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X-ray based computed tomography, a non-invasive approach in order to assess the damage caused by *Lamprodila festiva* of hidden lifestyle

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Abstract: The cypress jewel beetle *Lamprodila* (*Palmar*) *festiva* is a wood-boring pest posing a major threat to the phytosanitary conditions of several coniferous trees. Its unprecedented European expansion has triggered serious plant protection concerns in several new habitats. Parts of *Chamaecyparis lawsoniana* injured by *L. festiva* were collected and analysed by computed tomography in order to study the dimensions of the cavities caused by *L. festiva* larvae as well as the larval positions. It is concluded that computer tomography representing a non-invasive approach is a promising tool for the visual depiction of the position and the physical parameters of the cavities formed. According to our experimental data, the penetration into the cypress caused by larvae and, inherently, its depth depends on the diameter of the branch. Additionally, the developing larvae appeared to keep distance from each other, which also depended on the diameter of the attacked branch. Our approach provides new data to the biological traits of the species. The main benefit that our imaging method furnishes is the exact, stress-free measurement method of the hidden developing stages. Its additional advantage is the indirect pest identification, which is based on the predetermined pest-specific damage characters.

Keywords: computer tomography; cypress jewel beetle; damage assessment; larval position; non-invasive method

The cypress jewel beetle *Lamprodila* (*Palmar*) *festiva* (Linnaeus, 1767) (Coleoptera: Buprestidae) is originally a Mediterranean (Balkan peninsula and Asia Minor) (Hellrigl 1972) fauna element, where its primary hosts, *Juniperus* spp. and *Cupressus* spp. are widely distributed (Volkovitsh & Karpun 2017).

Hitherto it has been found in several countries of the Western part of the Mediterranean Basin through Northern Africa to the Middle-East (Volkovitsh & Karpun 2017; Ruicănescu & Stoica 2019). The unprecedented expansion of this pest has triggered grave plant protection problems in several countries, in which this rare and protected species has become a dangerous pest in recent years (Nitzu et al. 2016; Rabl et al. 2017).

The yearly generation numbers of *L. festiva* vary according to its distribution area. Consequently, it has acquired semi- and univoltine ecotypes whose consecutive development was interrupted by an overwintering larval stage (Hellrigl 1972; Evans et al. 2007). This diapausing larva will further develop

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into pupa and finally into adult-stage in the early spring as a result of the daily temperatures rising above its biological threshold (Hellriegl 1972; Volkovitsh et al. 2019).

L. festiva is typically an oligophagous pest because its larva develops in the wood of Cupressaceae such as *Chamaecyparis*, *Cupressus*, *Juniperus*, *Thuja* etc. (Volkovitsh 2017). Due to its larval mastication in the trunk and branches, individual parts of the attacked plant wither and turn brown in otherwise healthy-appearing coniferous trees. The primary cause of the damage is the tunnels formed by the larvae, which are wide, flat formations between the bark and the xylem. These cavities are partly filled with feed and excretion particles, thereby preventing the transpiration of the plant (Volkovitsh & Karpun 2017).

There is no practical method involving tissue destruction, which would be suitable to precisely measure in a quantitative way the injuries caused by hidden-lifestyle insect pests.

The purpose of this study was to accurately determine the scale of tissue destruction in coniferous plant trunks and branches caused by *L. festiva* by using computer-based (CT) three dimensional reconstruction technology. The employment of this sophisticated image analysis technique is expected to contribute to the knowledge of biological features of this dangerous pest. Besides, the imaging of features of the mining done by the insect in tree parts and information gained by capitalising on this non-destructive technology can assist to enhance our understanding as regards some biological characteristics of this insect.

MATERIAL AND METHODS

Sampling and experimental setting. Parts of *Chamaecyparis lawsoniana* (A. Murray bis) Parl. 1864 damaged by *L. festiva* were collected on 15 January both of 2019 and 2020. These samples derived from the city of Kaposvár, from a private house-yard located in the centre (Somogy county, Hungary; geographical coordinates: 46°20'53.7"N 17°45'51.1"E). The sampled trees formed 4–5 m high ornamental row, in which the separate withering branches were conspicuous. The collected five branches and trunks per tree (40–50 cm pieces originated from four trees/year) were placed in an isolator ensuring seasonal climatic conditions. These samples were conserved in these conditions until the end of investigations, and taken out only for the duration of the CT analysis (2 h). Identification of pests was realised by raising overwintering larvae in an isolator, which was carried out in both years of the experiment. In both years, the exact times of the emerging of the adult individuals were recorded.

