DOI 10.5958/0976-1926.2021.00006.1

RESEARCH ARTICLE

Interception of Insect Pests using X-Ray Radiography during Quarantine of Plant Genetic Resources

Kavita Gupta*, SP Singh, Shashi Bhalla and DS Meena
Division of Plant Quarantine, ICAR-National Bureau of Plant Genetic Resources, Pusa Campus, New Delhi-110012, India

(Received: 20 May, 2020; Revised: 17 January, 2021; Accepted: 08 March, 2021)

X-ray radiography is a tool used in quarantine processing of 340 plant genera for detection of hidden infestation. X-ray of 22,146 samples belonging to 55 plant genera from a total of 9,48,751 imported samples of various crops during 2007- 2016 revealed presence of hidden infestation caused by bruchids and phytophagous chalcids in 1971 samples. Seven exotic bruchid species viz., *Acanthoscelides desmanthi* in *Desmanthus* spp. from Colombia, *A. obtectus* in *Phaseolus vulgaris* from South Africa and USA and in *Lathyrus sativus* from Lebanon; *Bruchus atomarius* in *Lens culinaris* from Lebanon; *B. dentipes* in *Vicia* spp. from ICARDA (Syria) and Lebanon; *B. ervi* in *Pisum sativum* and *L. sativus* from Lebanon and *Lens culinaris* from ICARDA (Syria), Lebanon, Morocco and Syria; *B. tristis* in *L. culinaris* and *Lathyrus sativus* from Lebanon and ICARDA (Syria), respectively; *Callosobruchus subinnotatus* in *V. subterranea* from Ghana and one seed chalcid *Bruchophagus roddi* in *Trifolium pratense* from USA were intercepted. Many of these were intercepted repeatedly from the same/ different source(s) year after year. All the infested samples were salvaged either through X-ray radiography or by using suitable disinfestation treatments. Many of the intercepted pests are not yet reported from India, and are therefore, of high quarantine significance.

Key Words: Bruchids, Exotic, Germplasm, Interception, Quarantine, X-ray radiography

Introduction

A large number of plant germplasm samples are being exchanged throughout the world for crop improvement programmes, more so, under the liberalized WTO regime. Such exchanges carry the risk of inadvertent introduction of exotic pests. If established, these pests could prove more devastating in the new geographical areas in the absence of their natural enemies and competitors. Quarantine testing of the planting material under exchange reduces this risk.

ICAR-National Bureau of Plant Genetic Resources (NBPGR) is the nodal agency in India to undertake the quarantine processing of germplasm including that of transgenics introduced into the country for research purposes. Over the years, effective quarantine processing at ICAR-NBPGR has resulted in the interception of a number of exotic pests. These include *Acanthoscelides obtectus* in *Cajanus cajan, Phaseolus vulgaris* and *Vicia faba* from Brazil, Bulgaria, Malawi; *Anthonomus grandis* in *Gossypium* sp. and *Hibicus* spp. from USA; *Bruchophagus gibbus* in *Medicago* spp. and *Trifolium* spp. from France, Germany, Italy and USA; *Leptinotarsa decemlineata* in potato from Europe and USA; *Popillia japonica* in root stocks of nursery crops from Japan and USA.

Certain groups of insects infesting seeds, *viz.*, seed wasps (Hymenoptera: Chalcidoidea), pulses beetles (Coleoptera: Bruchidae), and a few weevils/borers (Coleoptera: Curculionidae/Scolytidae) infest the developing seeds without showing any external signs or symptoms of their presence within. The quarantine risk becomes much higher with the exchange of seeds with such hidden infestation because these are not detectable through routine visual inspection.

These are several examples of transboundary movement of insects causing hidden infestation *viz.*, The douglas fir seed chalcid *(Megastigmus spermotrophus Wachtl.)* has spread from its original home in United States to Scotland (Mac Dougali, 1926), France (Vayssiere, 1931), Germany (Echerich, 1938) and New Zealand (Gourlay, 1930) alongwith the shipment of douglas fir *(Pseudotsuga taxifolia*) seeds. The rose

**Email: kavita.gupta@icar.gov.in, kavita6864@gmail.com*

seed chalcid [*Megastigmus aculeatus* (Swedrus)] was introduced into New Jersey (USA) through the seeds of *Rosa multiflora* from Japan (Weiss, 1917). The crop losses reported to have been caused by various introduced chalcid pests include over 80% seed damage by *Bruchophagus roddi* in seeds of alfalfa (Stronge, 1962) and about 40% by *Systole albipennis* in various umbelliferous seeds (Gupta, 1962). Seed damage to the extent of about 40% has been reported in *Trifolium alexandrium* by bruchids, *viz., Bruchidius alfieri* and *Bruchidius trifolii* (Abou-Roya, 1954). Recently, an Indian biotype of *Acanthoscelides obtectus* was detected in French beans from Himachal Pradesh (Thakur, 2010). These are detected through employment of special detection techniques such as X-ray radiography and transparency test.

Use of X-ray radiography was initiated in 1983 in the Division of Plant Quarantine, ICAR-NBPGR, New Delhi, India for detection of hidden infestation caused by bruchids and phytophagous chalcids when an initial list of host plant genera was prepared. Bhalla *et al.*, 2002 reported 340 plant genera belonging to 75 families are known to carry hidden infestation. The present paper highlights the detection of insect pests through X-ray radiography and how this technique has helped in detection of exotic pests in imported germplasm many of which are legumes thereby prevented their introduction into India. The quarantine significance of the pests intercepted is also discussed.

