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# Antifeedants of Rice Planthoppers in Some Millets

Chul-Sa KIM,[1] Hen-Sik KOH and Hiroshi FUKAMI

*Pesticide Research Institute, Faculty of Agriculture, Kyoto University, Sakyo-ku, Kyoto 606–01, Japan*

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Feeding behaviors of three species of planthopper, *Nilaparvata lugens*, *Sogatella furcifera* and *Laodelphax striatellus*, were examined against some species of millets. Finger millet and Indian barnyard millet are resistant to *N. lugens* and *S. furcifera*, while serving as one of the host plants for *L. striatellus*. In the case of Japanese barnyard millet, *N. lugens* only can not select it as a host plant. Through the biological and chemical studies, we have come to the conclusion that the resistance of these millets to each planthopper species is due mainly to the presence of multiple antifeedants.

*Key words*: *Nilaparvata lugens*, *Sogatella furcifera*, millet, antifeedant(s), host selection

## INTRODUCTION

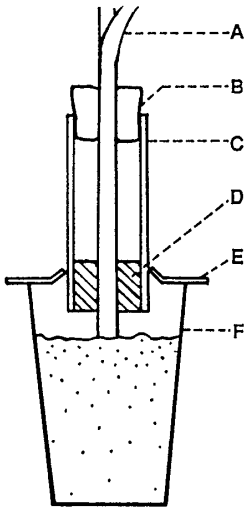
Three species of planthopper, the brown planthopper, *Nilaparvata lugens* (STÅL), the white-back planthopper, *Sogatella furcifera* (HORVÁTH), and the smaller brown planthopper, *Laodelphax striatellus* (FALLÉN), are the most destructive pests of rice plant in Asian countries, causing significant loss of yield either by sucking plant sap or by transmitting virus diseases. In the course of studies on the chemical basis of host selection of these planthoppers, we isolated the antifeedant of barnyard grass (*Echinochloa oryzicola* VASING) against *N. lugens* and identified it as *trans*-aconitic acid (KIM et al., 1975, 1976). In these studies, we have been interested in the feeding behavior of these planthoppers to the gramineous cultivated plants having the same cultivation period as that of rice plant. Of these plants, we have found that Japanese barnyard millet *Echinochloa utilis* OHWI et YABUNO is resistant only to *N. lugens*, while finger millet *Eleusine coracana* GAERTN and Indian barnyard millet *Echinochloa frumentacea* LINK are resistant to *N. lugens* and *S. frucifera*. These millets however still serve as one of the host plants for other species of planthopper. The present study was undertaken in order to find out why these millets are resistant to each planthopper species.

## MATERIALS AND METHODS

*Insects*. Three species of planthopper (*N. lugens*, *S. furcifera* and *L. striatellus*) have been reared successively on rice seedlings at 25°C, under 14 h illumination.

*Plants*. Rice plant (cv. Nihonbare), Japanese barnyard millet (cv. Suiraitan), finger millet (cv. Iyazairai 1) and Indian barnyard millet were grown in a green house. Millets used for extracting antifeedants were collected in a paddy field that had been treated with no pesticide.

[1] Present address: *Faculty of Agriculture, Kochi University, Nankoku, Kochi 783, Japan*




A: seedling of each plant species, B: absorbent cotton, C: glass tube (2.5 cm i.d. × 7 cm), D: polyurethane, E: plastic plate, F: polyethylene cup.

Fig. 1. Bioassay apparatus for feeding response of planthopper to each plant species.

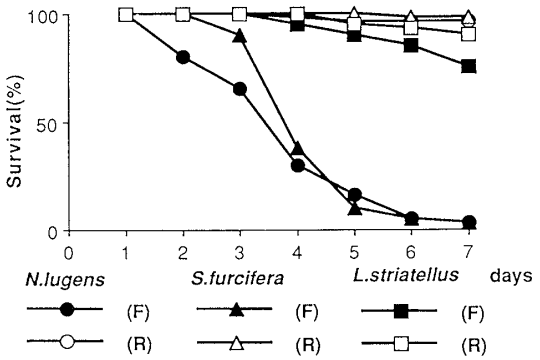



Fig. 2. Survival of the 3rd instar nymphs of three species of planthopper on each plant species. (F): finger millet, (R): rice plant.

