Rose Ring Pattern: A Component of the Rose-Mosaic Complex

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ABSTRACT

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A previously undescribed disease of roses, named rose ring pattern (RRP), which has symptoms similar to rose mosaic is described. Symptoms include rings, fine line patterns and chlorotic flecking of the leaves, and (in some cases) color-break rings in the petals. *Rosa multiflora* 'Burr' is a reliable indicator for RRP. Symptoms on this rootstock include severe stunting, and deformity, rugosity, and mottling of the leaflets. Infection of most other major rootstocks is usually without symptoms. Graft inoculation of Shiro-fugen cherry does not cause a necrotic reaction. Attempts to infect other woody and herbaceous plants have not been successful. No natural spread has been observed. Lateral buds propagated from RRP-affected plants held at 38 C for 3 wk or longer were free from infection based on index tests. Injections of Virazole at 200 μg/ml resulted in remission of symptoms in new growth. Rose ring pattern has characteristics distinct from other virus or viruslike diseases of roses, but the causal agent, which is readily transmissible by grafting, has not been identified. Diseased plants have been found in other states. Control is achieved by using clean propagating material.

Additional key words: viruslike rose disease, chemotherapy, graft transmission.

Roses are subject to a number of important virus and viruslike diseases (19, 25, 26, 27, 32). Rose mosaic, one of the most frequently encountered diseases of roses wherever they are grown, detracts from the quality and appearance of the plants. Rose mosaic, which is caused by a transmissible agent, is a general name for a disease with variable symptoms that may show as calico, ringspot, line pattern, chlorotic bands, or other mosaic symptoms. Determination of the specific cause of rose mosaic is difficult because mosaic symptoms may be induced by different disease agents.

Most reports (2, 3, 5, 19, 20, 25, 35) have associated rose mosaic, including yellow mosaic (6, 30) and yellow net (16), with infection by strains or isolates of Prunus ringspot virus (PRSV). Symptoms similar if not identical to rose mosaic are reported to be caused by other viruses. In one case, a virus that caused a rose mosaic was reported to be distinct from PRSV (11, 13), but subsequent work (7, 14) proved it to be an isolate of apple mosaic virus (ApMV). These same studies have shown that PRSV and ApMV are serologically related, but share a minor proportion of antigenic determinants and can be considered different serotypes of the same virus. These two viruses have other similarities: both have positive Shiro-fugen reactions (6), the same number of ribonucleic acid components, similar molecular weight of capsid proteins, activation of infectivity with homologous RNA-4 component (17), sensitivity to oxidized plant polyphenols, similar dilution infectivity curves, and particle classes differentiated by size but not by density (22).

Mosaic symptoms in rose also have been associated with infection by tomato ringspot virus (18), tobacco ringspot virus (24), and Arabis mosaic virus (19). Furthermore, other viruses have been reported to produce diseases in roses (15, 19), but symptoms are distinct from the rose mosaic type. Rose mosaic in California is caused primarily by PRSV. Traylor et al. (35) showed that for five symptom types of rose mosaic the agent was mechanically transmissible to cucumber, was graft-transmissible to peach, caused necrosis on *Prunus serrulata* Lindl. 'Shiro-fugen,' and was reactive with antisera to four strains of PRSV. Usually identification of PRSV is based upon these criteria.

In 1973, during routine indexing of hybrid tea roses for virus and viruslike diseases, a severe disease unlike anything previously encountered was observed in *Rosa multiflora* Thunb. 'Burr' (Burr multiflora). The symptoms of this disease were similar to rose mosaic in hybrid tea cultivars, but graft transmissions with tissue from affected plants did not cause a necrotic reaction in Shiro-fugen. Because rose-mosaic diseases are an important element in the rose improvement program in California, this observation led to further investigations. In this paper we report a previously undescribed disease of roses which we have named rose ring pattern (RRP). We discuss similarities and differences between this and other virus and viruslike diseases of roses. Information concerning diagnosis, host range, distribution, etiology, and control are included.

