Assessment of Forest Pests and Diseases in Native Boxwood Forests of Georgia

Final report

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The cover picture and photos in the next report were taken by the executor of this research.
ABBREVIATIONS AND ACRONYMS

APA  Agency of Protected Areas
BLAST  Basic Local Alignment Search Tool
Btk  *Bacillus thuringiensis* var. *kurstaki*
DNA  Deoxyribonucleic acid
DNB  Dothistroma needle blight
EDRR  Early detection and rapid response
EPPO  European and Mediterranean Plant Protection Organization
ENPI  European Neighbourhood and Partnership Instrument
EU  European Union
FHP  Forest Health Protection
FLEG  Forest Law Enforcement and Governance
FHS  Forest Health Service
FPRD  Forest Protection and Reforestation Department
FPS  Forest Policy Service of the Ministry of Environment and Natural Resources Protection of Georgia
ha  Hectares
HMG  High mobility group
IUCN  International Union for Conservation of Nature
IUFRO  International Union of Forest Research Organizations
ITS  Internal transcribed spacers
LEPL  Legal Entity of Public Law
MAS  2% Malt-agar with streptomycin
MEA  2% Malt-agar
MoENRP  Ministry of Environment and Natural Resources Protection of Georgia
NP  National Park
NFA  National Forestry Agency
NCBI  National Center for Biotechnology Information
SNR  Strict Nature Reserve
PARPH  Selective medium with antibiotics
PARPNH  Selective medium with hymexazol for *Phytophthora* species
PCR  Polymerase Chain Reaction
PDA  Potato-dextrose agar
PDS  Potato-dextrose agar with streptomycin
spp.  A short way of saying that something applies to many species within a genus
USFDA  US Food and Drug Administration
The WB  The World Bank
WWF  World Wide Fund for Nature
EXECUTIVE SUMMARY

This report is the final report of the researches conducted for the assessment of forest pests and diseases in the native boxwood forests of Georgia. The study was carried out from July 2015 through January 2016.

Background information

The majority of Georgia’s biodiversity is directly or indirectly connected with forest ecosystems. Forest occupies 31.4% of the territory of Georgia (http://geostat.ge/cms/site_images/_files/georgian/agriculture/Garemo_2014.pdf). Endemic and rare species of rich broadleaf and coniferous forests are the real treasure of Georgia. Forest ecosystems are true significant for the conservation of biodiversity in Georgia. Forests cover 2.17 mln ha (about 97% of these are of natural origin) and they are mostly mountain forests spread and located at the lower mountain belt up to the treeline ecotone. 81% of forest area is occupied by broadleaf forests and approximately 19% is covered by coniferous forests. The new schema of habitat types according to Natura2000 standards in Georgia was developed determining codes and natural habitat types of Europe (Akhalkatsi and Tarkhnishvili, 2012). Code of Georgia 92BCGE Boxwood Forest (Buxus colchica) is well described in Akhalkatsi (2015). Also, Buxus colchica could be found in Kolkheti broad-leaved mixed forest (Code of Georgia: 9BCGE Kolkhic broad-leaved mixed forest). Kolkhic boxwood (Buxus colchica) is the related species of the boxwood growing in Europe (B. sempervirens). In the East Georgia boxwood stands are in abundance in Aragvi gorge, Saguramo, Bulachauri, Navdaraant Kari. In Kakheti boxwood stands are encountered in several places. The boxwood hill is especially represented in Kvareli surroundings, on the Bursa riverbank; Devubani, Sviana Khevi, Chontis Khevi, Saborio Khevi and Didgori. Hornbeam, Georgian oak, lime and beech are compatible species of the mentioned boxwood forests in R. Stori gorge.

Climate Change have already reached South Caucasus region with all its features such as increasing temperatures, shrinking glaciers, rising sea level, reduction, and redistribution of river flows, decreasing snowfall and an upward shift of the snowline (Akhalkatsi, 2015). It is proved that forest formations will respond to changes in the climate and this model predicts that conditions in the South Caucasus will become less suitable for most forest classes that occur in the region (Akhalkatsi, 2015). Consequently, it should be emphasized that all of these impacts will increase the risk of outbreaks of pests and diseases and will create attractive conditions for invasive species (Akhalkatsi, 2015). Forests parasites are another threat to Georgia’s forests. The studies of pathologies of the forests have not been conducted in the recent years and according to 2004 data, 192,900 ha of forest suffer from various diseases (Akhalkatsi, 2010; 2015).

Literature review

The literature review and analysis of the existing information about the boxwood diseases and pests in Georgia and in other countries were conducted.

Buxus colchica Pojark (syn. B. hyrcana) (genus Buxus) is an evergreen Tertiary-period relict plant in the IUCN Red List of Threatened Species (http://www.iucnredlist.org/details/full/32177/0). Kolkhic boxwood is the related species of the boxwood growing in Europe (B. sempervirens). The name refers to the ancient landscape of Colchis on the Black Sea. Geographic range of Buxus
*B. colchica* is concentrated in the Caucasus from North Kolkheti in Russia to South Kolkheti in Turkey. It can be found in the north eastern part of Turkey (Trabzon) and along Russian Black Sea coast (http://kraevedenie.net/2009/03/19/samshit-buxus/2/) and in Azerbaijan (Talish) (Machutadze and Davitashvili, 2009). But mainly it could be found in west Georgia, limestone massifs, Abkhazia, Samegrelo and Racha-Lechkhumi. Amplitude of vertical distribution is 1300 – 1800 meters above the sea level.

The first signs of *B. colchica* damage were found in 2009. In 2011, a new disease (box blight) caused by invasive species *Calonectria pseudonaviculata* (anamorph *Cylindrocladium buxicola*) was found in boxwood natural population in the protected areas in Caucasus (Gorgiladze et. al. 2011; Meparishvili, 2013). Assessment of sanitary conditions the natural populations of boxwood was conducted in June and October 2014 on the territories of Mtirala National Park and Kintrishi Protected Areas (Matsiakh, 2014; Matsiakh and Tsiklauri, 2015). According to this study, the new threat of natural boxwood in Colchida lowland caused by the dangerous invasive species phylophage *Cydalima perspectalis* (Walker, 1859) [synonyms: *Diaphania perspectalis*, *Glyphodes perspectalis* (Walker, 1859)] (Lepidoptera: Crambidae: Pyraustinae) in the Caucasus has recently discovered (Matsiakh, 2014; Matsiakh and Kavtarishvili, 2015). The damage of box tree by bow tree moth was identified in green areas near the office of Kintrishi Protected Area (Kobuleti) and administration of Mtirala National Park (village Chakvi) as well as in the city of Batumi and Tikeri nursery farm (Administration of Kobuleti, Lepl Forestry Agency of Adjara). Also, in the literature review the information about two invasive species (boxwood blight pathogen and bow tree moth) was described in details as well as information about other possible pathogens and pests of *Buxus* spp. was presented.

**Methodology**

The methodology of study was divided into two parts: field research and laboratory work.

The field research was conducted in June-July and October-November 2015, in 48 and 13 boxwood locations respectively. The natural boxwood forests were examined in six regions (out of nine) of Georgia, the main area of sopecies distribution from the east to the west of the country: Kakheti, Imereti, Racha-Lechkhumi, Samegrelo Upper Svaneti Guria Forestry Services and L.E.P.L. Forestry Agency of Adjara.

The soil and tissues samples of symptomatic *Buxus colchica* were analyzed and processed in the laboratory of Forest Pathology at the Süleyman Demirel University (Faculty of Forestry, Isparta, Turkey) and different approaches were used. Isolation of boxwood blight pathogen was done directly from asymptomatic boxwood branches and leaves according to Henricot and Culham, 2002; Šafránková et al. 2013. Development stages of box tree moth were identified according to Mally and Nuss 2010. *Phytophthoras* were isolated from soil samples using the baiting technique (Jung et al. 1996).

**Results and Discussion**

According to the results of the field and laboratory studies, the intensive defoliation of boxwood plants in different ages is caused mainly by invasive species *Calonectria pseudonaviculata* and *Cydalima perspectalis* combining with the soil phytopathogens of *Phytophthora* and *Pythium* geniuses strong development, which caused root rot.
Invasive fungi *Calonectria pseudonaviculata* (Cy. buxicola) was detected in four Regions of Georgia: Imereti, Samegrelo Upper Svaneti and Guria Forestry Services and L.E.P.L. Forestry Agency of Adjara. Unfortunately, boxwood blight pathogen was also isolated from healthy looking plant material collected in summer 2015 from the native boxwood forests in Kakheti Region. Morphological structures, biology of disease and its life cycle were well studied and described. Also, the disease triangle as illustration tool of the infection caused by the fungus *C. pseudonaviculata* in Georgia was analysed and explained. The secondary opportunistic pathogen *Pseudonectria buxi* (asexual *Volutella buxi*), boxwood rust (*Puccinia buxi*) and other secondary pathogens were also isolated from infected boxwood leaves. 132 isolates of *Phytophthoras* (from soil of examined boxwood forests) were obtained using baiting technique. The most of them were isolated from the soil samples from Imereti and Racha-Lechkhumi Regions. Among the percentage of the total number of isolates obtained from boxwood forests soils *Phytophthora plurivora* was one of the most detected species. The wood decay fungi detected on the dead wood of boxwood trees were also identified.

In summer (July-August) 2015 the large damages caused by *Cydalima perspectalis* feeding on boxwood leaves in the native boxwood forests were found in three regions of west part of Georgia: Racha-Lechkhumi, Samegrelo Upper Svaneti (Zugdidi, Martvili, Tsalenjikha) and Guria Region. In autumn (October-November) 2015 the damage of box tree moth of *Buxus colchica* was revealed in Imereti Region in Zestaponi Forestry and in the natural boxwood populations in L.E.P.L. Forestry Agency of Adjara. During the summer period of field studies, the distribution of *Cydalima perspectalis* was observed in boxwood forests and green areas along the coast of Black Sea. This finding confirms that box tree moth is being spread into the native boxwood forests towards the central part of the country. Undoubtedly, the largest damages caused by *Cydalima perspectalis* feeding on boxwood trees on the territories of L.E.P.L. Forestry Agency of Adjara demonstrate the successful adaptation of alien pest in the natural forests of the Caucasus region, causing the great concern to the extinction of native boxwood forests in this region.

The developmental stages of *Cydalima perspectalis* recorded during field trips in Caucasus region were described and regulatory mechanisms to stabilize situation were analysed. Moreover, other boxwood pests were studied.

**Recommendations**

The recommendations and proposals for the conservation management of native boxwood forests in Georgia were developed taking into account strategic plan for forest protection in the United States. These recommendations are focused on the following four strategic areas:

- Prevention;
- Early detection and rapid response;
- Control and management;
- Rehabilitation and restoration.

Also, four themes for strategies of forest protection include:

- Partnerships and collaboration;
- Scientific basis;
- Communication and education;
- Organizing towards success.
All strategies were effectively developed with detailed explanations all activities that should be implemented to boxwood forest health. Also, Strategic Plan to improve and organize the work of Forest Protection and Reforestation Department (FPRD) under the NFA was proposed. The proposed plan includes developing priority operational activities supported by scientific research that will help to achieve results on the ground against the invasive species threat.

Additional studies within the project time

The aim of these additional studies within the project time was to carry out additional assessment and to provide a new data on the fungi in Georgia. Samplings were conducted from 30-40 years old and 40-50 years old *Pinus nigra* and 50-60 years old *Pinus ponderosa* forests in Georgia from three different Regions end of July 2015. They were analysed in in the laboratory of Forest Pathology at the Süleyman Demirel University (Faculty of Forestry, Isparta, Turkey) using molecular and mycological approaches. Our research provides two new findings of DNB on the territory of Georgia and *Pinus ponderosa* and *Pinus nigra* were added to host list of DNB.

*Keywords:* *Buxus colchica*, native forests, soil pathogens, boxwood blight, box tree moth, control activities.
INTRODUCTION

The assessment of forest pests and diseases in the native boxwood forests of Georgia has been conducted by Dr. Iryna Matsiakh (Assistant of Forestry Department at the National Forestry University of Ukraine, Lviv). The work has been carried out from July 2015 to February 2016. Dr. Volodymyr Kramarets, forest pathologist and entomologist at the Forestry Department, of National Forestry University of Ukraine (Lviv) has been voluntarily involved in carrying out of the field research, as well as in development of recommendations. We are very much grateful for his contribution and support to this complex work.

The work has been carried out in the frame of the regional programs “European Neighborhood and Partnership Instrument (ENPI) East Countries Forest Law Enforcement and Governance II Program” and “ENPI East Countries FLEG II: Complementary Measures for Georgia and Armenia Single Donor Trust Fund” are aimed at putting in place improved forest governance arrangements through the effective implementation of the main priorities set out in the St. Petersburg Ministerial Declaration and Indicative Plan of Actions for the Europe and North Asia Forest Law Enforcement and Governance (ENA-FLEG) process.

This Program specifically covers seven countries of the ENA Region, including six members of the European Neighborhood Policy Initiative (ENPI) – Armenia, Azerbaijan, Belarus, Georgia, Republic of Moldova, Ukraine – and the Russian Federation. The Program supports selected pilot activities to be implemented with the active involvement of governments, civil society and the private sector. Most activities are at a country level, complemented by strategically targeted sub-regional and regional actions. The program is supported by EC. The Austrian Development Agency is contributing to a same single-donor trust fund administered by the World Bank (WB) for the supplementary activities in Armenia and Georgia. Implementation of the Program is led by the World Bank, working in partnership with the International Union for Conservation of Nature and Natural Resources (IUCN) (the Client) and the World Wide Fund for Nature (WWF) (all three collectively referred to as the “Implementing Organizations” or “IO’s”) and in close coordination with governmental and nongovernmental stakeholders of the participating countries.
1. BACKGROUND INFORMATION

1.1. Biodiversity of Georgia

Georgia occupies the central and western part of the Caucasus region between Black and Caspian seas (Figure 1), covers 69.700 sq.km, and has a population of 3 729.5 thousand persons (http://geostat.ge/index.php?action=0&lang=eng). There are still a high proportion of undisturbed areas, not least because of poorly developed countryside infrastructure. (http://www.biodiversity-georgia.net/index.php?pageid=905).

The Caucasus region is characterized by a high diversity of landscapes and biological species, compared with other non-tropical regions and it was included to IUCN list of the planet’s 34 most diverse and endangered hotspots (and one of the four hotspots of the Europe and Central Asia). The Causasus region is also determined as a globally outstanding for biodiversity by Critical Ecosystem Partnership Fund and WWF (in a slightly different way from IUCN) because of a high proportion of the narrow-ranged and endemic species in the local Fauna and Flora and a high diversity of landscapes (from humid mountain forests and wetlands to deserts) (http://www.biodiversity-georgia.net/index.php?pageid=905).

Georgia covers far beyond two-thirds of the species found throughout the entire Caucasus and almost all kinds of the landscapes/biomes one could find throughout the area. Forests, freshwater and wetlands, marine and coastal, high-mountain, semi-desert and steppes are the main biomes in Georgia that are contributed by the complex of landscapes and variations in climatic conditions.

The majority of Georgia’s biodiversity is directly or indirectly connected with forest ecosystems. Forest occupies 31.4% of the territory of Georgia (http://geostat.ge/cms/site_images/_files/georgian/agriculture/Garemo_2014.pdf). Among these 15% are intensively used (arable land and perennial crops) agricultural fields and 28% with hay meadows and pastures. It is known, that 4,130 species of vascular plant are registered in Georgia and include 79 ferns, 17 gymnosperms, 4,034 angiosperms (http://www.biodiversity-georgia.net/index.php?pageid=905). Up to 900 species (around 21% of Georgian flora) are being endemic and among them around 600 (14% of all species) are Caucasus endemics and 300 (9% of all species) are endemic to Georgia (Akhalkatsi, 2010).

Endemic and rare species of rich broadleaf and coniferous forests are the real treasure of Georgia. Well-known Kolkheti refugium as well as limestone areas of the Western Caucasus and high mountainous vegetation complexes are especially notable for their species diversity and high levels of endemism (http://www.biodiversity-georgia.net/index.php?pageid=905). Also, the intact forest stands with the greatest conservation value have to be well preserved and managed in Georgia.
1.2. Forest Ecosystems

Forest ecosystems are significant for the conservation of biodiversity in Georgia. Forests cover 2.17 mln ha of Georgian territory (about 97% of these are of natural origin) and they are mostly mountain forests spread and located at the lower mountain belt up to the treeline ecotone (Figure 2) (Akhalkatsi, 2015). During the Soviet period, these forests were under the ownership of collective farmer households and mainly located near villages and prone to damage. In addition, Georgian forests were intensively exploited for economic purposes after 1990 and firewood has remained as one of the sources of energy. Nowadays, Georgian forests are entirely State owned, managed by the Legal Entity of Public Law - the National Forestry Agency (LEPL NFA, under the Ministry of Environment and Natural resources protection of Georgia) with forest-use licenses issued by the Ministry of Economic Development, via auction. Since 2005, 109,337 ha of mountain forests have belonged to resort forests, 270,340 ha are green zone forests, and the remaining 2,076,555 ha have soil protection and water regulative functions (Akhalkatsi, 2015).

There are two separate mountain systems in Georgia: the Greater Caucasus lying between the Black and Caspian Seas; and the Lesser Caucasus, which runs parallel to the greater range. According to Dolukhanov (2010), the Caucasus forest belt can be subdivided into four major elevation zones: broad-leaved forests (50–900 m), coniferous forests (900–1700 m), and High...
Mountain subalpine forests (1700–2000 m) and forests in treeline ecotone (2000–2800 m). About 400 arborescent wood species grow in the forests, including trees (153), high shrubs (202), low shrubs (29) and lianas (11). 26% (104 species) of the dendroflora here are either Georgian or Caucasian endemics (Akhalkatsi, 2015). Forested regions are presented on the Map (Figure 2).

Figure 2: Map of forest distribution in Georgia (data of National Environmental Action Programme of Georgia 2012–2016)

All forests lands in Georgia can be divided into mountain and lowland forest with special ecological features, water regulation, soil protection and climate stabilization which influences plant and animal habitats (Dolidze, 2013; Introductory Report on Nature Conservation in Georgia, 2010). 81% of forest area is occupied by broadleaf forests and approximately 19% is covered by coniferous forests. Beech (*Fagus orientalis*) (46.6%), oaks (*Quercus* spp.) (10.6%), hornbeam (*Carpinus caucasica*) (8.8%), Caucasian fir (*Abies nordmaniana*) (7%), alder (*Alnus barbata*) (5.5%), spruce (*Picea orientalis*) (4.5%), pines (*Pinus* spp.) (4%) and sweet chestnut (*Castanea sativa*) (3.2%) are dominated in forest compositions (Introductory Report on Nature Conservation in Georgia, 2010).

According to the data of Dolidze (2013), forest area is decreasing along the lower part of mountain slopes and in the west of Georgia up to 500 – 600 m.a.s.l., in the east of Georgia up to 700 – 800 m.a.s.l. and also in the sub Alpine region up to 1,800 – 2,500 m.a.s.l. At High Mountain levels and steep slopes there are areas still covered with high forests in a natural state and not damaged by farming activities. Moreover, Dolidze (2013) points that for main functional aims, the forests in Georgia are divided in two: Reserve forests (protected territories) 495, 900 ha (16.6%) and State farming forests fund 2,492,100 ha (83.4%).
1.3. Boxwood Forests in Forests Habitat Classification

The "Habitats" Directive (Council Directive 92/43/EEC of 21 May 1992 on the conservation of natural habitats and of wild fauna and flora, O.J. L206) is a Community legislative instrument in the field of nature conservation that establishes a common framework for the conservation of wild animal and plant species and natural habitats of Community importance. It provides for the creation of a network of special areas of conservation, called Natura 2000, to "maintain and restore, at favourable conservation status, natural habitats and species of wild fauna and flora of Community interest". According to the Interpretation Manual of European Union Habitats – EUR27, the habitat classification is based on plant community types and it is well studied by Georgian botanists in Georgia (Grossheim et al. 1928; Ketskhoveli, 1959; Kimeridze, 1965, 1966; Dolukhanov, 1989; Nakhutsrishvili, 1999, Kvachakidze, 2009).

It is well known, that Georgia is a country with very diverse habitat types and Soviet schools used the different methodology in nomenclature than in European Natura2000 habitat directives based on the CORINE biotope classification. It was very important to underline the Caucasus differs from European vegetation to the new classification. There are species, which are related to European ones but are endemic to the Caucasus: Abies nordmanniana, Picea orientalis, Pinus kochiana, Fagus orientalis, Quercus iberica, Betula nitida, and there are some habitats similar to the European habitat types should be considered as sub-types: 1) Beech forests without understory (Fageta sine fruticosa); 2) Dark-coniferous forest (Piceeta orientale-Abieta nordmanniana); 3) Pine forest (Pinus kochiana); 4) Yew forest (Taxus baccata); 5) Hornbeam forest (Carpinus caucasica); and 6) Boxwood forest (Buxus colchica). The new schema of habitat types according to Natura2000 standards was developed in Georgia, determining codes and natural habitat types of Europe, where new Georgian habitat types were involved (Akhalkatsi and Tarkhnishvili, 2012; Nakhutsrishvili, 2013).

Currently, twenty-four forest habitat types were identified in Georgia (Table 1). 18 of them belong to the forests of temperate Europe; 6 of them belong to Mediterranean deciduous forests; 7 - beech forest groups are found only in Georgia; 4 other habitats differ from European ones and 6 forest types of Mediterranean deciduous forests are typical only for the Caucasus: 1) chestnut forest (Castanea sativa); 2) zelkova forest (Zelkova carpinifolia); 3) boxwood forest (Buxus colchica); 4) Kolkheti broad-leaved mixed forests; 5) and open woodlands; 6) sub-alpine birch krummholz. (Akhalkatsi, 2015).