*Rearing experiments on plants.* Ten 3rd instar nymphs of each planthopper species were introduced into the apparatus illustrated in Fig. 1, and allowed to feed on stem part of plant at 25°C, 60–70% relative humidity and under 14 h illumination.

*Rearing experiments on plant extracts.* Eight 3rd instar nymphs of each planthopper species were introduced into the apparatus as described in the previous paper (KIM et al., 1975) and allowed to feed by sucking the test solution through a stretched thin film of polyethylene.

All rearing experiments were replicated five times. Survival of the insects was observed for seven consecutive days, but the calculation of survival rate was performed after 1 day in order to avoid counting artificially damaged insects.

*Instrument.* Atomic absorption spectrophotometric analyses were done with a Shimadzu AA-640-12.

## RESULTS AND DISCUSSION

*Survival rate of insects reared on either rice plant or three species of millet*

The 3rd instar nymphs of three species of planthopper were reared on 20 to 30 days old seedlings of rice plant or three species of millet. As shown in Fig. 2, *N. lugens* and *S. furcifera* reared on finger millet died within 6–7 days. On the contrary, *L. striatellus* developed normally on finger millet as well as on rice plant. The same results were also obtained when the 1st instar nymphs of each planthopper species were reared on the stem parts of each plant species at the 5 to 7 leaf stage.

The 3rd instar nymphs of *N. lugens* and *S. furcifera* reared on seedlings of finger millet were transferred to rice seedlings after the termination of the given rearing periods (1–3 days). The longer the nymphs were kept on finger millet, the lower was their survival rate. The survival, however, developed normally on rice seedlings because of the change in their food plant.

Mean survival rates of insects reared on Indian barnyard millet and Japanese

Table 1. Mean survival rate of the 3rd instar nymphs of three species of planthopper on each plant species[a]

| Plant | *N. lugens* | *S. furcifera* | *L. striatellus* |
|---|---|---|---|
| Rice plant | 95 | 98 | 90 |
| Japanese barnyard millet | 0 | 90 | 94 |
| Finger millet | 2 | 2 | 78 |
| Indian barnyard millet | 0 | 0 | 45[b] |

[a] Numbers indicate the percentage of survival 7 days after rearing.
[b] Survivals are grown to adults and many nymphs are emerged from the stem parts.

