
<td>3rd</td>
<td>686</td>
<td>4.58</td>
<td>49.7</td>
<td>57</td>
</tr>
<tr>
<td>4th</td>
<td>1200</td>
<td>2.04</td>
<td>38.7</td>
<td>26</td>
</tr>
<tr>
<td>5th</td>
<td>2415</td>
<td>1.32</td>
<td>21.3</td>
<td>10</td>
</tr>
</tbody>
</table>

** The number of stubbles on which at least one insect was observed.

of the larval colony is 10 in the second instar and 5 in the third instar, respectively. But the mean sizes increase respectively to 13~14 and 6~7 larvae in the second and the third instars with the exclusion of a case with only one larva present, on a ear (Table 2A).

HOKYO and KIRITANI (1962) reported that young larvae distribute in the paddy field in an overdispersed manner up to the third instar owing to their gregarious habit. While in a stage of the fourth instar, the degree of aggregation in the distribution abruptly decreased approaching to the Poisson type of distribution due to an increase in the ability of dispersal. It can be seen from Table 2B that the mean number of larvae present on a stubble was only 1~2 in the fourth and the fifth instars. This figure indicates that in many cases the larval colony after passing the third instar disintegrates if it is taken into account that the larvae feed on the ear and that the ear per stubble amount to about 20 in number.

THE SYNCHRONIZATION OF HATCHING IN RELATION TO THE SIZE OF AN EGG MASS

The cumulative percentage of hatching at 2 hr. intervals since the beginning of hatch was shown in Figure 1 with regard to the size of an egg mass. There existed an obvious tendency that the time required for the completion of hatch became short as the size of an egg mass increased. The mean times required for the completion of hatch were respectively 2, 3, 3½, and 4½ hrs. for the size of an egg mass of 10, 5, 2, and 1 in egg number. The first beginning of hatch, however, did not differ with the size of an egg mass.

OCCURRENCE OF MOLTING RELATIVE TO THE SIZE OF A LARVAL GROUP

The cumulative percentage curves of molting relating to the different group sizes were obtained assuming the first date as an original point on which any of the larvae reached the next stage among the sets of the same group size (Fig. 1). Molting of the first instar larvae (to the second instar) occurred almost simultaneously in all of the groups, but the larvae of 10 individual group required the shortest time for molting. While the reverse was the case among the isolated. This tendency was demonstrated more decisively in the second instar. On the contrary, the larvae of the fifth instar
reared in isolation or in medium group sizes rather synchronized in the occurrence of moulting than those reared in the group of 10 larvae. It can be said that hatching and moulting of the larvae occurred concurrently among the larvae of a large group, say 10 individuals, from the egg stage to the second instar inclusive, but at the late larval stages the larvae of the groups of one to five individuals tended to moult more simultaneously than those of the group of 10 individuals.

**EFFECT OF THE GROUP SIZE UPON THE DURATION OF LARVAL STAGES, MORTALITY AND THE WEIGHT OF ADULTS**

Difference in the duration of the first instar was not detected in regard to the group size. The duration of the second instar became shorter as the larval group increased in size, and the reverse relationship was observed for the fourth and

![Fig. 1. Cumulative percentage curves of hatching and moulting in relation to the size of a larval group.](image)

![Fig. 2. Duration (---) of each instar and daily mortality rate (--•--) relative to the rearing density.](image)
Table 3. The duration of developmental stages (egg+larva) in days and the total mortality in per cent relating to the group size.

<table>
<thead>
<tr>
<th>Group size</th>
<th>Male</th>
<th>Female</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No. observed</td>
<td>Mean ± s. d.</td>
</tr>
<tr>
<td>1</td>
<td>15</td>
<td>38.5 ± 4.86</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>37.8 ± 7.42</td>
</tr>
<tr>
<td>5</td>
<td>20</td>
<td>39.5 ± 5.67</td>
</tr>
<tr>
<td>10</td>
<td>30</td>
<td>38.8 ± 11.76</td>
</tr>
</tbody>
</table>

The fifth instars, crowded situation retarded their development. The third instar was the transitive stage from one stage to the other mentioned above (Fig. 2).