The “Forest Code of Georgia” was adopted in 1999 and established “legal grounds for conducting tending, protection, restoration and use of the Georgian Forest Fund and its resources”. With regard to biodiversity, the Forest Code aims to protect Georgia’s forests, maintain the integrity of primary forests, and to preserve endemic, relic and otherwise important species of plants.

Code of Georgian boxwood forests 92BCGE is described by Akhalkatsi (2015).

1) General description

Kolkh fie boxwood (Buxus colchica) is the related species of the boxwood growing in Europe (B. sempervirens). It is mainly found in west Georgia, limestone massifs, Abkhazia, Samegrelo and Racha-Lechkhumi. Amplitude of vertical distribution is 1300 meters above the sea level. It
participates in the formation of hornbeam and other broad-leaved forests. In forests of this type, the following endemic species characteristic to limestones are represented in large amounts - *Ruscus ponticus*, *Hedera helix*, *Asplenium adiantum-nigrum*, *Carex divulsa*, *C. transsilvanica*, *Veronica peduncularis*, *V. persica*.

Table 1. List of forest habitat types in Georgia. The code is based on the Interpretation Manual of European Union Habitats – EUR27. The Palaearctic classification (Pall. Class.) corresponds to the CORINE biotope classification (1988, 1991). “None” is indicated for 11 habitat types, which are absent in the list of habitat types of Europe. Subtypes and plant community types are determined for some habitats (Akhalkatsi, 2015)

In the East Georgia boxwood stands are in abundance in Aragvi gorge, Saguramo, Bulachauri, Navdaraant Kari. In Kakheti boxwood stands are encountered in several places. The boxwood hill is especially represented in Kvareli surroundings, on the Bursa riverbank; Devubani, Sviana Khevi, Chontis Khevi, Saborio Khevi and Didgori. Hornbeam, Georgian oak, lime and beech are compatible species of the mentioned boxwood forests in R. Stori gorge.

The boxwood here creates the secondary layer. There are different opinions on the primary origin of the boxwood in east Georgia. Some researchers consider that it is the tertiary relict, which
is preserved in refugiums (Troitsky, 1928; Matikashvili, 1953). Majority though thinks that the box was planted at the surrounding of churches and it has been naturalized into the natural habitat.

2) Species of plants

Asplenium adiantum-nigrum, Buxus colchica, Carex divulsa, C. transsilvanica, Carpinus betulus, Fagus orientalis, Hedera helix, Quercus iberica, Ruscus ponticus, Tilia begoniifolia, Veronica peduncularis, V. persica.

3) Corresponding categories

1. 42.A72 - Corsican yew woods - Formations of Taxus baccata, Ilex aquifolium, Buxus sempervirens

2. Code of Georgia: 91GE-FC beech forest with Kolkheti understory (Fageta fruticosa colchica)

4) Associated habitat

1. Hornbeam forest

2. Kolkhic broad-leaved mixed forest

Moreover, Buxus colchica could be found in Kolkheti broad-leaved mixed forest (Code of Georgia: 98CGE Kolhkic broad-leaved mixed forest) with Rhododendron ponticum, Laurus nobilis, Ruscus colchicus, R. ponticus, Daphne pontica, Ilex colchica, Rhododendron ungernii, Epigaea gaultherioides mainly distributed in west Georgia, non-marshy lowland places and lower zone of the forest.

1.4. Georgian Forests Habitat in the Context of Climate Change

It is known that Georgian climate differs from dry warm continental in the southeast of the country to the very humid temperate in the west (Mtirala mt. in the southwest has 4,000 mm annual precipitation, exceeding any other geographic point in the continental western Eurasia (Akhalkatsi, 2010).

Climate Change have already reached the South Caucasus region with all their features such as increasing temperatures, shrinking glaciers, rising sea level, reduction, and redistribution of river flows, decreasing snowfall and an upward shift of the snowline (Akhalkatsi, 2015). According to Rosenbaum et al. 2004, the world is becoming warmer because of anthropogenic emissions of carbon dioxide and other greenhouse gases-emissions from power stations, vehicles, domestic wood stoves, and clearance of forests, which alone contributes 30% of total emissions.

The air temperature has been changed on the average of 0.65 °C per 100 m altitude in Georgia. The annual precipitation varies from 1500 to 4500 mm in the Western, from 600 mm in Kolkhic part to 1000 mm in drier parts of eastern and southern regions (Neidze, 2003). The Greater Caucasus Mountains may be covered by snow up to 5–7 meters in northern Abkhazia region from November to April. The Lesser Caucasus Mountains are somewhat isolated from the moist influences coming from the Black Sea and therefore receive considerably less snow precipitation than the Greater Caucasus Mountains. The average winter snow cover in the Lesser Caucasus Mountains ranges from 10–30 cm (Neidze, 2003).
According to the results of Taghieyeva, 2006, from 1906 to 1995, the mean annual air temperature in Georgia increased in the eastern part of the country, whilst it has actually decreased in the west, including in the Greater Caucasus Mountain areas. The same change is determined for annual atmosphere precipitation sums for 1964–1990 periods relative to 1937–1964 periods (Akhalkatsi, 2015).

According to the UN Intergovernmental Panel on Climate Change’s (IPCC) forecasts26, the average temperature is expected to increase between 2 and 4ºC until the end of the century in the Southern Caucasus. Researches, conducted by Magnus et al. (2008) showed the western parts of Georgia may become colder in 1-1.5ºC by mid-century and IPCC also predicts that precipitation will decline in the Southern Caucasus region between 10 and 20% by the end of the century. Global warming may also degrade steppe ecosystems in the lowlands and may reduce the alpine vegetation at altitude 150-200 m in South Georgia with changing of the relict Colchic forest ecosystems (Magnus et al. 2008).

In 1994, Georgia parted to the United Nations Framework Convention on Climate Change (UNFCCC) and the second national communication was prepared in 2006-2009. This process included the possible scenarios of expected climate change in Georgia and vulnerability of various ecosystems and branches of economics to current and expected changes was assessed (Magnus et al. 2008).

It is proved that forest formations will respond to changes in the climate and this model predicts that conditions in the South Caucasus will become less suitable for most forest classes that occur in the region (Akhalkatsi, 2015). The biodiversity of forests ecosystems may reduce by 8% in the area of the South Caucasus. Of course, some of biological formations may do better but in any way, the range of suitability for the present day forest formations will change.

It is very important to consider the ecological effects of anthropogenic global climate change as well a gradual change in the climate brought caused by global warming, and now forests face other impacts. It will bring strong winds and frequent storms, with breaking and uprooting the stems of trees, soil erosion and landslides. Aslo, prolonged dry and hot weather will increase the risk of forest fires.

Consequently, it should be emphasized that all of these impacts will increase the risk of outbreaks of pests and diseases and will create favourable conditions for invasive species (Akhalkatsi, 2015). Many tree species may suffer from Climate Change by becoming more weakened and sensitive to the new invasive species and these new challenges may cause forest damage and decline.

Forest parasites are another threat to Georgia’s forests. The studies of pathologies of the forests have not been conducted in the recent years and according to 2004 data, 192,900 ha of forest suffers from various diseases (Akhalkatsi, 2010; 2015).
2. LITERATURE REVIEW

2.1. "Buxus Colchica" as a unique tree species in Georgia

*Buxus colchica* Pojark (syn. *B. hyrcana*) (genus *Buxus*) is an evergreen Tertiary-period relict plant in the IUCN Red List of Threatened Species (http://www.iucnredlist.org/details/full/32177/0). Geographic range of benefit: IUCN status Global Red list assessment: Category CR; Criteria String: A1a; B1b (i). Since 2006 *Buxus colchica* has been also included in the ‘Red list’ of Georgia under the category VU, criterion A2, i.e. due to the tendency of areal fragmentation and habitat loss.

Kolchic boxwood is the related species of the boxwood growing in Europe (*B. sempervirens*). According to the database The Plant List, it is regarded as synonymous with *B. sempervirens* L., and the NCBI database is identified as a form of *B. sempervirens*. The name refers to the ancient landscape of Colchis on the Black Sea.

Geographic range of *Buxus colchica* is concentrated in the Caucasus from North Kolkheti in Russia to South Kolkheti in Turkey. It can be found in the north eastern part of Turkey (Trabzon) and along Russian Black Sea coast (http://kraevedenie.net/2009/03/19/samshit-buxus/2/) and in Azerbaijan (Talish) (Matchutadze and Davitashvili, 2009). But mainly it could be found in west Georgia, limestone massifs, Abkhazia, Samegrelo and Racha-Lechkhumi. Amplitude of vertical distribution is 1300 – 1800 meters above the sea level. It participates in the formation of hornbeam and other broad-leaved forests. In forests of this type, the following endemic species characteristic to limestones are represented in large amounts - *Ruscus ponticus*, *Hedera helix*, *Asplenium adiantum-nigrum*, *Carex divulsa*, *C. transsilvanica*, *Veronica peduncularis*, *V. persica* (Akhalkatsi, 2015). In east Georgia boxwood stands are in abundance in Aragvi gorge, Saguramo, Bulachauri, Navdaraant Kari. In Kakheti boxwood stands are encountered in several places. The boxwood hill is especially represented in Kvareli surroundings, on the Bursa river bank; Devubani, Sviana Khevi, Chontis Khevi, Saborio Khevi and Didgori. Hornbeam, Georgian oak, lime and beech are compatible species of the mentioned boxwood forests in R. Stori gorge. The boxwood here creates the secondary layer. Moreover, *Buxus colchica* could be found in Kolkheti broad-leaved mixed forest with *Rhododendron ponticum*, *Laurus nobilis*, *Ruscus colchicus*, *R. ponticus*, *Daphne pontica*, *Ilex colchica*, *Rhododendron ungernii*, *Epigaea gaultherioides* mainly distributed in west Georgia, non-marshy lowland places and lower zone of the forest.

There are different opinions on the primary origin of the boxwood in east Georgia. Some researchers consider that it is the tertiary relict, which is preserved in refugiums (Troitsky, 1928; Matikashvili, 1953). Majority of scientists consider that the box tree was planted at the surrounding of churches and it has been naturalized into the natural habitat. (Akhalkatsi, 2015).

*Buxus colchica* is small-leaved and the most winter hardy of European boxwood which can withstand winter temperatures up to -10 °C and lives up to 600 years but it grows very slowly, under favourable conditions, reaches the height of 15 m (sometimes 20 m) and a diameter can have at the bottom of the trunk up to 30 cm (http://kraevedenie.net/2009/03/19/samshit-buxus/2/). The *Buxus colchica* branches are straight, sticking out, 4-sided and green. Its leaves are almost sessile, glabrous, shiny, dark green and light green below or even yellowish, opaque, highly variable in shape. The flowers are small, greenish and usually asexually stamineate in compact capitated inflorescences. The fruit is a small spherical box with processes disclosed in the maturation of the
seed wings. All parts of the plant, especially the leaves, are poisonous. In order to grow *Buxus colchica* the carbonate soils are required or it can grow on limestone rock sand in alluvial soil. The plant communities of *Buxus colchica* create a favourable wet microclimate (Matchutadze et al. 2013).

The first signs of *B. colchica* damage were found in 2009. In 2011, a new disease (box blight) caused by invasive species *Calonectria pseudonaviculata* (anamorph *Cylindrocladium buxicola*) was found in boxwood natural population in the protected areas in Caucasus (Gorgiladze et al. 2011; Meparishvili, 2013). Assessment of sanitary conditions the natural populations of boxwood was conducted in June and October 2014 on the territories of Mtirala National Park and Kintrish Protected Areas (Matsiakh, 2014; Matsiakh and Tsiklauri, 2015). According to this study, the new threat of natural boxwood in Colchida lowland caused by the dangerous invasive species phyllophage *Cydalima perspectalis* (Walker, 1859) [synonyms: *Diaphania perspectalis*, *Glyphodes perspectalis* (Walker, 1859)] (Lepidoptera: Crambidae: Pyraustinae) in the Caucasus has recently discovered (Matsiakh, 2014; Matsiakh and Kavtarishvili, 2015). The damage of box tree by bow tree moth was identified in green areas near the office of Kintrishi Protected Area (Kobuleti) and administration of Mtirala National Park (village Chakvi) as well as in the city of Batumi and Tikeri nursery farm (Administration of Kobuleti, Lepl Forestry Agency of Adjara).

2.2. Distribution of invasive species and their threat to natural populations of boxwood (*Buxus colchica* Pojark) in Georgia

2.2.1. Boxwood Blight – *Calonectria pseudonaviculata* (anamorph *Cylindrocladium buxicola*)

**HISTORY.** Cylindrocladium boxwood blight is one of the main diseases of *Buxus* spp. *Calonectria pseudonaviculata* (anamorph *Cylindrocladium buxicola*) is an asexually reproducing species in a genus of common ascomycete plant pathogens.

**Taxonomic Tree**
- Domain: Eukaryota
- Kingdom: Fungi
- Phylum: Ascomycota
- Subphylum: Pezizomycotina
- Class: Sordariomycetes
- Subclass: Hypocreomycetidae
- Order: Hypocreales
- Family: Nectriaceae
- Genus: Calonectria
- Species: Calonectria pseudonaviculata

It is known that this fungus was first found in the United Kingdom where the disease was observed and the scientific name was given (Ivors, Prevention and Management of Boxwood...
Blight). However, both current names of fungus *Cylindrocladium pseudonaviculatum* or *Calonectria pseudonaviculata* refer to the same fungus.

In the UK, some symptoms of blight disease of boxwood were found in the nursery in Hampshire at the end of 1994 (Henricot and Culham, 2002). Henricot B. (2002) indicated the misidentification because of the morphological characters variability of the genus. In 1998 in New Zealand *Cylindrocladium spathulatum* was isolated from *Buxus* sp. showing leaf and twig blight symptoms (Ridley, 1998) and Ridley didn’t rule out the possibility that it could be *C. ilicicola* which has a wider host range than *C. spathulatum* including *Buxus* (Crous and Wingfield, 1994). But finally, the name *Cylindrocladium buxicola* was offered and confirmed use of the morphological characters, sequencing of the ribosomal 5.8S RNA gene and the flanking internal transcribed spacers (ITS), the b-tubulin gene, and the high mobility group (HMG) of the MAT2 mating type gene (Henricot and Culham, 2002). British and New Zealand isolates were compared and no fertile perithecia was obtained suggesting that *C. buxicola* is heterothallic species.

However, the species *Calonectria pseudonaviculata* was discovered and described by Crous et al. (2002) a few months before Henricot and Culham (2002) who proposed the name *Cylindrocladium buxicola*. The study was determined by the examination of morphology and comparison of sequences of several regions of nuclear DNA of both new species and no teleomorph was obtained by mating of a single spore cultures on CLA. Historical facts indicate that the genera *Calonectria* 1867 used for sexual states, and *Cylindrocladium* 1892, used for the asexual states, are synonymized. *Calonectria* is the first name and thus has the priority. Therefore, the name *Calonectria* for species previously known as *Cylindrocladium* is listed in Lombard et al. (2010a).

Henricot et al. (2012) proposed to conserve the name *Cy. buxicola* against *Ca. pseudonaviculata* but it was has been filed.

The origin of this new fungal species remains unknown, but hypothetically, it was first introduced into Europe, then to New Zealand (EPPO, 2004) since now actions were requested uring this period. Scientific evidences of the spreading of *Calonectria pseudonaviculata*, often as *Cylindrocladium buxicola*, from the UK to mainland Europe are represented in several articles. In Europe, the pathogen is widespread in many countries including Austria, Belgium, Croatia, Czech Republic, France, Germany, Italy, Netherlands, Slovenia, Spain, and Switzerland (Crepel and Inghelbrecht, 2003; Brand, 2005; CABI, 2007; Henricot et al. 2008; Benko Beloglavec et al. 2009; Varela 2009; Cech et al. 2010; Šafránková et al. 2012). Based on the morphological, cultural and molecular characters the species *Cylindrocladium buxicola* was observed on potted box plants (*Buxus sempervirens* ‘Suffruticosa’) followed by a sudden and severe defoliation in a nursery located in the Como province (Lombardy, northern Italy) in spring 2008 (Saracchi et al. 2008). Boxwood blight pathogen was firstly isolated from the infected leaves and shoots of evergreen boxwood (*Buxus sempervirens*) in a private commercial nursery near Lviv on the west part of Ukraine (Matsiakh 2016, unpublished yet).

During the summer 2012 in Caspian hyrcanian forests, covering the Alborz mountain range of northern Iran, a sudden leaf and twig blight disease of *Buxus sempervirens* subsp. hircana was found which was caused by *Calonectria pseudonaviculata* (Mirabolfathy et al. 2013). Also in 2012, blight symptoms and severe defoliation of boxwood trees were seen in two colonies in Lirehsar and

In 2011, boxwood blight was first noted, in the province of Trabzon in the Black Sea region and later in the entire Eastern Black Sea region including forests around the province of Artvin (Turkey) (Akıllı et al. 2012). In November 2012 it was found that epidemic had spread 3–25 km along the river valleys near the Black Sea coast (Lehtijärvi et al. 2014).

The boxwood blight have been found in Delaware, Maryland, Massachusetts, New Jersey and the southeastern New York, US and in October 2011, shoot dieback and defoliation was revealed on Buxus sempervirens 'Suffruticosa' (dwarf English boxwood) and 'Green Balloon' outdoor, 10-cm pots at a wholesale nursery in Chilliwack, British Columbia, Canada (Malapi-Wight et al. 2014; Ivors, Prevention and Management of Boxwood Blight; Elmhirst et al. 2013).

First published information about the discovery of Cylindrocladium buxicola in Georgia was presented in 2011 (Gorgiladze et al. 2011). In November 2010, in Mtirala National Park such symptoms as dark brown spots on the leaves, narrow blackish streaks on the stems and the defoliation of the relict species of the box species Buxus colchica were observed. In addition, the latest research conducted by Gasich et al. (2013) showed the blight symptoms on Buxus colchica in ravine of Psyrtskha River (Novyi Afon, Abkhazia). The new diseases was identified as Calonectria pseudonaviculata and fungal pathogenicity to Koch's postulates was confirmed.

Ca. pseudonaviculata (syn. Cy. buxicola) was included on the EPPO alert list (EPPO, 2004), but has subsequently been removed (EPPO, 2008).

HOSTS. It is known that the three main boxwood species Buxus sempervirens, Buxus microphylla (littleleaf boxwood) and Buxus sinica var. insularis (Korean boxwood) are the most susceptible to Cy. buxicola. Buxus colchica and Buxus microphylla var. japonica (Japanese boxwood) are also hosts. Also, boxwood blight pathogen was found on genera such as Sarcococca and Pachysandra (Henricot et al. 2008; LaMondia et al. 2012). The ability to be infected by the fungus is determined due to the plant genetics, as well as physical features of the plant such as a dense and compact leaf canopy.

SYMPTOMS. The symptoms of the box blight are: dark or light circle spots on the leaves, black cankers on the stem and straw- to bronze-coloured blighted foliage and defoliation (Figure 3). Leaf spots may blend together eventually cover the entire leaf surface. The infected stems can have lots of dark brown or black lesions, either linear or diamond-shaped. The black streaks can be found on stems progressing from the bottom of the plant to the top.

New growth continues to develop on healthy stems, and often the root systems remain healthy and intact (Ivors and LeBude, A new pest to the U.S. Ornamental Industry: The “box blight” pathogen Cylindrocladium pseudonaviculatum = Cylindrocladium buxicola.). Blighting and defoliation can occur rapidly with complete leaf loss under warm (64 to 80°F) and humid conditions. Shady conditions contribute to disease development. Spores of the pathogen may sometimes be seen on the underside of the infected leaves. Under high humidity, white fuzzy masses comprised of a large numbers of spores, which are sometimes visible on infected stem and leaf tissue with the naked eye or with a hand-lens. However, these fuzzy masses are not often observed if the environmental conditions are not appropriately right (i.e. if relative humidity or temperature is too
low). The most boxwood cuttings are propagated in humid chambers or shaded structures and liners often grow in shade; conditions that promote disease development.

It is very important to know that the infected boxwood stems may remain green under the outer bark until a secondary invader or opportunistic pathogen attacks this tissue and eventually kills the plant.

![Symptoms of boxwood blight](image)

**Figure 3: Symptoms of boxwood blight: A - The dark or light circle spots on the boxwood leaves; B - Black cankers on the stem; C – The straw- to bronze-coloured blighted foliage; D – Defoliated plant**

**MOVEMENT AND DISPERSAL.** Pathogen can be easily dispersed by rain splash or wind for short distances (Crous 2002; Douglas, 2011). For long distance, it may spread through the movement of infected plant materials and while their walking from landscape to landscape. In addition, the spores can adhere to shoes, gardening shears, lawn mowers and can be dispersed via animals and birds (Ong et al. 2015). Undoubtedly, the spreading of this pathogen occurs via the movement of apparently healthy plant material between nurseries and countries. Also, the phenomenon “Trojan horse” takes place when some fungicides can suppress the disease but not kill or eradicate the fungus and plants look healthy for some time until the fungicide is no longer effective. As results, the

**MANAGEMENT.** Generally, it is very difficult to control boxwood blight diseases with fungicides because of application procedures and the inoculum levels of the pathogen. No fungicides provide complete protection from infection especially at high inoculum levels.

