モモアカアブラムシに適用される L-システイン殺虫剤(農薬生産用農産食料製造)(農芸化学科)

Pesticide-producing Agricultural Food Processing. L-Cysteine insecticides applicable to *Myzus persicae* SULZER (Hemiptera: Aphididae)*

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I INTRODUCTION

Using L-cysteine, an example of the possible pesticides derived from the products of agricultural food processing, the mechanisms of disturbing visual function at a level of molecular interaction with all-*trans*-retinal[4] and a photoreceptor model membrane[6], or of insect pigments[3] have been studied in authors' laboratory. In order to study the mechanisms of disturbing the visual functions of *Myzus persicae* SULZER by L-cysteine, the effects of the compound on phototaxis, phototactic daily rhythm, and color vision were studied in this work. Furthermore, assuming the secondary lethal effect of L-cysteine on the aphids as the consequence of the physiological processes initiated by the disturbance of their vision, a possible role of the pigments found in their body fluid was investigated.

II MATERIALS AND METHODS

1 Insect

The 3rd or 4th-instar lavae of *Myzus persicae*, which had been reared on leaf mustard, *Brassica juncea* CZERN. et COSS., in a green house, were used for the present study.

2 Administration of L-cysteine

Administration of L-cysteine was carried out with the set-up described in Figs. 1 after a modification of the set-up used by Mittler et al[8]. A round plastic container (2.8 cm in a diameter, 0.7 cm in a height) containing 25 aphids, was sealed with a sheet of the streched parafilm (Sealon film of Fuji Film, Tokyo) having air-holes at the edge area. At the central portion, 0.2 ml of L-cysteine solution (dissolved into 15%-sucrose solution) was contained under a cover glass and supped by the aphids from the under surface of the film. All administration procedures were carried out under a 20-W white fluorescent lamp at 23°C. Unless otherwise stated, these conditions were maintained for the observation of insect behaviors and mortality.

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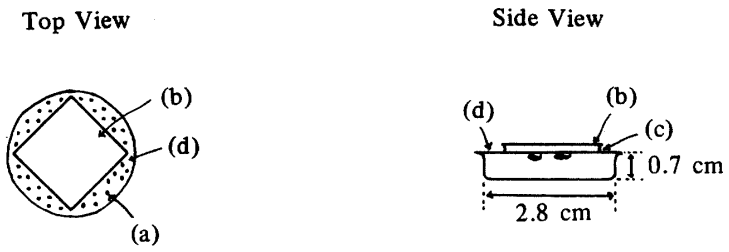
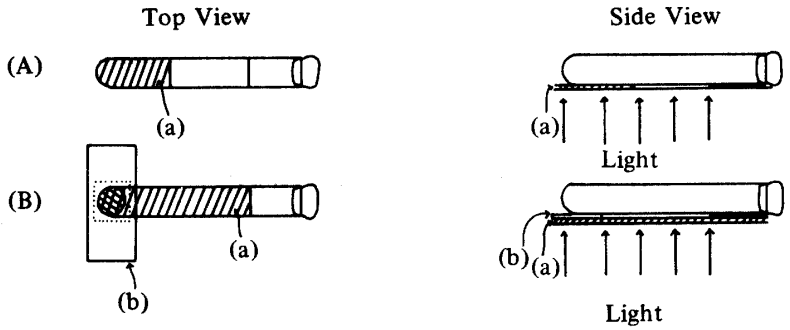


Figs. 1. The experimental set-up used for L-cysteine administration
Twenty five aphids were sealed up in a plastic container by parafilm. (a): Air hole, (b): Cover glass, (c): L-Cysteine solution or 15%-sucrose solution, (d): Parafilm

3 Behavioral evaluations

1) Phototaxis

The experimental set-up used is shown in Figs. 2 (A). The illumination surface was 1.2 cm in width



Figs. 2. The experimental set-ups used for phototaxis determination (A) and color-vision determination (B)
Twenty aphids were used. (a): Green leaf, (b): Hole slide glass

and 5.0 cm in length, and the portion corresponding to the bottom part was rounded. All other area was covered with black paper. And one half of the illuminated portion, the bottom side, was covered with the green leaf of the leaf mustard. The light source was located beneath the illumination surface. The insects were light-adapted up to the time of measurement. Twenty insects were contained in a test tube and closed with a cotton stopper and were collected at the bottom part of the tube. Then, immediately, the tube was placed holizontally on the illumination surface. The number of insects dispersed to the white-light portion was evaluated at 8, 10, and 12 min. The phototaxis was expressed as a percentage of the insects observed at the white-light portion to the total insects used.