Materials and Methods

Over the past decade from 2007-16, a total of 9,48,751 samples were processed of which 8,79,934 were seeds (Table 1). 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 bruchids and phytophagous chalcidoids were compulsorily subjected to X-ray radiography (Bhalla *et al*., 2002). A total of 22,146 samples of seeds of 55 plant genera of various crops listed in Table-2 were exposed to X-ray radiography or seed transparency to detect presence of seed beetles.

Seeds were arranged on small 12 × 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. It is a self-calibrated machine and depending on the seed structure, it automatically adjusts the exposure parameters to get a sharp image of the seed. 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. Alternatively, another manual Faxitron X-ray system was also used where the seeds are spread on a sheet of paper that is placed on a black envelope containing the X-ray film and are exposed to soft X-rays generated at 22 Kv, 3 mA, at a distance of 30 cm from the source for 15 seconds. The envelope containing the exposed film was removed gently without disturbing the seed geometry and the film is developed/ fixer prepared for the purpose in a dark room. In both the cases, infested seeds were marked/ identified on the X-ray and corresponding (infested) seeds were hand-picked from the seed sample with original seed geometry. The dose, however, was varied with the size, shape or thickness of the seeds for the better resolution of image on the X-ray plate/ computer monitor. The dose was also standardized for different seeds as and when required, as was done in case of mango stones (exceptionally large seeds) for detection of mango stone weevil, *Sternochaetus mangiferae* as 60KV, 3 mA at a distance of 30 cm from source for 30 seconds (Kapur *et al*., 1997). Composition of the solutions used in developing and fixing the image on X ray film used were as per Bhalla *et al.*, 2009)

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). Seeds were submerged in test tubes containing lactophenol-acid fuchsin and boiled for 10-20 minutes and left overnight. On next day, transparent seeds were observed for internal infestation with the help of stereo-binoculars.

The eggs and adult insects can be easily detected through naked eye observation or under stereo-binoculars. However, once the eggs hatch the first instar larva bores into the seed and the remaining development up to pupal stage is completed inside the seed, which can only be detected through X-ray radiography (Fig. 1). The adult insects were retrieved from the infested seeds either by

Table 1. Details of crop species X-rayed during 2007-16

Crop	Source	Year(s) of Import	Consignments (No. of Samples)	Samples Infested
Abelmoschus esculentus	Bangladesh, Germany, Taiwan, USA	2008, 2012, 2013, 2014	13(329)	3
Abelmoschus spp.	Niger, USA	2011, 2015	3(547)	20
Acacia sp.	Australia	2008	1(3)	02
Aeschynomene spp.	Ethiopia	2011	1(19)	
Arachis hypogaea	USA	2010, 2013	2(2)	
Arachis spp.	Ethiopia	2011	1(2)	
Brachypodium distachyon	USA	2010	1(1)	
Calopogonium spp.	Ethiopia	2011	1(5)	
Canavalia spp.	Ethiopia	2011	1(7)	
Caragana arborescens	USA	2013	1(10)	
Cassia angustifolia	USA	2011	1(5)	
Casuarina sp.	Australia	2008	1(1)	
Centrosema spp.	Ethiopia	2011	1(10)	
Cicer arietinum	Canada, ICARDA (Syria), Lebanon, Morocco, Spain, Syria, USA, Uzbekistan	2007, 2009, 2010, 2011, 2013, 2014, 2015, 2016	25(3782)	13
Cicer spp.	Syria	2011, 2009	2(7)	
Clitoria ternatea	Colombia	2012	1(102)	
Coriandrum sativum	Germany, Kyrgyzstan, UAE, Uzbekistan	2009, 2015, 2016	4(6)	3
Crotalaria juncea	USA	2016	1(1)	
Crotalaria spp.	USA	2013	1(33)	
Cuminum cyminum	UAE	2009	1(3)	
Desmanthus spp.	Columbia	2013	1(216)	16
Dolichos lablab	Taiwan	2013	1(35)	
Dolichos spp.	Ethiopia	2011	1(1)	
Elaeis guinensis	Malaysia	2015	1(20)	
Eucalyptus spp.	Australia	2008	1(1)	
Fagopyrum esculentum	Germany, Uzbekistan	2015, 2016	2(3)	
Foeniculum vulgare	UAE	2009	1(3)	
Gladiolus sp.	South Africa	2009	1(24)	03
Glycine max	USA	2013	1(3)	
Gossypium sp.	USA	2007, 2010	2(114)	
Gossypium anstral	Australia	2012	1(2)	
G. arboreum	Pakistan	2011	1(5)	
G. barbadense	USA	2008	1(80)	
G. hirsutum	Israel, Pakistan, USA	2007, 2008, 2009, 2010, 2011, 2012, 2013, 2014, 2015, 2016	32(1817)	15
Helianthus annuus	Egypt, France, Switzerland, UK, USA	2007, 2008, 2009, 2011, 2013	14(836)	01
Hibiscus spp.	USA	2013	1(20)	
Jatropha curcas	Italy	2010	1(23)	
Lathyrus odoratus	Switzerland, Syria	2008, 2011	2(28)	7
L. sativus	ICARDA (Syria), Lebanon, Nepal, Syria, UK	2008, 2009, 2010, 2011, 2013, 2014, 2015, 2016	17(933)	41
Lathyrus sp.	Ethiopia, ICARDA (Syria)	2007, 2011	2(14)	
Lens culinaris	Australia, Canada ICARDA (Syria), Lebanon, Morocco, Nepal, Syria, Turkey, USA	2007, 2008, 2009, 2010, 2011, 2013, 2014, 2015, 2016	24(4919)	1435
Lens spp.	Syria	2008, 2009	3(495)	05
Lotus japonicas	Japan	2010	1(02)	
Lupinus spp.	Australia, USA	2012, 2014	2(26)	
Macadamia integrifolia	USA	2015	1(10)	

