PEST SURVEY CARD

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Pest survey card on *Ceratitis rosa* and *Ceratitis quilicii*

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Abstract

This pest survey card was prepared in the context of the mandate on plant pest surveillance (EFSA-Q-2017-00831), upon request by the European Commission. The purpose of this document is to assist the Member States in planning annual survey activities of quarantine organisms using a statistically sound and risk-based pest survey approach, in line with the current international standards. The data requirements for such activity include the pest distribution, its host range, its biology, risk factors as well as available detection and identification methods. This document is part of a toolkit that consists of pest-specific documents, such as the pest survey cards and generic documents relevant for all pests to be surveyed, including, the general survey guidelines and statistical software such as RiBESS+.

Keywords: plant pest, survey, risk-based surveillance, *Ceratitis rosa, Ceratitis quilicii,* natal fruit fly, cape fruit fly

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Introduction

The information presented in this pest survey card was summarised from the factsheets on *Ceratitis rosa* and *Ceratitis quilicii* (Virgilio et al., 2014), the European and Mediterranean Plant Protection Organization (EPPO) datasheet on *Ceratitis rosa* (1997), the EPPO Global Database and the Centre for Agriculture and Bioscience International (CABI) datasheet on *C. rosa* (2018) and other documents.

Ceratitis quilicii has only recently been distinguished from *C. rosa* as a separate species; therefore this survey card is also relevant for that species and outlines the differences. However, risk factors, spread capacity and probable host range are common to both species and they can only be distinguished at the identification stage.

The objective of this pest survey card is to provide the relevant biological information needed to prepare surveys for *C. rosa* and *C. quilicii* in EU Member States (EFSA, 2018). It is part of a toolkit that is being developed to assist Member States with planning a statistically sound and risk-based pest survey approach in line with International Plant Protection Convention guidelines for surveillance (FAO, 2016). The toolkit consists of pest-specific documents and generic documents relevant for all pests to be surveyed:

i. Pest-specific documents:
   a. The pest survey card on *Ceratitis rosa* and *Ceratitis quilicii* [1]
ii. General documents:
   a. The general survey guidelines (to be finalised in 2019)
   b. The RiBESS+ manual available online[2]
   c. The statistical tools RiBESS+ and SAMPELATOR which are available online[3] with open access after registration.

1. The pest and its biology

1.1. Taxonomy

Class: Insecta, **Order**: Diptera, **Family**: Tephritidae, **Genus**: *Ceratitis* MacLeay, 1829 **Subgenus**: *Ceratitis (Pterandrus)* Bezzi, 1918	
Scientific names	**Common names**
Ceratitis rosa Karsch	Natal fruit fly
Ceratitis quilicii De Meyer, Mwatawala & Virgilio, 2016	Cape fruit fly

Ceratitis rosa and *C. quilicii* have distinct ecological requirements (Tanga et al., 2018): *C. rosa* is considered as the 'lowland type' (formerly *C. rosa* R1 type), whereas *C. quilicii* is considered as the 'highland type' (formerly *C. rosa* R2 type). The area predicted to be climatically suitable for *C. rosa* is narrower than that for *C. quilicii*, so *C. quilicii* might be more tolerant to a wider range of climatic conditions than *C. rosa*. The host range differentiation between the two species needs further investigation.

[1] The content of this EFSA Supporting Publication is reproduced as a live document available at https://efsa.maps.arcgis.com/apps/MinimalGallery/index.html?appid=f91d6e95376f4a5da206eb1815ad1489 where it will be updated whenever new relevant information becomes available.
[2] https://zenodo.org/record/2541541/preview/ribess-manual.pdf
[3] https://websso-efsa.openanalytics.eu/auth/realms/efsa/protocol/openid-connect/auth?response_type=code&client_id=shiny-efsa&redirect_uri=https%3A%2F%2Fshiny-efsa.openanalytics.eu%2Fsso%2Flogin&state=d6f7f997-d09f-4bb0-afce-237f192a72d5&login=true&scope=openid

1.2. EU pest regulatory status

Ceratitis rosa is regulated under Council Directive 2000/29/EC[4] in Annex I Part A Section 1 (a) 25 Tephritidae (non-European) such as: (n) *Pterandrus rosa*.