MATERIALS AND METHODS

Isolation, transmission, and host range studies.—Isolates of the RRP agent used in this study were obtained from hybrid tea cultivars from commercial sources. Negative results from indexing on a selected host

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range indicated the absence of any other disease-causing transmissible agents. The indicators used were those recommended for indexing stone fruits (10), pome fruits (1), and included also were the rose cultivars Madame Butterfly and Queen Elizabeth. Isolates of RRP agent were maintained in Burr multiflora, Queen Elizabeth, or Madame Butterfly rose plants. All rose cultivars were obtained from commercial sources. Rose rootstocks were obtained from The Foundation Seed and Plant Material Service, University of California, Davis, CA 95616. Virus-free *R. multiflora* seedlings were provided by Star Roses, West Grove, PA 19390. The herbaceous hosts used were grown in pots of UC mix (23) in the greenhouse with supplemental incandescent lighting. The tree seedlings used were started from seed and grown in plastic 8-liter containers. Bramble plants were vegetatively propagated and grown in UC mix in the greenhouse. Trees of Shiro-fugen flowering cherry, Nemaguard peach, and Mahaleb cherry were inoculated in the field. Plants of other tree species were grown in containers in a lathhouse.

Standard grafting methods were used for tissue-grafting and approach-grafting trials. To be considered successful, grafts had to remain alive for at least 4 days. For the Shiro-fugen test, only tissue implants that lived longer than 10 days were scored for a reaction. Woody plants graft-inoculated with tissue from RRP-affected plants were indexed to Burr multiflora 30-60 days later to detect latent infections. Mechanical transmission studies always involved the use of 0.05 M phosphate buffer pH 7.0 and either Celite or corundum in the inoculum. Several mechanical inoculation methods reported to successfully transmit viruses from rose were used (5, 8, 16, 21, 36). Leaves, petals,and roots were used as sources of inoculum. A total of 23 herbaceous plants were tested by mechanical inoculation for susceptibility to the RRP agent, including such common virus indicator plants as cucumber, *Nicotiana* sp., *Chenopodium* sp., *Gomphrena globosa, Vinca rosea,* and others. Thirteen woody species were tested for susceptibility to the RRP agent by tissue-grafting and approach-grafting, including four species of *Rubus,* four cultivars of cherry, a *Malus* sp., two cultivars of peach, and one species of plum.

Etiology.—Chemotherapy was investigated as a means of obtaining evidence of the nature of the causal agent. Oxytetracycline (Chas. F. Pfizer and Co., 235 E. 42nd St., New York, N Y 10017) and Virazole (ICN Pharmaceuticals, Irvine, CA 92664), a broad spectrum antiviral compound (33), were injected into plants with a gravity-flow device illustrated in Fig. 1.

Xylem sap was collected from infected leafy canes of Burr multiflora rose using a pressure-chamber method (31). Sap components applied to electron microscope grids were stained with 4% potassium phosphotungstate (KPT) and viewed with an RCA EMU-3H electron microscope.

Leaf-dip preparations and the products resulting from attempted partial purification were stained with 2% KPT, 4% uranyl acetate, or 1% uranyl formate. For all ultrastructural studies, small pieces of tissue were fixed in 3% glutaraldehyde, postfixed in 2% osmium tetroxide, dehydrated in acetone, and embedded in low viscosity epoxy resin. Ultrathin sections were cut with a Porter-Blum MT-2 microtome and stained with uranyl acetate and lead citrate. In one test, Burr multiflora leaflets were wilted for 3 hr prior to fixation and further processing. That technique (28) reportedly facilitates the detection of small spherical virus particles present in low concentrations by concentrating them in crystalline arrays.

Attempts were made to concentrate and partially purify the causal agent by using hydrated calcium phosphate (12). Serological analyses using Ouchterlony double-diffusion tests were conducted in 1% Noble agar containing 0.05 M Tris-HCl (pH 7.0) and 0.85% sodium chloride. Antiserum to PRSV was the same as used in a previous study (35). Antiserum to tobacco-streak virus (PVAS-56) was purchased from the American Type Culture Collection, 12301 Parklawn Dr., Rockville, MD 20852.

The identity of the causal agent, presumably a virus, was investigated by analysis of extracted nucleic acid, specifically to determine if an unusual double-stranded RNA (either RF or viroid) was present in RRP-infected rose tissue. The method of Morris (29) was used for extraction and detection of double-stranded RNA, except that the volume of extraction buffer was doubled for rose tissue.

