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Exotic pulse beetles intercepted in imported legume germplasm

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ABSTRACT

Quarantine examination of 13,29,901 imported seed samples of various crops during 1999- 2014 revealed presence of exotic bruchids commonly called seed or pulse beetles in 2,819 samples which were detected by X-ray radiography. Thirteen exotic bruchid species *viz.*, *Acanthoscelides desmanthi* in *Desmanths* spp. from Colombia, *A. obtectus* in *Phaseolus vulgaris* from Argentina, Colombia Mexico, Peru; *Bruchidius atrolineatus* in *Vigna unguiculata* from Nigeria; *Bruchus affinis* in *Vicia faba* from Afghanistan; *B. dentipes* in *Vicia* spp. from Afghanistan, ICARDA (Syria) and Syria; *B. ervi* in *Lens* spp. from Afghanistan, Chile, Cyprus, Ethiopia, Germany, Greece, ICARDA (Syria), Iran, Iraq, Italy, Jordan, Lebanon, Mexico, Morocco, Russian Federation, Syria and Turkey; *B. nubilis* in *V. faba* from Ukraine; *B. rufimanus* in *V. faba* from Afghanistan, Canada, Spain and Syria; *B. signaticornis* in *L. culinaris* from ICARDA (Syria); *B. tristis* in *Lathyrus odoratus* from ICARDA (Syria); *B. tristiculus* in *V. narborensis* from Portugal; *Callosobruchus rhodesianus* in *Vigna unguiculata* from Nigeria and *C. subinnotatus* in *V. subterranea* from Ghana were intercepted. Many of the pulse beetles were intercepted repeatedly from the same/ different source(s) year after year. All the infested samples were salvaged using suitable disinfestation treatments. None of the intercepted beetles are yet reported from India, and are therefore, of high quarantine significance.

Key words: Bruchids, Exotic, Germplasm, Interception, Legumes, Seed beetles.

INTRODUCTION

ICAR- National Bureau of Plant Genetic Resources (ICAR-NBPGR), New Delhi, India is the nodal agency to undertake quarantine processing of germplasm including transgenic planting material introduced into the country for research purposes both for public and private sectors. Several pulse beetles of great economic significance have been intercepted in imported seed material at ICAR-NBPGR over the years, many of which have yet not been reported from India (Khetarpal *et al.* 2006, Gupta *et al.* 2005, 2010).

The bruchids (Coleoptera: Bruchidae) commonly called seed beetles, belong to a moderate-sized family represented by about 1,600 species in 71 genera found worldwide (Southgate 1979, Gupta *et al*., 2011)). About twenty species are major pests of legumes in field and/or in storage. *Bruchus* and *Bruchidius* species usually attack only field crops, ovipositing on the young pods. The larvae burrow into the green pods and develop within the seeds. Adults of these species emerge in the store, after the crop has been harvested. Since they produce a single generation and are usually unable to breed further on these dry seeds, they are considered less harmful than genera like *Callosobruchus, Zabrotes, Acanthoscelides, Caryedon,* and some *Bruchidius* species which are able to breed successively, producing many generations on the same stored legumes until the food resources are exhausted.

The pulse beetles feed in a wide variety of seeds and cause huge losses to stored grains. Storage species often have a dramatic multiplication leading to over 80% damage within 6-8 months. However, not all bruchids are considered pests; some species of *Tuberculobruchus decelle* and *Acanthoscelides mankinsi* have been used as biocontrol agents of various Acacia and Leucaena weed species, respectively.

The present work is a critical analysis of the samples processed over the previous fifteen years (1999-2014), the pulse beetles intercepted and their level of infestation, the significance of the interceptions and how their interception is a success story in prevention of introduction of such destructive beetles into India.