On the CT images, the depth of the larval cavity measured from the surface was determined, together with the diameter of the attacked parts of the branch and the distance of the larvae from each other as well as the extent of the cavity system was visualised by means of generating 3D-rendered images.

CT imaging and post-processing. The image acquisition was performed by a Siemens Somatom Definition AS+ CT scanner (Siemens Healthcare GmbH, Germany). The scanning parameters were set as follows: tube voltage 120 kV, current 120 mA, spiral data collection mode with Pitch factor of 0.6. The overlapping scans were reconstructed by the Somaris/7 syngo CT software programme (version VA48A). The reconstruction parameters were set in convolution kernel I70h, diameter 307 mm, with slice thickness of 0.6 mm. The images were archived in DICOM (Digital Imaging and Communications in Medicine) files.

The image post-processing was carried out by the 3D Slicer software (www.slicer.org). The fiducial module was used to mark the location of the larvae and the closest tree surface position from the larvae. The distances of the two marked points were registered. The cross-section of the tree was segmented at the positions of the larvae.

Python (version 3.6) programming language was used for the calculations. The distances were calculated based on the marked positions between the larvae. The area, circumference and diameter of the segmented cross sections of the trees were calculated.

Statistical analysis. In order to assess the depth measured from the surface of cypress branch of the cavities made by the larvae and the diameter of the injured parts and the length of larvae ($n > 50$), the Kolmogorov-Smirnov test was used. For the survey of the normal distribution of data ($P < 0.05$), the method of Ghasemi and Zahediasl was employed. The effects of the diameter of the cypress branch on the depth of *L. festiva.* Larvae as well as the distance of the larvae were statistically analysed by one-way ANOVA. Mean values were separated by using the Tukey (HSD) test, at $P \leq 0.05$.

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The tendencies of these relationships were surveyed by regression analysis via using the SPSS for Windows software package (version 11.5)

RESULTS

Imagoes identified as *L. festiva* emerged at almost the same time period of the year in both examined years (on June 5–11, 2019 and on June 13–15, 2020). It is confirmed, that non-invasive computer tomography is a useful tool for the detection of hidden-developing larval stage of *L. festiva* and that of the position and the extent of cavities that are formed (Figure 1).

Computer-based three-dimensional (3D) reconstructions of cypress branch damaged by *L. festiva* can be seen in Figure 2. The inner xylem tissues always remained intact. The destruction of the bark, which is located in the external part of the branch, is discernible based on the location of apparent cavities revealed by CT. The volume of the examined cavity triggered by *L. festiva* was 171.12 ± 35.835 mm^3. The mean depth of larvae was 3.15 ± 0.365 mm into the cypress tissues as estimated by the position of 48 larvae in the injured branch parts. The diameters of the infested branches varied from 40.27 mm diameter (side branch) to 171.23 mm (trunk).

The Kolmogorov-Smirnov normality test indicated that the nutrient content data obtained were of normal distribution, $P > 0.05$. Significant relationship ($df = 1$; $P < 0.001$) between the diameters of the injured parts and the depth of larvae were revealed by statistical analysis. The regression analysis unequivocally confirmed that the thicker the branch, the deeper the larva penetrating it (Figure 3). The correlation between the presence of the larvae and the thickness of the attacked parts of the tree could not be statistically proven.

According to our experimental data, the developing larvae strive to keep distance from each other. The mean distance was 27.529 ± 3.31 mm. The larva-distances in the substantially damaged samples ranged from 3.73 mm to 108.81 mm. The statistical analysis proved that the distance of the larvae from each other highly depends on the diameter of the branch ($df = 1$; $P < 0.001$). As the diameter of the branch increases, so does the distance between the larvae.