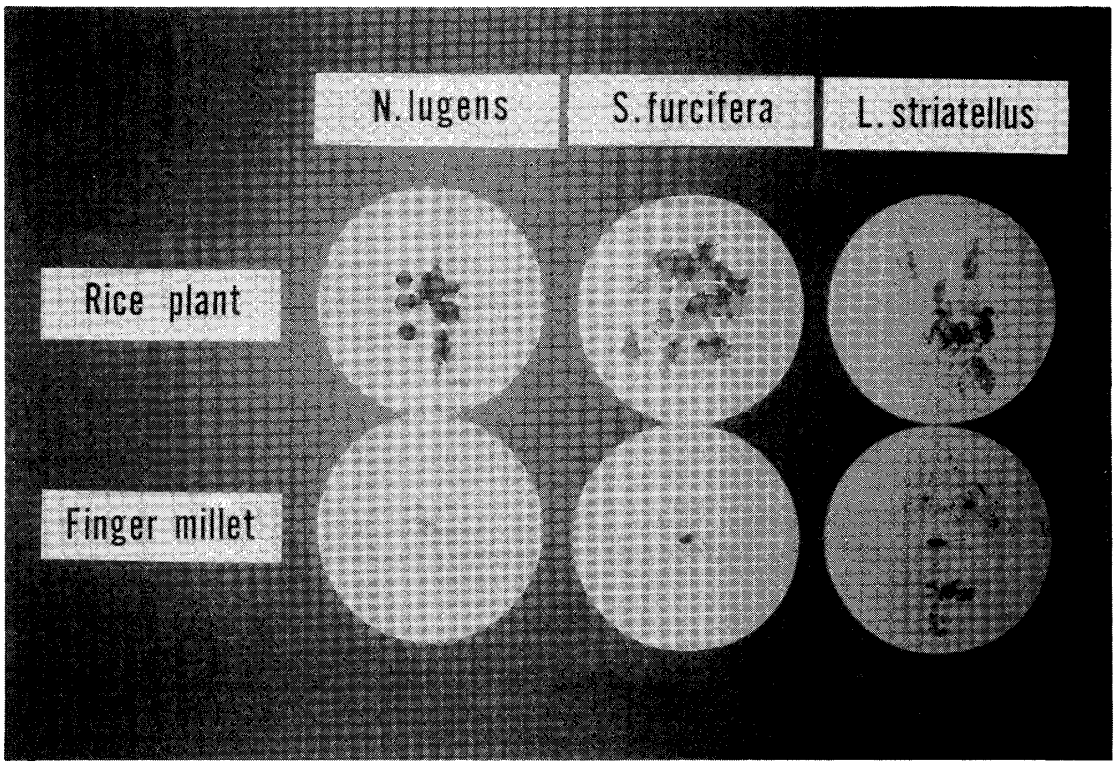



Fig. 3. Honeydew excreted onto filter papers by three species of planthopper fed on each plant species (treated with ninhydrin reagent).

barnyard millet are summarized in Table 1. Indian barnyard millet is resistant to *N. lugens* and *S. furcifera* as in the case of finger millet, while Japanese barnyard millet is resistant to *N. lugens* only. Host plant changing experiments were also conducted with these millets. The nymphs which were kept on non host plant developed normally on rice seedlings as in the case of finger millet.

*Experiments on honeydew*

According to SOGAWA's procedure (SOGAWA and PATHAK, 1970), the amount of honeydew excreted by female of each planthopper species was examined. As shown in Fig. 3, *N. lugens* and *S. furcifera* fed on rice plant excreted a large amount of honeydew,

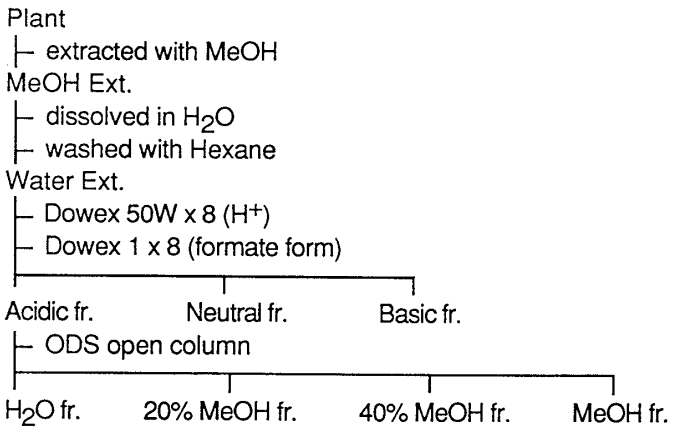



Fig. 4. Fractionation procedure of the antifeedant(s) in each plant species.

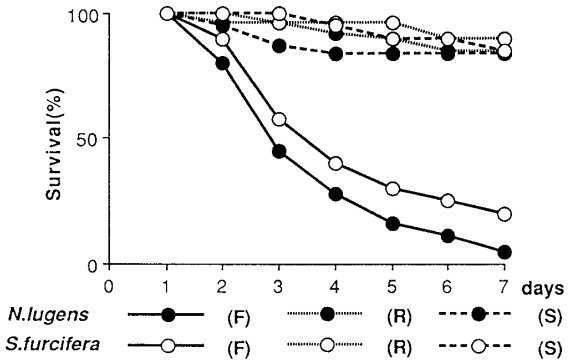



Fig. 5. Effect of each plant extract on survival of the 3rd instar nymphs of *N. lugens* and *S. furcifera*. (F): 10% finger millet extract+15% sucrose, (R): 10% rice plant extract+15% sucrose, (S): 15% sucrose.

while those on finger millet excreted only a small quantity. In the case of *L. striatellus*, however, there was no remarkable difference in the amount of honeydew excreted by those fed on rice plant and those on finger millet.

The same results were obtained when either Japanese barnyard millet or Indian barnyard millet was used. Planthopper excreted a large amount of honeydew only when they fed on plant suitable for feeding and excreted a small amount of honeydew on the non host plant.