MATERIALS AND METHODS

Over the past decade, a total of 15,17,177 samples were processed of which 13,19,901 were seeds. All the seed samples were examined visually and under stereo-binocular microscope for any external symptom of insect infestation i.e. holes, rotting, swelling, deformity, etc. or presence of dead or alive insects/ mites, eggs/ egg shells, immature stages, exuviae or excreta thereof. Seed samples belonging to the 340 plant genera known to carry hidden infestation of

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bruchids and phytophagous chalcidoids were compulsorily subjected to X-ray radiography (Bhalla *et al*. 2002). A total of 33,378 samples of seeds of plant genera of *Abelmoschus, Arachis, Casuarina, Cicer, Eucalyptus, Glycine, Gossypium, Helianthus, Lathyrus, Lens, Leucaena, Medicago, Phaseolus, Pisum, Trifolium, Vicia* and *Vigna* were exposed to X-ray radiography or seed transparency to detect presence of seed beetles. Seeds were arranged on small 12 X 12 cm tray kept over the window in the X ray cabinet and exposed to soft X-ray (Cabinet X-ray Systems, Faxitron Series MX 20, USA) kept at a distance of 60 cm from the source. The seed geometry on the plate was left undisturbed. This is a real-time computer controlled X-ray system; hence the image as visualized on the monitor was saved for removal of seeds suspected to carry infestation from the seed geometry mechanically. Internal infestation in samples of small seeds of *Casuarina, Eucalyptus, Medicago* and *Trifolium* spp. was difficult to detect through X-ray radiography, hence, these were subjected to transparency test by heating in lactophenol-acid fuchsin (Kaura 1959).

The insects were retrieved from the infested seeds either by keeping them at 28±1°C and 60±5%RH or soaking overnight in water. The insect pests thus retrieved were identified on the basis of published identification keys, digitized keys (Gupta *et al.*, 2011) and reference collection at ICAR-NBPGR.

The infested samples were salvaged using mechanical cleaning, X-ray radiography (by removing infested seeds) and fumigation. Seeds found infested through X-ray radiography were salvaged by handpicking the infested seeds from the seed geometry as seen on the developed X-ray film.

RESULTS AND DISCUSSION

The year-wise details of seed samples imported X-rayed and found infested during 1999- 2014 are presented in Table 1. A total of 13,19,901 seed samples were imported of which 33,378 were subjected to X-ray and 2,819 samples showed insect infestation. Exotic seed beetles were intercepted in the infested samples from 25 different source countries.

The details of interception of exotic seed beetles, the average % infestation caused by them, hosts on which intercepted, year when imported, per cent infestation and the source/ country of import is presented in Table 2.

More than 85 % of the total samples imported were those of seeds. About 2.5 per cent of the total seed samples subjected to X-ray radiography were infested with bruchids. The percentage infestation varied from 3-100 % in the samples X-rayed during the period.

Upon comparing the samples imported vis-à-vis the total infested seed samples by each intercepted seed beetle, it is clear that the maximum number of samples infested were of *Lens* spp. due to *B. ervi* followed by *Phaseolus* spp. due to *Acanthoscelides obtectus*, followed by *V. faba* due to *B. dentipes* and *B. rufimanus*. However, despite few numbers of samples, 100% average infestation by *B affinis* in *Vicia faba* from Afghanistan and *C. subinnotatus* in *V. subterranea* from Ghana was observed. The average % infestation due to other exotic seed beetles varied from 8-60%.

An analysis of Table 2 indicates that many of these beetles were repeatedly intercepted year after year on the same host and from the same or different source country which is indicative of its pest status there. Four of the pulse beetles intercepted were in material received from ICARDA, Syria, a CGIAR Centre which supplies germplasm material from different source countries. In such a case the infestation could be either from Syria or from the source country of the material which remained undetected due to its hidden nature. Besides, four of the seed beetle species have been intercepted in material received from Afghanistan and Lebanon followed

Table 1: Year-wise details of seed samples processed through X-ray radiography in quarantine.

Year	Seed samples imported	Seed samples X-rayed	Samples infested with seed beetles	Infestation (%)
1999	87,230	4,598	294	6.48
2000	99,624	2,741	333	12.15
2001	85,615	703	92	13.08
2002	83,527	2,214	216	9.75
2003	1,05,676	2,435	134	5.50
2004	76,907	4,572	219	4.79
2005	64,684	1,480	103	6.95
2006	63,599	1,110	91	8.19
2007	91,523	1,130	91	8.05
2008	80,301	1,454	82	5.64
2009	75,475	566	38	6.71
2010	86,766	1,120	72	6.42
2011	1,16,876	1,120	231	20.62
2012	1,01,064	3,997	184	4.60
2013	1,00,579	2,324	274	11.79
2014	1,06,031	1,814	265	14.06
Total	**13,19,901**	**33,378**	**2819**	**Average 8.45%**

Table 2: Seed beetles intercepted during 1999-2014 in different crops.