RESULTS

Diagnosis and indexing.—Rose ring pattern is so-called because of the distinctive symptoms produced in hybrid tea roses. Symptoms always include rings and fine-

Fig. 1. Rose ring pattern affected Burr multiflora rose 60 days after injection of Virazole at 200 μg/ml. The injection apparatus and remission of symptoms in the newly developed shoot is clearly shown.

line patterns of various numbers and sizes (Fig. 2). Often these symptoms are difficult to see or occur only on a few leaves. Chlorotic flecks or larger spots often occur with or without the rings and fine-line patterns (Fig. 2). Some Queen Elizabeth plants experimentally infected with the RRP agent developed chlorotic blotches on the leaflets and color-break rings in the petals (Fig. 2). Bright yellow patterns often seen in plants affected with yellow or common rose mosaic rarely occur in plants affected only by RRP.

A reliable indicator for RRP is Burr multiflora, a thornless rootstock. The indicator host is graft-inoculated, stripped of leaves 10 days later to force new growth, and the new growth is observed for symptoms 30 days after inoculation. Dormant Burr multiflora cuttings also can be chip-grafted with tissue of plants to be tested, and rooted after inoculation. Symptoms in the greenhouse on Burr multiflora remain constant and include severe stunting (Fig. 3), deformity, rugosity, and mottling of the leaflets (Fig. 3). Field symptoms in the spring are similar on this indicator, but disappear during the summer. Later in the season, recurring symptoms in the field consist of an intense ring pattern without the severe leaf deformity. Known diseases of rose, including PRSV-incited rose mosaic, do not have these symptoms in Burr multiflora.