Since *C. quilicii* has only recently been distinguished as a separate species, the regulation is also relevant for that species.

Annex IV, Part A Section 1 requires that fruit of *Citrus* L., *Fortunella* Swingle, *Poncirus* Raf., and their hybrids, originating in third countries where Tephritidae (non-European) are known to occur on these fruit, originate in areas known to be free from the relevant organism; or that no signs of the relevant organism have been observed at the place of production and in its immediate vicinity or the fruit has been shown to be free from the relevant organism in all stages of their development; or have been subjected to an appropriate and efficient treatment without damaging the fruit.

1.3. Pest distribution

The two species are not known to occur in the EU. Both fruit flies, *C. rosa* and *C. quilicii*, are distributed in the south-eastern countries of the African continent as shown in Figure 1 and Figure 2.

Figure 1: Global distribution of *Ceratitis rosa* (Source: Belgian Biodiversity Information Facility, accessed on 26 November 2018)

Figure 2: Global distribution of *Ceratitis quilicii* (Source: Belgian Biodiversity Information Facility, accessed on 26 November 2018)

[4] Council Directive 2000/29/EC of 8 May 2000 on protective measures against the introduction into the Community of organisms harmful to plants or plant products and against their spread within the Community. OJ L 169, 10.7.2000, p. 1–112. Consolidated version of 01/04/2018

1.4. Life cycle

Since there was no distinction between *C. rosa* and *C. quilicii* before 2015 in the scientific literature, biological information published before that year may be relevant for both species.

At 15–30°C, *C. rosa* can complete its immature development in 17–68 days. In comparison, at the same temperatures, *C. quilicii* can complete its immature development in 23–65 days. Adult females of both species lay their eggs under the fruit skin (Tanga et al., 2015). Surveys need to be timed when the fruit of host plants is present, since the larvae are found in the fruit itself. This depends on the host plants and locations and can differ between Member States. Trapping should be carried out once the adults have emerged. In general, surveys should be conducted between May and October.

1.5. Host range and main hosts

The host range information for both species is incomplete, as the distinction between the two species has only been made very recently. Further studies would be needed on this.

Based on De Meyer et al. (2016) and Virgilio et al. (2014), the following host plants can be confirmed:

Outdoors: Apple (*Malus domestica*), pear (*Pyrus communis*), peach (*Prunus persica*), apricot (*Prunus armeniaca*), Citrus and possibly grapevine (*Vitis vinifera*), coffee (*Coffea arabica*), mango (*Mangifera indica*), avocado (*Persea americana*), lychee (*Litchi chinensis*), guava (*Psidium guajava*).

Indoors: Tomato (*Solanum lycopersicum*) is possibly a host but probably not a major one.

As main host plants for the survey, apple, pear, peach, apricot and citrus are suggested.

1.6. Environmental suitability

The potential range of the two species is similar to that of *C. capitata*, though correlative ecological niche modelling demonstrated that *C. rosa* (still combining both species at that time) prefers climatic conditions with lower temperatures in comparison to *C. capitata* (De Meyer et al., 2008).

Ceratitis rosa and *C. quilicii* have different climate requirements. The proportion of the regions predicted to be climatically suitable is narrower for *C. rosa* than for *C. quilicii*, so that *C. quilicii* is more tolerant to a wider range of climatic conditions than *C. rosa*. Tanga et al. (2018) found that the highest fecundity, intrinsic rate of increase and reproduction rate for *C. rosa* was at 25°C and for *C. quilicii* at 30°C. This is in line with the known distribution of *C. rosa* and *C. quilicii* in Africa and the islands of La Réunion and Mauritius, and demonstrates a risk of introduction posed by the two species to cropping regions in the Americas, Australia, India, China, Southeast Asia, southern Europe, and west and central Africa.

Given the models presented by Tanga et al. (2018) there appears to be a marginal risk of *C. quilicii* becoming established in Mediterranean climates with a hot or moderately warm summer (Csa and Csb according to the Köppen–Geiger climate classification). Furthermore, taking into account the current known distribution in southern Africa, semi-arid climates (BSk) could also be vulnerable, in particular if irrigation is being applied. For *C. rosa*, the whole of Europe seems to be unsuitable or only marginally suitable, since it is a tropical pest (hot and humid climate).