Insect Pest	Host	Year of Import	% Infested Samples	Source/ Country
Acanthoscelides desmanthi	*Desmanthus* spp.	2013	45.50	Colombia
A. obtectus	**Lathyrus sativus**	***2013***	***12.25***	***Lebanon***
		2014	14.03	
	Phaseolus vulgaris	***2002***	***8.72***	***Argentina, Colombia, Mexico and Peru***
		2003	5.90	Colombia
		2005	8.15	
		2010	***10.22***	***USA***
Bruchidius atrolineatus	*Vigna unguiculata*	2003	33.33	Nigeria
		2004	63.63	
Bruchus affinis	*Vicia faba*	2002	100.00	Afghanistan
B. dentipes	*V. faba*	1999	23.07	ICARDA (Syria)
		2000	59.33	Syria
		2003	50.00	
		2004	11.11	
		2006	40.00	Afghanistan
		2006	12.06	Syria
		2008	3.84	ICARDA (Syria)
		2014	14.05	Lebanon
	V. narbonensis	2006	60.00	Afghanistan
B. ervi	*Lathyrus sativus*	2014	15.05	Lebanon
	Lens spp.	2003	20.18	ICARDA (Syria)
		2004	8.05	ICARDA (Syria)
	L. culinaris	1999	11.00	Afghanistan, Chile, Cyprus, Ethiopia, Iraq, Jordan, Mexico, Syria
		2000	16.10	Chile, Germany, Greece, Iran, Italy, Lebanon, Morocco, Russian, Federation,Syria, Turkey
		2009	20.08	ICARDA (Syria)
		2010	10.00	Syria
	Pisum sativum	2013	12.25	Lebanon
B. nubilis	*V. faba*	2002	38.46	Ukraine
B. rufimanus	*V. faba*	2000	100.0	Syria
		2002	70.00	Canada
		2005	47.05	Spain
		2006	20.68	Afghanistan
		2008	3.84	Syria
B. signaticornis	*L. culinaris*	2004	25.00	ICARDA (Syria)
B. tristis	**Lathyrus odoratus**	***2008***	***39.02***	***ICARDA (Syria)***
	Lens.culinaris	2014	38.08	Lebanon
B. tristiculus	**V. narborensis**	***2006***	***60.00***	***Portugal***
Callosobruchus rhodesianus	**Vigna unguiculata**	***2003***	***55.55***	***Nigeria***
C. subinnotatus	**V. subterranea**	***2008***	***100.00***	***Ghana***

by three species from Syria and two from Mexico and Colombia. In addition, one species each of exotic seed beetle was also intercepted from Argentina, Canada, Chile, Cyprus, Ethiopia, Germany, Ghana, Greece, Iran, Iraq, Italy, Jordan, Morocco, Nigeria, Peru, Portugal, Russian Federation, Spain, Turkey, Ukraine and USA.

Seeds of *Vicia* spp. imported several times over the years were found to be infested by five different species of seed beetles *viz., B. affinis, B. dentipes, B. nubilis, B. rufimanus* and *B. tristiculus*. Three species viz., *Bruchidius atrolineatus*, *Callosobruchus rhodesianus* and *C. subinnotatus* were intercepted on different species of *Vigna*. Two species *viz., B. ervi* and *B. signaticornis* were intercepted on *Lens* spp. and one species each viz., *Acanthoscelides obtectus* and *B. tristis* on *Phaseolus vulgaris* and *Lathyrus odoratus*, respectively.

The year-wise analysis shows that a maximum of five exotic beetles were intercepted in different imports during 2003, four each exotic species were intercepted in imported material during 2002, 2004 and 2008 and three each were intercepted during 2000, 2006, 2013 and 2014, while two exotic pulse beetles were intercepted during 1999, 2005 and 2010, and only one exotic pulse beetle was intercepted in 2009. However, no exotic pulse beetles were intercepted in years 2001 and 2007 (Gupta *et al.*, 2002). The maximum number of interceptions in the year 2003 could be due to the fact that the large number (>1,00,000) and a vast variety of seed genera from different countries were imported that year (Table1).