*Rearing experiments on plant extracts*

The plant extract was obtained as follows (Fig. 4): Fresh leaves and stems of finger millet (3.1 kg) were cut in rather large pieces (about 10 cm long), immersed in methanol (7 *l*) for 4 days, and decanted. This procedure was repeated three times. The combined methanol extract was evaporated under reduced pressure. The residue was dissolved in water (2.5 *l*) and washed three times with hexane (1.5 *l*). The aqueous layer was evaporated to dryness, leaving a brownish oil (94.05 g), which was referred to as "finger millet extract". Rice plant, Japanese barnyard millet and Indian barnyard millet were extracted through the same procedure.

Rearing experiments of the 3rd instar nymphs of *N. lugens* and *S. furcifera* were carried out with 15% sucrose solution containing either the finger millet extract or the rice plant extract at a concentration of 10%. As shown in Fig. 5, mortality did not increase when the insects were allowed to feed on the 15% sucrose solution or on 15% sucrose plus 10% rice plant extract, while 80–95% mortality occurred within 7 days in the case of the insects fed on 15% sucrose plus 10% finger millet extract which was similar to the case where insects were reared on intact plant.

The finger millet extract was submitted to the probing behavior test of *N. lugens* and *S. furcifera* according to the procedure described in the previous paper (KIM et al., 1985). *N. lugens* and *S. furcifera* deposited quite a lot of long, branched stylet sheaths in the finger millet extract, as they do in the rice plant extract.

Rearing experiments with Indian barnyard millet extract and Japanese barnyard millet extract were also conducted at a concentration of 10% and the results are summarized in Table 2. The extract of non host plant caused 80–100% mortality within 7 days, similar to the case when insects were reared on intact plant. In the probing behavior test, the extract of non host plant showed the same behavior as that of the rice plant extract.

These experimental results support the premise that resistance of finger millet and Indian barnyard millet to *N. lugens* and *S. furcifera*, and also resistance of Japanese barnyard millet to *N. lugens* is not based on the presence of toxic substance or probing inhibitor, but is based mainly on the presence of some antifeedants which inhibit feeding of the insect and result in death by starvation.

*Separation of the antifeedants against N. lugens*

The finger millet extract (8.45 g) was separated into neutral (4.07 g), acidic (1.59 g), and basic (0.44 g) fractions by column chromatography on cation exchange resin (Dowex 50W×8, $H^+$ form, 200–400 mesh, 25 mm i.d.×300 mm) eluted with 2 N $NH_4OH$ (1.5 *l*) and subsequently on anion exchange resin (Dowex 1×8, formate form, 200–400 mesh, 25 mm i.d.×400 mm) eluted with 20 N formic acid (2 *l*) as shown in Fig. 4. In the feeding experiment of sucking insects, the test solutions must be neutralized to pH 7 (AUCLAIR, 1967; SAKAI and SOGAWA, 1976). The main inorganic cationic group of the millet extracts was, therefore, examined prior to bioassay, the results of which are shown in Table 3.

The content of four inorganic cation species differed in each millet extract, but potassium cation is contained in large quantities as expected in plant extract. The

Table 2. Mean survival rate of the 3rd instar nymphs of *N. lugens* and *S. furcifera* on each plant extract[a]

| Culture | *N. lugens* | *S. furcifera* |
|---|---|---|
| 15% sucrose | 84 | 85 |
| Rice plant extract[b] | 85 | 90 |
| Japanese barnyard millet extract | 3 | 75 |
| Finger millet extract | 5 | 20 |
| Indian barnyard millet extract | 0 | 11 |

[a] Numbers indicate the percentage of survival 7 days after rearing.

[b] All extracts contain 15% sucrose.

Table 3. The content of four inorganic cationic species in each plant extract (ppm)

| | $K^+$ | $Na^+$ | $Ca^{2+}$ | $Mg^{2+}$ |
|---|---|---|---|---|
| Rice plant | 810 | 5.6 | 1.1 | 12 |
| Japanese barnyard millet | 940 | 368 | 7.8 | 54 |
| Finger millet | 1400 | 5.5 | 3.4 | 15 |
| Indian barnyard millet | 850 | 312 | 10 | 83 |