2) Phototactic daily rhythm

A vial (1.4 cm in a diameter, 3.2 cm in a height), containing 20 aphids and being closed with a cotton stopper, was placed in a sample chamber of a turbidimeter (Nihon Seimitsu Kogaku K. K., Type SEP-TW). The extent of the insects attracted to white light was expressed as a decrease of transmittance due to the scattering of the light on the insect bodies and was continuously recorded by Yokogawa pen

recorder from 8 hr to 20 hr. Food and water were not given over the period. The administration of L-cysteine and sucrose was carried out for 12 hr previous to the measurements. The insects were conditioned to 11-hr light and 13-hr darkness.

3) Color vision

The detection of color vision was carried out by the method described elswhere[5]. A modification of the previous method was made on the pigment-solution container; hole slide glasses were used in the present experiment as shown in Figs. 2 (B). The pigments were water soluble dyes obtained from Dylon International, Limited, London (Cold A24 green and A26 purple).

4 Mortality

For the aphids treated with sucrose or L-cysteine and the non-treated aphids as stated above the number of dead insects was determined at proper times.

5 The measurement of pH of the body fluid

The pH of the body fluid was evaluated on the aphids immediately after removal from their host plant, leaf mustard, after 30-min dispersion-activities, after L-cysteine treatment over 5 hr, or after sucrose treatment for 5 hr in light or darkness. The color change of the Toyo pH test paper (B. T. B.) was utilized after pressing several aphids on the test paper by a glass rod.

6 Pigment in the body fluid

Wingless aphids were immersed in boiled water (90°C) for 1 min and were washed with 60% acetone. Then, the aphids were grounded in a mortar and utilized for the pigment extraction with 60% acetone. After removing oil soluble fraction by petroleum ether completely, the acetone extract was concentrated by evapolation under reduced pressure. By adding charcoal powder into the concentrated extract, a yellow solution was obtained upon filteration. The stability of the pigment was evaluated by a spectrophotometric method after 5-min immersion in boiled water (90°C) or after 30-min illumination with white fluorescent light.

7 The crude extract of the enzyme hydrolizing protoaphins

Living aphids were grounded with 60% acetone in a mortar. The paste was filtered. The filterate was fractionated with petroleum ether to remove oil soluble fraction and used as the crude extract of the enzyme hydrolyzing protoaphins[2].

8 The absorption spectra of the pigments of body fluid

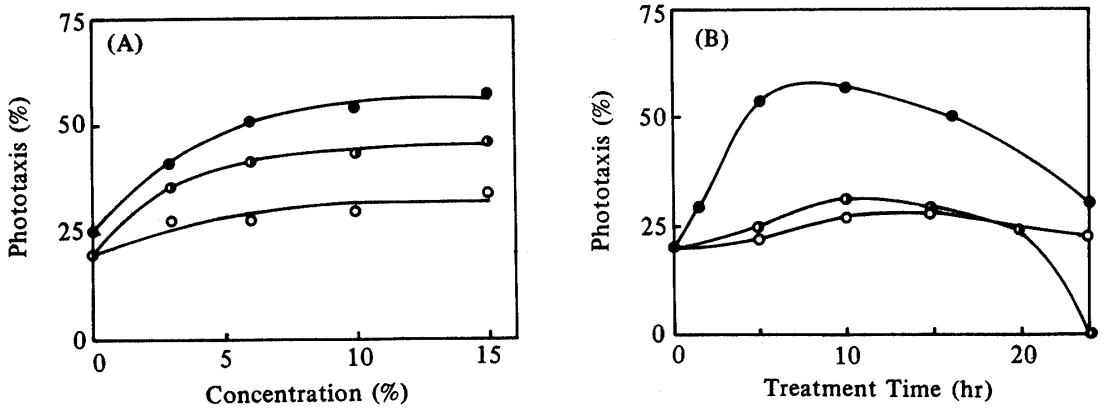
The crude pigment solution obtained before or after the treatment with charcoal powder was adjusted to a desired pH by adding an equivalent volume of 0.2 M phosphate buffer of the desired pH value. The absorption spectra were obtained with Toshiba Beckman DB-GT recording spectrophotometer.