**Chemical control.** Several fungicides had been proposed to control boxwood blight diseases in the past. They included chlorothalonil, copper compounds, MBCs and prochloraz (Crous, 2002). Signum (boscalid + pyraclostrobin) and Octave (prochloraz) show variable control of natural infections in the nurseries (Polizzi and Azzaro, 1996; Crous, 2002; Polizzi and Vitale, 2002). In these field trials, Signum can be the best fungicide when used as a protectant and a curative in the single-sprays treatment. A satisfactory activity against *Calonectria* spp. was disclosed by fludioxonil (Haralson et al. 2007), fosetyl-Al, prochloraz + cyproconazole, trifloxystrobin, azoxystrobin and K phosphate, whose efficacy, however, varied with the type of infection (Aiello et al. 2013). The phytotoxicity risks must be monitored in the case of repeated treatments with high concentrations.

**Biological control.** Knowledge about biological control of boxwood blight is very limited as it is not always effective. Several studies explained inconsistency of efficacy of various biocontrol agents when introduced under commercial field conditions (Bardin et al. 2015):

- being less effective or completely ineffective - even though their efficacy was very good in controlled conditions: climatic variations (temperature, humidity, radiation) in field conditions, ecological survival and colonization ability of the biocontrol agent, intrinsic traits of the antagonistic microbe (variable production of required metabolites or enzymes) and/or an unstable quality of the formulated product (Shtienberg and Elad, 1997; Guetsky et al. 2001; Mark et al. 2006; Nicot et al. 2011b);
- genetic diversity of the plant pathogen (mutation, population size, recombination, gene flow and selection);
- using of biocontrol agents in practice (surface treated, doses of application etc.);
- using of chemical methods in parallel or in combination with biological control (Ajouz et al. 2010; Fillinger et al. 2012).

Currently, bioformulates containing different *Trichoderma* spp. (*T. asperellum* TV1, *T. harzianum* strains Rifai T22 and ICC 012, *T. viride* ICC 080) that are widely adopted and notably succeeded and applied exclusively as soil treatments, are commercially available for ornamental plants. Fresh research, conducted by Rivera et al. (2015) were aimed to determine the complexities of individual phytobiomes and explored the fungal component of the boxwood rhizosphere to identify resident natural enemies of boxwood blight pathogen. They collected rhizosphere soil samples from 40 mature boxwood plants from two arboreta collections and using metagenomic analyses performed from 454 pyrosequencing of the ITS region. They investigated that Fusarium and Mortierella were the most widespread and dominant fungal genera in the boxwood soils but also, Trichoderma. Trichoderma was cultured and in dual-culture experiments showed 99.4% reducing of *Ca. pseudonaviculata* cultural growing. It was suggested that indigenous fungal communities in the native phytobiome may be powerful weapons for disease control (Hébert, 2014; Rivera et al. 2015).
Biopesticides are the most perspective way to suppress pathogen populations. They are living organisms or natural products derived from these organisms. Also, there are several advantages compared to the chemical products (Cawoy et al. 2011):

• It decomposes more quickly in the environment than chemical products;
• It is less toxic (Thakore, 2006);
• It can often help suppress resistant pathogens;
• It can be applied in alternation with other pesticides to avoid resistance development;
• As they are obtained from aerial or underground parts of plants that are naturally less or not at all affected by a pathogen that devastates a neighboring (Cook and Baker, 1983; Ryan et al. 2009);
• Multiplicity of their ways of actions: 1. competition for nutrients and space; 2. direct antagonism of plant pathogen growth; 3. host plant immunization (Cawoy et al. 2011).

**Bacillus as biopesticides against plant pathogens**

Bacillus genus includes a large genetic biodiversity of microbial organisms from sea water to soil and even found in extreme environments like hot springs (Cawoy et al. 2011; Hoch et al. 1993). They are well-studied organisms which facilitates their rational use, for example *Bacillus subtilis*. Secondly, *B. subtilis* was recognized non-pathogenic (Harwood and Wipat, 1996) and granted the "generally regarded as safe" by US Food and Drug Administration (USFDA). It can be apply as biopesticide because of huge capacity to produce spores and resistant dormancy forms capable to withstand high temperatures, unfavourable pH, lack of nutrients or water, etc. (Piggot and Hilbert, 2004). Under unfavorable environmental conditions, bacteria helps these microorganisms to survive in the phytosphere. Sporulation can be induced at the end of cultures and this phenomenon is exploited in industrial production (Monteiro et al. 2005). Moreover, *B. subtilis* can survive in the rhizosphere and thus it is effective as a biopesticide (Losick and Kolter, 2008; Rosas-Garcia 2009) as well as it can live in aerobic environments under low oxygen concentration (Nakano and Hulett, 1997). This is a real advantage in the rhizosphere as oxygen availability may fluctuate during time and is generally low. Finally, *B.subtilis* is a motile bacterium, which can be pioneer and colonize new ecological niches, it readily moves towards and on the root surface (Cawoy et al. 2011). *B.subtilis* can play important role in all mechanisms of biocontrol, biostimulation/fertilisation and also may act through several mechanisms. Bacteria is able to be effective in many conditions (variety of pathogens, plants, environmental conditions) while one mechanism may act instead of another (Cawoy et al. 2011).

In literature there is an evidence involving *B.subtilis* for the control of *Cylindrocladium spathiphylli* (Wit et al. 2009). Consequently, commercial products based on *B.subtilis* can be proposed to use as biopesticide against boxwood blight pathogen.

2.2.2. Box tree moth – *Cydalima perspectalis*

**HISTORY.** *Cydalima perspectalis* (Walker, 1859) [synonyms: *Diaphania perspectalis*, *Glyphodes perspectalis* (Walker, 1859)] (Lepidoptera: Crambidae: Pyraustinae) is a dangerous invasive species phyllophage, larvae of which feeds on the leaves of box trees, *Buxus* spp., resulting in defoliation and killing the trees.
Taxonomic Tree
- Domain: Eukaryota
- Kingdom: Metazoa
- Phylum: Arthropoda
- Subphylum: Uniramia
- Class: Insecta
- Order: Lepidoptera
- Family: Pyralidae
- Genus: Cydalima
- Species: Cydalima perspectalis

According to the data of Russian experts, firstly Cydalima perspectalis was found on September 22, 2012 in Sochi on boxwood bushes in temporary nursery where the planting material was imported from Italy for the planting area of the Basic Olympic Village in Imeretian Valley. Conducted control measures failed as well as applying of insecticide «Aktelik» (non-systemic organophosphorus insectoacaricide product with enteric-contact action) has not led to the death of detected larvae. It was the cause of the subsequent rapid resettlement and spread of the pest to the green stands of Sochi and later entered the natural population of boxwood on the territory of yew-box grove in Caucasian Biosphere Reserve (short unpublished information in Georgian by Eskin and Bibin, 2014; Trokhov and Kaurova, 2015; http://www3.syngenta.com/country/ru/ru/crop-protection/products/insecticides/Pages/actellic.aspx).

Since October 2013, Cydalima perspectalis has penetrated the relict native boxwood forests on the territory of the Sochi National Park (Shchurov et al. 2013). The expert group meeting of forest EPPO quarantine objects, which took place from 9 to 13 March 2014 in Vienna (Austria) announced that box tree moth, revealed in Sochi in 2012, is a particular concern because of the season this pest can destroy all native population of Buxus colchica forests in Northern Caucasus (http://mvl-saratov.ru/samshtovaya-ognevka-na-chernomorskom-poberezhe-rossii).

Nowadays, it is known, that the box tree moth Cydalima perspectalis is native to subtropical regions of eastern Asia (India, China, Korea, Japan and the Russian Far East) (Walker, 1859; Hampson, 1896; Inoue, 1982; Kirpichnikova, 2005; Leraut, 2012; Park, 2008) but simultaneously it is an invasive species on box tree Buxus spp., in Europe that has been spreading and establishing across the continent during the last decade. Firstly box tree moth was recorded in south-western Germany and in the Netherlands in 2007 (Krüger 2008; van der Straten and Muus 2010). Currently many well-documented evidences of rapid spread of this species in Europe are provided: Switzerland (Leuthardt et al. 2010), France (Feldtrauer et al. 2009), England (Salisbury et al. 2012), Belgium and Austria (Lepiforum, 2013), Croatia (Koren and Crme, 2012), Czech Republic (Šumpich, 2011) Hungary (Sáfián and Horváth 2011), Italy (Lepiforum 2013), Romania (Székely et al. 2011), Slovenia (Seljak 2012), Slovakia (Slamka 2010) and Turkey (Hızal et al. 2012).

The damage of box tree by bow tree moth was identified in 2014 in green areas near the office of Kintrishi Ptotected Area (Kobuleti) and administration of Mtirala National Park (village Chakvi) as well as in the city of Batumi and Tikeri nursery farm (Administration of Kobuleti, Lepl Forestry Agency of Ajara) (Matsiakh, 2014).
HOSTS. The main host plants of *C. perspectalis* are *Buxus* species, including *B. sempervirens* L., *B. microphylla* Siebold & Zucc., *B. sinica* (Rehder and Wils.) M. Cheng and *B. colchica* Pojark (Buxaceae). In its origin countries, the pest has also been reported on *Euonymus japonicus* Thunb., *E. alatus* (Thunb.) Siebold (Celastraceae), *Ilex purpurea* Hassk. (Aquifoliaceae), *Pachysandra terminalis* Siebold & Zucc. and *Murraya paniculata* (L.) Jack (Rutaceae), but still there are no reports of these plant species being attacked in Europe (Wang, 2008; Hizal et al. 2012; Bella, 2013). Nevertheless, infestation of *C. perspectalis* also was discovered on *Ruscus colchicus*, *R. aculeatus*, *Eriobotrya japonica*, *Acer campestre*, *Fraxinus excelsior* and *Rubus* spp. in Sochi (Trokhov and Kaurova, 2015).

MANAGEMENT. Mechanical control such as removal of larvae by hand or by shaking or water-spraying the infested trees is proposed by Kenis et al. (2013) but may be extremely time-consuming and impossible to be done in the native boxwood forests.

**Chemical control** of *C. perspectalis* on ornamental *Buxus* spp. in Europe include the use of insecticides but there is still not much published information about available control of invader. Due to the webbing that the older larvae form around them, applying control treatment is very difficult task. Synthetic pyrethroid insecticides that have enteric contact action can be used in ornamental nurseries: Sumi-alpha (Esphenvalerat, Senpai); Decis, Decis prophi (Kotryn, Oradelt, Polytoyx,) Fastak (Alphatoks, Geletrin, Kinimks, Fury). Difenbuturon (Dimilin) should be the alternative to the synthetic pyrethroid insecticides. Dimilin refers to a low-hazard insecticides, has a low toxicity to mammals, birds and fish, long period of aftereffect (up to 40 days) and lack of bioaccumulation. Dimilin is the most effective if applied when caterpillars are very small, ideally just after egg hatch.

**Biological control.** Preferably entomopathogenic bacteria, *Bacillus thuringiensis* var. *kurstaki* (Btk) is one of the biological option and easily can be implemented in the forest as part of a good resistance management program. *B. thuringiensis* produces the proteins Cry as a part of its spores and Cyt proteins that act through pore formation in the gut wall of the animal and allow the bacteria to emerge from the spores and feed on the contents of the insect’s body cavity. Both of these proteins are highly toxic to insects but not to mammals or for the environment (Cawoy et al. 2011). When the insects are killed, the new bacterial population as a new source of spores are formed and generated (Sanahuja et al. 2011).

First, this biopesticide was applied in 1938 (Sanahuja et al. 2011). The commercial products Btk had been used for control of forest defoliators Lepidoptera across North America and Europe: *Thaumetopoea processionea*, *T. pityocampa*, *Lymantria monacha*, *Dendrolimus sp.*., *Bupalus piniaria*, *Panolis flammea*, *Tortrix viridana*, *Operophtera brumata*, *Dioryctria abietella*, *Lambdina fiscellaria fiscellaria*, *Choristoneura occidentalis*, *C. pinus pinus*, *Orgyia leucostigmata*, *O. pseudotsugata*, and others (Fuxa et al. 1998; van Frankenhuyzen, 2000; Cawoy et al. 2011).

Fungi also can be potentially alter pathways toward their development as microbial control agents (Lacey et al. 2015). There are many examples of performed laboratory trials with showing a “great potential” of fungi control but actually failed in the field (Vega et al. 2012). It is still needed to understand fundamental ecology of these organisms in the natural environment, post application value and behaving of insects that can attract or repel fungal metabolites as well as extend or deter their activity (Meyling and Pell, 2006; Rohles and Churchill, 2011). Moreover, more researches
under field conditions should be required to identify effects of biotic and abiotic factors (sunlight, humidity, and microbial activity on the phylloplane etc) on efficacy and persistence of fungal treatments applied against foliar pests (Jaronski, 2010). Fungi *Beauveria* and *Metarhizium* spp. were used for biological control of different groups of insect (Table 2), they can be dispersed passively via wind or rain splash. Their transmission occurs while contact susceptible insects with infected individuals but also their spores can affect other arthropods bodies (Meyling et al. 2006; Roy et al. 2007; Vega et al. 2007).

| Table 2. An overview of the entomopathogenic fungi *Beauveria* and *Metarhizium* spp. developed for microbial control of insect pests (by Lacey et al. 2015) * |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|
| Species names                   | Targeted insects                | Produced in                     | Selected references            |
| *Beauveria bassiana* sensu lato | Acari, Coleoptera, Diplopoda, Diptera, Hemiptera, Hymenoptera, Isoptera, Lepidoptera, Orthoptera, Siphonoptera, Thysanoptera, | Africa, Asia, Australia, Europe, South & North America | Wekesa et al. (2005), Brownbridge et al. (2001, 2006), Jaronski and Jackson (2012), Lacey et al. (2011) |
| *Metarhizium anisopliae* sensu lato | Acari, Blattoidea, Coleoptera, Diptera, Hemiptera, Isoptera, Lepidoptera, Orthoptera, | Africa, Asia, Australia, Europe, South, Central & North America | |


The insecticidal activity of essential oils of cloves (*Syzygium aromaticum*), Atlas cedar (*Cedrus atlantica*) and European silver fir (*Abies alba*) against box tree moth were tested in Sochi in 2014 (Dvoretska, 2014). The oils effect in different concentrations on the adults of *C. perspectalis* was checked in glass container (10 imago per 1 container with volume 3000 ml) and in the native *Buxus colchica* forests. All butterflies died within 1.5-6 hours at a concentration of 10 drops of essential oils on the containers volume and after 6 days in control box. In containers with vapours of essential oil from *Abies alba*, the moth butterfly fell to the bottom of the container with convulsive twitching wings and then died for 1.5-2 hours. In containers with vapours of essential oil from *Syzygium aromaticum*, butterfly died for 2-3.5 hours with a minimum concentration of 1 drop in volume. For field test, the emulsions were made of each type of essential oils from the rate of 10 drops of an essential oil per 500 ml of water and plants were sprayed in the massive fly period of imago. During seven days under maintaining favorable weather conditions, trees have not been damaged by box tree moth at all. Over the next three days after heavy rains and cooling weather, the majority of butterflies disappeared. However, the treated of oils emulsions boxwood Colchis trees are still remained intact (Dvoretska, 2014). This research gives a new tool for improved development of alternative control management against *C. perspectalis*.

Finally, classical biological control as possibility of eco-friendly regulation using predators and parasitoids (*Chrysoperla carnea*, *Harmonia axyridis*, *Orius majusculus* and *Trichogramma* wasps) was experienced by Herz and Göttig (2015) and Tabone et al. (2015). This work is not resulted yet. Acceptance tests by Herz and Göttig (2015) with eight *Trichogramma* species showed that there is a present acceptance of *C. perspectalis* as a host especially for *T. dendrolimi*.
Matsumura but parasitism was low for all species (maximum mean 44%) (Göttig, 2012). Tachinid Pseudoperichaeta nigrolineata (Walker) has emerged from late larval instars but none from pupae collected during the surveys was parasitized (Nacambo, 2012). Additionally, further investigations should be carried out to improve application techniques and using Asian host specific natural enemy has to exclude their potential negative effects on non-target species native to Europe (Wan et al. 2014).

For the period from 03/23/2015 to 04/20/2015, more 1,100,000 individuals of entomophagy Chouioia cunea were introduced to the native boxwood forests on the territories of Sochi National Park (http://www.dendrarium.ru/dendrarity/lesa-ecology/195-2015-05-15-11-48-08). In the Adler Forestry district, for the period from 04/17/2015 to 29/04/2015, eight beehives of Fabra populated by wasp Euodinerus posticus were established (http://www.interfax-russia.ru/Crimea/print.asp?id=602019&type=main). Documented results of these researches are not available yet.

2.3. Other Boxwood Diseases

*Phytophthora* Root Rot

The XXI\textsuperscript{th} century has already shown as a major paradigm shift in our understanding of the biology, evolution, and genetics of the genus *Phytophthora* as well as they continue to emerge at an accelerated rate due to increased global travel and trade (Roy and Grünwald, 2014). Invasive plant pathogens, especially those of the *Phytophthora* genus are recognized as an emerging threat to natural ecosystems, as well as to horticultural production systems (Hansen, 2015, Jankowiak et al. 2015, Rahman et al. 2015).

*Pythium* and *Phytophthora* species are considered as “fungus-like organisms” or “pseudo-fungi” and are placed in the kingdom Chromista or kingdom Stramenopilia, distinct from the kingdom Fungi (Martin et al. 2012). The species of the genus Phytophthora (from Greek: φυτόν (phyton) - "plant" and φθορά (phthora), "destruction" - "destroyer of plants") are primary parasites of fine roots causing the root rot and the bark lesions of young shoots and stems, as well as mature trees and shrubs of many species (). The genus *Phytophthora* is large, with over 100 described species, and disease symptoms include blights, cankers, dieback, wilts, root rots, and decline. Also, genus *Pythium* include some of the world’s most destructive plant pathogens which account collectively for multibillion dollar losses in forestry and agriculture (van West et al. 2003). Some *Phytophthoras* cause multiple symptoms on a single host, whereas other different symptoms on different hosts (Forest Phythophthoras of the World 2015).

The special concern is due to the rate of *Phytophthoras* spread, a wide range of host plants and the lack of effective control measures. The research recently conducted shows that the cause of the infections of different plants in different parts of the world is often the same aggressive pathogenic *Phytophthora* species.

*Phytophthoras* influence includes some visible symptoms on the trees such as thinning of the crown, delay of the plant growth and drying of lateral branches. The leaves may become abnormally small and change their color to straw-yellow and green. Over the time, the branches and crowns of trees become completely dead.
Species of the genus *Phytophthora* can be airborn, soilborn or waterborn (Erwin and Ribeiro, 1996). First ones have an advantage of transferring their zoospores through air, but they can survive in soil too. The biological life cycle of the second group is connected with soil and most of these organisms are fine root pathogens infecting plant tissues via zoospores especially when the soil is saturated with water. The third group of *Phytophthora* is linked to the water ecosystems and its life cycles, as well as their sexual behavior. They can be transferred from tree to tree by direct contact of zoospores with the bark tissue take place (Oszako and Zakrzewska, 2005). Significant precipitation, the increase of soil moisture and optimal temperatures cause favor its development and further spread. In addition, structures such as oospores and chlamydospores survive in infected seedlings and root rhizosphere in the nursery, they stick to the tools, wheels of the cars, tractors and people’s shoes.

Natural watercourses can be an important but often neglected source of *Phytophthora* pathogens. One of the main pathways of *Phytophthora* spreading is water carrying spores. The first record of such a spread dates back to 1921, when *Phytophthora cryptogea* was found in water used for watering the plants in a nursery greenhouse (Bewley and Buddin, 1921). Since the 19th century more than 20 species of *Phytophthora* and representatives of the genus of *Pythium, Fusarium, Rhizoctonia* have been isolated from water (Themann et al. 2002; Hong and Moorman, 2005). Hong and others believe that water used for watering of plants is the main source of *Phytophthora* in nurseries causing damage to orchards and vegetables (Hong 2005). High diversities of *Phytophthora* species have also been found in natural waterways all over the world (Sims et al. 2015, Nagel et al. 2015, Huberli et al. 2013). In Poland, the possibility of spread of zoosporic organisms via water courses has been demonstrated several times in the horticulture sector, especially in ornamental plant cultivation (Orlikowski, 2006, Orlikowski et al. 2007, 2008, Nowak et al. 2015, Ptaszek and Orlikowski, 2015). The first report of *Phytophthora* species in water sources in the Ukraine and the occurrence of *Phytophthora* species in the rivers Stryj and Yasenytysya and in the lake situated in the National Park of „Skolivskoi Beskid“ was documented in 2012 (Matsiakh et al. 2012). Three *Phytophthora* species (*P. gonapodyides, P. lacustris and P. cactorum*) were detected in Ukrainian rivers presumably moving naturally towards the EU border. Species of the *Pythium* genus were detected in Polish rivers, out of which the most common species were *Py. lycopersicum, Py. sylvaticum, Py. citrinum and Py. terrestris* (Matsiakh et al., unpublished yet).

*Phytophthoras* most commonly infected genera of ornamental plants, including *Rhododendron* spp., *Pieris* spp., *Buxus* spp., and *Ilex* spp. (Bienapfl and Balci, 2014). According to the study of Hasen Mary Ann (Major Diseases of Boxwood http://www.mastergardenproducts.com/gardenerscorner/boxwooddisease.htm), *Buxus sempervirens* cv. ‘Suffruticosa’ and *B. sempervirens* cv. ‘Arborescens’ are susceptible to *Phytophthora* disease, which is caused by *Phytophthora parasitica*. In addition, this disease was found in Virginia on *Buxus microphylla*.

American Boxwood Company shows that *Phytophthora* is a plant destroyer and it gives some information how serious a problem can be (American Boxwood Company Boxwood Diseases http://www.americanboxwood.com/index.php/plantcare/56-boxwood-diseases) and that a soil-borne fungus can affect all cultivars of *B. sempervirens* at any age or size. *Phytophthora parasitica* can damage root, stems and leaves. Infection may begin in wet and cool soil about 58°F to 70°F (14°C to 21°C), usually in spring and autumn. The progress of the disease and first damage appears when
the temperature of the soil is higher and then it occurs at 75°F (24°C). The greatest effect is observed at 85°F (29°C). When Phytophthora infects the boxwood, it seldom survives. Produced spores in the water that flows in the soil, Phytophthoras can damage the boxwood growing in poorly drained soils very well.