The pulse beetles that were intercepted repeatedly over the years i.e., eight times were *B. dentipes* and *B ervi*, followed by *Acanthoscelides obtectus* intercepted six times, and *B. rufimanus* which were intercepted five times each, and, *Bruchidius atrolineatus* and *B. tristis* were intercepted twice and the remaining species were intercepted only once during 1999-2014.

Some details on the biology, losses caused, geographic distribution, host range and other information of phytosanitary significance during transboundary movement for each of the intercepted exotic pulse beetle is presented below.

Acanthoscelides desmanthi was intercepted in *Desmanthus* spp. from Colombia is oligophagus pest feeding only on the species belonging to Genus *Desmanthus.* It has been reported from Colombia, Mexico and USA.

A. obtectus was repeatedly intercepted on *Phaseolus vulgaris* from several countries of the Central South America and USA is indicative of the severity of pest problem in the region (Table 2). It has a high potential for population growth due to its wide temperature tolerance i.e., it occurs in cool highland areas, warmer lowland tropics and some temperate regions. Eggs are lodged under cracks in the bean testa and on ripening pods (Howe and Currie, 1964, Meirleire, 1967). It is reported from several countries including a recent report of an Indian biotype and has a wide host range (Thakur, 2012).

Bruchidius atrolineatus intercepted twice in *V. unguiculata* from Nigeria has yet not been reported from India and is a serious pest of *Vigna* spp. and *Lens* spp. from African countries like Kenya, Niger, Nigeria, Togo and from parts of West Africa has unconfirmed records of being present in Central Asia and Australia (CABI, 2007). It has been reported to cause upto 100% loss within few months of storage in cowpea (Kossou *et al.*, 2001).

Bruchus affinis which was detected on seeds of *Vicia faba* from Afghanistan is a pest reported on *Lathyrus* sp. *Lens esculenta*, *Phaseolus* sp., *Pisum sativum* from Algeria, France, Iran, Italy, Sweden and the erstwhile USSR. It is reported to be univoltine and undergoes imaginal reproductive diapause for nine months in the southwest of France (CABI, 2007). A temporal synchronization has been reported between pod dehiscence and adult emergence from the seeds (Bashar *et al.*, 1994).

B. dentipes detected in various species of *Vicia* is a pest specific to faba bean causing upto 76% damage (Hariri and Tahhan 1983) and has been reported from very few countries in Europe (Greece), Asia (Syria and Turkey), and Australia and is yet not reported from India (Table 3). Infestation ranges from 10- 90%, depending on location in the Mediterranean region and with an average of about 42%. Gamma irradiation is found effective as a quarantine disinfestation treatment for faba bean seeds infested with *B. dentipes* in Syria where it is a serious pest (Mansour and Al-Bacheer 1995).

B. ervi intercepted in various *Lens* species from several countries including Syria has unconfirmed records of being present in Central Asia and Australia (CABI, 2007). It has been reported on *Lathyrus latifolius*, *Lens esculenta*, *Ulex europaeus*, *Vicia ervilia* and *V. sativa*, and has been recorded as a serious pest of lentil in Algeria, Iran, Lebanon and Turkey with infestation level reaching upto 80% and loss of germination upto 100% (Hariri, 1981). It is a univoltine species which overwinters in seeds with higher survival, spread and establishment potential i.e., poses a higher quarantine risk. The pest was intercepted at ICAR-NBPGR on *Acacia brachystachya* imported from Australia (Verma *et al.*, 1991) on which it was never reported earlier.

B. rufimanus intercepted from *Vicia faba* is reported to infest several species of grain legumes viz., *Cicer arietinum*, *Lathyrus* spp., *Lens* spp., *Lupinus angustifolius*, *Phaseolus* spp., *Pisum* spp., *Vicia* spp. and *Vigna subterranea* and has yet not been reported from India but is widely distributed in Europe, some parts of Asia and Africa and hence , poses a higher quarantine risk. It is a univoltine species and *Vicia faba* seeds, sampled from 29 different areas of Morocco revealed an average infestation level of 33% (Boughdad and Lauge, 1997).