Crop	Source	Year(s) of Import	Consignments (No. of Samples)	Samples Infested
Macroptilium astropurpure	Ethiopia	2011	1(10)	01
Macrotyloma spp.	Ethiopia	2011	1(12)	
Mangifera indica *Mangifera* sp.	Israel	2012, 2016, 2009	5(2862)	02
Medicago lupulina	Switzerland	2010	1(05)	
M. sativa	Japan, USA	2008, 2013	2(09)	
Medicago spp.	New Zealand	2011	1(23)	01
Nenolonia spp.	Ethiopia	2011	1(05)	
Onobrychis vicifolia	Russia	2010	1(03)	03
Panicum maximum	USA	2008	1(01)	
Pennisetum glaucum	USA	2008	1(02)	01
P. purpureum	Ethiopia	2012	1(27)	
Phaseolus lunatus	Taiwan	2013	1(18)	
Phaseolus spp.	Ethiopia, Taiwan	2011, 2013	2(18)	
P. vulgaris	Canada, Colombia, Egypt, Kazakhstan, Nepal, Philippines, South Africa, USA, Uzbekistan	2007, 2009, 2010, 2012, 2013, 2014, 2015, 2016	16(438)	17
Phoenix dactylifera	Egypt	2007	1(04)	
Pisum sativum	Australia, Canada Spain, Switzerland, USA	2007, 2009, 2010, 2014, 2015, 2016	9(684)	28
P. fulvum	Spain	2009	1(08)	04
Pisum spp.	Germany	2016	1(03)	
Pueraria spp.	Ethiopia	2011	1(05)	
Rhynchosia spp.	Ethiopia	2011	1(16)	
Ricinus communis	USA, Uzbekistan	2015, 2016	2(95)	
Sesbania spp.	Ethiopia	2016	1(17)	06
Stylosanthes spp.	Ethiopia	2011	1(37)	
Theobroma cacao	Malaysia	2007	1(02)	02
Trifolium alexandrinum	Egypt, USA, Uzbekistan	2007, 2008, 2010, 2015	4(37)	04
T. pratense	USA	2007	1(76)	02
Trigonella foenum graecum	Syria	2010	2(54)	
	UAE	2009	1(03)	
Trigonella spp.	USA	2014	1(41)	
Vicia faba	Cyprus, Lebanon, Syria	2008, 2011, 2013, 2014, 2015, 2016	10(903)	115
V. narborensis	Lebanon	2014	1(01)	
V. sativa	Lebanon, USA	2013, 2014	2(07)	
Vicia spp.	Ethiopia, ICARDA (Syria), Syria	2007, 2009, 2011	3(48)	2
Vigna mungo	Nepal, USA	2009, 2014	2(34)	07
V. radiata	Australia, Nepal, Taiwan, Thailand	2007, 2008, 2009, 2015	4(321)	11
Vigna spp.	Ethiopia, Taiwan	2008, 2011	2(38)	
V. subterranea	Ghana	2008	1(02)	2
V. umbellata	Taiwan	2008	1(07)	
V. unguiculata	Belgium, Italy, IITA (Nigeria), Taiwan, Uzbekistan	2007, 2010, 2011, 2012, 2013, 2015	9(1698)	203
V. unguiculata subsp. *sesquipedalis*	Philippines, Taiwan	2010, 2015	2(24)	
Zornia spp.	Ethiopia	2011	1(19)	
			280 (22,146)	1971