1.7. Spread capacity

According to CABI (2018) the flies are usually introduced accidentally by the importation of infested fruit, either with consignments or in the luggage of passengers. Adults usually remain in the area where they emerged, and normally do not fly longer distances than a few hundred metres. Eggs are laid inside the fruit where the larvae also develop. Mature individuals emerge from the fruit and pupate in the soil. Introduction of the pupae via soil has not been reported so far.

1.8. Risk factor identification

A risk factor is a biotic or abiotic factor that increases the probability of infestation by the pest. The risk factors that are relevant for surveillance are those that result in different effects on different parts of the target population depending on its structure and its variability.

Identification of the risk factors and their relative risk estimation is essential when performing a risk-based survey. It needs to be tailored to the situation in each Member State. The proportion of the target population for each risk factor needs to be known or estimated by each Member State. This section presents examples of risk factors. Different Member States may need to consider different risk factors.

The most likely pathway for dispersal and introduction is as larvae in infested fruit with commercial shipments or in the luggage of travellers (CABI, 2018).

The pack houses, nurseries, fresh fruit markets and processing industries handling the host plants are considered as locations in the production areas with a higher risk, particularly those facilities that process imported commodities originating from areas where the pest is present, i.e. *Citrus*, *Malus domestica*, *Prunus armeniaca*, *Prunus persica* and *Pyrus communis* orchards.

2. Detection and identification

2.1. Visual examination

Specimens can be found either by trapping adults using particular attractants and traps or by examining infested fruit showing immature stages (mainly larvae). An identification process is proposed in Figure 4 of Virgilio et al. (2018), providing a whole identification protocol for species within the FAR complex, including *C. rosa* and *C. quilicii* as well as *C. fasciventris* and *C. anonae* (for males, females and immatures).

2.2. Pest identification

Eggs are usually white to creamy yellow in colour. Where eggs are laid, the fruit skin usually becomes discoloured (Tanga et al., 2015).

Both species, like other *Ceratitis* spp., have banded wings, and a swollen scutellum, which is marked yellow and black. The pattern of grey flecks in the basal wing cells distinguishes *Ceratitis* spp. from most other genera of tephritids.

Ceratitis rosa and *C. quilicii* belong to the FAR complex (De Meyer et al., 2015). While male specimens can be easily differentiated from *C. fasciventris* and *C. anonae*, female specimens of *C. fasciventris*, *C. rosa* and *C. quilicii* cannot be differentiated morphologically. The differences with *C. anonae* are minute and subtle and these can be easily confused. Male specimens of *C. rosa* and *C. quilicii* can be differentiated by the shape and ornamentation of the mid tibia (Figure 4).

A detailed description of *C. rosa* can be found at http://projects.bebif.be/fruitfly/taxoninfo.html?id=62 and for *C. quilicii* at http://projects.bebif.be/fruitfly/taxoninfo.html?id=434.

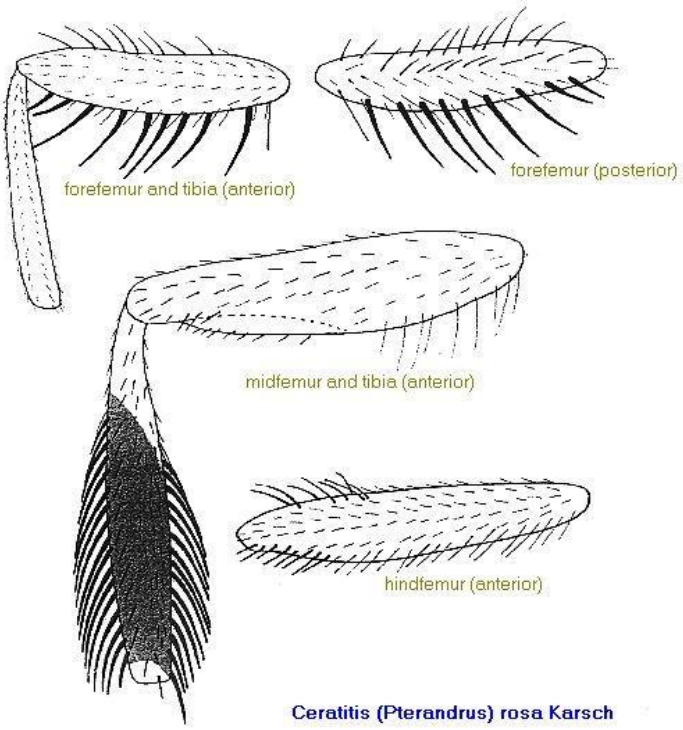

Figure 3: Male leg of *C. rosa*. (Copyright: De Meyer and Freidberg, 2006)