A perusal of literature shows that not much is reported on the biology and lifecycle of *B nubilis*, *B tristis* and *B tristiculus* except their host range and distribution across the world (Table 3). However, all three bruchids causes hidden infestation which is of high quarantine risk. *B. signaticornis* intercepted on *Lens culinaris* from ICARDA (Syria) has been reported on several hosts from countries in European and Mediterranean region and USSR (erstwhile) (Table 3). It is reported to be highly injurious to lentil in France (Coutin and Moreau, 1978). Together with *B. lentis*, it causes loss of 35% of lentil seeds attacked in the field in Spain and germination was reduced by upto 64% (Monoz, *et al.*, 1992).

Callosobruchus rhodesianus intercepted on *Vigna unguiculata* from Nigeria has been reported on cowpea,

Table 3: Geographical distribution and host range of intercepted exotic seed beetles.

Insect Pest	Host Range	Geographical Distribution
Acanthoscelides desmanthi	*Desmanthus covillei, D. virgatus, D. virgatus* var. *depressus, Desmanthus* spp.	Colombia, Mexico, USA
A. obtectus	Albizzia *sp*., Astragalus *sp*., Cajanus indica, C. sativus, Cicer arietinum, Cicer *sp*., Dolichos melanophthalmus, Erythrina *sp*., Fagopyrum esculentum, Glycine hispida, Lathyrus sativus, Lens esculenta, Lupinus albus, Mucuna pruriens, Phaseolus aconitifolius, P. aconitifolius latifolius, P. aureus, P. calcaratus, P. carcacalla, P. coccineus, P. latifolius, P. lunatus, P. lunatus macrocarpa, P. macrocarpus, P. multiflorus, P. mungo, P. vulgaris, Pisum arvense, P. sativum, Sesbania aegyptica, Tephrosia cuspidate, T. virginica, Vicia faba, V. sativa, Vigna catjang, V. ribra, V. sesquipedalis, V. sinensis, V. subterranea, Zea mays	*Widely distributed*
Bruchidius atrolineatus	*Astragalus* sp., *Cicer arietinum, Medicago acutellata, Medicago* spp., *Pisum arvense, P. sativum, Trigonella corniculata, Vicia faba, Vigna unguiculata V. villosa, Vigna* spp.	Algeria, Canary Islands, Egypt, France, Germany, Iraq, Israel, Italy, Lebanon, Syria, Turkey,, USSR (erstwhile), Yugoslavia
Bruchus affinis	*Lathyrus* spp., *L. latyfolius, Lens esculenta, Phaseolus* sp., *Pisum sativum, Sarothamnus scorarius, Vicia* spp.	Algeria, France, Iran, Italy, Sweden, USSR (erstwhile) Afghanistan, Iraq, Syria, USSR (erstwhile)
B. dentipes	*Vicia faba, V. hyrcana, V. lutea, V. sativa, Vicia* sp.	Algeria, Australia (Verma *et al*.,
B. ervi	*Acacia brachystachya, Lathyrus latifolius, Lens esculenta, Ulex europaeus, Vicia ervilia, V. sativa*	1991), Cyprus, Egypt, France, Germany, Hungary, Iran, Israel, Italy Lebanon, Martinique Island, Reunion Island, Syria, Turkey
B. nubilis	*Calycotome spinosa, Coronilla emerus, Cyticus, sessilifolius, C. triflorus, Lathyrus angustatus, L. angustifilius, L. aphaca, L. ciceria, L. sativus, Lathyrus* sp. , *Lens esculenta, Pisum arvense, P. sativum, Vicia angustifolia, V. cracca, V. ervilia, V faba, V hirsute, V. lutea, V. macrocarpa, V. peregrine, V. sativa, V. sepium, V. sicula, V. tenuifolia, V. villosa, Vicia* sp.	Algeria, Canary Islands, France, Greece, Israel, Italy, Turkey, USSR (erstwhile), Yugoslavia
B. rufimanus	*Cicer arietinum, Lathyrus sativus, Lathyrus* sp., *Lens esculenta, Lens* sp., *Lupinus angustifolius, Phaseolus* sp., *Pisum sativum, Pisum* sp., *Vicia faba, Vicia* sp., *Vigna subterranea*	Widely distributed
B signaticornis	*C. arietinum, Lathyrus* sp., *Lens esculenta, Lupinus albus, L. termis, Pisum arvense, P. sativum, Trigonella corniculatus, Vicia* spp., *Vigna subterranea*	Algeria, Azores Islands, Canary Islands, France, Hungary, Iran, Israel, Italy, Syria, Turkey, USSR (erstwhile)
B. tristis	*Calycotome spinosa, Calycotome* sp., *Lens esculenta, Lens* sp., *Pisum sativum, Pisum* sp., *Ulex* spp., *Vicia ervilia, V. sativa*	Algeria, Canary Islands, Crete, Cyprus France, Greece, Italy, Malta, Spain, Syria, Turkey, USA, USSR (erstwhile)
B. tristiculus	Cicer arietinum, Lathyrus *spp*., Lens esculenta, Lupinus termis, Pisum arvense, P. sativum, Vicia ervilia, V. faba	*Algeria, Egypt, France, Hungary, Iran, Israel, Italy, Sicily, Syria, Turkey*
Callosobruchus rhodesianus	Arachis hypogea, Vigna subterranea	*Cameroon, Gabon, Guinea, Nigeria, Senegal*
C. subinnotatus	*Rhynchosia* sp., *V. subterranea*	South Africa, Zambia