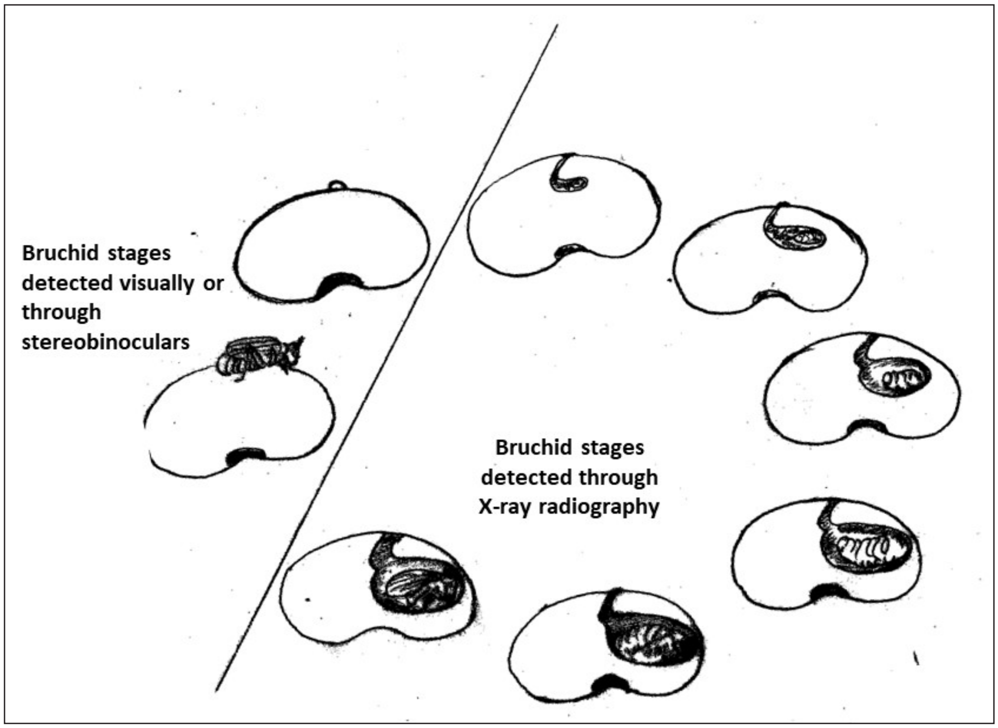

Fig. 1. Representative diagram of bruchid life stages detected through X-ray radiography

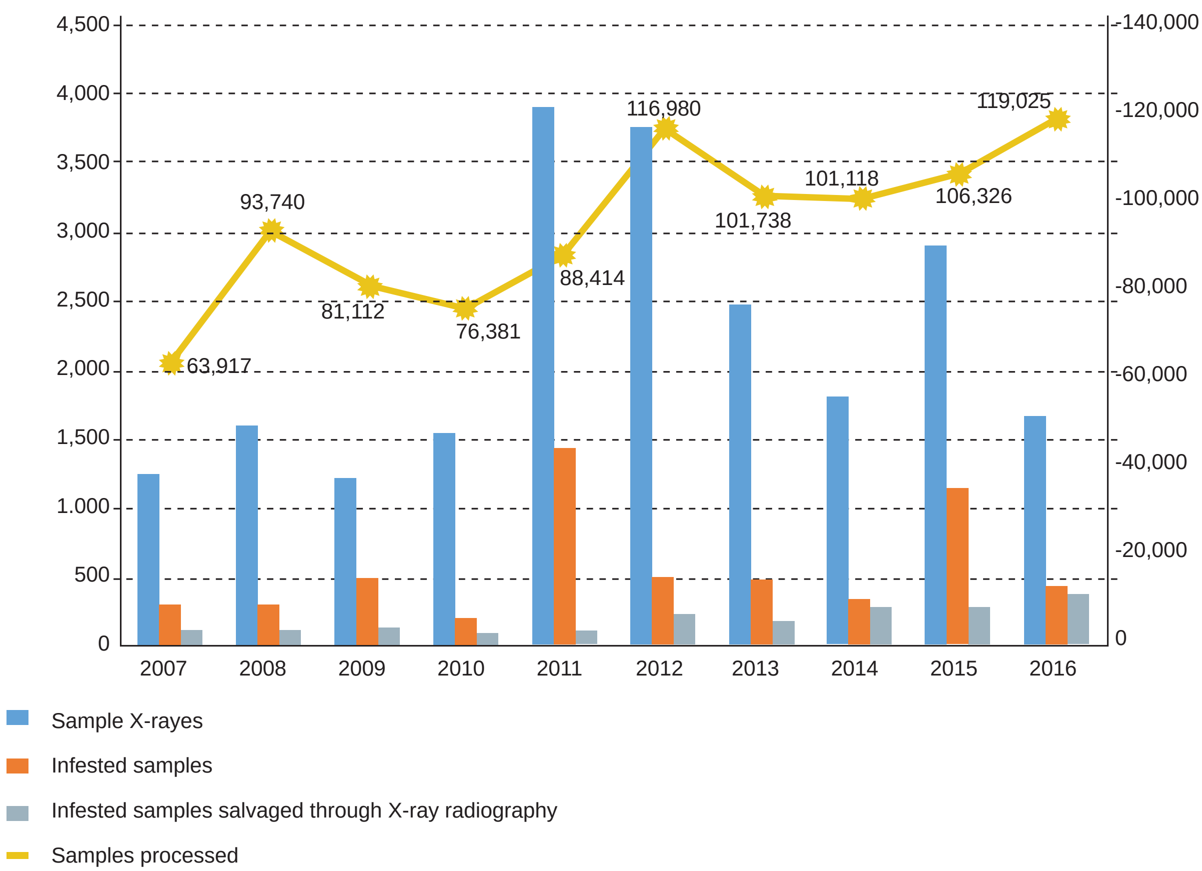

Fig. 2. Detection of insect infestation through X-ray radiography

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 either or in combination- 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 X-ray.

Results and Discussion

The year-wise details of imported seed samples X-rayed and found infested during 2007- 2016 are presented in Figure 2. A total of 9,48,751 seed samples were imported of which 22,146 were subjected to X-ray and 1,971 samples showed insect infestation through X-ray (Figure 2). More than 90% of the total samples imported were those of seeds. About 2.5% of the total seed samples belonged to one of the 340 plant genera earmarked for X-ray radiography of which approximately 35% were infested with bruchids.