Daily mortality of each instar relative to the size of a larval group was shown in Figure 2. The daily mortality of the second instar larvae was the highest among the isolated and decreased successively to the lowest with the increase of the size of a rearing group. A high level of daily mortality among the isolated larvae persisted up to the fourth instar. Excepting the isolated larvae, the daily mortality increased with the increase of the size of a rearing group from the third instar on. The intermediate group sizes, say two or five, were the most favourable in respect to the larval survival during the third and fourth instars. In the group of 10 larvae, the daily mortality became the highest at the fifth instar being accompanied with the prolongation of the pertinent stage. Again, it was confirmed in the daily mortality, though not so decisive as in the duration, that aggregation works in favour of the larvae in the early stages and becomes deleterious eventually in the later stages.

It is interesting to note that when the size of a larval group is evaluated by the total duration of larval stage as well as the total mortality, the following order of decreasing favourability can be suggested for the sizes of group employed; 2, 5, 10, and 1 (Table 3). The difference in the duration between the extremes, however, was only about two days largely due to the inverse relation between the duration of instar and the size of the rearing group among larvae of different stages. The total mortality, on the contrary, differed greatly between the rearing groups of 1 and 2, and the mortality of isolated larvae was three times as high as that of the larvae in the group of 2. An addition of one individual as a partner leads to a remarkable shift from one extreme to the opposite in the mortality as well as the duration of the larval stage.

Generally, the male emerged earlier than the female by one or two days (Table 3). The weight of the newly emerged adults became lighter with the increase in size of the larval group (Table 4). Its coefficient of variation in the body weight became large as the size of a group increased. The female was invariably heavier than the male. The decrease in the body weight was significant between the groups of 2 and 5, and of 5 and 10 with a 99 per cent level of confidence irrespective of sex, but there was no significant difference between the groups of 1 and 2. The difference between sexes in weight was also significant with

Table 4. The fresh weight of newly emerged adults in regard to the rearing density.
a 99 per cent level of confidence except a group of 10 individuals.

COLOUR VARIATION RELATIVE TO THE SIZE OF A LARVAL GROUP

The first generation: All of the colour types from A to H appeared at the fourth instar in the first generation in every larval group involved, but the value of I.C. showed a decreasing tendency or darkening with the increase in size of a group. Particularly, the change in the proportion of the darkest type A among the fourth instar larvae was remarkable. The percentages of type A were 44, 36, 71, and 79 per cent in the groups of 1, 2, 5, and 10, respectively. On the other hand, there was no difference of colour types in the fifth instar among the groups except one with 10 individuals, and they belonged exclusively to the types G and H. The larvae reared in the group of 10, however, expressed all of the range of variation from types A to H showing darkening of the population (Fig. 3, left).

The third generation: Larvae of the third generation showed a strong tendency to a light colour as compared with those of the first generation irrespective of instar and group size. The dark type, e.g. type A, only appeared in the cultures of the fourth instar larvae which were reared in groups of more than two individuals. It is noticeable that the relative frequencies of type G and H were reversed in the largest group where the frequency of G was twice as high as that of H, and this was thought to be responsible for the decreasing trend in the I.C. value with an increase in size of the group in the fourth instar. The fifth instar larvae almost belonged to the type

Fig. 3. Percentage frequency of the colour type in relation to the rearing group size in the first (left) and the third generation (right). White column: 4th instar larva; black column: 5th instar larva.
H even in the group of 10 and the value of I.C. was almost constant irrespective of the group size (Fig. 3, right).

**DISCUSSION**

As Allee (1938) has pointed out, aggregation is due in the first place to the eggs being laid together. The mode of life of the ensuring larvae hatched from the egg mass, however, is very different according to the species. Some species form into a group or groups throughout their larval life. In others, hatchlings disperse as soon as they hatch. Between the both extremes, there are many species whose larval colony persists until some stages followed by a solitary life. Hence, in the study of insect aggregation, it should be understood in terms of the mode of life specific to the species. The ecological meaning of aggregation among insects with a colonial habit was studied recently by several workers, e.g. Mizuta (1960) and Sugiuira (1961), with tea tussock moth, *Euproctis pseudoconspersa*; Ghent (1960) and Lyons (1962), with sawflies of *Neodiprion*; Sugimoto (1962), with *Artona funeralis*; Sato and Morimoto (1962) and Morimoto and Sato (1962), with rice stem borer, *Chilo suppressalis*; Hitchcock (1961), with orange striped oakworm, *Anisota senatoria*; Kiritani and Kimura (unpublished), with cabbage stink bug, *Eurydema rugosa*.