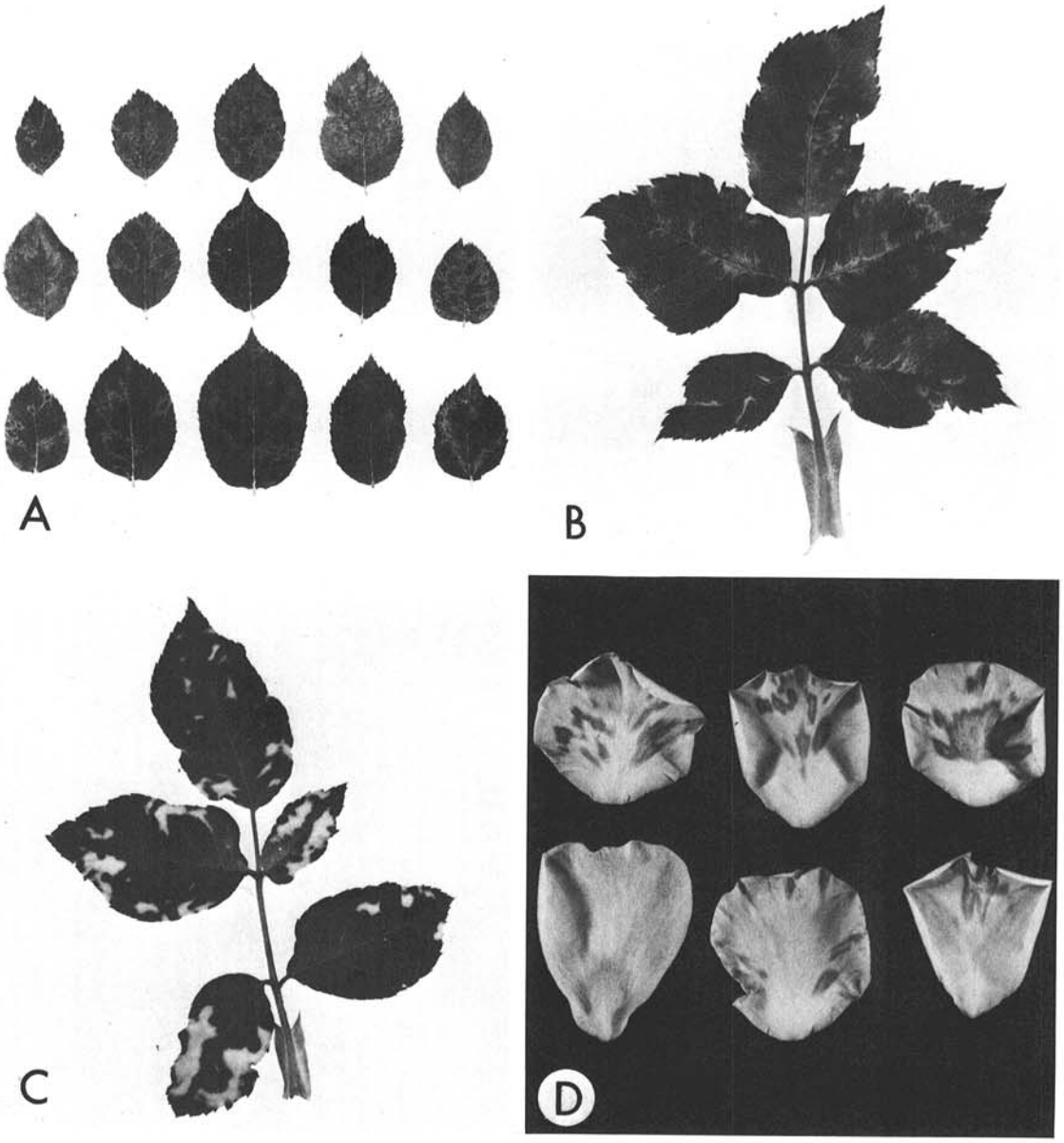

Fig. 2-(A to D). Disease symptoms of rose ring pattern in hybrid tea roses: **A)** a composite of typical ring- and fine-line patterns; **B)** chlorotic flecking; **C)** chlorotic patches; and **D)** dark pink color break rings on the petals and healthy petal at lower left.

The agent causing rose ring pattern was transmitted to roses only by grafting. All rose cultivars tested were susceptible, but reaction to infection was variable. Hybrid tea, climber, miniature, and floribunda cultivars react to infection with rings and fine-line patterns and chlorotic flecks. All rootstocks tested, except Burr multiflora, showed no symptoms. However, an occasional ring or line pattern may develop in Dr. Huey, Manetti, or *Rosa odorata* Sweet., but infection of *R. rugosa* Thunb. is totally symptomless. The RRP agent is readily transmissible to other roses by grafting from all the rootstocks mentioned above. Hybrid tea seedlings develop the typical symptoms seen on hybrid tea cultivars when inoculated with RRP, but five seedlings of *R. multiflora* inoculated with the RRP agent reacted with ring and line patterns instead of the severe symptoms that occur on Burr multiflora.

Distinctive characteristics of rose ring pattern (RRP).—The symptoms in roses induced by the infectious agent of RRP are unique. No agents other than that associated with RRP were detected during indexing on a standard minimum host range for stone and pome fruits and rose cultivars.

The Shiro-fugen test commonly is used to detect PRSV in rosaceous and some other species. A test is considered positive when a gummy, necrotic area develops around the test tissue 30 days after insertion into Shiro-fugen twigs. The results of tests conducted for 2 successive years using material from both hybrid tea roses and Burr multiflora showed that grafting with RRP-affected tissue does not cause this reaction in Shiro-fugen.

Most virus-like infectious agents associated with diseases of roses have one or more hosts in addition to rose. To identify the causal agent of RRP or separate it from other viruses, attempts were made to transmit the infectious agent to a number of herbaceous and woody plants. We were unable to transmit the RRP agent mechanically from rose plants with symptoms to any test plant. Of the woody and herbaceous plants inoculated by grafting, only 2 of 6 Nemaguard peach seedlings, 1 of 6 Mazzard cherry seedlings, and 1 of 4 Fresno strawberry plants became infected. Throughout a 1-yr period, no symptoms were observed in these plants, although the RRP agent was recovered from them by return-graft inoculation of Burr multiflora. No symptoms were detected in herbaceous hosts inoculated with extracts from RRP-affected Nemaguard peach seedlings and Fresno strawberry plants.