Upon comparing the samples imported vis-à-vis the total infested seed samples by each intercepted seed beetle, it was clear that the maximum number of samples infested were of *Lens* spp. due to *B. ervi* followed by *Vigna unguiculata* and *Vicia faba*. However, despite few numbers of samples, 100% average infestation was observed in *Onobrychis vicifolia, Theobroma cacao* and *Vigna subterranea*. The average per cent infestation due to other exotic seed beetles varied from 8-60% (Table 1). Exotic insects were intercepted in the infested samples from 19 different source countries (Table 2).

It is clear that many of these crops were repeatedly imported year after year or several consignments in a year from the same or different source country which is indicative of its importance and demand among the indentors. The largest number of samples X-rayed (4,919) were of lentil divided in 24 consignments imported from nine source countries/ CG Centres, followed by five consignments of mango stones (2,862) from three countries; followed by 32 consignments of cotton (1,817) from three countries. The maximum consignments were for *Gossypium hirsutum* (32); followed by *Cicer arietinum* (25); followed by *Lens culinaris* (24). *G. hirsutum* was the only crop species that was imported every year from 2007-2016 while *Lens culinaris* was imported during nine of the ten years and *Lathyrus sativus* and *Phaseolus vulgaris* were imported during eight of the ten years under analysis.

The details of interception of exotic seed beetles, hosts on which intercepted and the source or country of import is presented in Table 2. A closer look at the Table 2 shows that as many as seven bruchid species were intercepted from the material received from ICARDA, CGIAR Centre, which supplies germplasm from different source countries. Four of these bruchids *viz*., *Bruchus dentipes, B. ervi, B. rufimanus* and *B. tristis* are not yet reported from India. In such a case, the infestation could be either from Syria or from the source country/ station of the material, which remained undetected due to its hidden nature. Six of the bruchid species have also been intercepted in material received from Lebanon of which five are exotic and not yet reported from India and hence, of great quarantine significance. Two exotic bruchid species were intercepted in material from USA. In addition, one species each of exotic bruchid was also intercepted from Colombia, Ghana, Morocco and South Africa (Table 2).

Seeds of *Lens* spp. including International Trial Nurseries imported several times over the years were found to be infested by five different species of bruchids, three of which are exotic *viz*., *B. atomarius, B. ervi* and *B. tristis*. *Lathyrus* spp also imported several times during 2007-16 showed infestation by five different species of bruchids, three of whom were exotic *viz*., *Acanthoscelides obtectus*, *B. ervi* and *B. tristis*. One exotic species each *viz*., *A. obtectus* on *Phaseolus vulgaris, B. ervi* on *Pisum* spp. and *Bruchophagus roddi* on *Trifolium pratense,* respectively were intercepted (Table 2).

Some details on the biology, losses caused, geographical distribution, host range and other information of phytosanitary significance during transboundary movement for each of the intercepted insect pest 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.

Acanthoscelides obtectus was repeatedly intercepted on *Phaseolus vulgaris* from several countries *viz*., Lebanon, South Africa and USA is indicative of the severity of pest problem in the region (Table 2). It has

Table 2. Interceptions of pests in imported seeds examined through X-ray radiography

Insect pest	Host	Source
***Acanthoscelides desmanthi**	*Desmanthus* spp.	Colombia
***A. obtectus*	*Phaseolus vulgaris*	South Africa, USA
	Lathyrus sativus	Lebanon
Bruchidius trifoli.	*Trifolium alexandrium*	Egypt
**Bruchophagus roddi*	*T. pratense*	USA
Bruchophagus sp.	*Medicago* spp.	New Zealand
Bruchus atomarius	*Lens culinaris*	Lebanon
B. dentipes	*Vicia faba*	ICARDA (Syria), Lebanon
B. emarginatus	*Pisum sativum*	Spain
B. ervi	*P. sativum, Lathyrus sativus*	Lebanon
	Lens culinaris	ICARDA (Syria), Lebanon, Morocco, Syria
B. lentis	*L. culinaris*	ICARDA (Syria), Lebanon
B. rufimanus	*V. faba*	ICARDA (Morocco)
B. tristis	*L. culinaris*	Lebanon
	Lathyrus sativus	ICARDA (Syria)
Callosobruchus analis	*L. sativus*	ICARDA (Syria)
C. chinensis	*L. sativus*	Taiwan
	Lens culinaris	ICARDA (Syria)
	Macroptilium atropurpurius	Ethiopia
	Vigna unguiculata	Italy
C. maculatus	*V. mungo, V. radiata*	Nepal
	V. unguiculata	Italy
**C. subinnotatus*	*V. subterranea*	Ghana
Chalcids	*Daucus carota, Onobrychis vicifolia*	Russian Federation
Pectinophora gossypiella	*G. hirsutum*	Israel, USA
Systole coriandri	*Coriandrum sativum*	UAE
Immature stages of bruchids	*V. mungo*	USA
	L. culinaris	Morocco
	Crotolaria juncea	Ethiopia
	P. sativum	Australia
	P. vulgaris	USA
	Gossypium hirsutum	Israel

* Quarantine pest not yet reported from India
** Regulated pest under PQ Order 2003 and its amendments

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, 2010).

Bruchidius trifolii is not yet reported to be a pest of *Trifolium* spp. in India although the genus *Bruchidius* has been reported on other host plants from India. Three species of *Bruchidius* are reported on *Trifolium* spp. from Egypt *viz*., *B. alfierii, B. poupillieri* and *B. trifolii* and none of them are reported from India (Gupta *et al*., 2011).