The research in hardy ornamental nursery stocks with Buxus sempervirens was conducted during three years in Poland (Orlikowski et al. 2008). The first symptom of disease was yellowing of individual stems spreading slowly to other shoots. Strongly invaded stems were straw coloured. The bark started to separate from the wood which turned bluish. Phytophthora citricola was determined as a new pathogen on Buxus sempervirens in Polish ornamental nursery stocks.

In spring 2008, some gradual decline was revealed on 6-year-old Buxus rotundifolia plants in a garden in central Italy (Vetraino et al. 2010). Among 150 boxwood plants, 70% of them had symptomatic damage and 25% among them were completely wilted. The symptoms of disease included stunting, reduced growth, leaf chlorosis when leaves were light green at first and then became yellow, bronze or straw coloured and necrotic bark lesions at the base of the stem. First foliar symptoms were observed only on several branches and later it extended to the whole crown. After using immunological field tests (Pocket Diagnostic, CSL Diagnostics, Milan, Italy) and the isolation on PARHP (2) from necrotic tissues, P. citrophthora was determined (Vetraino et al. 2010).

Moreover, at the end of September in 2012 in the commercial nurseries in Italy the collar and root rot associated with the severe wilting and desiccation of foliage on Buxus sempervirens was discovered (Luongo et al. 2013). About two hundred boxwood plants were detected and 50% of them showed the change from green to straw colour and diffused desiccation resulting in 20% mortality. The leaves symptoms were the same as on Buxus rotundifolia described above. Phytophthora nicotianae was detected by plating small pieces of stem and root tissues on P5ARPH selective medium, from soil samples baited with azalea and camellia leaves and using DNA analysis.

Phytophthora species (P. taxon Buxus) closely resembling the recently described species P. himalsilva, colonized roots, collars and rhizosphere soils from diseased boxwood saplings (Buxus sempervirens) from German and Romanian nurseries (Nechwatal et al. 2015). The high similarity between P. taxon Buxus and P. himalsilva was confirmed by comparison of both taxa using morphological, phylogenetic and pathogenicity data. No differentiation was found regarding growth patterns, morphological characters and breeding behaviour. These findings highlighted the increasing importance of exotic pathogens causing plant diseases in commercially important plants, especially in nurseries. The box isolates may belong to a group of species potentially endemic to Asia, the Phytophthora taxa involved in this disease might represent another important threat to European boxwoods with an exotic origin (Nechwatal et al. 2015).

First h. occultans was found in 1998 in the Netherlands, then went unnoticed for more than 10 years, to re-emerge in 2010 (Man in 't Veld et al. 2015). As Buxus sempervirens is one of the main host of Phytophthora occultans known in Europe in Roman times and it is likely that movement of this plant was responsible for the introduction of P. occultans. A possible candidate for the introduction of P. occultans is Acer palmatum, currently imported from Japan and China in large quantities. After its introduction the pathogen could have spread through horticultural centers (Man in ’t Veld et al. 2015).
Venediyapina (1985a) gave the first information about *Phytophthora* disease in Abkhazia in 1985. It was the discovery of *Phytophthora cinnamomi* in soils of chestnut woods of Abkhazia. But in 2014, using baiting and molecular techniques *Phytophthora cinnamomi*, *Phytophthora plurivora* and *Phytophthora gonapodyides* and many other *Pythium* spp. were isolated the soil samples from the native boxwood forests in Mtsirala National Park and Kintrishi Protected Areas (Matsiakh, 2014).

According to the data of the USA Plant Pathology Department, even the boxwood has suffered from several defoliations and has been infected by the box blight but the root systems remained healthy, intact and without damages (Ivors, Prevention and Management of Boxwood Blight). Nevertheless, the roots infected by *Phytophthora* may look different.

**Symptoms.** *Phytophthoras* symptoms are very specific because they include visible and underground features. Aboveground symptoms have gradual signs such as poor growth, off-color foliage, wave edges and changing colour of leaves from light green to yellow, bronze and straw-color. The leaf symptoms may appear on just a few branches or on the entire plant, depending on the extension of the infection of the roots and they can remain attached to the twigs. The bark of the infected boxwoods dies and can be easily removed from the stem. The reduction of functioning roots is preceded by yellowing and the death of the plant top. Aboveground symptoms of the stem include dark brown and black coloration of the vascular tissues under the bark at a ground level to a few inches (7 cm to 18 cm) above and this dark colour is a response of the vascular tissue to the fungus. The internal symptoms of stem become apparent in partial or complete blockage of nutrient and water movement in the stem (American Boxwood Company Boxwood Diseases http://www.americanboxwood.com/index.php/plantcare/56-boxwood-diseases).

There is a very important feature when foliar symptoms are visible on the plant, roots may be in brown colour and complete dull whereas healthy roots have a bright light tan colour.

**Disease features.** *Phytophthora* diseases may be described as a slow progressive process that may last for a long period generally occurring in large plants of 20 years old or more. Nevertheless, *Phytophthora* root rot prefers a wet soil (a large amount of moisture 70%) and the temperature of about 20 degrees. At this temperature sporangia form and the mass floating zoospores mature. They have the ability to mix in the soil, to touch the most sensitive roots, surround and grow into the tissue forming penetrable hyphae. This process is ideal in peaty soil with high level of humidity (Łabanowski et al. 2000).

**Volutella box blight - *Volutella buxi* (teleomorph *Pseudonectria rouselliana*)**

The infected boxwood stems may remain green under the outer bark until secondary opportunistic pathogens attack this tissue and eventually may kill the plant. The fungus *Volutella buxi* is often associated with boxwood blight pathogen as secondary cancer pathogen and is known to cause *Volutella* blight, but it does not cause the box blight on its own (Ivors, Prevention and Management of Boxwood Blight). *Volutella* blight symptoms usually appear in spring, as individual shoots or entire plants exhibit poor growth (Douglas, Boxwood blight – A new disease for Connecticut and the U.S.). *Volutella buxi* can be distinguished from box blight easier because it produces masses salmon-coloured spores on the undersurfaces of infected leaves that are visible. The bark of infected branches may be peeled and darkened and discoloured (Douglas, Boxwood
blight – A new disease for Connecticut and the U.S.). A very important feature is that the fungus does not cause black circle spots on the leaves. Mat infects only one branch of plant whereas boxwood blight develops on the bottom and gradually moves upwards on the stems often making the plant look “top heavy” and leaving foliage only at the branch tips (Ivors, Prevention and Management of Boxwood Blight).

**Macrophoma leaf spot – Macrophoma candollei**

*Macrophoma candollei* is considered to be a secondary pathogen of *Buxus* spp. that causes raised small black spots on the undersurfaces of dying leaves (Ivors, Prevention and Management of Boxwood Blight). *Macrophoma candollei* can cause leaf blight, but it usually acts as a weak pathogen following root diseases or environmental stresses. The main disease diagnostics include change of the color of the leaves to yellow-straw and the influence of several other factors, such as nematodes and environmental conditions (winter, frost, drought) (Douglas, Boxwood blight – A new disease for Connecticut and the U.S.). The presence of *Macrophoma candollei* indicates that the plant is damaged by some other factors and any recommendations are developed but for predisposing factors which should be addressed (Hansen, Major Diseases of Boxwood).

As a result of many years of infections by *Volutella buxi* and *Macrophoma candollei*, boxwood plants wholly or partially lost the upper part of the crown, as well as the distal portions of the large branches, which dry out after defeat leaves. Eventually the whole plant dies in some time.

**Box Rust or Boxwood Rust - Puccinia buxi**

*Puccinia buxi*, box or boxwood rust is particularly troublesome in the nurseries but seldom causes serious problems regarding disease in the forests. It wide spreads throughout Europe and rarely reported on native hosts in China and Japan and also was discovered in Pennsylvania on recently received nursery stock of boxwood from Greece (Yun, 2015). Mainly, it affects *B. sempervirens*, traditionally used for boxwood hedges, but also native species in Asia, *B. microphylla* and *B. sinica*. It can be seen as thickened rusty blister-like pustules on both sides of the leaves. *Puccinia buxi* forms only one type of spore (black telia) which develops in autumn and winter and breaks through the epidermis of the leaves in spring. The new leaves usually infect in the spring and early summer. The fungus continues to grow during the summer and autumn and, as a result, the infected spots become thicker (https://www.rhs.org.uk/advice/profile?PID=851).

*Puccinia buxi* is the subject of a Phytosanitary Alert issued by the USDA-APHIS (http://www.pestalert.org/oprDetail.cfm?oprID=202) but it is indigenous species to the Caucasus *Buxus colchica* plants (http://www.czl23.ru/news.php?extend.106).

### 2.4. Other Boxwood Pests

The state of boxwood can also be influenced by the development of certain species of insect-phytophagous.

The threat of boxwood green spaces and natural populations may be represented by the superfamily Coccoidea (Hemiptera): *Lepidosaphes ulmi* (L.), *Parthenolecanium corni* (Bouché), *Eriococcus buxi* Fonsc (Łabanowski et al. 2000).
Boxwood Scale *Eriococcus buxi*

It is the most dangerous among these species. It characterizes the narrow specialization of feed and inhabits the branches and leaves of *Buxus* species. During the year, two generations of this pest may develop. The first generation can last from early May to November, the second from August to May or June next year. The larvae of first or second age overwinter. After its appearance, the young female is covered with a waxy bloom, which eventually turns into a felted bag where the female lays the eggs. Mass reproduction and nutrition of *Eriococcus buxi* is accompanied by yellowing and shedding of the leaves on individual branches first, and then the whole bush that leads to significant damage and dieback in boxwood green spaces (Yatskova, 2012).

The other two species *Lepidosaphes ulmi* and *Parthenolecanium corni* are characterized as a wider feeding specialization and may inhabit the different trees and shrubs but can also damage the boxwood (Łabanowski et al. 2000).

Boxwood Leafminer *Monarthropalpus buxi*

This is the most serious insect pest that attacks boxwood. The leaf miner *Monarthropalpus buxi* may develop on the leaves of the ornamental boxwood plants. Females lay eggs on the surface of the leaf. From this egg hatches the tiny yellow larva called the boxwood leafminer. During summer and autumn, as the leafminer grows, the plant produces a small circle of cells around the developing larva. These cells serve as a source of food for the maggot. Larvae are orange and about ⅛-inch in length. They form a cell for overwinter (survive the winter) inside the leaves. There is one generation per year. *Monarthropalpus buxi* causes serious damage to American boxwood, English and Japanese boxwoods (*B. microphylla* var. *japonica*) (http://www.clemson.edu/extension/hgic/pests/plant_pests/shrubs/hgic2052.html). The damaged leaves are slightly deformed and swollen and are not noticed immediately. Then leaves turn yellow, cover with spots and fall. A heavy infestation can cause serious loss of leaves and result in death of the boxwood (Kluepfel and Scott, 2004).

Boxwood Mite *Eurytetranychus buxi*

It is not an insect but is more closely related to spiders. The adult is green to yellowish brown in color and very small. That is why the early symptoms are not distinctive and it is easy to overlook the problem until a heavy infestation occurs and greater damage has occurred. All developed stages of boxwood mite feed on both leaf surfaces. Boxwood mites usually feed on young leaves, but damage is most obvious on second- and third-year leaves. It develops eight or more generations per year and inject toxic saliva while feeding and as a result forming stippled, tiny, yellow spots the leaf’s upper surface (http://www.clemson.edu/extension/hgic/pests/plant_pests/shrubs/hgic2052.html).

Boxwood Psyllid *Psylla buxi*

It is a very small, greenish insect and looks like tiny cicada with clear wings and strong legs adapted for jumping. Both - the adult and nymph (the immature insect stage which resembles the adult) feed by piercing leaf surfaces and sucking plant sap. Nymphs hatch from eggs in the spring. They produce a white, waxy material that often covers their bodies. Nymphs feed from buds and young leaves. This feeding results in the typical cupping of leaves and stunted twig growth that are
seen with this pest. Plants tend to outgrow the injury by midsummer
(http://www.clemson.edu/extension/hgic/pests/plant_pests/shrubs/hgic2052.html).

Moreover, the boxwood can be damaged by winter or sunscald and the results of these
injuries are associated with dieback of leaves, twigs and sometimes-even plants. Under the
influence of the different weather conditions leaves may start to reddish, may become brownish
and bronze, the bark of the stems and branches may be peeled and later diebacked.

Analogously, this known disease complex is called “boxwood decline” and it includes
nematodes (Meliodogyne and Pratylenchus) and fungus Clonostachys buxi (=Paecilomyces buxi
=Verticillium buxi) which may contribute to this complex. This complex may influence the boxwood
decline for several years which is manifested by some insignificant defoliation, stunting, wilting, loss
of the vigor and chlorosis (Douglas, Boxwood blight – A new disease for Connecticut and the U.S.).
3. METHODOLOGY

3.1 Field Research

In summer and autumn 2015, the natural forests of boxwood were examined in six regions (out of nine) of Georgia. The field works took place in the species' distribution area from the east to the west of the country: Kakheti, Imereti, Racha-Lechkhumi, Samegrelo Upper Svaneti Guria Forestry Services and L.E.P.L. Forestry Agency of Adjara. The sanitary assessment of health conditions of tree stands was carried out:

- Infected plant materials (twigs, leaves, branches) were collected in a paper bags and kept dry until processing in September-October 2015.
- They were processed in the laboratory of Forest Pathology at the Süleyman Demirel University (Faculty of Forestry, Isparta, Turkey).
- All recorded developmental stages of *Cydalima perspectalis* were collected during the field trips. The adults were identified according to Mally and Nuss (2010).
- According to the GPS coordinates, the areas of spread the box tree moth and boxwood blight pathogen were assigned to the map using program GPS Map.

The pictures and photos of the next chapters are personally were photographed by Canon G15 camera by the executor of these researches.

3.2 Laboratory Work

Small pieces of symptomatic tissues were surface-disinfected and incubated on moistened sterile filter paper in Petri dishes at 18°C to induce the pathogens sporulation (Lehtijärvi *et al.* 2014). Fresh-growing colonies on Petri dishes were checked daily using a microscope LEICA ICC50 HD and transfected onto 2% Malt-agar (MAS) (MEA, Germany) with streptomycin. Isolation of boxwood blight pathogen directly from infected plant material was made using sterilization: 75% alcohol - 30 seconds, 0.5% sodium hypochlorite - 1 minute, again 75% alcohol - 30 seconds and then washed four times in pure distilled water. After sterilization, samples were plated on the surface of potato-dextrose agar (PDS) with streptomycin and 2% Malt-agar (MAS) with streptomycin in a Petri dish. After 7 days growth on MAS and PDS at 24°C obtained isolates were transferred onto PDA and MEA in Petri dish (Henricot and Culham, 2002; Šafránková *et al.* 2013) (Figure 4).

![Figure 4: Methods of isolation of boxwood blight pathogen for infected samples of Buxus colchica: A – Incubation of affected leaves on the moistened sterile filter paper in Petri dish; B - sterilization of infected plan material.](image-url)
Phytophthoras and Pythiums were isolated from soil samples collected in the native boxwood forests using the baiting technique (Figure 5). Young leaves from healthy different hosts (e.g. oaks, beech) and carnation petals were used as baits according to Jung et al. (1996). Infected leaflets were cut into small pieces and were plated onto selective PARPNH agar. Sporangia production was performed into sterile distilled water (ca. 20 mL) or 10% soil solution. Isolates were characterized morphologically with published keys for genus Phytophthora (Waterhouse 1963, Stamps et al. 1990, Erwin and Ribeiro, 1996).

Figure 5: The baiting technique for Phytophthoras isolation
4. RESULTS AND DISCUSSION

4.1. Boxwood blight disease in the native boxwood forests of Georgia

As mentioned already, field works took place during the summer and autumn season in 2015 (48 and 13 boxwood locations respectively), in six regions (out of nine) of Georgia. Detailed information with descriptions of sanitary conditions of boxwood forests is shown in and Annex 1 on Figure 6.

![Research areas map](image)

**Figure 6: Map of visiting all research areas in summer and autumn 2015**

After conducting laboratory study with summarizing of field trip results, it was investigated that the intensive defoliation of boxwood plants of different ages is caused mainly by invasive species *Calonectria pseudonaviculata* and *Cydalima perspectalis* combining with the soil phytopathogens of *Phytophthora* and *Pythium* genera species strong development, which cause root rot.

Invasive fungi *Calonectria pseudonaviculata* (*Cy. buxicola*) was detected in four Regions of Georgia: Imereti, Samegrelo Upper Svaneti and Guria Forestry Services and L.E.P.L. Forestry Agency of Adjara (Figure 7).
Boxwood forests in Kakheti Region were in the best conditions (Figure 8). However, unfortunately, boxwood blight pathogen was isolated from healthy looking plant material collected in Kakheti (Kvareli Forestry Service) in summer 2015.
Typical symptoms of boxwood blight include dark or light brown spots on the leaf surface and then lesions which coalesce with a concentric pattern or zonate lines (yellow arrow). The box trees with the above described symptoms were detected in Zestafoni, Tkibuli, Zugdidi, Tsalenjikha, Martvili and also Lanchkhuti in summer (Figure 9). Infected leaves were turned brown or straw coloured and infected plants looked “blighted”. It was investigated that defoliation occurred very quickly after initial symptoms and heavily infected plants dropped down most of their leaves. Also, some plants were attempted to regrow new branches but after repeated infections and defoliations probably their root system were weakened and it led to the death of plants near the water (rivers) where humid conditions fastened the spread of pathogen. In autumn, the high level of infection was still revealed and sporulation of fungus was detected on undersurfaces of asymptomatic leaves (Figure 9).
Figure 9: Boxwood blight symptoms in Georgian boxwood forests: A - Dark or light brown spots on the leaf surface; B – The concentric pattern or zonate lines (yellow arrow) on the leaf surface; C - “Blighted” plant; D - Sporulation of fungus on under surfaces of asymptomatic leaves; E - Defoliation with typical symptoms and infected plants dropped down the leaves; F - Regrow new branches after repeated infections; G - Heavily infected plant.

Morphological structures of boxwood blight pathogen were described by the laboratory analysis. The fungus mycelium grows slowly, but on the maltose agar (MEA) its growth is doubled compared with potato agar (PDA). The colony reverse was brown in the centre surrounded by a creamy mycelial growth. The colony surface was covered by aerial cottony mycelium in 2-3 weeks and fruiting bodies of the fungus started to develop after seven days (Figure 10).
On hosts, fungus produces spores and fruiting structures called sporodochia which are usually seen on the undersides of infected leaves and in the black lesions on the stems. Huge numbers of spores called conidia are formed in the sporodochia which show the typical crystalline appearance due to the hyaline color. Spores are cylindrical in shape with one septum. Vesicles, the terminal swollen hyphal structure of conidiophore, are also produced in the fruiting structures (Figure 11).

**Disease biology.** The pathogen can survive as mycelium in cankers on infected stem tissues and in leaf debris for at least 5 years. In addition, the fungus can produce and survive in resting structures as microsclerotia (hardened spores or mass of hyphal structures in host tissue that can survive as long as 15 years in soil) and chlamydospores in infected leaves (Figure 12). Spores may
also be formed with chlamydomospores as microsclerotia and have the ability to affect other host plants (Ong et al. 2015).

The disease cycle of *Calonectria pseudonaviculata* can be completed in one week (Figure 13). Infection occurs very quickly in warm (64 to 77°F) and humid conditions and with the help of free water. Germination occurs 3 hours after inoculation. The mycelium of fungus can penetrate the leaf through the cuticle in 5 hours without forming an appressorium (a specialized organ of many fungal pathogens used for infection). The pathogen colonizes the host tissues intercellularly and conidiophores are produced on the leaf surface after 7 days. Conidiophores aggregate and develop into white asexual fruiting structures called sporodochia (easily recognized with the naked eye). The spores are then released to infect other healthy hosts and plant defoliation occurs within one week (Ong et al. 2015).
The disease cycle of boxwood blight pathogen. (Figure 13)

The disease triangle can be used to illustrate the infection caused by the fungus *C. pseudonaviculata* in Georgia (Figure 14). For the development of diseases caused by biotic agent it is needed in one place to interact the plant susceptible to pathogens action, aggressive pathogen and environmental factors that weaken plants and promote the development of pathogen (Stevens, 1960; Agrios, 2005). The massive destruction of plants, which reaches the epiphytotic level, is observed when these three factors of the disease overlay. (Figure 14: The disease triangle)
In the case where there are aggressive pathogen and vulnerable plant, the local diseases may occur in the most favourable conditions for the pathogen. In the case of absence of aggressive pathogen, environmental factors can lead to the non-parasitic damage of the plants.

The environmental factors, in our opinion, are one of the most important conditions and determine the course of the disease process. They enhance the pathogenicity of biotic agent (aggressive pathogen) and weaken the plants on the large areas by creating favourable conditions for the spread of the disease (the nature of the interaction between the components that cause plant disease is shown by arrows on Figure 14). Such close interaction of these three components in plant diseases explained by the fact that plants (unlike animals) and still can not escape from unfavourable conditions (Francl, 2001). Deteriorating external conditions inevitably displays on the condition and resistance of plants as well as the pathogen (a living organism) is highly dependent on environmental conditions.

The spread of the boxwood blight pathogen in boxwood forests in Georgia is observed as the interaction of all these three components. There are the sufficient present of aggressive alien pathogen, the significant areas of boxwood plantations involving or advantage as part of Buxus colchica (sensitive to the action of the pathogen) and, perhaps the most important, the regional environmental conditions (temperature, humidity and soil) that promote the development and spread of the pathogen with intensifying the disease.