Etiology.—Light- and electron-microscope examination and attempted culture from infected tissue on common bacteriological and mycological media did not reveal bacteria or fungi associated with infected roses. Furthermore, when thin sections of vascular tissues were examined by electron microscopy, no mycoplasma- or rickettsia-like organisms were observed, and injections of 5 ml of oxytetracycline at rates of 100 and 200 μg/ml failed to cause remission of RRP symptoms in Burr multiflora. Insects or mites were not detected in or on buds or leaves of infected plants, nor was there any evidence of natural spread between diseased and healthy plants growing in the greenhouse or field.

The product resulting from attempted purification of the RRP agent from Burr multiflora leaves was not infective when inoculated mechanically on a selected host range, did not react with antisera to three strains of PRSV or tobacco streak virus, and did not contain viruslike particles when viewed with the electron microscope.

No virus or unusual particles were seen in the xylem sap or in leaf-dip preparations of infected rose tissue when observed with the electron microscope. Electron microscopy of thin sections of various rose tissues, including meristem, lamina, petal, midrib, and petiole, did not reveal the presence of recognizable virus particles or any unusual cytological features. No virus or viruslike particles were seen when wilted leaf tissue was similarly examined.

Double-stranded RNA was detected in extracts of apparently healthy, RRP-affected, and rose spring dwarf-affected (34) Burr multiflora. The electrophoretic patterns of the dsRNA were identical in all three cases, which indicated that no dsRNA was present in RRP-affected Burr multiflora which was not present also in healthy Burr multiflora. Double-stranded RNA was not detected in extracts of healthy or RRP-affected Queen Elizabeth. Even though the method used to detect dsRNA

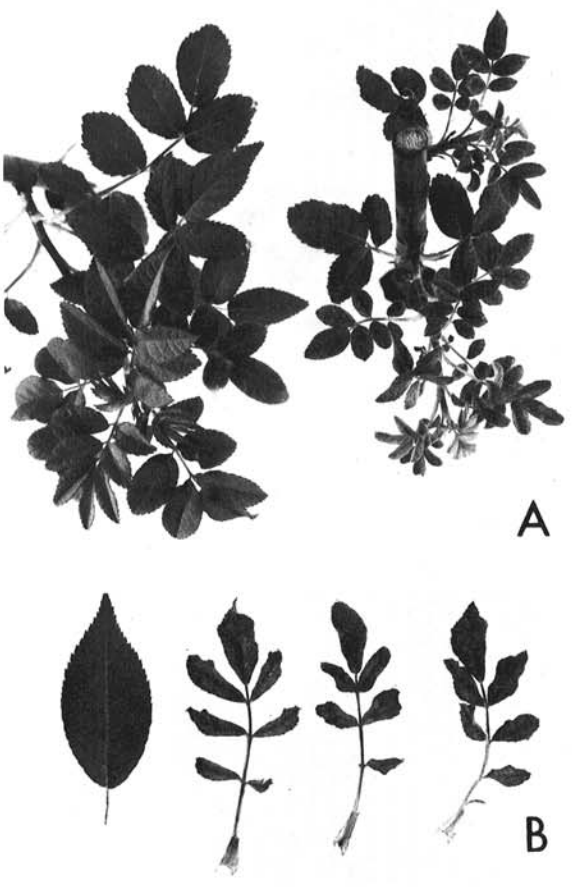

Fig. 3-(A, B). Disease symptoms caused by the rose ring pattern agent in Burr multiflora roses: **A)** stunting of rooted cuttings (right) and healthy (left); **B)** leaf symptoms compared to healthy leaflet (left).

also will detect viroids (Jack Morris, *personal communication*), no abnormal, low-molecular-weight bands suggestive of viroid nucleic acid were observed in extracts of the rose tissue that we tested.

Heat and chemical therapy.—Experiments were conducted to determine heat sensitivity of the RRP agent and to investigate that means of eradicating the agent from infected rose cultivars. Burr multiflora roses systemically infected with RRP agent were placed in a heat chamber at 38 C. Lateral buds were removed at 3-day intervals and propagated on healthy Burr multiflora. These plants were stripped of leaves after 10 days and the regrowth was observed for symptoms. Results of these tests revealed that after 3 wk of heat treatment, the RRP agent was no longer transmissible by grafting. Additionally, shoots arising from the heat-treated grafted buds also were free of RRP symptoms. Similar heat treatment experiments were conducted using RRP-affected hybrid tea roses and buds from heat-treated plants propagated on Burr multiflora. The results of these tests were similar to the results obtained with heat treatment of Burr multiflora. Heat treatment of RRP-affected roses at 38 C for 3-4 wk followed by propagation of lateral buds onto clean rootstocks in the field or greenhouse is now routinely used to produce rose plants free of this disease.