Figure 4: f. *Ceratitis quilicii*: mid tibia, anterior view. g. *Ceratitis rosa* s. str.: midtibia, anterior view. Scale bars = 1 mm (Source: De Meyer et al., 2016)

Figure 5: Female of *C. quilicii* (left) and male of *C. rosa* (right). (Copyright: NHM and RMCA. Source: De Meyer et al., 2016)

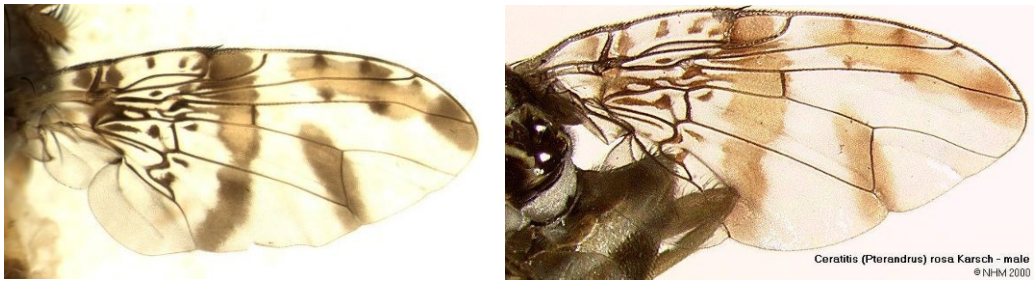

Figure 6: Wing of *C. quilicii* male (left) and of *C. rosa* male (right). (Copyright: NHM and RMCA. Source: De Meyer et al., 2016)

2.2.1. Symptoms

Fruit can be examined for puncture marks that are caused by the female flies puncturing the fruit's skin in order to lay eggs. Puncture marks can be recognised by discolouration of the fruit skin and sometimes also by fruit juices exuding from the puncture hole. In a more advanced stage, the area around the puncture marks becomes soft (the larval feeding behaviour causes the fruit structure to disintegrate). Upon opening the fruit one can detect the larvae, especially if they are in the advanced third instar (De Meyer, EFSA Working Group of Experts of 2018 on surveillance; CABI, 2018).

2.2.2. Traps

Both species and sexes are attracted by protein bait products, e.g. liquid protein baits (Torula yeast), protein bait capsules (Questlure) three-component biolure, and two-component biolure (ammonium acetate and trimethylamine) (Virgilio et al., 2014). Male flies are attracted by trimedlure (t-butyl-4(or 5)-chloro-2-methyl cyclohexane carboxylate) and enriched ginger oil (EGO) lure. Mwatawala et al. (2012, 2015) showed that EGO lure attracted a significantly higher number of males than trimedlure in an experimental setup in central Tanzania, catching significantly more specimens than trimedlure (Mwatawala et al., 2015). Manrakhan et al. (2017) demonstrated that the probability of a trapping grid of five EGO-baited traps per 2.59 km² capturing one or more flies of *C. rosa* (s.l.) for a population consisting of 1 000 males was estimated at over 95%, and flies released at a distance of 200 m from the EGO-baited trap could be captured within one week. Male attractant lures can be applied on a cotton wool wick posed in the centre of a plastic trap with small openings at both ends. Commercially available controlled-release formulations exist for trimedlure and EGO lure, providing a longer-lasting attractant that remains active for a month or longer. Food-based synthetic attractants are also available (see IAEA (2013) for more details). Traps should be placed in fruit trees at a height of about 2 m and be emptied on a regular basis. If the pest is present, hundreds of flies may be caught in one single trap over only a few days.

General information on trapping, types of traps, lures and required density of trapping stations can be found in IAEA (2013), Shelly et al. (2014) and Manrakhan (2016). Specific trapping information can be found in Mwatawala et al. (2015).