4.2. Other boxwood diseases in the native boxwood forests of Georgia

The secondary opportunistic pathogen Pseudonectria buxi (asexual Volutella buxi) that attacks boxwood tissue and eventually may kill the plant was also isolated from the asymptomatic plan material. The fungus is often associated with boxwood blight pathogen as secondary cancer pathogen and is known to cause Volutella blight, but it does not cause the box blight on its own (Ivors, Prevention and Management of Boxwood Blight). In any way, its danger might be underestimated due to enhanced aggressiveness of Cy. buxicola. V. buxi produces pink to orange spore masses on infected tissues and on MEA or PDA media (Figure 15).
Boxwood rust (*Puccinia buxi*) was detected in the boxwood forests in Kakheti, Imereti, Racha-Lechkhumi Regions. One type of spore (black telia) which develops in autumn and winter and breaks through the epidermis of the leaves in spring were detected during both field trips and morphological structures of fungus were observed under light microscope (Figure 16).
Other secondary pathogens were isolated from infected boxwood leaves (Figures 17, 18, 19).
Also, the bark infections of boxwood trees were found as large cankers with brown to black discoloured outer tissues on the lower trunk that seep or, ‘bleed’ a dark red sap (Figure 20). Typically, these cankers can occur on the lower part of the trunk. When the outer bark is removed, as we see on picture below, the mottled areas of necrotic, dead and discoloured inner bark tissue with black zonal lines around the edge may be observed. When cankers girdle the trunk, death of the tree occurs resulting in wilting and a rapid change in foliage colour.

132 isolates of *Phytophthora* (from soil of examined boxwood forests) were obtained using baiting technique. The most of them were isolated from the soil samples from Imereti and Racha-Lechkhumi Regions. Carnation petals, azalea and rhododendron were used as baits for *Phytophthora* and mostly isolates were obtained from necrotic lesions of rhododendron leaves. Baiting is commonly used method for detection of *Phytophthora* spp. in soil. The technique involves floating pieces of susceptible tissue on a soil water slurry with a high water/soil ratio (Erwin and
Ribiero 1996; O’Brien Philip et al. 2009). According to the data of O’Brien Philip et al. (2009) the efficiency of detection can range from 0 to more than 90% and depends on the time of year at which the sample was taken. The host species from which the bait tissue is derived also influences on the efficiency of detection (Marks and Kassaby, 1974).

The localised spreading of *Phytophthora* spp. is by (asexual) spores (sporangia) produced on the surface of infected leaves and shoots (*Phytophthora ramorum* A practical guide for the nursery stock and garden centre industry, 2005.). The leaf hosts such as *Rhododendron* and possibly ash, holm oak, sweet chestnut and *Vaccinium* are the important sources of inoculum for initiating and maintaining epidemics of the tree mortality. *Rhododendron ponticum*, which growth everywhere, is a principal environmental leaf host contributing inoculum to trees (causing lethal bark infections) though some tree species with susceptible leaves may also have a role.

Among the percentage of the total number of isolates obtained from boxwood forests soils *Phytophthora plurivora* was one of the most detected species (Figure 21). Nowadays *Phytophthora plurivora* (in the past *P. citricola*) is a very aggressive soil-borne plant pathogen, with worldwide distribution and a wide variety of hosts. First, it was detected in 2014, using baiting and molecular techniques with *Phytophthora cinnamomi*, *Phytophthora gonapodyides* and many other *Pythium* spp. from soil samples of the native boxwood forests in Mtirala National Park and Kintrishi Protected Areas (Matsiakh, 2014).

![Figure 21: Morphological structures of Phytophthora plurivora: A – Mature ovoid sporangia of P. plurivora; B – Mature oogonia of P. plurivora with oospores; pure culture of P. plurivora on P. plurivora on V8 media.](image)

It was also described as the main reason for ericaceous and coniferous ornamental plants dying as well as the cause of stem base rot of forest trees (Orlikowski et al. 1995; Orlikowski and Szkuta, 2003a, b; Orlikowski et al. 2004, 2006; Orlikowski and Ptaszek, 2010; Orlikowski et al. 2011b). *Phytophthora plurivora* was analysed in water sources and it was suggested that is probably connected with the presence of this species on deciduous, coniferous and ericaceous plants as well as on trees growing along river banks (Orlikowski et al. 2012). As zoospores can be transported for a long distance in river water (Miligroom and Peever, 2003), they can infect others, new host plants
such as alder, poplar or willow growing along banks. Hong and Moorman (2005) confirmed that water is a primary if not the sole, source of inoculum for *Phytophthora* diseases of numerous nursery, fruit and vegetable crops.

*Phytophthora gonapodyides* and many other *Pythium* spp. were also isolated from soil samples of the native boxwood forests. *Phytophthora gonapodyides* belongs to *Phytophthora* clade 6, which includes aquatic species that are found in natural waterways also beyond agricultural areas, and do not seem to be harmful to residents until suitable conditions occur (usually floods), being probably opportunistic pathogens. While *Pythium* species are not considered forest pathogens, at least some of them cause damping off in nursery settings (Weiland *et al.* 2013). Many species are usually present in nurseries, but damping off usually occurs only when conditions are too wet. However, they are most likely spread by irrigation water.

Determining and assessing *Phytophthora* community structure in the boxwood forests is challenging. Plant diseases caused by *Phytophthora* species will remain an ever increasing threat to agriculture and natural ecosystems. There is always a chance that endemic *Phytophthora* species discover new potential hosts. This may result in new diseases and likely in the expansion of the pathogen population. Also, contributing factors as Climate Change plays a significant role in increasing aggressiveness of *Phytophthora* species in the Caucasus region. The means to control *Phytophthora* diseases are limited but more research should be done in this scientific direction with technologies to detect and identify these plant pathogens.

There are other wood decay fungi detected on the dead wood of boxwood trees are shown in Annex 2.

### 4.3. Box tree moth in the native boxwood forests of Georgia

In summer (July-August) 2015, the large damages caused by *Cydalima perspectalis* feeding on boxwood leaves in the native boxwood forests was found in three regions of west part of Georgia: Racha-Lechkhumi, Samegrelo Upper Svaneti (Zugdidi, Martvili, Tsalenjikha) and Guria Region (Figure 22).
Figure 22: Map of distribution the box tree moth in the native boxwood forests of Georgia

The plants were completely defoliated by box tree moth and lots of *C. perspectalis* imagos were discovered in boxwood forests in Djumari Forestry (Figure 23).
In autumn (October-November) 2015, the damage of box tree moth of *Buxus colchica* was revealed in Imereti Region in Zestaponi Forestry and in the natural boxwood populations in L.E.P.L. Forestry Agency of Adjara. During the summer research, the distribution of *Cydalima perspectalis* was observed in boxwood forests and green areas along the coast of Black Sea region. This finding confirms that box tree moth is being spread into the native boxwood forests towards the central part of the country (Figure 24).

Undoubtedly, the largest damages caused by *Cydalima perspectalis* feeding on boxwood trees on the territories of L.E.P.L. Forestry Agency of Adjara demonstrate successful adaptation of alien pest in the natural forests of the Caucasus region, causing the great concern to the extinction of native boxwood forests in this region (Figure 25). Heavy infestation by larvae of *C. perspectalis*
was observed on plants of *Buxus colchica* grown as a tree close to the Mirveti village and along the rivers. The plants had a dry appearance, covered by dense webs or were absolutely defoliated.

During autumn visit, no single tree was found with a green leaves (Figure 26) and many trees had debarked trunks. Unfortunately, there is no chance for them to be recovered. Also, trees with intact bark can resprout but it is likely they won’t be able to survive next spring under new attracts of overwintering larva.

The damage caused by *Cydalima perspectalis* on native box trees in the Caucasus region was found to be critically serious. The morphological description of the different life stages and
damage of box tree moth is well studied by Korycinska and Eyre (2011). Followed this study, the biological and morphological features of box tree moth were examined and described.

The greenish yellow eggs with black heads were found on the underside of box leaves, overlapping each other (Figure 27). Newly hatched larvae were greenish yellow coloured with a pattern of thick black and thin white stripes along the length of the body and could have reached a length up to 40 mm. It is known that the box tree moth has six larval stages and the last stage of larva can retain a yellowish green ground colour but sometimes can be more brownish. First instar larvae feed by ‘windowing’, eating the lower surface of leaves only and leaving the upper epidermis intact. Study conducted by Leuthardt (2013) shows that young larvae contain twice as much alkaloids as larvae in later instars. The concentration of alkaloids doubles between one-year-old leaves and older leaves in box tree leaves that may explain why damage on a box-tree most often starts in the lower part of tree, where the oldest leaves are found. The older larvae were fed inside silk webbing and skeletonised the leaves of host plants, leaving only the midribs, and occasionally the outer margin, intact. Webbing, frass and moulted black head capsules were also observed.

Two-colour form of imagoes were detected in native boxwood forests of Caucasus region. First, one the most common has a thick dark brown border of uneven width around the edges of white-coloured wings with a wingspan of around 4 cm. Another one, less common, has the wings completely brown except for a small white streak on the forewing. The pupa was found in cocoons of white webbing spun among the foliage on the boxwood trees. Winter stage of box tree moth as larva protected in a cocoon spun between *Buxus* leaves, was observed in November 2015 (Figure 27).
Figure 27: The developmental stages of Cydalima perspectalis recorded during field trips in Caucasus region: A – Mature C. perspectalis larva feeding on the boxwood leave; B - The pupa of C. perspectalis in a cocoon of white webbing among the leaves and twigs of box trees; C - Imago of box tree moth with the typical brown and semi-transparent white wing pattern; D - Imago of box tree moth with almost totally brown wings; E - Overwintering larva spinning a cocoon between box leaves in autumn; F - Overwintering opened larva in a cocoon on the surface of box leaves in autumn.

During this short period of research, it was not able to define the exact number of generations of box tree moth. The flight period was observed in summertime (late July) as well as in late October with active 1st instars of the next “wintering” generation. It is known that, two generations per year occur in Central Europe (Nacambo et al. 2013) and 3–5 generations per year in China (Chen et al. 2005; She and Feng 2006). The initial observations in Sochi region show that the pest produces 2-4 generations per year (Gninenko et al. 2014). They found 2nd and 3rd instars under natural conditions of Sochi in late October 2013 and larvae actively crawled but only a fraction of them fed in mid-November. Also, Gninenko et al. (2014) summarized that the latest generation of C. perspectalis may develop with timing of certain phases being partially overlapped. The late pupae and third-generation females occurred in nature, as well as 1st and 3rd instars of the next “wintering” generation were observed in late October. In the local Lepidoptera fauna of the Northwest Caucasus, species developing in winter evergreens are known to occur (Gelechia senticetella (Staudinger, 1859); Gelechiidae).

It is very important to establish the development cycle of this species and the total number of complete generations per season in the Caucasus. Due to its multivotine to develop several
generations in its native conditions per year (Maruyama and Shinkaji 1993; Zhou et al. 2005), in subtropical climate of Caucasus that contributes to the development and nutrition of larvae increases its spread capacity. Moreover, large size of adults, the ability to migrate (5 km/year) and huge feed base (the presence of a sufficient number of boxwood trees in the natural populations in the forests as well as in landscaping of cities, villages, churches, cemeteries) the bow tree moth provides additional threat to native boxwood forests in Caucasus region.

It is known that one of the features of an altered state of biocenosis is a violation of forest layering. During researches, large amount of excrement was found on the surface of soil that might be a mineral nutrition for the grass and in some places the direct sun's rays could have reached the lower tiers of shade tolerance plants. Presumably, this is beneficial to the growth of the grass cover, as air circulation do not violate the dense mats of decaying leaves. In this case, the displacement of weakened boxwood by plants and shrubs from the lower vegetative layers will be observed. Consequently, an absolutely new succession and biocenosis will change the exiting boxwood forests.

In case of destroying the primary source of food base, the pest will begin to parasitize on other plants and tree species. The main host plants of *C. perspectalis* are *Buxus* species but in Imereti Region in Zestaponi Forestry and in Adjara Region where *C. perspectalis* destroyed all boxwood plants, larva of box tree moth was found on *Rubus* spp., *Ruscus colchicus*, *Ruscus fruticosus* and *Smilax excelsa*. Invader destroyed the integrity the leaf blade, plants were weakened and in future even might be dead. Thus, this invasive species has an impact not only on individual tree species but also on the whole phytocoenosis.

There is an alarming situation with the penetration of new pest-invader (*C. perspectalis*) into the boxwood forests in Georgia. In this case interaction of natural conditions (in particular, climate that contribute to the development of this multivotine pest), the availability of sufficient fodder species (composition and structure of boxwood forests are favourable to the nourishment of box moth) is important.

The regulation of mass reproduction of insects in ecosystems is implemented at different levels (Viktorov, 1967):

I level – polyphagous entomophagous-predators;
II level - specialized entomophagous;
III level - pathogens (fungi, bacteria, viruses);
IV level - intraspecies competition for feed resources.

Due to the biological features of box tree moth, these regulatory mechanisms currently does not give the desired effect in case of mass distribution of *Cydalima perspectalis* in boxwood forests in the Caucasus region (Figure 28).
**Polyphagous entomophagous-predators.** They can regulate the amount of herbivores insects between outbreaks and on the early growth stages of populations in ecosystems. This group includes the predatory insects of the families *Formycidae*, *Vespidae*, *Carabidae* est., which feed different species of herbivores from the Lepidoptera. With increasing amount of some of the herbivores, predators entomophagous more actively try to obtain the food that is accessible. However, the further sharp increase of the amount of herbivores does not allow the predators to destroy such excessive growth of herbivores population.

Currently, this level of regulation does not provide a protective effect in the boxwood forests populated by box tree moth. It requires additional research, because it is still unknown if predatory insects in the Caucasus can feed larvae and adults of *Cydalima perspectalis*. The situation is complicated by the fact that the box moth larvae accumulate the toxic alkaloids of boxwood leaves in their body of (Leuthardt, 2013). Therefore, any insectivorous birds and other vertebrates do not eat box moth caterpillars.

**Specialized entomophagous.** This group includes entomophagous parasites (mono- or oligophagies) which are adapted to nourishment of certain species of herbivorous insects. In the natural populations, the high population density of herbivores allows parasites quickly find a hosts and populate more of them. But, with further growth of the amount of herbivores the regulatory role of parasites is decreased.

However, the parasites also can not completely eliminate the herbivorous population. In this case, they would destroy their food source. At present, this level of herbivorous regulation does not
provide the required effect in the Caucasus. There are no specialized parasites of *Cydalima perspectalis* in the region. One of the directions may be searching specialized entomophagous in natural habitats and their introduction into the territory of the Caucasus. This may be the one of the solution aimed to reduce the negative impact of herbivores-invader. This type of work is time-consuming and requires huge investments and a thorough analysis of all possible risks from the introduction of new parasites and their influence of other native local insects.

**Pathogens (bacteria, viruses, fungi).** Entomopathogenic organisms can quickly distribute into the herbivorous populations and with a large numbers of host larvae, the probability of contact the patients and healthy individuals are doubled. It is known that the biological insecticide produced from soil bacterium *Bacillus thuringiensis* var. *kurstaki* is used to successfully control the caterpillars *C. perspectalis* (Cawoy *et al.* 2011; Lacey *et al.* 2015).

Entomopathogenic fungi are likely to affect different types of herbivorous larvae. A new opportunity to control box tree moth with the baculovirus *Anagraphe falcifera* nucleopolyhedrovirus (AnfaNPV) was recently investigated (Rose *et al.* 2013). The performed laboratory experiments indicated the susceptibility of *C. perspectalis* to AnfaNPV. There are no studies connected with searching fungi or bacteria that can cause disease of box three moth in Georgia. In our opinion, the search for effective special biological agents will be potentially promising pathways towards their development as microbial control agents against *C. perspectalis*.

**Intraspecies competition for feed resources.** With the lack of effectiveness of mechanisms for regulation the amount of herbivores outbreaks, their massive development can lead to strong or even complete destruction of their fodder. The intraspecies competition for food as the most radical level of influence is included in this case. With a shortage of food, some part of larvae die while others are moving towards the nourishment of other inappropriate or unsuitable for them host plants. It provides the deterioration of larvae survival and sharply reduce the fecundity of adults and finally it leads to very rapid attenuation of herbivores outbreak.

This can be expected in the Regions of mass distribution of *C. perspectalis* in Caucasus. As it was mentioned before, in Imereti Region (in Zestaponi Forestry) and in Adjara Region where *C. perspectalis* destroyed all boxwood plants, no single tree was found with a green leaves and many trees had debarked trunks. Also, the displacement of weakened boxwood by plants and shrubs from the lower vegetative layers was observed. An absolutely new succession and biocenosis will change the exiting boxwood forests. Also, larvae of box tree moth were found on new host plants *Rubus* spp., *Ruscus colchicus*, *Ruscus fruticosus* and *Smilax excelsa*.

That massive development of box tree moth and depletion of fodder leads them to actively search for new forage plants. The adaptation to new nourishment can ensure survival of *C. perspectalis* in Caucasus conditions. Consequently successful acclimatization of this species is also enhanced: adaptation to new forage plants with the absence of natural mechanisms of regulation of its amount is significant threat to the natural vegetation of the region.

Regulatory mechanisms in the natural populations are formed for a long time and it is the result of joint development of plants, insects and other components of ecosystems. Penetration of new species into the new territory in the presence of suitable conditions for its development and the
absence of natural enemies leads to its excessive development and was called "explosion" (Elton, 1958).

The natural mechanisms for regulating C. perspectalis in the native boxwood populations (polyphagous entomophagous-predators, specialized entomophagous, pathogens and intraspecies competition for feed resources) are absent or not effective enough. The stabilization may occur only when acclimatization of the specialized entomophagous, naturally or as introductive species, will reproduce the biocenotical regulation system regarding the amount of this species.

In summer 2015 (end of July) we visited Botanical Garden in Zugdidi. The massive damages by box tree moth Cydalima perspectalis were detected in green areas around Dadiani Castle as well as on the territory of Botanical Garden (Figures 29, 30).

![Figure 29: Damages by box tree moth Cydalima perspectalis of Buxus colchica Alley near Dadiani Castle](image)

![Figure 30: Damages by box tree moth Cydalima perspectalis in Botanical Garden of Zugdidi](image)

The letter concerning situation with distribution of box tree moth, describing the important biological and ecological features of pest, was send 10.08.2015 to Administration of Botanical Garden and National Forestry Agency of Georgia. It was advised to organize the work connected with preventing further spread of this dangerous pest into natural populations of Buxus colchica.
and carrying out some activities was purposed. After getting this letter, Administration of Botanical Garden and Municipality of Zugdidi managed to conduct chemical treatment of all green areas in Botanical Garden several times. During second visit in autumn 2015, the new branches regrow was found on the green alley near Dadiani Castle. It means that chemical treatment should be repeated in spring to prevent feeding of these new green leaves by overwintered larvae (Figure 31).

![Figure 31: New green branches of Buxus colchica on the green alley near Dadiani Castle](image)

### 4.4. Other insects in the native boxwood forests of Georgia

The life cycle of boxwood leafminer *Monarthropalpus buxi* was observed in Imereti and Racha-Lechkhumi Lower Svaneti Regions. Female flies puncture foliage during egg laying creating a small yellow hole on the underside of leaves. Larvae mine leaves by feeding on the mesophyll layer, forming a blister on the surface of foliage. The blisters are often yellow in the centre and have a translucent emergence hole created by mature larvae. There can be multiple larvae in each blister (Johnson and Lyon, 2003). Larvae are yellow to orange legless maggots and are approximately 1.5 mm long (Figure 32). Boxwood leafminer attacks result in irregularly shaped swellings on the leaf. Infested leaves typically turn yellow or brown in splotches, are smaller and drop sooner than healthy leaves.
Also, other boxwood leaves insects were detected in the native boxwood forests. Boxwood spider mite (*Eurytetranychus buxi*) overwinters as eggs on the underside of leaves. The eggs hatch in the spring. From a short distance, the infested boxwood appears unhealthy with a dingy silvery color. Boxwood spider mite creates small, silver-coloured stripes and dots on the leaves, usually old leaves (Figure 33). Adult of boxwood sucker (*Psylla buxi*) have a length of 3 to 5 mm, their larvae are covered with a white waxy coat and they prick and suck out dry young shoots. They create spoon-shaped curly shoots.
Figure 33: Other boxwood insects: A - Boxwood spider mite on the boxwood leaves in summer; B - Boxwood spider mite on the boxwood leaves in autumn; C - Adult of boxwood sucker on the boxwood leave.
5. Recommendations and Proposals for the conservation management of native boxwood forests in Georgia with improved forest protections approaches

5.1. The future of native boxwood populations - strategic plan of forest protection

According to the results of the field works in six regions of Georgia and the laboratory studies, the intensive defoliation of boxwood plants in different ages is caused mainly by invasive species *Calonectria pseudonaviculata* and *Cydalima perspectalis* combining with the soil phytopathogens of *Phytophthora* and *Pythium* geniuses strong development, which cause root rot.