III RESULTS AND DISCUSSION

1 Behavioral modifications

1) Enhancement in phototaxis

The effects of L-cysteine concentration and treatment time on the phototaxis of the aphids used are



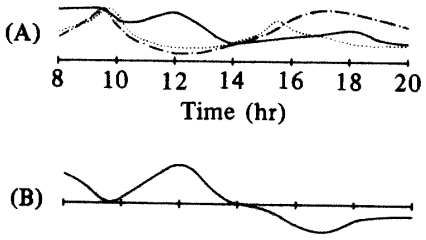
Figs. 3. The effect of L-cysteine on the phototaxis
(A): The effect of L-cysteine concentration on the phototaxis, ○ ; 1.5-hr Treatment, ◑ ; 3-hr Treatment ● ; 5-hr Treatment, (B): The effect of duration of various treatments on the phototaxis, ○ ; 15% Sucrose, ◑ ; Starvation, ● ; 10% L-Cysteine

shown in Figs. 3 (A). Within the range of reaction time 1.5-5 hr, phototaxis increased with an increase in reaction time or L-cysteine concentration. In each treatment time, the phototaxis reached its maximum at the concentration range of 10-15%. Since 5-hr treatment at 10-15% concentrations gave the phototaxis two times greater than that of non-treatment, L-cysteine treatment was carried out at 10% concentration for 5 hr in the subsequent experiments.

Relationships between treatment time and phototaxis in sucrose treatment, L-cysteine treatment, or non-treatment are shown in Figs. 3 (B). The phototaxis of sucrose treatment was constant at 20-29%, while that at non-treatment somewhat increased up to 20-hr treatment. In L-cysteine treatment, 5 or 10-hr treatment increased to their maxima 54% and 57%, respectively, reaching to the values 2.4 or 2.1 times greater than sucrose treatment. This may indicate an increase in the activities of the insect in the light by L-cysteine administration. A decrease in phototaxis after a treatment for 20 hr or longer may be related to an increase in the number of the aphids being affected to the secondary death.

2) Modification of phototactic daily rhythm

The phototactic daily rhythm of the insects obtained from the host plant, fed on sucrose or L-cysteine are shown in Figs. 4 (A). The non-treated insects fed on the host plant and sucrose-treated insects had two peaks, one in the morning and the other in the evening. The morning peak was observed at 9-10 hr and the evening peak at 17-18 hr in non-treated insects and just before 16 hr in the sucrose-treated insects. The ratios of the minimum to the maximum were about 0.25 and 0.33, respectively. The insects treated with L-cysteine, on the other hand, showed higher phototaxis from the



Figs. 4. The phototactic daily rhythm of the aphids used
(A): The effect of various treatments on the phototactic rhythm of aphids, ---: Host plant, ···· : 15%-Sucrose treatment, ——: 10%-L-Cysteine treatment, (B): Difference between L-cysteine treatment and feeding on the host plant of the aphids used

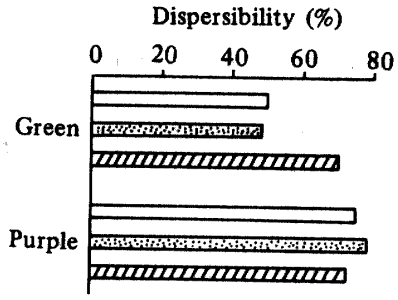


Fig. 5. The green or purple dispersibility obtained under various treatments
□ : Feeding on the host plant, ▨ : 15%-Sucrose treatment, ▨ : 10%-L-Cysteine treatment

outset of the measurement and maintained such an active state even after 12 hr, at which the non-treated insects showed an activity valey. The ratio of the valey to the peak in the morning was 0.88, being about 2.7 times greater than the former two treatments.

The difference between L-cysteine treatment and the non-treatment is shown in Figs. 4 (B). L-Cysteine treatment extremely increased phototaxis in early morning and at the valey at 12 hr. It is noteworthy that the rate of enhancement of phototaxis was the greatest at 12 hr when the non-treated insects hide themselves from the sun light in its highest degree since the secondary leathal effect of L-cysteine could be effectively induced by the inversion of the phototactic daily rhythm, resulting in reception of the hazardous sun light of early afternoon.