2.3. Laboratory testing and pest identification

Specific genetic markers of *C. capitata* and *C. rosa* could be identified and isolated with the amplified fragment-length polymorphism technique. For this method, a repetitive DNA sequence was isolated from the genome of *C. capitata* and then used as a probe. It identified *C. capitata* and *C. rosa* fast and reliably among a collection of other fruit fly species and other insects (Kakouli-Duarte et al., 2001).

Multiple reference DNA barcodes from *C. rosa* distribution are available on the Barcode of Life Data Systems (BOLD) at:
http://www.boldsystems.org/index.php/Taxbrowser_Taxonpage?taxon=Ceratitis+rosa&searchTax=

However, the molecular identification of *C. rosa* with DNA barcoding is problematic because the species cannot be properly differentiated from the closely related species of the FAR (*C. fasciventris*, *C. anonae*, *C. rosa*, including the recently described *C. quilicii* (De Meyer et al., 2016) complex (De Meyer et al., 2015).

An advanced method is proposed by Virgilio et al. (2014), using a reduced set of microsats. This allows differentiation between *C. rosa* and *C. quilicii* at all developmental stages and both sexes.

3. Key elements for survey design

Based on the analyses of the information on the pest–host plant system, the different units that are needed to design the survey have to be defined and tailored to the situation of each Member State. The size of the defined target population and its structure in terms of the number of epidemiological units need to be known. Table 1 shows an example of these definitions. When several pests have to be surveyed in the same crop, it is recommended to use the same epidemiological and inspection units for each pest in order to optimise the survey programme as much as possible.

Table 1: Example of definitions of the target population, epidemiological unit and inspection unit for *C. rosa* and *C. quilicii*

	Definition	**Unit**
Target population	*Citrus* spp., *Malus domestica*, *Prunus armeniaca, Prunus persica*, *Pyrus communis* orchards including backyards/gardens in Member States with a Mediterranean climate	Total number of half hectares
Epidemiological units	Orchards, backyards/gardens, with host plants	Half hectare*
Inspection units	Fruit (young and mature) and traps	

*In Spain, half a hectare of citrus orchard is assumed to represent the average size of a farm area in which the cultivar (citrus species and variety), the cultural practices and the ownership are similar or the same.

The general guidelines for the survey of *C. rosa* and *C. quilicii* are presented in a separate document and describe the process of the survey design step by step:

1/ the choice of the type of survey to develop depending on the objectives of the survey

- Illustration with an example

2/ a description of the different surveillance components required to determine statistically sound sample sizes

3/ a manual for guiding the user through the tools

4/ calculation of the sample size

5/ essential considerations when:

- choosing the sampling sites and taking the samples
- collecting the data
- reporting the data and the survey results

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Glossary

TERM	DEFINITION*
Component (of a survey)	In the general framework of surveillance, with the goal of demonstrating pest freedom, a component is an activity characterised by a given sensitivity of the method of detection and identification. The overall confidence of the survey for pest freedom will result from the combination of the different components. Two components of the same survey could have different target populations. E.g. Survey on an insect performed by trapping of the pest (component 1) and sampling the host plants for visual examination of signs or symptoms (component 2).
Confidence	Sensitivity of the survey. Is a measure of reliability of the survey procedure (Montgomery and Runger, 2010).
Design prevalence	It is based on a pre-survey estimate of the likely actual prevalence of the pest in the field (McMaugh, 2005). The survey will be designed in order to obtain at least a positive test result when the prevalence of the disease will be above the defined value of the design prevalence. In 'freedom from pest' approaches, it is not statistically possible to say that a pest is truly absent from a population (except in the rare case that a census of a population can be completed with 100% detection efficiency). Instead, the maximum prevalence that a pest could have reached can be estimated, this is called the 'design prevalence'. That is, if no pest is found in a survey, the true prevalence is estimated to be somewhere between zero and the design prevalence. (EFSA, 2018)
Diagnostic protocols	Procedures and methods for the detection and identification of regulated pests that are relevant to international trade (ISPM 27: FAO, 2016).
Epidemiological unit	A homogeneous area where the interactions between the pest, the host plants and the abiotic and biotic factors and conditions would result in the same epidemiology should the pest be present. The epidemiological units are subdivisions of the target population and reflect the structure of the target population in a geographical area. They are the units of interest, on which statistics are applied (e.g. a tree, orchard, field, glasshouse, or nursery) (EFSA, 2018).
Expected prevalence	In prevalence estimation approaches, it is the proportion of epidemiological units expected to be infected or infested.
Identification	Information and guidance on methods that either used alone or in combination lead to the identification of the pest (ISPM 27: FAO, 2016).
Inspection	Official visual examination of plants, plant products or other regulated articles to determine whether pests are present or to determine compliance with phytosanitary regulations (ISPM 5: FAO, 2018).
Inspection unit	The inspection units are the plants, plant parts, commodities or pest vectors that will be scrutinised to identify and detect the pests. They are the units within the epidemiological units that could potentially host the pests and on which the pest diagnosis takes place. (EFSA, 2018).
Inspector	Person authorised by a national plant protection organisation to discharge its functions (ISPM 5: FAO, 2018).
Method sensitivity	The conditional probability of testing positive given that the individual is diseased (Dohoo et al., 2010) The method diagnostic sensitivity (DSe) is the probability that a truly positive epidemiological unit will give a positive result and is related to the analytical sensitivity. It corresponds to the probability that a truly positive epidemiological unit that is inspected will be detected and