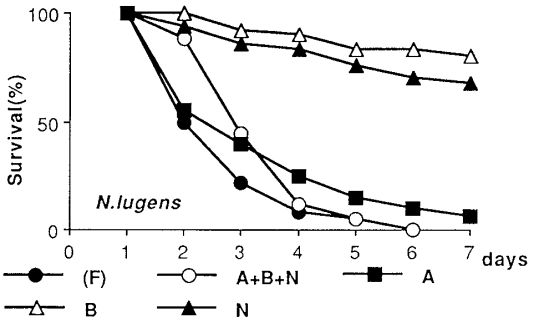



Fig. 6. Survival of the 3rd instar nymphs of *N. lugens* on each fraction of the finger millet extract. (F): 10% original finger millet extract+15% sucrose, A: acidic fraction adjusted to pH 7 with KOH+15% sucrose, B: neutral fraction+15% sucrose, N: basic fraction+15% sucrose.

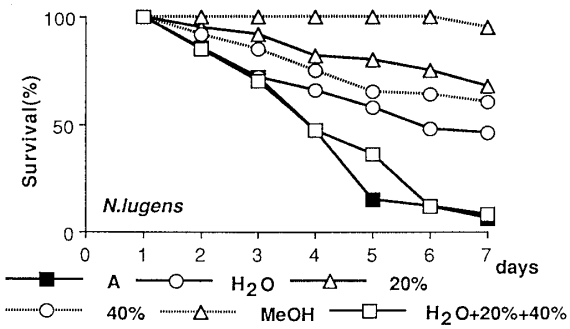



Fig. 7. Survival of the 3rd instar nymphs of *N. lugens* on each fraction of the finger millet acidic fraction. A: original acidic fraction+15% sucrose, $H_2O$: water fraction+15% sucrose, 20%: 20% aqueous methanol fraction+15% sucrose, 40%: 40% aqueous methanol fraction+15% sucrose, MeOH: methanol fraction+15% sucrose.

acidic fraction was first neutralized to pH 7 with KOH and subjected to bioassay. As shown in Fig. 6, only the acidic fraction showed feeding inhibitory activity at the concentration equivalent to 10% solution of the original active extract. The acidic fraction was then neutralized to pH 7 with KOH, NaOH, $Ca(OH)_2$ and $Mg(OH)_2$ at the concentration similar to the cation ratio as analyzed above. Rearing experimental result of this fraction was the same as that neutralized with KOH only. Subsequent rearing experiments of the further fractionation part of acidic fraction were, therefore, neutralized with KOH.

The total acidic fraction (1.47 g) was then chromatographed with a reverse phase open column (50 g of ODS, 100–200 mesh, 25 mm i.d. $\times$ 205 mm, Fuji Davison Chemical Ltd.) eluted with water, 20% and 40% aqueous methanol and methanol successively (1 *l* each, Fig. 4). The combined solution of water, 20% and 40% aqueous methanol fractions shows the same level of activity as the original acidic fraction, while each fraction alone is much less active as shown in Fig. 7. This indicates that the inhibitory activity for feeding is not attributable to a single component but to several components combined.

The Japanese barnyard millet extract and the Indian barnyard millet extract were treated with the same procedure and the active fraction was followed by testing the survival rate of insects. The same level of activity as the original active extract was found only on the acidic fraction, the activity of which was moreover recovered

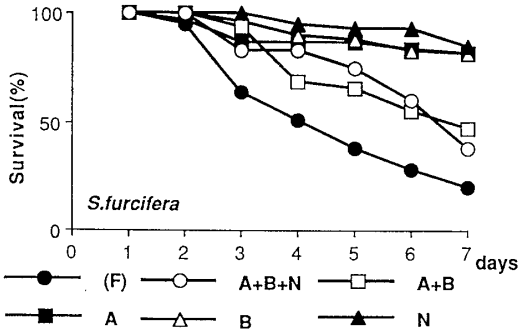



Fig. 8. Survival of the 3rd instar nymphs of *S. furcifera* on each fraction of the finger millet extract. (F): 10% original finger millet extract+15% sucrose, A: acidic fraction adjusted to pH 7 with KOH+15% sucrose, B: basic fraction+15% sucrose, N: neutral fraction +15% sucrose.