Bruchophagus roddi, a Eurytomid chalcidoid has been reported only on *Medicago sativa*. The larvae develop within a single alfalfa seed and they are easy to spread through the world in the seed trade. The seed wasp has continuing, overlapping generations, which means that all stages of the life cycle are present at any one time in any one area. *B. roddi* is closely related to its host plants and it can be found wherever alfalfa is grown. It is said to have a cosmopolitan distribution. In alfalfa seed-producing areas of North America, up to 80-90% of the harvested seed may be infested and this can cause great financial losses (CAB International, 2007).

Bruchus atomarius, which was detected on seeds of *Lens culinaris* from Lebanon is a pest reported on

Table 3. Exotic pests, their host range and geographical distribution

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 trifolii	*Medicago sativa, Trifolium alexandrinum, T. incarnatum, T. maritimum, T. ochraceum, T. pretense, Trifolium* sp.	Algeria, Egypt, Israel, Libya
Bruchophagus roddi	Medicago arabica, *M. falcata, M. minima, M. polymorpha, M. sativa, M. sativa* subsp. *caerulea* and *M. sativa* subsp. *glomerata*	Argentina, Australia, Austria, Brazil, Bulgaria, Canada, Chile, Czechoslovakia (former), France, Germany, Hungary, India, Iran, Iraq, Israel, Kazakhstan, Kyrgyzstan, Moldova, Mongolia, New Zealand, Peru, Poland, Romania, Russian Federation, Serbia, Slovakia, South Africa, Spain, Sweden, Turkey, Turkmenistan, Ukraine, USA, Uzbekistan and Yugoslavia (Serbia and Montenegro)
Bruchus atomarius	*Lathyrus niger, L. pisiformis, L. pratensis, L. sphoericus, L. tuberosus, L. vernus, Lens esculenta, Lens* spp., *Phaseolus* sp., *Pisum sativum, Sarothamnus scoparius, Vicia angustifolia, Vicia cracca, V. dumetorum, V. faba, V. narbonensis, V. pisiformis, V. sativa, V. sapium, V. tenuifolia*	Algeria, France, Iran, Italy, Lebanon, Sweden, USSR (erstwhile)
B. dentipes	*Vicia faba, V. hyrcana, V. lutea, V. sativa, Vicia* sp.	Australia, Afghanistan, Greece, Iraq, Syria, Turkey, USSR (erstwhile)
B. emarginatus	*Cicer arietinum, Lathyrus angulatus, L. angustifolius, L. hirsutus, L. ochrous, Pisum arvense, P. sativum, Vicia peregrina, V. sativa*	Algeria, France, Germany, India, Israel, Italy, Lebanon, Syria, Turkey, USSR (erstwhile), Yugoslavia (former)
B. ervi	*Acacia brachystachya, Lathyrus latifolius, Lens esculenta, Ulex europaeus, Vicia ervilia, V. sativa*	Algeria, Australia (Verma *et al*., 1991), Cyprus, Egypt, France, Germany, Hungary, Iran, Israel, Italy, Lebanon, Martinique Island, Reunion Island, Syria, Turkey
B. rufimanus	*Vicia faba, V. hybrida, V. leucosperma, V. lutea, V. macrocarpa, V. minor, V. monanthos, V. narbonensis, V. pannonica, V. peregrina, V. sativa, V. tenuifolia, V. vestita, V. villosa, Vicia* sp., *Voandzeia subterranea*	Widely distributed
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)
C. subinnotatus	*Rhynchosia* sp., *V. subterranea*	South Africa, Zambia

Lathyrus sp. *Lens esculenta, Phaseolus* sp., *Pisum sativum* from Algeria, France, Iran, Italy, Lebanon, 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).

Bruchus 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 Australia, Greece, Syria and Turkey is yet not reported from India (Table 3). Infestation ranges from 10- 90% with an average of about 42%, depending on location in the Mediterranean region and 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).

Bruchus emarginatus although reported from India has a wide host range including species of *Cicer, Lathyrus, Pisum* and *Vicia.* It is reported from Algeria, France, Germany, Israel, Italy, Lebanon, Syria, Tunisia, Turkey, USSR (erstwhile) and Yugoslavia (erstwhile) (Gupta *et al.,* 2011).

Bruchus 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 *Acacia brachystachya, Lathyrus latifolius, Lens esculenta, Ulex europaeus, Vicia ervilia* and *V. sativa,* and has been recorded as a serious pest of *Lens* spp. 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 NBPGR on *Acacia brachystachya* imported from Australia (Verma *et al.,* 1991) on which it was never reported earlier.

Bruchus lentis a specific pest of *L. culinaris* has been recorded to be serious pest in Algeria, Iran, Lebanon and Turkey. It is reported from 14 countries including India (Bhalla *et al*., 2004). Infestation level of up to 80% and loss of germination of up to 100% has been reported. It is univoltine and overwinters in seeds with high survival, spread and establishment potential. The pest has its distribution limited to a few countries from Europe, Central and South Asia and Africa, although reported from India, has been intercepted several times from different countries (Gupta *et al*., 2004, 2010, 2013)

Bruchus 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).