	confirmed as positive.
Pest diagnosis	The process of detection and identification of a pest (ISPM 5: FAO, 2018).
Pest freedom	An area in which a specific pest is absent as demonstrated by scientific evidence and in which, where appropriate, this condition is being officially maintained (ISPM 5: FAO, 2018).
Population size	The estimation of the number of plants in the region to be surveyed (EFSA, 2018).
Relative risk	The ratio of the risk of disease in the exposed group to the risk of disease in the non-exposed group (Dohoo et al., 2010).
Representative sample	A sample that describes very well the characteristics of the target population (Cameron et al., 2014).
RiBESS+	An online application that implements statistical methods for estimating the sample size, global (and group) sensitivity and probability of freedom from disease. Free access to the software with prior user registration is available at: https://shiny-efsa.openanalytics.eu/
Risk assessment	Evaluation of the probability of the introduction and spread of a pest and the magnitude of the associated potential economic consequences (ISPM 5: FAO, 2018).
Risk factor	A factor that may be involved in causing the disease (Cameron et al., 2014). It is defined as a biotic or abiotic factor that increases the probability of infestation of the epidemiological unit by the pest. The risk factors relevant for the surveillance should have more than one level of risk for the target population. For each level, the relative risk needs to be estimated as the relative probability of infestation compared to a baseline with a level 1. Consideration of risk factors in the survey design allows the survey efforts to be enforced in those areas where the highest probabilities exist to find the pest should the pest be present.
Risk-based survey	A survey design that considers the risk factors and enforces the survey efforts in the corresponding proportion of the target population.
Sample size	The number of sites that need to be surveyed in order to detect a specified proportion of pest infestation with a specific level of confidence, at the design prevalence (McMaugh, 2005).
Survey	An official procedure conducted over a defined period of time to determine the characteristics of a pest population or to determine which species are present in an area (ISPM 5: FAO, 2018).
Target population	The set of individual plants or commodities or vectors in which the pest under scrutiny can be detected directly (e.g. looking for the pest) or indirectly (e.g. looking for symptoms suggesting the presence of the pest) in a given habitat or area of interest. The different components pertaining to the target population that need to be specified are: • Definition of the target population – the target population has to be clearly identified • Target population size and geographic boundary. (EFSA, 2018)
Test	Official examinations, other than visual, to determine whether pests are present or to identify pests (ISPM 5: FAO, 2018).
Test specificity	The conditional probability of testing negative given that the individual does not have the disease of interest (Dohoo et al., 2010). The test diagnostic specificity (DSp) is the probability that a truly negative epidemiological unit will give a negative result and is related to the analytical specificity. In freedom from disease it is assumed to

	be 100%.
Visual examination	The physical examination of plants, plant products, or other regulated articles using the unaided eye, lens, stereoscope or microscope to detect pests or contaminants without testing or processing (ISPM 5: FAO, 2018).

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