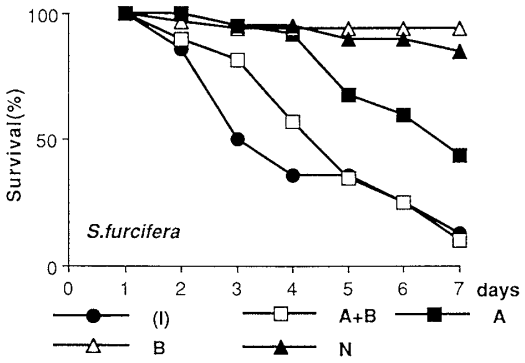



Fig. 9. Survival of the 3rd instar nymphs of *S. furcifera* on each fraction of the Indian barnyard millet extract. (I): 10% original Indian barnyard millet extract+15% sucrose, A: acidic fraction adjusted to pH 7 with KOH+15% sucrose, B: basic fraction+15% sucrose, N: neutral fraction+15% sucrose.

on the combined fraction of water, 20% and 40% methanol eluate on the chromatography with a reverse phase open column as in the case of the finger millet extract.

These experimental results suggested that the antifeedants of these species of millet against *N. lugens* are acidic compounds and composed of multiple components.

*Separation of the antifeedants against S. furcifera*

Separation of the antifeedants against *S. furcifera* was also tried using the finger millet extract and Indian barnyard millet extract. The finger millet extract, as well as the acidic, neutral and basic fractions obtained by resin treatment as described above, were assayed at a concentration equivalent to 10% solution of the original active extract. As shown in Fig. 8, the acidic fraction alone is much less active in the case of *S. furcifera* and differed in the case of *N. lugens*. The combined solution of three fractions obtained by resin treatment shows moreover the low level of activity as the original active extract. This indicates that the feeding inhibitory activity against *S. furcifera* is not attributable to all fractions obtained by resin treatment and that another separation methods must be adopted.

The Indian barnyard millet extract and three fractions obtained by resin treatment were assayed at the concentration equivalent to 10% solution of the original active extract. As shown in Fig. 9, the acidic fraction alone is much less active and the basic fraction does not have any feeding inhibitory activity, but the same level of activity as the original active extract was recovered when the basic and acidic fractions were combined together.

Three species of planthopper, *N. lugens*, *S. furcifera* and *L. striatellus* showed different feeding behaviors to the tested three species of millet. The polyphagous *L. striatellus* can select these millets as the host plants, while the monophagous *N. lugens* fails to feed and does not survive adequately on these millets. The oligophagous *S. furcifera* well survives on Japanese barnyard millet, but can not select Indian barnyard millet and finger millet as the host plants. Through the biological and chemical studies,

we have come to the conclusion that resistance of these millets to each planthopper species is due mainly to the presence of multiple antifeedants. It was moreover concluded that the antifeedants of these millets against *N. lugens* are multiple acidic compounds and those of Indian barnyard millet against *S. furcifera* are composed of acidic and basic compounds.

There is a major problem in nomenclature on the millets, especially on the species of *Echinochloa*. On the basis of morphological, cytological and distributional evidences, *E. frumentacea* was separated from *E. utilis* (YABUNO, 1962, 1966). YABUNO (1971) also proposed names to the two cultivated strains as Indian barnyard millet to *E. frumentacea* and Japanese barnyard millet to *E. utilis*. In this paper, we adopted his nomenclature.

We isolated the antifeedant of barnyard grass (*E. oryzicola*) against *N. lugens* and identified it as *trans*-aconitic acid (KIM et al., 1975, 1976). Through the preliminary examination of each millet extract, it was recognized that *trans*-aconitic acid exists in large quantities both in the Japanese barnyard millet extract and the Indian barnyard millet extract. The finger millet extract, however, does not contain any detectable amount of this acid as in the case of rice plant extract. In connection with the host selection of *N. lugens*, it is interesting to note that *trans*-aconitic acid has been shown to be common in many species of plants (STOUT et al., 1967; BURAU, 1969; CLARK, 1969), but that it is not detectable in host plant, rice, and also in non host plant, finger millet. Through the present study, it may be mentioned here that the antifeedant of barnyard grass against *N. lugens* must be reinvestigated and the possibility of participation of another component other than *trans*-aconitic acid must be researched.

The structure elucidation of these antifeedants contained in these millets is important to clarify the host selection of planthoppers in connection with the difference of origin of *Echinochloa* species and also to research into useful tools to prevent rice plant injury by these planthoppers.

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