The decline process of boxwood trees is compounded by the combined influence of several species of pathogenic organisms and the climatic conditions of boxwood growth. The growth conditions of *Buxus colchica* are confined to the valleys and river banks where it forms small groves or growing under the canopy of the other species stand (beech, alder). There are different opinions on the primary origin of the boxwood in Georgia. Some researchers consider that it is the tertiary relict, which is preserved in refugiums (Troitsky, 1928; Matikashvili, 1953). Majority of scientists has an opinion that the box tree was planted at the surrounding of churches and it has been naturalized into the natural habitat (Akhalkatsi, 2015). The plant communities of *Buxus colchica* create a favourable wet microclimate (Machutadze et al. 2013). Wet conditions, the water level raising in the spring after the snow melts and in the summer-autumn period during heavy rains, promote the distribution of *Phytophthora* and *Pythium* pathogens. Invasive fungal spores of *Calonectria pseudonaviculata*, which is parasitic on the leaves of the boxwood, spread by the airflow. The wide distribution of this pathogen within Georgia can be contribute by anthropogenic factor because of boxwood twigs are still used in religious ceremonies (Palm Sunday). Due to the fact that people may use the infected boxwood twigs for the ceremonies, spores of *Calonectria pseudonaviculata* may be spread on the long distance. Later these infected branches are often cultivated near the church, and this is one way how this disease can be spread. Nowadays, fungus *Calonectria pseudonaviculata* is spread in natural tree stands at the attitude more than 1000m above sea level.

In summer (July-August) 2015 the large damages caused by *Cydalima perspectalis* feeding on boxwood leaves in the native boxwood forests was found in three regions of west part of Georgia: Racha-Lechkhumi, Samegrelo Upper Svaneti (Zugdidi, Martvili, Tsalenjikha) and Guria Region. In autumn (October-November) 2015 the damage of box tree moth of *Buxus colchica* was revealed in Imereti Region in Zestaponi Forestry and in the natural boxwood populations in L.E.P.L. Forestry Agency of Adjara. During the summer period of field studies, the distribution of *Cydalima perspectalis* was observed in boxwood forests and green areas along the coast of Black Sea region. This finding confirms that box tree moth is being spread into the native boxwood forests towards the central part of the country. Undoubtedly, the largest damages caused by *Cydalima perspectalis* feeding on boxwood trees on the territories of L.E.P.L. Forestry Agency of Adjara demonstrate the
The successful adaptation of alien pest in the natural forests of the Caucasus region, causing the great concern to the extinction of native boxwood forests in this region.

That is why it’s a very difficult task to implement the treatment measures and conducting control of pathogens development in the natural boxwood tree stands. It is prohibited to use fungicides or insecticides on the territories near the water and actually on the protected areas. The boxwood creates the secondary layer in the forest. It participates in the formation of hornbeam, and other broad-leaved forests as well as it may have a negative impact on the environment (particularly on mycorrhizae, soil invertebrates and aquatic organisms) and water.

The recommendations and proposals for the conservation management of native boxwood forests in Georgia are developed taking into account strategic plan for forest protection in the United States. They may play keystone role in improving Georgian forest protection approaches (Keefer 2010; http://www.forestryimages.org). This work strategy will expand the awareness beyond standard protective measures and boxwood forests will be used as illustrative tools (demonstration object).

To move toward the vision of improving boxwood forest health, these recommendations will focus on the following four strategic areas:

1. **Prevention** — Stop invasive species before they arrive.
2. **Early detection and rapid response** — Find new infestations and eliminate them before they become established.
3. **Control and management** — Contain and reduce existing infestations.
4. **Rehabilitation and restoration** — Restore native habitats and ecosystems.

**Four themes for strategies of forest protection include:**

1. Partnerships and collaboration.
2. Scientific basis, which includes conducting appropriate research activities to develop scientific information and technology to ensure that assessment and management programs are effective and science based. A scientific basis also includes setting priorities based on risk assessments.
3. Communication and education.
4. Organizing towards success—incorporating the themes of improving capacity, procedural streamlining, and funding flexibility with long-term commitment.

Interwoven with these program elements is the need to employ a science-based approach, work collaboratively and expand our partnerships, apply a prioritized system for taking action, and improve our efficacy and accountability. Moreover, there four themes that are common for all strategies but some activities embodied will receive priority depending on the situations in the forests.

1. **Prevention.** The most effective strategy against invasive species is to prevent them from ever being introduced and established. Preventive measures typically offer the most cost-effective means to minimize or eliminate environmental and economic impacts. Prevention relies on a diverse set of tools and methods, including education.

   - The integrated and effective preventive strategies should be developed to reduce risk of invasive species invasion in boxwood forests in Georgia. It is recommended to use the risk map, site-
specific information, and other factors to help determine where treatments should take place. According to the GPS coordinates, the areas of spread the box tree moth and fungus *Calonectria pseudonaviculata* were assigned to maps that will provide an appropriate solution for the storage, display, and sharing of occurrence and distribution data on known and new areas where introduced invasive species were found. For future, it is recommended to update these maps after monitoring and visiting new places to complete boxwood forests maps with determining treatment and health areas. Also, the risk map has to be differentiated considering the location and accessibility of boxwood forests; the category of boxwood forests and their significance (protected areas, national parks, nature reserves, forests along rivers, recreational areas, places of grazing, municipal territories or private gardens) and the possibility of applying the various protective measures against pests.

The emphasis will be on identifying and protecting forests that have not been invaded by invasive species. For example, the boxwood forests in Kakheti Region have to be kept under control and prevention strategy should be focused on conducting researches in these forests. In autumn (October-November) 2015, the damage of box tree moth of *Buxus colchica* was revealed in Imereti Region in Zestaponi Forestry and it is recommended to apply the protective measures against *C. perspectalis* to stop its penetration into the deeper forests areas.

Without **continuous monitoring** of rural and urban forests as well as studying of disruptive insects, pathogens, and invasive plants that provides information to effectively respond to situations, the health of the forests and trees is threatened, it is not possible to prevent and protect forests. It is recommended to help targeted survey and monitoring efforts to areas where the most serious threats to forest health exist and where corrective action is feasible.

At this time, the monitoring system of boxwood forests in Georgia does not allow to detect timely the emergence of box tree moth or fungi pathogens during their penetration into the new forest areas. The same problem exists in the case of diseases distribution and with the detection of mass development of insect herbivores. It is needed to organize the monitoring network in all Forest Regions in Georgia that will provide observation and control for the health situation in their forests including boxwood forests. Monitoring of boxwood forests may be based on the assessment of tree health conditions emphasizing the value of the combined assessment of defoliation and damaging agents provided by ICP Forests monitoring (Technical report of ICP Forests, 2015).

The monitoring system will not be successfully developed without cooperation with scientists of Research Forest Universities or Institutes, and international partner institutions to understand the biology and develop tools for detecting and controlling high-priority pests in their countries of origin and especially without carrying out laboratory and field trip researches. The national guide should be developed for best management practices for boxwood pests. For example, field guides or brochures should be prepared with identification pests’ information in Georgian.

**Preventive activities of distribution boxwood invasive pests into new areas also should include these measures:**

- explanatory work among the local population;
- monitoring of the health condition of boxwood seedlings which are planted in nurseries as ornamental plants;
- control the variety boxwood places (shops, garden centres, nurseries, botanical gardens and private entrepreneurs);
• prohibition of the boxwood branches sale and use in religious rituals (it needed to ask the Church representatives for collaboration).

2. Early detection and rapid response (EDRR). “Every person working or recreating in a national park or forests has the potential to serve as an early detector” (Williams et al. 2007). Early detection and rapid response efforts minimize ecological damage by preventing habitat fragmentation and ecosystem degradation associated with large or widespread invasive species populations and related management activities (Smith et al. 1999, Timmins and Braithwaite, 2001).

It is considered that the early detection and rapid response (EDRR) is a critical component of any effective invasive species management program and may be the “second line of defense” after prevention. This strategy results in lower cost and less resource damage than implementing a long-term control program after the species is established.

Before beginning any effort to develop an early-detection program it is important to evaluated that if there is little hope of supporting the efforts needed to synthesize the information into an implementation plan or to implement the plan itself (Keefer, 2010). Time and money are obvious limitations, but so too are institutional support and the personal commitment of staff. There are many examples of resources expended to develop elaborate sets of management recommendations that have little chance of being implemented because they require funding that is unlikely to be available. Early detection of new infestations requires vigilance and regular monitoring of the managed area and surrounding ecosystems. The early detection involves planned activities with using monitoring reports to locate newly introduced alien species in a given area before their populations become established.

Planed activities for early detection of boxwood pests may include:

- to develop and maintain a list of target boxwood species that occur in forests areas, are extremely rare, or are not currently present within the forests, but have the potential to cause major ecological, cultural, or economic problems if they were to become established. After conducting laboratory and field trip researches this type of list with boxwood fungi and insects was made where two invasive species Cydalima perspectalis and boxwood blight Calonectria pseudonaviculata have a high level of treatment of native boxwood populations;
- to focus on educating field staff and interested co-operators, resource managers, and volunteers on invasive species identification because knowledgeable crew members provide an additional “set of eyes and ears” to detect incipient species. It is important to collect primary monitoring data even from local people that will help to react rapidly before the pests become established. That is why field guides or brochures with identification pests’ information have to be shared around the country with the support of the activities in the media (TV, press). Also, the guidelines and tools for forest guards to monitor the health conditions of boxwood forests have to be developed. All new detections of box tree moth or symptoms of fungus should be immediately reported through the appropriate chain of command to the main office (for exp. Forest Service) with photographs (originals or copies) that will help to complete the risk map. Due to the boxwood as tree species is under threat of extinction it has to be built functioning EDRR system.
- to continue to conduct the studies needed to provide scientifically based information on basic biology of priority invasive species. The biology, distribution and symptoms caused by boxwood blight as well as the morphological description of the different life stages and damage of box tree
moth are well described in Chapters 2 and 4. In any way, it still request increasing knowledge about distributions these two invaders in climate conditions of Caucasus. Observations in boxwood stands should be done during the year (especially winter and early spring):

- Biology and genetics: e.g. phylogeny, conditions for development and infection. When early symptoms caused by boxwood blight appear on the leaves and how fast the infection can spread under forest canopy on the boxwood trees. Disease development is also likely to occur all winter but it is not well studied in Caucasus Region. Diagnostic tools: e.g. epidemiological model to predict development of the fungus, symptomatology, molecular detection.

- Box tree moth should be monitored by using sex pheromone traps for better understanding its phenology in different climatic conditions. Also, it is important to detect colonization and dispersal methods, life cycle, number of generations, hosts and varietal preferences in Caucasus Region. Larva observation should be focused on the overwintering larva development and starting their feed to provide the first treatment measures and for future to knowing the appropriate time for using biological control insecticides. Egg-stage and adults-stage monitoring should be done for looking native and non-native egg parasitoids as well as entomopathogenic bacteria and nematodes, entomopathogenic viruses and fungi.

- prioritizing sites (also with help of risk map) should focus on areas where boxwood can be saved and the potential for invasive species becoming established is relatively low. At the same time, this combined approach allows to focus on areas within the management unit where invasions are most likely to occur or where resources of greatest value for protection are located. According to the summarized information connected with detection of two boxwood invaders, Kakheti and Racha-Lechkhumi Lower Svaneti Regions can be chosen as the most priority areas for winter and spring monitoring to assess the presence the box tree moth and spread of boxwood blight (Table 3).

Among the other Regions, Region Imereti (especially Zestaponi) should be visited during this winter and early spring for conducting treatment measures to prevent spreading of invaders to other uninfected by fungus and not damaged by moth boxwood tree stands. It is recommended to value the level defoliation in the boxwood areas (using five defoliation classes and UNECE and EU standardized criteria (Table 4) (Eichhorn et al. 2010).

Table 3. Summarized information about research localities in Georgian boxwood forests and detection of Cydalima perspectalis and Calonectria pseudonaviculata in them

<table>
<thead>
<tr>
<th>Forest Service</th>
<th>Researches areas and numbers of examined places</th>
<th>Observation perspectalis</th>
<th>Observation pseudonaviculata</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>summer 21.07. - 03.08.2015</td>
<td>summer 21.07. - 03.08.2015</td>
</tr>
<tr>
<td></td>
<td></td>
<td>autumn 27.10. - 03.11.2015</td>
<td>autumn 27.10. - 03.11.2015</td>
</tr>
<tr>
<td>Region Kakheti</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Kvareli</td>
<td>Telavi, Akhmeta (totally 6 places)</td>
<td>-</td>
<td>not visited</td>
</tr>
<tr>
<td>Region Imereti</td>
<td></td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Kutaisi</td>
<td>Zestafoni, Tkibuli (totally 15 places)</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Region Racha-Leckhumi Lower Svaneti</td>
<td>Ambrolauri, Taqari (totally 12 places)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Region Samegrelo Upper Svaneti</td>
<td></td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 4. Defoliation classes according to UNECE and EU classification

<table>
<thead>
<tr>
<th>Defoliation class</th>
<th>Degree of defoliation</th>
<th>Percentage of leaf loss</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>not defoliated</td>
<td>0–10%</td>
</tr>
<tr>
<td>1</td>
<td>slightly defoliated</td>
<td>&gt;10–25%</td>
</tr>
<tr>
<td>2</td>
<td>moderately defoliated</td>
<td>&gt;25–60%</td>
</tr>
<tr>
<td>3</td>
<td>severely defoliated</td>
<td>&gt;60–90%</td>
</tr>
<tr>
<td>4</td>
<td>dead tree</td>
<td>100%</td>
</tr>
</tbody>
</table>

A defoliation of 10 to 25% is considered a warning stage, and a defoliation of more than 25% is taken as a threshold for damage. Boxwood tree stands in Imereti Region can be established as slightly defoliated or damaged and requires the rapid response in early spring. The boxwood forests in Racha-Lechkhumi Lower Svaneti and Samegrelo Upper Svaneti Regions can be assessed as moderately defoliated or severely defoliated (detailed information about health conditions of each visited boxwood locations is presented in Annex 1), there is also demand to apply protective measures. As it was mentioned, the boxwood forests in Kakheti Region have to be under eye and prevention strategy should be focused on conducting researches in these forests. The health conditions of these areas were the best ever among visited boxwood forests. Nevertheless, after laboratory analyses, boxwood blight pathogen was isolated from leaves material without typical infective symptoms. That is why early detection work should be implemented in this Region. The boxwood forests located in Guria and Adjaria Regions were established as the totally defoliated (dead) stands. Unfortunately, is it not easy to save these boxwood forests but prioritizing boxwood sites around them have to be considered as high-risk areas.

Prioritizing sites has also include the priority of boxwood stands connected with the forest structure. Mostly of visited boxwood areas were grown under broad-leaves trees canopy (alder, oak, sweet chestnut, beech and picea), only some places in Tkibuli Forestry where boxwood planted on the open spaces. This information will be used for established points of response biological control procedures in place.

- To work with partners to develop a Web-based database system to identify and communicate information on invasive species from any source. It is advisable to efficiently organize forest health information on Web sites of National Forestry Agency of Georgia that are easy to navigate and contain information pertinent to forest health decision-making and will be available for any interested sides.
- **Rapid Response** to invasions is effective and can prevent the spread and permanent establishment of invasive species. Early detection and rapid response go hand in hand. The effort invested in early detection has limited value if management action is not taken to eliminate targeted populations before they spread. Response efficiency is successfully working in case of having
designated response personnel (the National Forestry Agency of Georgia is limited to a single person that it is responsible for forest protection) or response teams depending on the resources available and the potential need. It is proposed to organize a network of forest protection departments in the nine Regions of Georgia with further training of forest guards how to protect forests including boxwood. The stricter requirements and responsibility for the late detection of foci of pests and diseases can be also established.

Undoubtedly, the partnerships and networking is one of the main goals of guidance and technical rapid response assistance. To save boxwood forests in Georgia, it is proposed to develop an “early alert” system to enable all entomologists, pathologists, botanists, weed scientists, invasive species specialists, and forest health specialists in Georgia, and local governments; profit and nonprofit organizations; and educational institutions to communicate and learn about new infestations. Also, coordination with other Caucasian countries, on whose territories the populations of Colchis boxwood of natural origin are located is strongly recommended (Turkey, Armenia, Azerbaijan and other).

The detection protocols of the existing forest protection systems in Georgia using for rapid response capabilities have to be improved considering the distribution new invasive species and developed new ones where these systems do not exist. For saving boxwood forests, the emergency authority and funding mechanisms to increase capability for rapid research and management response to boxwood blight and box tree moth have to be developed and implemented. Of course, no response plan can be implemented without flexible funding because detections of plant pests do not always occur at predictable times.

There is concern that an appropriate level of forest health expertise in Georgia may not be available to resource managers in the near future. Unfortunately, no modern phytopathological laboratory with mycological, entomological and molecular equipment is available in National Forestry Agency and even at the Ministry of Environment and the Natural Resources protection of Georgia. In addition to this, substantial investment and training of highly skilled specialists is required. Nowadays, it is not easy to find flexible money for early detection or rapid response but it is possible to conclude the contract with the existing scientific Institutions and Universities to conduct the necessary field studies and laboratory analyses with encouraging Georgian scientists to support EDRR and ensure the timely progress from detection to response. Also, the building strong linkages between Forest Protection Service in Georgia and science will not be effective without continuing education and experience-based learning for FHP specialists.

3. Control and management. Control and management activities are based on integrated pest management principles that may include any combination of physical/mechanical, biological, cultural, and chemical techniques.

The aim of this strategy is to synthesize available information, identify information gaps and develop new or improved control, management, and monitoring technology including biological controls for priority species (boxwood blight and box tree moth) based on the latest research and adaptive management feedback and transfer this technology to users.

Rapid spread of invasive species in the Caucasus Region requires conducting the easy reacting control activities. At the same time, in different categories of boxwood stands it is
recommended to use different strategies and tactics. Methods of active control must be differentiated considering the features of growing native boxwood forests in Georgia:

- Boxwood plantations located on the natural protection areas (protected areas, nature reserves, national parks, wildlife sanctuaries);
- Tree stands near the places of worship (monasteries, churches);
- Tree green areas of cities and towns, botanical gardens;
- Stands near the rivers and water sources;
- Boxwood plantations near the places of grazing;
- Boxwood plantations on the municipal territories or in private gardens.

Also, location and the accessibility to reach the boxwood forests with the possibility of application control measures there can display a key role in maintaining of boxwood plantings. Some control activities were developed in 2014 after assessment the health conditions of Buxus colchica on the Protected Areas in Georgia and some of them were designed for Zugdidi Botanical Garden in summer 2015 (Matsiakh 2014, 2015).

The biological controls for priority species (boxwood blight and box tree moth) as well as for other detected boxwood pathogens are focused on the fast-track implementation of cost-effective management solutions.

Boxwood blight control measures.

*There is no control for this disease once it is present.* The only control is preventing its introduction and preventive fungicide applications to protect non-infected plants. To limit spread and movement of the pathogen, all confirmed infected plants should be destroyed in the gardens or nurseries but not in the forests.

At present, there is no accessible information how to control disease in the forest and it should be understandable as extremely difficult task because for boxwood blight situation is the most complicated and worrying. There two scenarios with control actions recommended for boxwood stands.

**Scenario 1 (for boxwood stands near monasteries, churches, in green areas of cities and towns, botanical gardens, on the municipal territories or in private gardens).**

- Minimize leaf wetness and promote good air-circulation in boxwood plantings: avoid overhead irrigation, provide adequate spacing between plants.
- Mulch boxwood plantings to reduce the spread of boxwood blight inoculum to foliage by splashing water.
- It is advisable not to work with the plants when they are wet because this creates conditions suitable for the pathogen to spread.
- Cut out infected twigs and remove and destroy any fallen leaves, debris and topsoil to reduce the amount of inoculum.
- Avoid moving infested soil or plant material to other landscape locations where boxwood are located.
- Use of disinfecting pruners and tools frequently within and between different blocks of plants, especially between different field locations or landscapes: to dip tools for TEN SECONDS
into these products and then to dry them: ethyl or isopropyl alcohol at 70-100% (most Lysol formulations, grain/rubbing alcohol), sodium hypochlorite (10% Clorox or other brands - 1 part bleach to 9 parts clean water, made fresh each day), phenolics at 0.4-5% (trade name Pheno-Cen), or quaternary ammonium at 0.5–1.5% (trade names Greenshield, Consan Triple Action 20, Physan 20) (Ivors__). These products are corrosive, so oil tools after treatment.

- Do not compost boxwood debris or plant material. Remove leaf litter from soil surface by raking, or sweeping. If it is possible, a propane push flamer (Red Dragon, La Crosse, KS) can be used to scorch leaf material colonized with *C. pseudonaviculatum* left behind on the soil surface. The flaming soil will help to to destroy or remove infected leaf debris with microsclerotia as soon as they are blown by wind, buried by erosion or begin to decompose (Dart *et al.* 2012). But this control practice has to be studied before implementation.

Control via fungicides has to be conducted to assess the effectiveness of fungicides as a preventive and curative treatment. Repeat fungicide applications (7- to 14-day intervals, according to product label) to susceptible boxwood throughout the growing season for the life of the boxwood plants. Fungicides for controlling boxwood blight:

- Active ingredients: Boscalid + pyraclostrobin, FRAC code 7 + 11, Formulation Signum* (BASF), Action(s): Systemic, protectant and curative fungicide - compatible with some biological controls.
- Active ingredient: Chlorothalonil, FRAC code M5, Formulations Bravo 500* (Syngenta, Action(s): Protectant fungicide for selected outdoor crops - compatible with some biological controls.
- Active ingredients: Cyprodinil + fludioxonil, FRAC codes 9+12, Formulation Switch (Syngenta), Action(s): Systemic, translaminar fungicide with long residual activity - incompatible with biological controls.
- Active ingredient: Azoxystrobin, FRAC code 11, Formulations Various including Amistar* (Syngenta), Action(s): Systemic, translaminar, protectant broad-spectrum fungicide - compatible with biological controls.
- Active ingredients: Cyprodinil + fludioxonil, FRAC codes 9+12, Formulation Switch (Syngenta), Action(s): Systemic, translaminar fungicide with long residual activity - incompatible with biological controls.