Pearl and Parker (1922) has elucidated that under some conditions the strength of biological process such as longevity is greatest with the smallest population, and decreases as the numbers increase (Drosophila type). Allee (1938) has made an assertion that, however, under other conditions there is a distinct decrease in the strength of measured biological process with overcrowding as well as overcrowding (Allee type). Therefore, it can be considered that the mode of response to the population density in the early larval stage of *Nezara* accords with the condition of overcrowding of the Allee type and that of the late stage with the Drosophila type.

It has been observed on *Euproctis pseudoconspersa* and on *Chilo suppressalis* that the degree of synchronization at the time of hatch is influenced by the size of an egg mass (Sugiuira, 1961; Mizuta, unpublished; Morimoto and Sato, 1962). Furthermore, in the present experiment, a good synchronization was observed in the molting of young larvae at a high density and the reverse was the case among the mature larvae. The mechanism of such synchronization is uncertain but it is probably due to mutual stimulus.

The deleterious effect of isolation has been confirmed by many workers on the insect with a colonial habit. But the degree of depression in the survival rate is considerably different from species to species. Isolation was decisively fatal in the larvae of *Euproctis*, as reported by Mizuta (1960). But the difference in mortality between the solitary and the grouped was rather statistic in a sawfly, *Neodiprion pratti banksianae*, as reported by Ghent (1960). On the other hand, those species that show the gregarious habit in the early part of larval life and pass the late stage more or less in the solitary life, e.g. *Chilo suppressalis*, *Artona funeralis*, *Anisota senatoria* suffer ill effects when reared in isolation in the early stages but become advantageous in the late period of their larval life (Morimoto, 1960; Sugimoto, 1962; Hitchcock, 1961). Among these species including *Nezara viridula*, it is conceivable that isolation does not cause the inevitable death of the larvae.

The rearing number of grouped larvae employed in the present experiment was not unusually high or low as compared with the mean size of a larval colony under natural conditions. It must be noted, however, that the maximum size of the colony that was formed on a ear often exceeded sixty individuals in the second instar. Accordingly, it is necessary to study how far the adaptive value of the colonial life would be improved by the increase in the size of a colony at the young larval stage. This point, however,
is left for the future study.

KARIYA (1960) has reported on Nezara viridula that the individuals bearing a melanic pattern occur more frequently with the fall of the temperature within the range from 20°C to 30°C. The percentage of the black type in larval population of the third generation increases with the approach of the end of the generation (unpublished data), and this may be explicable by the result of KARIYA. But the fact that the same tendency was also observed in the first generation when it becomes warm with the approach of the end of the generation rejects the explanation by the effect of temperature. It has been shown by the present experiment that population density is one of the factors that determine the colour type of the larva. Sufficient evidence is ubiquitous among other species of insects, such as locusts (UVAROV, 1921, 1928; UVAROV and ZOLOTAREVSKY, 1929) (ref. UVAROV, 1961), noctuid larvae (FAURE, 1943; MATTHEE, 1946; LONG, 1953; IWAO, 1962) and a planthopper (JOHNO, unpublished) showing that the high population density produces individuals with dark colouration as compared with those reared under sparse populations. The quantity of food and the rearing temperature are responsible for larval colouration of armyworms (IWAO, 1962; HIRATA, 1962).

The survival value of aggregation of young larvae expressed in terms of both the survival and the duration of instar is feasible to be significant in the operation of natural control agents. As stated in the introductory, the mortality rate is considerably high in the early developmental stages of the Nezara population, accordingly it may be stated that the acceleration of the development in the early stages by grouping may raise the possibility of survival as a result of shortening the duration of vulnerable stages.

The daily mortality was the highest among the isolated larvae of the second instar and decreased successively to the lowest with the increase of the group size. This suggests that the mortality factors that bring about a partial destruction of the members of a colony such as egg parasites, predators and strong wind may affect the survival of the ensuing survivors. But the fact that the larval colony in its nature of aggregation is liable to be destroyed by the mortality factors as a whole (HOKYO and KIRITANI, 1963) should be taken into consideration in appreciation of any mortality factor.

SUMMARY

Experiments were conducted to assess the effect of aggregation of larvae upon the developmental rate and mortality in the southern green stink bug, Nezara viridula. Each experiment was set up with eggs from one batch of eggs within 24 hrs. after deposition. One egg mass was divided into the following groups, 1, 2, 5, and 10 eggs with replications of 10, 5, 2, and 1, respectively. Larvae were fed with the potato plant or the pods of the common haricot beans.