groundnut and bambara groundnut from some of the north African countries. Low temperature has been tested for disinfesting stored products against *C. rhodesianus* as a quick and safe alternative to methyl bromide fumigation for quarantine.

C. subinnotatus intercepted on Bambara groundnut *(V. subterranea)* from Ghana has been reported on *Rhynchosia* sp. and *V. subterranea* from parts of Africa. High mortality of different developmental stages of *C. subinnotatus* has been reported due to simulated solar heat in Bambara groundnut seeds (Lale and Vidal, 2000). While studies on modified atmosphere revealed that pupae of *C. subinnotatus* are most tolerant to hypercarbic and hypoxic atmospheres than adults (Mbata *et al*., 2000).

All the above intercepted pulse beetles are not yet reported from India and hence are of very high quarantine significance. Had they not been intercepted in quarantine they could have gained entry into the country. It is important to note the distribution and host range of each of the intercepted species which indicates the potential pathways for the entry of these seed beetles into the country (Table 3). Several pulse beetles infesting various crops have moved across the world which include *A. obtectus* indigenous to North and South America but which got introduced into Asia, Africa, Europe and Australia; *C. analis* indigenous to Asia got introduced in Africa; *C. chinensis, C. maculatus* and *Caryedon serratus* indigenous to Asia and Africa got introduced in Europe, North and South America, Europe, North and South America and Australia; and South America and Australia, respectively; *Zabrotes subfasciatus* indigenous to North and South America got introduced in Asia, Africa and Europe (Southgate, 1978; Bhalla *et al*., 2006).

All the seed samples infested with pulse beetles were salvaged using various methods *viz.*, mechanical cleaning done by removing infested/ deformed seeds, X-ray radiography and fumigation treatment. Two thousand, eight hundred and nineteen samples found infested through X-ray were salvaged by handpicking the infested seeds from the seed geometry as seen on the developed image of X-ray on the screen and/ or were fumigated with ethylene dichloride-carbon tetrachloride (EDCT) mixture @ 320 mg/ l for 48 h or 640 mg/ l for 24 h at 30°C in an airtight container at normal atmospheric pressure.

The Plant Quarantine (Regulation of Import into India) Order 2003 also requires freedom of seeds of certain tree species like elm, oak, pine and grain legume seeds (both for planting and consumption) from certain crop/ species specific pulse beetle/ bruchids in the Phytosanitary Certificate and the special conditions of freedom from soil, quarantine weed seeds, prior approval from Department of Agriculture Cooperation and Farmers Welfare and fumigation are fulfilled during their import (Plant Quarantine (Regulation of Import into India) Order, 2003). In view of the interception of several exotic seed beetles of quarantine significance from more than 25 countries in the past fifteen years it is essential to pay due attention to the regulations and the requirements thereunder. They are especially meant to prevent the entry of such exotic pulse beetle species that could become serious economic threats in India, if they get introduced and find favorable areas where the climatic conditions are suitable for their establishment. Therefore, effective quarantine processing is of paramount importance for the safe exchange of seeds.

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