Slow injection of Virazole into RRP-affected Burr multiflora growing in the greenhouse resulted in remission of symptoms. Administration of 10 ml of aqueous Virazole solution (200 μg/ml) by gravity flow into eight infected plants resulted in vigorous growth of shoots free of symptoms after 10-20 days. Prior observation of these plants had shown that no such growth had occurred during a 6- to 12-mo period. Infection of Burr multiflora rose by the RRP agent normally stunts growth so severely that the plants seldom grow any further. Remission of symptoms did not occur in five nontreated RRP-affected Burr multiflora or in five RRP-affected plants injected with water alone. Healthy Burr multiflora plants injected with Virazole (200 μg/ml) were not affected. Injections of 10 ml of aqueous Virazole (500 μg/ml) were phytotoxic. Virazole did not completely cure the disease or eliminate the causal agent from the symptomless shoots, since the infectious agent was transmissible from these symptomless shoots to healthy Burr multiflora 60 days after treatment. The shoots from five of the Virazole-treated plants were indexed and all carried the RRP agent.

DISCUSSION

Rose ring pattern has most likely been confused with rose mosaic, or obscured by it, because of the similarity of symptoms, particularly since rose mosaic exhibits a wide range of symptoms (3, 6). Our results show that RRP is a distinct component of the rose-mosaic complex in California and most probably elsewhere in locations to which infected rose plants have been shipped. The presence of RRP can be detected consistently by indexing on Burr multiflora, which also is useful for detection of the rose spring dwarf disease. Rose mosaic induced by Prunus ringspot virus and related viruses can be detected by indexing on Shiro-fugen cherry, although some precautions are necessary (9). Madame Butterfly is the most reliable indicator for rose-streak infection (4).

Even though RRP resembles rose mosaic, it is not caused by any of the viruses previously reported to be associated with the rose mosaic complex. Distinctive symptoms on hybrid tea roses and Burr multiflora, absence of reaction on Shiro-fugen, and restricted host range are characteristics that distinguish RRP from other virus and virus-like mosaic diseases of rose.

Although we have not identified the causal agent, RRP seems to have a viral etiology. This opinion is based on symptoms, lack of association with microorganisms, graft transmissibility, thermal inactivation, and remission of symptoms when infected plants were treated with Virazole. To our knowledge, this is the first report of remission of disease symptoms as a result of treatment of diseased plants with Virazole. The presence in rose tissue of virus inhibitors, polyphenols, tannins, and polysaccharides, may account for the difficulties in mechanical transmission of the causal agent to an herbaceous host. Also the causal agent may be present in low concentration or irregularly distributed in rose tissues.

Like most of the virus and viruslike diseases of roses, the RRP agent appears to be spread only by propagation. We have not observed natural spread in the greenhouse or field; thus the use of clean propagating stocks in conjunction with heat treatment should control this disease. Alteration of current propagating practices to include an index of new or introduced material for RRP should prevent its spread to other areas.

Rose ring pattern was encountered frequently in California, often occurring in high percentages of plants in selected lots, and has been found in several other western states, including some that also grow and ship roses. Thus, it is likely that the disease is present in many other states that import roses from growers in the western states.

We do not know the economic impact of this disease, but its prevalence threatened implementation of the California clean-stock program, because RRP is not detected by the Shiro-fugen test and symptoms of RRP can be mild and easily overlooked. As a result, the rose industry in California has modified the existing clean-stock program to eliminate RRP, as well as other diseases. Briefly, the program involves indexing on Burr multiflora, Madame Butterfly, and Shiro-fugen cherry, heat therapy of RRP-affected and rose mosaic-affected plants, and establishment of cultivar and rootstock mother blocks free from graft-transmissible agents.

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