3) Color vision

Shown in Fig. 5, the responses to purple were 75-78% in dispersibility for the aphids obtained from the host plants and treated with sucrose or L-cysteine and the responses to green were 50% for the non-treated insects and the sucrose-treated insects and increased to 70% by L-cysteine treatment. When the color responses are expressed by the ratio of the purple dispersibility to the green dispersibility, the non-treatment and the sucrose treatment gave 1.5 and 1.6, respectively, indicating a green preference or ability of distinguishing the purple from the green, while the L-cysteine treatment gave 1.0, indicating a decrease in a green preference or inability in distinguishing the two colors. From this, it is clear that the L-cysteine treatment decreased the sensitivity to the light in the visible range. That is, L-cysteine may competitively inhibit formation of visual pigments and may form UV receptor pigments[4]. This may result in a relative decrease in visible light sensitivity, leading to the disturbance of the color vision. Since L-cysteine decreases insect's ability of green-leaf identification as observed in this work, the compound would be used as a protectant of green plants against attacks by phytophagous insects.

2 Mortality

The mortality of the aphids under sucrose, L-cysteine, or non-treatments are shown in Fig. 6.

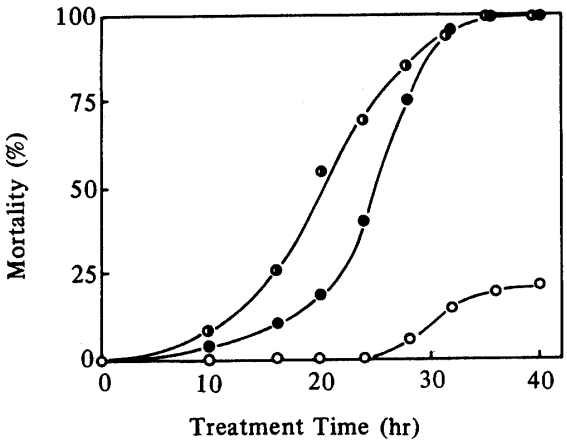


Fig. 6. The effect of the duration of various treatments on the mortality of the aphids used
○ : 15%-Sucrose treatment, ◑ : Starvation,
● : L-Cysteine treatment

L-Cysteine or non-treatments gave similar S shaped curve with half-mortality ratio of 25 hr or 20 hr. Inspite of this general similarities, L-cysteine treatment curve increased more steeply than non-treatment curve after 20 hr and reached to 90% mortality, the same value with the non-treatment at 30 hr and 6 times greater than the sucrose treatment. Furthermore, an increase in phototaxis was quite different between the two treatments as shown in Figs. 3 (B). From these observations, the cause of the leathal effect observed in L-cysteine treatment may be distinct from simple starbation of the non-treatment.

3 Changes in the pH of the body fluid

Changes in pH of the body fluid due to L-cysteine treatment, sucrose treatment or dispersion activities were studied since, as shown in the latter section, the pigment of the body fluid can be affected by pH of the solution. Composing 25-30% of the body weight in soft insects, the body fluid has pH being variable to species, environmental conditions, and physiological conditions, indicating a low buffer capacity[1,7]. The aphids staying on the host plants gave pH values of 7.0-7.2. When these aphids were forced to dispersion activities for 30 min, the pH decreased to 6.2-6.4. That of the L-cysteine treatment for 5 hr was 6.2-6.4. The aphids treated with sucrose for 12 hr in darkness gave 7.0-7.2 while those in light gave 6.2-6.4. It is known that the pH of the insects is affected by the concentration of not only amino acids but also bicarbonates and inorganic phosphates. From this, the lowering of the pH value of the body fluid can be caused easily by an increase in the concentration of these acids. From the above discussion it can be said that the decrease in pH values due to L-cysteine treatment may be partially due to an increase in amino acid concentration and partially due to an increased activity caused by an enhancement in phototaxis.