A good synchronization was observed in both hatching and moulting among the first and the second instar larvae reared in a group of 10. Difference in the duration of each instar was observed in regard to the group size except the first instar. The duration of the second instar became short as the group increased in size, and the reverse relationship was observed for the fourth and the fifth instars. The daily mortality of the second instar was the highest among the isolated larvae and decreased successively to the lowest with the increase of the group size. On the contrary, the daily mortality became the highest in the fifth instar in the crowded culture being accompanied with a prolongation of the pertinent stage. The total duration of the larval stage, however, differed little due to the inverse relation with the grouping between early and late stages. But the total mortality among isolated larvae was three times as high as that of the group of 2 individuals.
which had the least mortality. In most cases, the decrease in the body weight of the newly emerged adults was significant at a level of 99 per cent confidence with the increase in size of a group.

Factors involved in the determination of the colouration of the larvae seemed to be multiple, and it was suggested that at least the population density besides temperature was responsible for the variation of the body colour. The survival value of larval aggregation under natural conditions was briefly discussed.

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REFERENCE


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摘 要

ミナミアオカメムシの幼虫集団の大きさと死亡率、令期間おおよび化・脱皮の斬一性

桐谷泰治

和歌山県農業試験場朝来試験地

ミナミアオカメムシの卵は卵塊でなく、17令幼虫は化・脱皮後に卵殻上に集団し、2令では2側以上の集団の総当たりの大きさは13～14頭、3令は6～7頭で4令以後では集合性はみられない。この集合の意義を明らかにするため産卵後24時間以内の卵塊を1, 2, 5, 10卵区に分割して25℃下で培養し、またはインゲンのやを与えて調べた。

（1）化および脱皮の斬一性は、卵および若令期で
抄

昆虫体液の抗細菌性について

ハチミツバの体液には Pseudomonas aeruginosa にたいする抗菌力があり、これは免疫応答によって獲得されたものであり、この病原菌に感染していない幼虫は、この抗菌力をもない。これに反してハチミツバにとって病原性のない Shigella dysenteriae には、ハチミツバはもともと抗菌力をもっている。そこで昆虫の種類によって体液の抗菌力が異なるかどうかを知るため、P. aeruginosa にたいする各種昆虫の体液の抗菌力を測った。

野外で採集した類図目、膜翅目、鞘翅目などいろいろの種類のうち、Vespula rufa および La orthopopuli を除くほとんどの種類の体液は P. aeruginosa にたいして抗菌力を示さなかった。そしてこのように抗菌力をもたない種類の昆虫にたいして、P. aeruginosa は強い病原性を示した。

一方 Shigella dysenteriae や Salmonella typhosa にたいする抗菌力は、昆虫の種類によって異なっている。一般的にやって、昆虫体液はその昆虫にたいして病原性をもたない微生物にたいしてのみ抗菌力をもつようである。

（農技術 平野千尋）

錄

コロモジラミにおける in vitro の DDT の解毒

DDT は、in vivo においては、感受性コロモジラミ成虫では解毒されず、抵抗性のシラミによってのみ解毒される。この論文では、in vitro における解毒力をしつらすために、濃元型ガラタチオンを補酵素として加えたシラミのホメオジェネートに C₁₄-DDT を加えて反応を行なった。反応物は、n-ヘキサン画分と水溶性画分にわけ、後者についてのみ代謝生成物を分析した。まず水層を強塩酸にて分解し、分解生成物を中性及び酸性物質に分離し、さらにカラムクロマトグラフィーにより精製した。それらは、ペーべークロマトグラムの Rf 値、及び赤外線紫外吸収曲線により、DDE, DBP, DDA と同定された。感受性と抵抗性ホメオジェネート間で代謝生成物の交代比が変化することを示した。さらに C₁₄-DDE, C₁₄-DDA, C₁₄-DBP を反応液に加えて代謝経路の推定をおこない、D²₁₄-DDA→DBP 及び D₂₁₄-DDD の二つの経路を確認した。これらの物質が、水溶性画分の強酸分解により生じたことから、これらが何らかの形の複合体として存在していることがうかがえる。

（農技術 藤條純夫）