There are many other commercial fungicides produced by different companies but Signum (boscalid + pyraclostrobin) and Octave (prochloraz) show variable control of natural infections in the nurseries (Polizzi and Azzaro, 1996; Crous, 2002; Polizzi and Vitale, 2002). In these field trials, Signum can be the best fungicide when used as a protectant and a curative in the single-sprays treatment. A satisfactory activity against *Calonectria* spp. was disclosed by fludioxonil (Haralson *et al.* 2007), fosetyl-Al, prochloraz + cyproconazole, trifloxystrobin, azoxystrobin and K phosphite, whose efficacy, however, varied with the type of infection (Aiello *et al.* 2012, 2013). The phytotoxicity risks must be monitored in the case of repeated treatments with high concentrations. The fungicides used for the boxwood blight treatment have to be registered and approved for apply on the territory of Georgia.
• It is needed to monitor boxwood weekly during the growing season for symptoms of boxwood blight. Remove any symptomatic plants/debris/soil as outlined above.

• If temperatures are over 15-16°C and a rain is expected in post-growing season, a preventative fungicide spray should be in place post-season as well.

Scenario 2 (for native boxwood forests, protected areas, nature reserves, national parks, wildlife sanctuaries and plantations near the water).

Currently no effective control and management solution can be found (neither fungicide nor manure-based treatments) to protect the native boxwood forests against boxwood blight. The great concern to the extinction of native boxwood forests because of the spreading disease more than 1000m above sea level is confirmed and the invader actually reached the east part of Georgia (Kakheti Region). Also, there are many restrictions in Georgia legislation concerning applying chemical treatments in the forests especially on the protected areas. Nevertheless, control and management should be conducted on the prioritizing sites (see 2. Early detection and rapid response) where there is chance to maintain native boxwood forests.

• It is needed to identify boxwood areas where is possible and reasonable to provide preventive and curative treatment and how to get there. It was noticed that disease is mainly spread on the boxwoods near the water and on the smaller plants.

• Early diagnosis and monitor of *Cylindrocladium buxicola* (boxwood blight) is crucial if the infestation is to be contained, particularly when climate conditions are favourable to the pathogen’s development (periods of rain or moisture and temperatures above 12–15°C). Unfortunately, symptoms often do not tend to be noticed until the final development stage when the disease is already widespread and leaves have started to drop.

• It is recommended to remove any ill boxwood (branches with symptoms) to help prevent spread of disease to healthy plants. Of course, in the forests it might be difficult to cut out infected twigs and destroy any fallen leaves but on the prioritizing sites this type of work should be done.

• Sanitize all tools, equipment, tarps, shoes, gloves, clothing, etc. (see Scenario 1).

• Control via fungicides in the forests has to be conducted only on the territories located far away from rivers and water sources and on the open spaces. Preventative treatments should focus on efforts to implement non-chemical alternatives such as biological control of *Calonectria* spp. in the forests.

**Biofungicides for controlling boxwood blight:**

• Active ingredient: *Bacillus subtilis* strain QST713, Formulation Serenade (Bayer), Action(s): biofungicide.

• Active ingredient: *Bacillus subtilis*, Formulation Rhapsody (Bayer), Action(s): biofungicide.

• Active ingredient: *Streptomyces lydicus*, Formulation Actinovate (Bayer), Action(s): biofungicide (antibiosis, enzymes, competition, parasitic, growth, promotion).

• Active ingredient: *Trichoderma* spp. (*Trichoderma asperellum* TV1, *Trichoderma harzianum* strains Rifai T22 and ICC 012, *Trichoderma viride* ICC 080), Formulation Various including Antagon, RootShield, Binad T (BioWorks, Inc., USA; BINAB Bio-Innovation AB, Sweden; De
CeusterMeststoffen N.V. (DCM), Belgium), Action(s): biological formulations (also for soil treatments) (Harman, 2000).
The same biofungicides may be used for controlling soil pathogens (*Phytophthora* and *Pythium* pathogens, *Puccinia buxi* and *Vollutela* blight) because they are available as foliar spray and as soil drench.

**Box tree moth control measures.**
Short-term and the long-term strategies were currently being represented by Mark Kenis (Kenis, 2015) for the controlling *Cydalima perspectalis* in the Eastern Black Sea coat.
But, the mail aim of our plan is developed to provide available and easily understandable activities should be done for rapid management and maximum effectiveness:

- Observations and monitoring should start in winter on all examined boxwood locations. According to the results (Chapter 4, Annex 1) it is important to set up priority areas e.g. Zestaponi Forestry.
- Collecting caterpillars, cutting, destruction of litter and damaged branches, remove and burn them may be extremely time-consuming but possible to be done.
- It is important to monitor first larva developing because first control treatment must be implemented parallel.
- Also, in winter and later during the whole year, monitoring should be done using pheromone traps. The water traps can be still needed for monitoring since it will be easy to count the number of trapped moths and therefore establish flight curves. Funnel traps are better for monitoring than glue traps. New large-capacity traps as effective as the funnel trap but easier to use were developed INRA and is marketed under the brand name Buxatrap®. This non-saturable trap uses no water. Once installed, it can remain in place for the entire season. The traps should be installed approximately six feet above ground since installing the traps at ground level risks trapping more non-targeted organisms such as lizards and small rodents. The pheromone has to remain effective throughout the season and not to be changed every 4–6 weeks.

**Chemical and biological control.**
As a one the ways of treatment against larva of *Cydalima perspectalis* in green areas and in the forest is using the insecticides of enteric contact action, particularly synthetic pyrethroids: Sumi-alpha (Esphenvalerat, Senpai); Decis, Decis prophi (Kotryn, Oradelt, Polytox,) Fastak (Alphatoks, Geletrin, Kinimks, Fury) and others. Chemical control can be implemented with an interval of 5-7 days: 1 generation-from March 20 to April 10, 2nd generation-early-mid June, 3-generation-beginning-middle of August, 4 generation-early-mid October.
However, the most of these synthetic insecticides do not give the proper effect at high temperatures of air and they are toxic to bees and are banned for use near the water. But, it is possible to implement them in boxwood stands near monasteries, churches, in green areas of cities and towns, botanical gardens, on the municipal territories or in private gardens. The insecticides used for the box tree moth have to be registered and approved for apply on the territory of Georgia.
Dimilin may be used for destroying the caterpillars of *Cydalima perspectalis* in in green areas of cities and towns, botanical gardens, on the municipal territories or in private gardens and the
forest plantations. It is an inhibitor of chitin and has no effect on other organisms, except insects. This insecticide has been well established against different species of phyllophagous insects. Other invertebrates (as water and land) are resistant or weakly resistant to Dimilin. Dimilin refers to a low-hazard insecticides, has a low toxicity to mammals, birds and fish, long period of aftereffect (up to 40 days) and lack of bioaccumulation. It is rapidly degraded in soil and water. It destroys the larva during their food in forest plantations. However, to determine the feasibility and efficiency of use this preparation against caterpillars of Cydalima perspectalis is needed to conduct several additional tests.

It would be the optimal solution to use the biological insecticide (biopesticide) produced by fermentation of the naturally occurring soil bacterium Bacillus thuringiensis var. kurstaki (Examples of Trade Names: Dipel, Foray, Bioprotec, Vectobac, Thuricide, Lepidocide, Btk) in the native boxwood forests. Types of Formulation: granules, wettable powder, aqueous concentrate, powder. Toxicity based on pure active ingredient: No infectivity or toxicity to the mammal and bird after 63 day feeding trial and Practically non toxic to the bees and fish. Btk is degraded very rapidly when exposed to UV light and is unstable in water pH greater than 8; does not affect other non-target insects or aquatic invertebrates; no threat to groundwater.

Timing of Btk applications is critical to successful control of caterpillars. Because Btk is a stomach poison, it must be eaten by the insect in order for it to work. Btk is most effective against young, actively feeding caterpillars. The first spray on the tree foliage after caterpillars have hatched from their eggs, and apply again 2 weeks later while larva are still less than 3/8-inch long. Make two applications of the spray over the course of 2 weeks to ensure that susceptible caterpillars are treated. When Btk is properly applied, it kills 80%- 85% of moth caterpillars with each spray and after two Btk consecutive sprays (2 weeks) approximately 99% of caterpillars might be killed. If necessary during the season, Bt-based treatments can be applied 10 days after flight peaks are reported.

The use of high-pressure sprays to dislodge box tree moths is not recommended. This technique creates a suffocating surface-sealing crust on the soil. Furthermore, it encourages moisture at the stem of the boxwood which can promote the development of boxwood blight symptoms. Also, the leaves of some boxwood varieties are too fragile to withstand the force of the spray.

Fungi also can be potentially alter pathways toward their development as microbial control agents (Lacey et al. 2015). Fungi Beauveria and Metarhizium spp. were used for biological control of different groups of insect including Lepidoptera, they can be dispersed passively via wind or rain splash. Their transmission occurs while contact susceptible insects with infected individuals but also their spores can affect other arthropods bodies (Meyling et al. 2006; Roy et al. 2007; Vega et al. 2007). That is why available commercial products based on these fungi may be tested and used in the boxwood forests.

The insecticidal activity of essential oils of cloves (Syzygium aromaticum), Atlas cedar (Cedrus atlantica) and European silver fir (Abies alba) against box tree moth were tested in Sochi in 2014 (Dvoretska, 2014). Methodology and results of study are described in Literature Review (2.2.2). This research gives a new tool to develop alternative control management against C. perspectalis should be improved and checked for applying in nature.
Undoubtedly, classic biological control, which involves introducing natural enemies collected from the moth’s native geographical region where the moth is kept naturally, is one of the best and the most optimal solution for controlling box tree moth. This work in under control and parasitic wasps in Asia were recorded. It is time-consuming and expensive research and nowadays probably is not available in Georgia but should be developed in the future.

4. Rehabilitation and restoration. It is aimed to restore or rehabilitate degraded areas to their ecological function to prevent invasive species infestations or to prevent reoccurrence after invasive species removal.

Success of this strategy consists in the fact if ecosystems impacted by invasive species have been effectively restored or rehabilitated. During implementation previous strategies, other types of action should be done to protect ecosystems (boxwood forests and boxwood as s species) and restore degraded if possible.

- Synthesize multiscale monitoring, control and management results and assess effectiveness of restoration action.

Depending on boxwood forests locations, some boxwood plants were infected by boxwood blight, while others remained in perfect health. Planting conditions and microclimate seemed to play a role in whether or not the disease took hold. The fragments of preserved boxwood trees were found upper the riverbanks and on the open spaces. Even on the areas where decline of box tree reached an irreversible process stage, there are still growing not damaged plants had been found also.

The restoration of box trees in these areas is only possible with the active and long-term human intervention and well thought out forest management. The artificial boxwood trees restoration is only possible with combination measures to prevent the escalation of blackberry, alder and other shrubs. In fact, it might be seeing genetic variability in boxwood but this is still at the very early stages of researches. Increasing the biological resistance of boxwood plantings should be done with developing strategic and tactical goals.

**Strategic goals**

- Conduct the study of the genetic characteristics of the native populations of *Buxus colchica* in Georgia.
- Improve the sustainability of natural boxwood populations by searching resistant plants to the boxwood blight pathogen with their artificial plantings.

**Tactical goals**

Good nursery management practices that minimize pests’ presence using Guide to implementation of phytosanitary standards in forestry (Guide to implementation of phytosanitary standards in forestry, 2010).

- Provide the best possible growth conditions (e.g. nutrients, water, light, appropriate spacing and weed control) to raise healthy, vigorous and resistant plants.
- Collect or obtain seed from good quality genetically superior boxwood plants.
- Locate the nursery producing the seedlings away from commercial stands to prevent contamination and the subsequent spread of pests around the country.
- Keep new plant material isolated from main growing areas, where it can be monitored for pests without risk of them spreading to the whole nursery.
- Treat soil if necessary to eliminate pests before planting.
- Use appropriate preventative silvicultural, chemical or biological control methods.
- Ensure irrigation water is free of pathogens and other contaminants such as pesticides.
- Avoid leaving leaves wet, especially when watering at night, as this can allow pathogens to infect plants.
- Limit the entry of visitors to reduce the risk of pests and pathogens moving on their clothing and footwear. Measures to limit the entry of animals and birds, which may spread pests, should also be considered.
- Disinfect all tools and collect and remove dead plants and debris every week to decrease the probability of infestation.

Some of the nursery practices can be useful in managing planted boxwood forests.

Some coordinating activities have to be organized at the national and regional levels to address the need for and supply of native plant materials (for example, seed and seedlings) for restoration.

Coming back to the four themes for strategies of forest protection (partnerships and collaboration, scientific basis, communication and education, organizing for success), success of their implementation depends on the National Forestry Agency of Georgia and its partners' capabilities and sufficient knowledge for invasive species management in boxwood forests. Some examples of practice activities were describe in strategy plan.

Several important summarized measures that should be undertaken are described below:

**Partnerships and collaboration**
Saving boxwood forests and strategy plan can not be coordinated without activities at all levels of the organization and across all programs: state and local governments, tribal interests, nongovernmental organizations and others in the private sector and international stakeholders.

**Scientific basis**
Achieving appropriate results is not possible without conducting appropriate research, development activities to ensure management programs are effective, and science-based. Scientists have to be included in monitoring system, developing methods and modifying activities as well as in control and management programs. Prioritization must be a dynamic and flexible process that enables decisions to be made by using the best available scientific information and knowledge connected with boxwood pests. Risk assessments will be used to set priorities.

**Communication and education**
The clear communicate information and ensure that it is understood properly for the public to gain understanding that boxwood will be saved for future generations (work in schools, with students and average people).

**Organizing for success**
Finally, if we have strategic plan how to maintain boxwood in Georgia, day-to-day activities could be facilitated through appropriate agency policy, guidance, and direction, including manuals, handbooks, and technical guides. It is not possible without internally and externally long-term funding support. It also may require budgeting process and structural changes that allow and secure long-term and continuing funding.

5.2. Strategy plan to improve and organize the work of Forest Protection and Restoration Department (FPRD) under the NFA in Georgia

Disadvantages in the existing FPRD in Georgia

- There is no early detection and rapid response program as well as prediction plan of pathological processes in the Georgian forests.
- Insufficient forest protection staff knowledge on issues of forest protection.
- The issues of forest protection deals with one entomologist specialist in Forestry Agency who physically can not cover all forests area of the country.
- Laws and regulations to protect the forest should be revised and improved.

This strategic plan focuses on developing priority operational activities supported by scientific research to achieve results on the ground against the invasive species threat. It includes several blocks and some of them were mentioned as activities in 5.1.

1. Improvement of the legal documents and methodical support of the FPRD.
   - Review and improvement the documents regulating activities in the field.
   - Develop the clearly understandable instructions and guidance on the procedure and methods of conducting surveillance for pests, diseases and other adverse factors affecting the health status of forests.

2. Organization of monitoring and forecasting the development of forest pests and diseases.
   - Identify the most dangerous pathogens and pests. Develop the techniques and rapid response methods for identifying and assessing the degree of threat economically significant diseases and potential pests.
   - Form a forest pathological monitoring network in nine Regions of Georgia.
   - Improve methods of collecting and analyzing information connected with sanitary situation in the forests (unification of documentation, the timing to survey and submit information from Forest Enterprises).
   - Use of synthetic pheromones and create a network of observation points for the most dangerous insect as box tree moth.
   - Develop and implement the software for processing information on the health conditions of forests. Forest pathological forecast should be organized in the regions and in a whole country.
3. Work with the forest protection staff

- There is urgent need to introduce the new category of specialists - forest pathologist to the existing forest protection staff. It is especially important for the regional offices and aimed to form a network of forest protection staff in the regions.
- To provide education of such specialists (forest pathologists and entomologists).
- To support the phytopathological surveys by satisfactory laboratory research and analysis and create forest protection laboratories, purchase modern equipment with parallel education and training of employees.
- To organize the training course for forest protection team with preparation of guidelines and manuals for fieldwork and surveys.

4. Financial and technical support to FPRD.

- Purchasing the laboratory equipment that would have made possible to carry out the mycological, entomological and genetic analyses.
- Creating and training groups for implementation of preventive measures in forests. It is not possible without internally and externally long-term financial support of these groups. Purchasing of the moto sprayers, aerosol generators as well as clothes, shoes and chemical protective tools for providing successful control and management of forest pests and diseases.
- Developing and publishing manuals and guidelines, posters, atlases (in Georgian) with describing the development stages of the most dangerous pests and pathogens in Georgia for forest protection co-workers and security officers.

Active control measures should be focused on the highest priority areas:

- Use of biological control fungicides and biopesticides.
- Regulate the sanitary cuttings in the country. For example, felling and removal of affected trees and burning of damaged branches and bark on the areas with strong sweet chestnut decline must be urgently conducted.
- Use the bacterial fungicides and biopesticides against diseases and insects (described in 5.1).
- Integration of all protection measures conducted in the forests in Georgia (not only in boxwood forests):
  - During applying chemicals and spraying them;
  - When chemical and bacterial control prepares may be combined;
  - During entering new search on the covered forests areas.

There are also priority directions, which should be implemented more actively:
• Work with municipalities and the broad public (e.g. Zugdidi and Batumi Botanical Gardens, private farms in the villages).
• Protected territories. Can the introduction of boxwood blight pathogen on the territories of PA be assessed as caused by human activities? In our opinion, it is not the natural process. It is strictly recommended to decide if control measures have to be conducted on the territories of PA with some changes to the Law or boxwood forests will die because of delayed actions.
Additional studies within the project time

*Buxus colchica* is evergreen plan and its leaves is hold on the branches for long period. Under favorable adverse conditions, lichens from the families *Gyalectidium*, *Fellhanera*, *Sporopodium*, *Pilocarpon* may be developed on the leaf surface. During field trips, the thalli of lichens on the boxwood leaves were found in Zugdidi and Guria Forests. Of course, the identification of the lichens requires special additional studies and analyses. Due to the defoliation of boxwood forests caused by boxwood blight pathogen and box tree moth, this group of organisms will disappear from the natural ecosystems in Caucasus. The lichen *Graphis scripta* Ach. was found on the boxwood trunk (L.) (Figure 34).

![Figure 34: Boxwood lichens: A - The lichen *Graphis scripta* on the boxwood trunk; B - The thalli of lichens on the boxwood leaves.](image)

Dothistroma needle blight (DNB) is one of the most serious foliar diseases of *Pinus* spp. in several European countries and continents, showing a continuously widening range, caused by quarantine fungi: *Dothistroma septosporum* and *Dothistroma pini*. The first documented information about DNB in Georgia was in 1966 where it was identified on different pine species (Shishkina and Tsanava, 1966). In addition, in Georgia, it was confirmed on infected needles of *Pinus pithyusa* Fox-Strangw. (synonym of *Pinus brutia* var. *pityusa* (Steven) Silba) (The Plant List) (Zhukov and Gninenko, 2002) and *Pinus kochiana* Klotzsch ex C. Koch (synonym of *Pinus sylvestris* var. *hamata* Steven) (The Plant List) on the northwest of Caucasus region (Zhukov and Zhukov, 2004). The latest data provided by G. Meparishvili (pers. communication, autumn
2015) confirms that DNB is spread along Black Sea region in Autonomous Republic of Adjara (Georgia).

The aim of these additional studies within the project time was to provide new data of the fungi in Caucasus region in Georgia.

Sampling was conducted at the end of the July 2015 from 30-40 years old and 40-50 years old *Pinus nigra* and 50-60 years old *Pinus ponderosa* forests in Georgia from three different regions: Kvareli Forest Service (Laliskuri and Sagarejo), Kutaisi Forest Service (Tkibuli Forestry, Sochkheti) and Racha-Lechkhumi Forestry Service Lower Svaneti (Ambrolauri Forestry) (Figure 35). Samples were collected in a paper bags and kept dry until processing in September-October 2015. They were investigated under light microscope (LEICA ICC50 HD) in the laboratory of Forest Pathology at the Süleyman Demirel University (Faculty of Forestry, Isparta, Turkey). All samples were classified as symptomatic and asymptomatic.

![Figure 35: Symptoms of DNB on *Pinus nigra* in Georgia: A - Infected *P. nigra* mature tree. B - Characteristically, needles with red necrotic bands and as the sign of disease.](image)

The needles used in DNA extraction were photographed by Canon G25 camera. Each sample was manually ground in a 2 mL Eppendorf tube with the aid of liquid Nitrogen. The DNA was extracted using DNeasy Plant Mini Kit (Qiagen, Hilden, Germany) and OMEGA E.Z.N.A. Bio-Tek Fungal DNA Mini Kit D3390-02 (USA) according to the manufacturer's instructions. PCR was carried out on a Mini Option Real-Time PCR System (MJ Mini BIORAD), with the following conditions: an initial denaturation step for 10 min at 95°C, followed by 35 cycles of denaturation for 30 seconds at 95°C, annealing for 30 seconds at 60°C and extension for 1 min at 72°C. A final elongation step was carried out for 10 min at 72°C. Species-specific priming PCR (SSPP) was performed using D. septosporum species-specific primers DSTub2-Forward (CGAACATGGACTGAGCAAAAC) and DSTub2-Reverse (GCACGGCTCTTTCACAATGAC) (Ioss *et al.* 2009); D. pini species-specific primers DPtef-Forward (ATTTTTTCGCTGCTCGTCGCT) and DPtef-Reverse (CAATGTGAGATGTTCGTCGT) (Groenewald *et al.* 2007).
Our research provides two new findings of DNB on the territory of Georgia. *D. septosporum* was directly detected using DNA extraction and PCR amplifications from infected needles of 40-50 years old *P. ponderosa* from the eastern part of Georgia and *D. pini* was found on 30-40 years old *Pinus nigra* in Racha-Lechkhumi Lower Svaneti Forestry Service (Ambrolauri Forestry). Our data shows that geographical range of DNB in Georgia has increased and reached the eastern part of the country and *Pinus ponderosa* and *Pinus nigra* were added to host list.