Although *Callosobruchus analis C. chinensis and C. maculatus* are reported from India, they pose a quarantine risk during import due to their high economic significance and possibility of presence of new strains (Applebaum *et al*., 1968). New strains or biotypes are also included in the category of pests according to the definition of 'pest' by IPPC (http://www.ippc.int/IPP/En/standards.htm).

Callosobruchus 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).

Pectinophora gossypiella larvae intercepted in seeds of *Gossypium* were kept under controlled conditions for adult emergence. *P. gossypiella* is an oligophagous pest and has been reported on cotton, okra, Deccan hemp (*Hibiscus cannabinus* [kenaf]) and roselle (*H. sabdariffa*) and few other members of family Malvaeceae. It is distributed throughout tropical America, Africa, Asia, Australasia, including subtropical regions, Pakistan, Egypt, USA (Arizona) and Mexico. *P. gossypiella* is a worldwide pest of cotton and in some regions of the world is a major economically important cotton pest. It is a quarantine pest in the USA and Russia (CAB International, 2007).

Systole coriandri, a chalcidoid reported on *Angelica archangelica* (Angelica) and *Coriandrum sativum* (coriander) from Hungary, Chile and Russia has been reported from India only from endemic pockets. *S. coriandri* is a specific seed and has been repeatedly intercepted in quarantine (Gupta *et al.*, 2005).

Salvaging and Quarantine Significance of In tercepted Pests

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. One thousand, nine hundred and seventy one 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/ for 24 h at 30°C or Aluminium phosphide @ 2g/ cu m for 72 h in an airtight container at normal atmospheric pressure.

Several of the above intercepted bruchids viz., *Acanthoscelides desmanthi, A. obtectus, Bruchophagus roddi, Bruchus atomarius, B. dentipes, B. ervi, B. rufimanus, B. tristis* and *Callosobruchus subinnotatus* are not yet reported from India, and hence, are of very high quarantine significance. Had they not been detected through X-ray radiography and 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). 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 or species specific pulse beetles or 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 and its subsequent amendments). In view of the interception of several exotic seed beetles of quarantine significance from more than 19 countries in the past ten years, it is essential to pay due attention to the regulations and the requirements thereunder. Several interceptions of exotic bruchids and chalcids of quarantine significance have been made as a result of X-ray screening of the listed plant genera. The advantages of X-ray radiography technique are that it is non-destructive (seeds remain viable), economically feasible, sufficiently rapid and reliable over a wide range of environmental conditions.

Conclusion

A total 1673 bruchid species belonging to 71 genera are reported from the world of which only 11 genera having 72 species are reported from India (Gupta *et al*., 2011). Thus, India has a risk of introducing >60 bruchid genera with >1500 species along with transboundary movement of plant species known to carry hidden infestation. Therefore, it is suggested to expose the listed plant genera to X-ray radiography at all the ports of entry (Bhalla *et al*., 2002). This is especially important to prevent the entry of such hidden infestation which could become serious economic threats in India. Therefore, effective quarantine processing is of paramount importance for the safe exchange of seeds.

Acknowledgements

The authors gratefully acknowledge Directors, ICAR-NBPGR for providing the necessary facilities and the Heads, Division of Plant Quarantine, ICAR-NBPGR for their critical comments and to the retired senior colleagues of the Entomology Section for their suggestions.

References

Abou-Raya AK (1954) *Bruchides alfieri* Pic a biologic race of *Bruchidius trifolii* Motsch. (Coleoptera: Bruchidae). *Bull. Society Fouad Ier Entomol.* **38:** 193-203.

Applebaum SW, Southgate BJ, Podoler H (1968) The comparative morphology, specific status and host compatibility of two geographical strains of *Callosobruchus chinensis* (L.) (Coleoptera: Bruchidae). *J. Stored Prod. Res.,* **4**: 135-46.

Bashar MA (1994) Pupal period and adult emergence of *Bruchus affinis* Froel. (Col: Bruchidae) in autumn in the south-west of France. *Bangladesh J. Zool.,* **22:** 101-108.

Bhalla S, Kapur ML, Gupta K, Lal B and Khetarpal RK (2006) *Check-list of bruchids associated with different plant genera*. National Bureau of Plant Genetic Resources, New Delhi, India.42p.

Bhalla S, Kapur ML, Lal B, Verma BR and Singh C (2002) Quarantine risk associated with exchange of plant genera carrying hidden infestation. *Indian J. Plant Genet. Res.,* **15:** 160-163.

Bhalla S, Kapur ML, Singh C, Gupta K, Kumar N and Lal B (2004) Interception of bruchids in imported lentil (*Lens* spp.) germplasm. *Indian J. Agric. Sci.* **74**: 332-33.

Bhalla S (2009) Plant Quarantine: Entomological Aspects. In: Bhalla S, Chalam VC, Lal A and Khetarpal RK. (eds)

Practical Manual on Plant Quarantine, National Bureau of Plant Genetic Resources, New Delhi, India, pp 56-61.

Boughdad A and Lauge G (1997) *Vicia faba* L. seed infestation and losses due to *Bruchus rufimanus* Boh. (Coleoptera, Bruchidae) in Morocco. *Al-Awamia Public,* **97:** 27-39.

CABI (2007) *Crop Protection Compendium*. CAB International, Wallingford, UK, CD Rom.

Echerich K (1938) Die phytophagen Megastigmus-Arten (Chalcididae) als Zerstörer von Nadelholzsamen, *Zeitschrift für angewandte Entomologie* **25:** 363–380.