4 Pigment in the body fluid

The crude pigment extract before charcoal treatment gave yellow-green color quite similar to the body color of the aphid. From this, the extracted pigments could be considered as the major pigments

representative of the body pigments. The pigments were stable to the heat treatment and light illumination carried out in this work. The color of the pigment at pH 7.2 was yellow green while that at pH 6.2 was light green. This pH dependent color variation was reversible. The absoption spectra were shown in Fig. 7. Within the spectral range of 375-700 nm, the pigment had the absorption peaks at 390, 550, and 668 nm and a shoulder at 430 nm. The water soluble pigments in the body fluid of the aphids were studied by Human et al.[2] and termed as protoaphin. The protoaphins were yellow at acid and

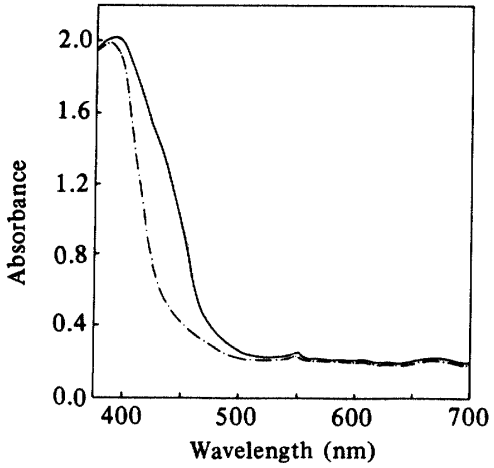


Fig. 7. The absorption spectra of the water soluble pigment obtained from the body fluid of the aphids used
—— : At pH 7.2, — - —: At pH 6.2

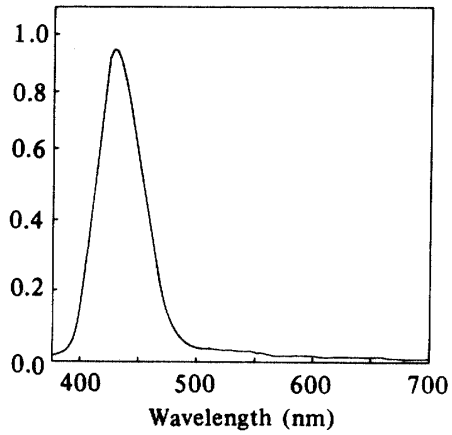


Fig. 8. The difference spectrum of the water soluble pigment obtained from the body fluid between the preparation at pH 7.2 and that pH 6.2

purple red at pH 5.5 without having fluorescent. The pigment was changed to strongly fluorescent red pigment, being soluble to oil, on the death of the aphids. Since the pigment of *Myzus percicae* showed the color different from that of protoaphin, the pigment was treated with active charcoal, resulting in the removal of a large fraction of the pigment, leaving a dilute yellow solution. The spectrum of this solution had no characteristic peak in the visible range. Neverthless, in alkali yellow color intensified and in acid it was decolorized as in a quite similar way to the non-treated solution. The addition of crude exzyme solution to the pigment solution treated with active charcoal gave brown precipitant, having blue-green fluorescent. The changes may be regarded as formation of lipid soluble aphins by the enzyme action on protoaphins. Although the pigment of *Myzus persicae* was considered as protoaphin-like substance from the observation of pH dependency of the pigment color and the presence of enzymatic interaction, further works were required for its caracterization. The difference spectrum of the pigment between pH 7.2 and 6.2 is shown in Fig. 8. Having a single absorbance peak at 430 nm indicated that a single pigment was involved in the pH dependency of the color of the solution. Lowering OD at 430 nm to its half value and, simultaneously, giving 75% increase in the light transmittance at the wavelength range of 400-500 nm may indicate a greater effect of the sun light on the body of the aphids by decreasing pH in insect's body fluid via an increase in L-cysteine concentration and phototactic activity. From this point, the usual behavior of aphids of hiding themselves from

the sun light by localizing at the undersurface of green leaves seems to be a proper adaptive measure.

5 The action mechanisms of L-cysteine

From the above results, the action mechanisms of L-cysteine was systematized as shown in Fig. 9. That is, L-cysteine administration results in (1) an increase in phototaxis (an increase in UV sensitivity),