The results of this study will be published.
REFERENCES


Boxwood Diseases & Insect Pests http://www.clemson.edu/extension/hgic/pests/plant_pests/shrubs/hgic2052.html.


Colchis boxwood (Buxus colchica) - review of materials on care of plants and timber processing, [http://kraevedenie.net/2009/03/19/samshit-buxus/2].


Crous P.W., 2002. Taxonomy and pathology of *Cylindrocladium* (Calonectria) and allied genera. APS Press, St. Paul, MN, USA.


Dolukhanov A. 2010. Lesnaja Rastitel’nost’ Gruzii (Forest Vegetation of Georgia). Universal, Tbilisi. (Russ.).


Jung T. Life cycle and pathological importance of the genus Phytophthora. [www.baumkrankheiten.com/.../phytophthora].


### Annex 1

**Detailed description of the studied native boxwood forests in summer 2015**

<table>
<thead>
<tr>
<th>Region Kakheti</th>
<th>Research areas</th>
<th>Coordinates X</th>
<th>Coordinates Y</th>
<th>Altitude, m above sea</th>
<th>Summer (data)</th>
<th>Brief description of forests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Telavi</td>
<td>Kvareli</td>
<td>38534796</td>
<td>4662619</td>
<td>583</td>
<td>21.07.2015</td>
<td>2.5 ha, compartment 94, subcompartment 16, 30 C. Carpinus 10 Hornbeam 10 Oak, 10 Ash + Sweet Chestnut, relative stand density 0.5, volume of growing stock 280 m³/ha, young growth -10 years, Maple, hornbeam, 2000 sht/ha, the undergrowth – hazel nut. Exposure - northeast, 31°. This is a site where more than 300 years ago, there was a church, and typically, boxwood was planted near churches and cemeteries. Shrubs and trees are in good condition, <em>Puccinia buxi</em> on the leaves.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38534752</td>
<td>4662544</td>
<td>608</td>
<td>21.07.2015</td>
<td>This is top of boxwood area, trees look healthy without damage or black spots on the leaves caused by boxwood rust.</td>
</tr>
<tr>
<td></td>
<td>Akhmeta</td>
<td>38526381</td>
<td>4668257</td>
<td>707</td>
<td>22.07.2015</td>
<td>It was not found any inventory characteristic of boxwood stands. Boxwood tree are mainly planted below the cemetery in good conditions. The abundant black spots on leaves by <em>Puccinia buxi</em> were observed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38526423</td>
<td>4668247</td>
<td>722</td>
<td>22.07.2015</td>
<td>Boxwood plants look healthy and recovery well, especially in the gaps where there is getting light. Maple and hornbeam create a first vegetation layer with ash trees that show ash dieback symptoms.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38568696</td>
<td>4643894</td>
<td>408</td>
<td>22.07.2015</td>
<td>These are inventory characteristic of boxwood stands in 1989. 7.5 ha, compartment 95, subcompartment 2. 70 Hornbeam 30 Beech + Georgian Oak, relative stand density 0.4, age -90 years, volume of growing stock 680 m³/ha, young growth 10 Hornbeam 1000 sht/ha, exposure - northeast 35°. This place is named &quot;Boxwood Mountain&quot; where boxwood trees are healthy and grow in appropriate conditions.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38568740</td>
<td>4644035</td>
<td>423</td>
<td>22.07.2015</td>
<td>Another side of &quot;Boxwood Mountain&quot;. 70 Oak 30 Hornbeam, age - 90 years, relative stand density 0.5, volume of growing stock 120 m³/ha, young growth 10 Hornbeam 2500 sht/ha, exposure – southwest. Sanitary conditions of boxwood tress are the same.</td>
</tr>
<tr>
<td>Region</td>
<td>Village</td>
<td>Phone</td>
<td>Number</td>
<td>Date</td>
<td>Description</td>
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<tr>
<td>Imereti</td>
<td>Kutaisi</td>
<td>38417182</td>
<td>4650260</td>
<td>656</td>
<td>23.07.2015 Forests had been artificially established on the old plowed soils or naturally regenerated on abandoned agricultural lands near river and electric power station. It is known that in 2013 there was very dry weather and boxwood defoliated. Currently, boxwoods are in a good condition and get enough sunlight. Boxwood leafminer <em>Monarthropalus flavus</em> was found sporadically. It is known that 2-3 years ago there was a significant defoliation of boxwood trees. Some fresh regrow stems are found, 10-15% of crown are living. Sweet chestnut (infected by <em>Cryphonectria parasitica</em>), hornbeam, wild pear and maple are in the main vegetation layer. Boxwood leafminer <em>Monarthropalus flavus</em> was rarely found.</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>38335223</td>
<td>4684104</td>
<td>739</td>
<td>23.07.2015 It is known that 2-3 years ago there was a significant defoliation of boxwood trees. Some fresh regrow stems are found, 10-15% of crown are living. Sweet chestnut (infected by <em>Cryphonectria parasitica</em>), hornbeam, wild pear and maple are in the main vegetation layer. Boxwood leafminer <em>Monarthropalus flavus</em> was rarely found.</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>38335218</td>
<td>4684148</td>
<td>746</td>
<td>23.07.2015 This research spot is near river where there is wet and shade. Boxwoods growth rarely without sporulation and restoration under moisture.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>38335258</td>
<td>4684275</td>
<td>752</td>
<td>23.07.2015 This place is located along the forest path with boxwood trees on the both sides of path. Boxwood blight <em>Calonectria pseudonaviculata</em> (<em>Cylindrocladium buxicola</em>) is found on the leaves and <em>Eriococcus buxi</em> and boxwood leafminer <em>Monarthropalus flavus</em>. This is a native old boxwood forest (200 years) completely dead with boxwood blight symptoms on the leaves and with necrosis on the branches. This forest is grown under infected sweet chestnut. Several boxwood tress are evidently renewed. The native old-grown boxwood forests located down the forest road growing under a hornbeam, sweet chestnut, <em>Oak iberica</em> and <em>Tilia caucasica</em> layer. It is available information about dry weather 3 years ago that effected by the trees conditions.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>38335383</td>
<td>4684331</td>
<td>717</td>
<td>23.07.2015 This forest was complete dead 2-3 years ago and currently is being regenerated. Tree stands is located on the sides of Sagvelitbe River. There were found several old dead trees and several undamaged young plants. This boxwood forest is located on the other side of Sagvelitbe River. According to the foresters’ data, 3 years ago they were totally yellow but nowadays the trees started to regrow, that’s why on the top of hill old trees are dead and young shrubs look unhealthy but alive.</td>
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<tr>
<td></td>
<td></td>
<td>38337613</td>
<td>4688745</td>
<td>959</td>
<td>24.07.2015 This is very nice native boxwood stands located under the base of mountain on the southwestern slope exposure. Boxwoods grow outdoors, low shrubs, sprawling, green, with beautiful colour of the leaves and without damages on them. According to the foresters’ data, boxwoods were sick 7-8 years ago, but quickly renewed. Symptoms of <em>C. buxicola</em> were found on the leaves. Local people evidently cut many branches. Generally, the sanitary conditions for the first look are good, but boxwood blight symptoms and</td>
<td></td>
</tr>
</tbody>
</table>
Boxwood leafminer were often observed. The area is close to the previous two but located on the open space. Boxwood shrubs are infected by boxwood blight and damaged by boxwood leafminer. This area is very snow-covered in winter (up to 6 meters of snow) and boxwood shrubs are damaged by snow. Boxwood is grown under layer of sweet chestnut and beech and the black spots of boxwood blight with boxwood leafminer and coccida were found. The same situation as in previous one.

Boxwoods grow on the south side of hill and complete dried on the edge of area where there is a lot of snow in winter with always strong blowing wind. In spring snow quickly melts ad boxwood suffers under wet conditions.

Region Racha-Lechkhumi Lower Svaneti

Ambrolauri  Ambrolauri 38326952 4710839 497 26.07.2015 Compartment 15, sub-compartment 5, relative stand density 0.4. First layer: 50Hornbeam30Tilia20Acer, volume of growing stock 1200 m$^3$/ha, young growth 4000 sht/ha. Second layer: 10Boxwood, age - 30 years, The first point of Shareula river and native boxwood trees are grown on the banks of river. Sanitary conditions of trees are good, only boxwood leafminer and coccida were rarely found with black dots of boxwood rust. The tree trunks tightly covered with moss.

Next point above the bank of the river in v.Gogoletti. Boxwood trees are generally in good condition, rarely with black spots on the leaves, and Eriococcus buxi, boxwood sucker Psylla buxi on the leaves. The tree trunks tightly covered with moss.

2.9 ha, compartment 25 sub-compartment 8 and 9 (between them), 60Beech20Acer20Hornbeam, age - 130 years, relative stand density 0.3, volume of growing stock 500. m$^3$/ha. This place is located along the path down to the river. The intensive boxwood dieback is found (80-90%) with many black dots on the leaves. When the water rises, the flooding of the trees are seen, also rubbish sticks to the bottom of the trunks, old bark completely removed. Mass dieback of trees is visible close to the river and on the upper parts of the banks, boxwoods are still alive and have green tops. The tree trunks tightly covered with moss. Compartment 25, subcompartment 8. Condition of boxwoods is good enough, single black spots of boxwood blight are visible and white symptoms of Mycosphaerella buxicola on the edges. The tree trunks tightly covered with moss.

7.4 ha, compartment 17, subcompartment 3, 40Chestnut40Hornbeam10Beech10Tilia+Acer, age - 90 years, volume of growing stock 1330 m$^3$/ha, young growth 40Chestnut40Beech20Picea, 5000 sht/ha. Next point is closer to the river and at the bottom of the left bank is mass of boxwood dieback. The trees general look healthy above the forest path but young trees complete dried and only many older are good with alive crowns. The tree trunks tightly covered with moss. 11 ha, compartment 17, subcompartment 8, 60Hornbeam10Chestnut10Beech10Oak10Picea,
age - 90 years, relative stand density 0.7, volume of growing stock 1980 m$^3$/ha, young growth 50Hornbeam30Beech20Picea, 5000 sh/t ha. The next point of Shareula river and native boxwood trees are grown on the banks of river. Boxwood trees are generally in good condition, rarely with black spots on the leaves, and Eriococcus buxi, boxwood sucker Psylla buxi on the leaves.

8.4 ha and 10 ha, compartment 39, between subcompartments 15 and 16, 100ak, age - 70 years, relative stand density 0.6, volume of growing stock 1500 m$^3$/ha.

The next area is located near the St. George monastery on the banks of the same river. It is suspected that boxwood trees are artificial origin. Generally, they look very nice with no signs of damaging. But, boxwood rust and Eriococcus buxi were observed. Due to the growing of rare tree species, this place is unique and valuable.

Region Samegrelo Upper Svaneti

Zugdidi

<table>
<thead>
<tr>
<th>Code</th>
<th>Date</th>
<th>Age</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>37731769</td>
<td>29.07.2015</td>
<td>37731769</td>
<td>166</td>
<td>The boxwood trees are planted near the monastery. Distributed of imago and larva Cydalima perspectalis and lots of typical damages were detected there. Also, boxwood blight and Volutella blight symptoms were found. Cattle is grazing everywhere.</td>
</tr>
<tr>
<td>37737541</td>
<td>29.07.2015</td>
<td>37737541</td>
<td>86</td>
<td>Boxwood and hawthorn bushes grow together and on the open areas the cattle is grazing. Wood decay fungi were found on the trunks of old trees. The flying period of box tree moth was observed.</td>
</tr>
</tbody>
</table>

Tsalenjikha

<table>
<thead>
<tr>
<th>Code</th>
<th>Date</th>
<th>Age</th>
<th>Volume</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td>38272475</td>
<td>30.07.2015</td>
<td>38272475</td>
<td>328</td>
<td>The boxwood stands are located in Luchela Gorge near Khobistskhal river. The strong defoliation of green-yellow leaves were detected 5 years ago but plants started restoring 2 years ago. There are now many new leaves and new branches.</td>
</tr>
<tr>
<td>38270891</td>
<td>30.07.2015</td>
<td>38270891</td>
<td>338</td>
<td>Next place upper Khobistskhal river. Typical symptoms of C. buxicola were detected with a high level of defoliation.</td>
</tr>
<tr>
<td>38270178</td>
<td>30.07.2015</td>
<td>38270178</td>
<td>352</td>
<td>Next place in the middle of right side of Khobistskhal river. Many leaves are infected by boxwood blight and first damaged trees were found. There are any healthy looking trees were seen on the opposite side of river.</td>
</tr>
</tbody>
</table>
This boxwood area is located close to the vil. Skuri near Tsiokvilara river. 70-90% of the young boxwood plantations under huge black alders trees are declined with fragments of green areas infected by *C. buxicola*. Trees started to defoliate 3 years ago, some part of them are regenerating, but in the place for swimming people they are complete dried. These are boxwood plantations of artificial origin on the both side of Chanickali river. The trees are infected by boxwood blight and Vollutea blight. This place is located upper the Chanickali river. Boxwood grows under the layer of sweet chestnut, black alder, hornbeam with rhododendron, blackberries and hawthorn. All trees near the rivers are dried, higher are still green. They try to regrow but is quite difficult. Black spots of boxwood blight are found everywhere. The boxwood stands are located on the banks of Tehuri river. The strong defilation of tress was observed 5 years ago when 80-90% of old trees were dead. There are still may old dried trees and 70% young plants are infected by boxwood blight. The boxwood stands with first feeding by box tree moth that makes ‘windowing’, eating the lower surface of leaves. This boxwood place is located to the monastery of Fathers near Abasha river. Boxwoods are planted along the river banks under the hornbeam and linden layer. 60-70% of plants were defoliated and infected by *C. buxicola*. First feeding by box tree moth was also detected. The next point downstream of the river near the monastery. Many old and young shoots of tress are affected by boxwood blight and strong degree of bow tree moth settlement is found.

Boxwood shrubs planted as ornamental plants near Guria Forest Service Administration. In 2014, the first signs of *Cydalima perspectalis* damage were discovered there. This year the damage has progressed, many larva of moth were found on the leaves. Nursery of Guria Forest Service. *C. perspectalis* destroyed all small plants. Compartment 4, total defoliation of all plants by box tree moth. Fist symptoms of damage started 2 years ago, and nowadays none green trees. There are many *Ilex* spp. species. Area suffers from grazing cattle. Next area is located in Djumari forestry where boxwoods grow on wetlands under alder and ash trees. The plants are completely defoliated by box tree moth and lots of *C. perspectalis* imagoes were seen.

Totally 48 examined boxwood areas in 5 Regions
**Detailed description of the studied native boxwood forests in autumn 2015**

<table>
<thead>
<tr>
<th>Region Imereti</th>
<th>Forest Service Researches areas</th>
<th>Coordinates X Y</th>
<th>Altitude, m above sea level</th>
<th>Summer (data)</th>
<th>Brief description of forests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kutaisi</td>
<td>Tkibuli</td>
<td>38338947 4688857</td>
<td>1034</td>
<td>27.10.2015</td>
<td>Boxwood shrubs growing on the open space where there is a lot of sun and wind. There is a high level of boxwood rust damage. The color of the leaves yellow, orange, but there are no signs of dieback on the shoots. <em>Macrophoma candoldei</em> was found on the fallen leaves and <em>Mycosphaerella buxicola</em> caused dieback of the leaves edges. Boxwood plantation is located on top of the hill, severely damaged by <em>Puccinia buxi</em>. <em>Macrophoma candoldei</em> was found on the fallen leaves and fly holes of <em>Monarthropalpus flavus</em> and <em>Puccinia buxi</em> affected leaves. Some shrubs are infected by boxwood blight.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38337966 4688497</td>
<td>1047</td>
<td>27.10.2015</td>
<td><em>Macrophoma candoldei</em> was found on the fallen leaves and fly holes of <em>Monarthropalpus flavus</em> and <em>Puccinia buxi</em> affected leaves. Some shrubs are infected by boxwood blight.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38337384 4688665</td>
<td>949</td>
<td>27.10.2015</td>
<td>Since summer time, the situation has not substantially changed. In this area, previously, lime had quarried and there are still ruins where he was burned. Boxwood is planted outdoors, shrubs for the first glance, look very nice without damages. <em>Macrophoma candoldei</em> was found on the fallen leaves, fly holes of <em>Monarthropalpus flavus</em> and <em>Puccinia buxi</em> affected leaves. Some shrubs are infected by boxwood blight.</td>
</tr>
<tr>
<td>Zestafoni</td>
<td></td>
<td>38331539 4680635</td>
<td>685</td>
<td>28.10.2015</td>
<td>Forests had been artificially established on the old plowed soils or naturally regenerated on abandoned agricultural lands near river and electric power station. Boxwoods grow on the both banks of river. <em>Macrophoma candoldei</em> was found on the fallen leaves, fly holes of <em>Monarthropalpus flavus</em> and <em>Puccinia buxi</em> affected leaves. Boxwood blight and Vollutea blight symptoms were found on the shrubs growing close to water, and first feeding by <em>C. perspectalis</em> on the lower part of plants and cobweb nests overwintering larva was discovered.</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Region Racha-Lechkhumi Lower Svaneti</th>
<th>Forest Service Researches areas</th>
<th>Coordinates X Y</th>
<th>Altitude, m above sea level</th>
<th>Summer (data)</th>
<th>Brief description of forests</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ambrolauri</td>
<td>Ambrolauri</td>
<td>38328860 4709035</td>
<td>575</td>
<td>29.10.2015</td>
<td>The first point of Shareula river. The native boxwood trees are grown on the banks of river. First layer: sweet chestnut, black alder, beech, hazelnut. Second layer: 10Boxwood. Sanitary conditions of trees are deteriorated compared to summer observation. Many boxwoods are declining hear the river (85%), upper to the hills only young trees (15%) and the thickest branches of old trees dry. The tree trunks tightly covered with moss. When the water rises, the flooding of the trees are seen, also rubbish sticks to the bottom of the trunks, old bark completely removed and root rot signs are visible.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38329006 4707877</td>
<td>658</td>
<td>29.10.2015</td>
<td>Next point is above the bank of Shareula river. The situation is the same in a previous area.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>38351549 4706548</td>
<td>916</td>
<td>30.10.2015</td>
<td>Boxwood plantation is located near the old church (built in XX century) in Kvemo Krikhi. Boxwoods aged min 200 years, are in good conditions without defoliation and damages. This is a big group of trees artificially planted around old cemetery with old <em>Taxus bacata</em> and <em>Tilia</em> spp. <em>Mycosphaerella buxicola</em> was rarely found.</td>
</tr>
</tbody>
</table>
Boxwood area is located near the vil. Larchvali on the right bank of Jonouri river. The young growth of boxwood is rarely damaged by Monarthropalpus flavus. The high level of Puccinia buxi damage on the leaves was observed.

The young boxwood plantations under huge black alders trees, 90% of them are dead, many plants are infected by C. buxicola together with Vollutea blight. Some plants complete defoliated. First feeding by C. perspectalis on the lower part of plants, silk webbing and skeletonised leaves was discovered. Also overwintering larva protected in a cocoon spun between Buxus leaves were found.

Typical symptoms of C. buxicola were detected with a high level of defoliation. Also, silk webbing and skeletonised leaves made by box tree moth were found everywhere.

These boxwoods were planted as ornamental decoration but in 2010 were defoliated by boxwood blight. In spring 2015, first infestation of C. perspectalis were discovered and currently there are 90% dead trees. Owners did some chemical treatment and several plants started to resprout new branches.
Annex 2

Wood decay fungi detected in the native boxwood forests

*Phellinus laevigatus* (Fr.) Bourdot & Galzin

*Armillaria cepistipes* Velen

*Kuehneromyces mutabilis* (Fr.)
Schizophyllum commune Fr.

Trametes versicolor (L.) Lloyd

Trechispora mollusca (Pers.) Liberta

Mycena alba (Bres.) Kühner

Mycena alba (Bres.) Kühner

Stereum hirsutum (Willd.) Pers
ACKNOWLEDGEMENTS

Firstly, I would like to thank the European Neighborhood and Partnership Instrument East Countries Forest Law Enforcement and Governance II Program for the opportunity to conduct this research as an international consultant. As it is my second work within FLEG project in Georgia, I really appreciate the confidence to me for carrying out these studies in boxwood forests.

I want to thank everyone who assisted me to do this work, for the help to organize my field trips and to those, who contributed in solving all work related tasks. They did it perfectly.

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My personal scientific improvement would not have happened without care and love of my Mother and daughter. Finally, I am grateful my boss and friend V. Kramarets who believed in me and continuously supported all my extremal ideas.
Last page in sweet memories…
About the Program

The ENPI East Countries FLEG II Program — Complementary Measures for Georgia and Armenia is being implemented by the World Bank in partnership with WWF and IUCN. It complements the EU-funded FLEG II Program. The objectives of the Program are to support Georgia and Armenia in strengthening forest governance through improving implementation of relevant international processes, enhancing their forest policy, legislation and institutional arrangements, and developing, testing and evaluating sustainable forest management models at the local level on a pilot basis for future replication. The three specific Program objectives are: Implementation of the 2005 St. Petersburg FLEG Ministerial Declaration and ensuring continuation of the process launched in 2005 (regional level); Formulation and implementation of sustainable forest sector policies, including legal and administrative reforms for sustainable forest management and protection (national level); and demonstration of best sustainable forest management practices in targeted areas for further replication (sub-national level). The overall objective of the complementary EU-funded Program is to promote sustainable forest governance, management, and protection of forests in the participating Program countries, ensuring the contribution of the region’s forests to climate change adaptation and mitigation, to ecosystems and biodiversity protection, and to sustainable livelihoods and income sources for local populations and national economies.

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http://www.entwicklung.at

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