Gourlay ES (1930) Some parasitic hymenoptera of economic importance in New Zealand. *New Zealand J. Sci. Tech.,* **11:** 319.

Gupta K, Bhalla S, Kapur ML, Lal B and Singh C (2013) A Decade of Interception of Insect Pests during Quarantine Processing of Imported Seed Germplasm *Indian J. Plant Prot.* **41**: 116-126.

Gupta SC (1962) Occurrence of exembryonate seeds in the umbelliferae. *Curr. Sci,* **31:** 203-205.

Gupta K, Bano R, Gupta CS, Bhalla S, Kapur ML and Lal B (2011) *Digital Library on Bruchids*. Available at http://202.141.12.150/bruchid-library/ accessed on 20.6.2019.

Gupta K, Bhalla S, Kapur ML, Lal B, Singh C, Meenakshi, Baloda RS and Kumar N (2004) Insect pests intercepted in exotic planting material during quarantine processing in 2002. *Indian J. Plant Prot.* **32:** 45-48.

Gupta K, Bhalla S, Kapur ML, Singh C, Kumar N, Baloda RS, Meenakshi and Lal B (2005) Insect-pests intercepted in introduced planting material during quarantine processing from 2000- 2004. *Indian J. Plant Prot.* **18:** 133-136.

Gupta K, Bhalla S, Lal B, Kapur ML, Singh C, Bano R, Singh S, Thakur M and Khetarpal RK (2010) Interception of insect pests in imported seed germplasm during 2007-08. *Indian J. Agric. Sci.,* **81:** 100-103.

Hariri G (1981) Insects and other pests. *Lentils*, Webb, C & G Hawtin (eds.), CABI, UK. pp. 173-189.

Hariri G and Tahhan O (1983) Insect damage and grain yield of fababean, lentil and chickpea. *Proceedings 10th International Congress of Plant Protection* Aleppo University, Syria. p 127. Volume 1. November 20-25, 1983, Brighton, England.

Howe RW and Currie E (1964) Some laboratory observations on the rates of development, mortality and oviposition of several species of Bruchidae breeding in stored pulses. *Bull. Entomol. Res.* **55:** 437-477.

http://www.ippc.int/IPP/En/standards.htm

Kapur ML, Verma BR and Bhalla S (1997) Quarantine detection of mango stone weevil, *Sternochaetus mangifera* (F.) in mango through X-ray radiography. In: *Souvenir and Abstracts: Symposium on Integrated Pest Management for Sustainable Crop Production*, IARI, New Delhi, pp. 59.

Kaura S (1959) A new transparency method for detecting internal infestation in grains. *Grain Storage Newslett.* **1:** 12.

Lale NES and Vidal S (2000) Mortality of different developmental stages of *Callosobruchus maculatus* F. and *Callosobruchus subinnotatus* Pic. (Coleoptera Bruchidae) in bambara groundnut *Vigna subterranea* (L.) Verdc. seeds exposed to simulated solar heat. *Zeitschrift fur Pflanzenkrankheiten und Pflanzenschutz,* **107:** 553-559.

Mac Dougall RS (1926) The *Megastigmus* of the douglas fir seed. *Trans. Royal Scottish Arboricul. Soc.* **19:** 52-65.

Mansour MY and Al Bacheer M (1995) Gamma irradiation as a disinfestation and quarantine treatment for faba bean seeds infested with *Bruchus dentipes* Baudi (Col., Bruchidae), *J. Appl. Entomol.,* **119:** 631-636.

Mbata GN, Shu S and Ramaswamy SB (2000) Sex pheromones of *Callosobruchus subinnotatus* and *C. maculatus* (Coleoptera Bruchidae): congeneric responses and role of air movement. *Bull. Entomol. Res.* **90:** 147-154.

Meirleire De H (1967) La bruche du haricot. *Phytoma,* **19:** 11-133.

Plant Quarantine (Regulation of Import into India) Order. (2003) (http://plantquarantineindia.nic.in/pqispub/pdffiles/pqorder2015.pdf).

Southgate BJ (1978) The importance of the bruchidae as pests of grain legumes, their distribution and control. Singh, SR, Van Embden HF and Taylor AT (eds) *Pests of Grain Legumes: Ecology and Control.* Academic Press, UK, pp 220-229.

Strong FE (1962) Laboratory studies on the biology of the alfalfa seed chalcid, *Bruchophagus roddi* Guss. (Hymenoptera: Eurytomidae). *Hilgardia,* **32:** 229-249.

Thakur DR (2010) Taxonomy, Distribution and Pest Status of Indian Biotypes of *Acanthoscelides obtectus* (Coleoptera: Chrysomelidae: Bruchinae) – A New Record. *Pak. J. Zool.,* **44:** 89-195.

Vayssiere P (1931) Appartition en France du *Megastigmus spermotrophus* Wachtl. Parasite des semences du *Pseudotsuga douglasii* Harr. *Review of Appl. Entomol.* (A), **19:** 586.

Verma BR, Kapur ML and Lal B (1991) A new host record for *Bruchus ervi* Froelich (Coleoptera: Bruchidae), *Indian J. Entomol.* **53:** 171-172.

Weiss HB (1917) *Megastigmus aculeatus* Swedrus-introduced into New Jersey from Japan. *Indian J. Entomol.* **6:** 402-403.