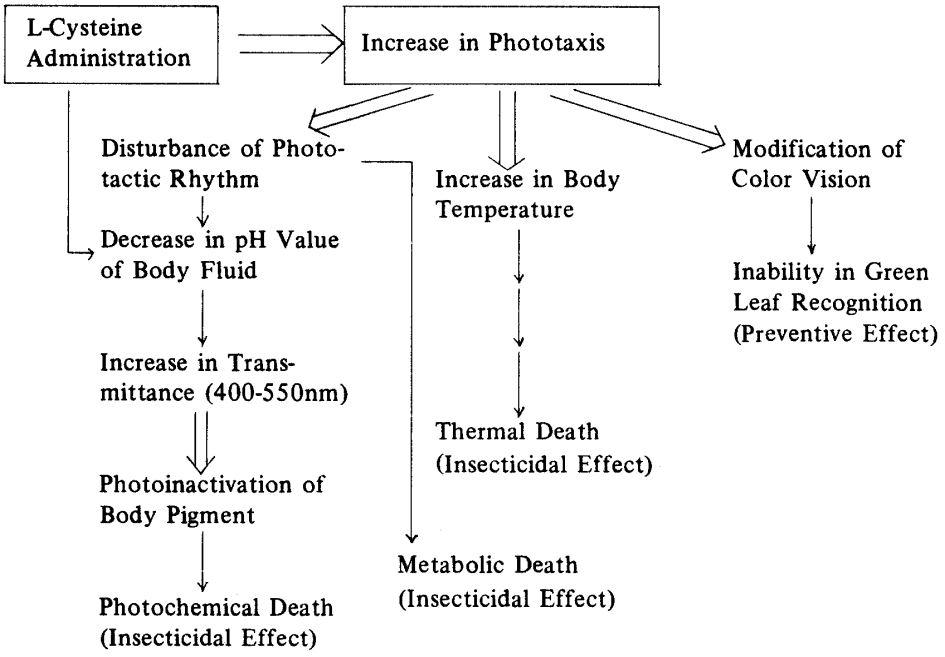


Fig. 9. A schematic diagram showing the pathways of the action of L-cysteine on *Myzus persicae*

(2) modification in color vision (a decrease in visible light sensitivity), and (3) disturbance of the identification of green leaf. This effect may lead to the development of a plant protectant from insect attacks by its repellent effect. On the otherhand, an increase in the phototaxis results in (4) disturbance of phototactic daily rhythm, then, this leads to (5) a decrease in the pH of body fluid via an increase in phototactic activities, and (6) an increase in the light transmittance of the pigment of the body fluid at 400-500 nm. As a result of this, (7) by the photochemical action of the sun light, other physiologically important pigments (e.g. riboflavine or pterines) can be affected, leading to the lethal effects. Furthermore, because of insect's low ability of controlling body temperature, effective photo-thermal energy conversion within the body may well result in a similar lethal effect. That is, L-cysteine as a food-originated insecticide, utilizing the solar-energy, may be developed as the plant protectant for its disturbance of the identification of green leaf by insects and as the insecticides for its secondary lethal effects.

IV SUMMARY

The purpose of the present work was to elucidate the action mechanisms of L-cysteine, one of the possible food-originated pesticides, on *Myzus persicae* SULZER. The results indicated that L-cysteine administration resulted in (1) an enhancement in phototaxis, (2) modification of color vision, (3) disturbance of phototactic daily rhythm, (4) decrease in the pH value of the body fluid via increased activities, and (5) an increase in light transmission of the pigment in the body fluid at the range of 400-500 nm, lending an enhancement in photochemical reactions by the sun light and, finally, leading to the altimate mortality. The pigment involved in this secondary mortality was shown to be one pigment species having its absorption maximum at 430 nm. Since the absorption spectrum of the pigment was pH dependent, the pigment was expected to play a role of controlling the transmission of the sun light into the body of the aphids. Having a wide distribution in the agricultural food products, amino acids, including L-cysteine used in this work, would be developed as highly safe pesticides by an appropriate use of them.

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農薬生産用農産食料製造－モモアカ

アブラムシに適用される
L－システイン殺虫剤

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要　　約

食料起源の農薬の一例としてのL－システインのモモアカアブラムシへの投与による致死作用の機構を解明することを目的とした。その結果，L－システインの投与により，(1)趨光性の増強，(2)色覚の変革が起こり，(3)趨光性リズムの撹乱を経て，(4)運動量の増大による体液pHの低下を引き起こし，(5)体液色素の400～500nmでの光透過度が増加し，太陽エネルギーの光化学的作用により，致死することが推測された。これに関与する色素は，430nmに最大吸収を持つ一種の物質であることが示された。この色素の吸収スペクトルはpH依存性を有することから，体内への太陽光の透過量を調節する役割を持つものと推測された。本研究で使用したL－システインを含めたアミノ酸は農産食料に広く存在するため，それらの適切な利用により，安全性の高い農薬を開発することが可能になった。

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