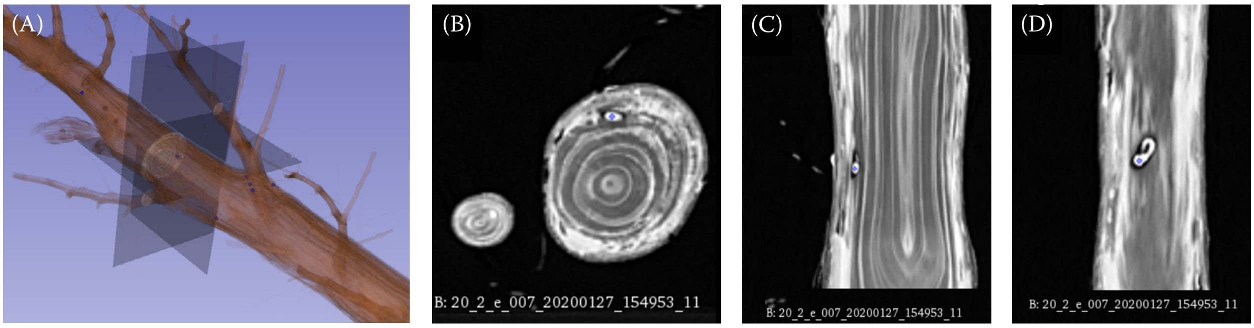

Figure 1. Different image reconstruction planes of examined cypress parts containing *Lamprodila festiva* larvae (A) Three-dimensional reconstruction, (B) axial, (C) coronal, (D) sagittal. Larvae are indicated by blue points

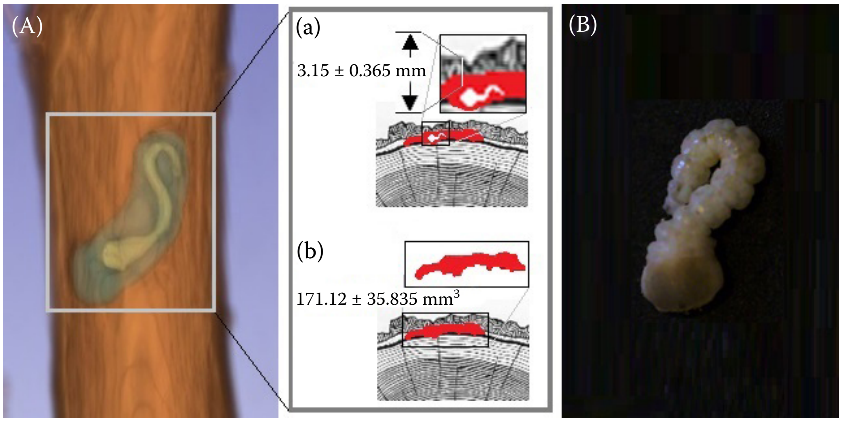

Figure 2. Non-invasive and traditional imaging of the hidden lifestyle *Lamprodila festiva*
(A) Computed tomography-based 3D-rendered image of the cavity triggered by *L. festiva* larvae in cypress plant parts and its position (a) average distance of the larvae from the surface, (b) average volume of the formed cavity; (B) *L. festiva* larvae removed from its natural habitat by using traditional ion

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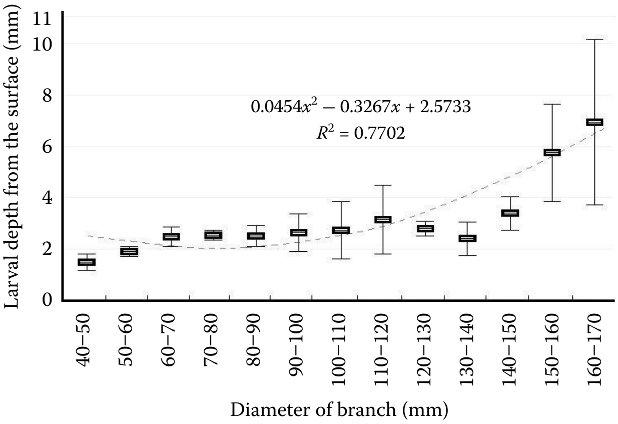

Figure 3. Depth of *Lamprodila festiva* larvae (mean ± SE) from the surface as a function of the diameters of the injured cypress plant parts

DISCUSSION

There had been previous studies on the survey of hidden-lifestyle arthropods in non-invasive ways (Cruvinel et al. 2003; Pavlović et al. 2018), but our experimental investigation supplies new data due to the novel approximation as to the location of the larvae as well as their 3D-rendered images. The employed method is suitable for determining the position of the developing larvae and it can provide data for the extent and volume of the cavities triggered by *L. festiva*. The limitation of this technique is posed by the size of the tree analysed since the bore diameter is 78 cm and the maximum acquisition section forms a circle with 500 mm diameter. On the other hand, the resolution of the scans is capable of visualising objects greater than 0.6 mm, if the radiodensity of the investigated object differs from that of the adjacent environment. Theoretically, the method also provides an opportunity to follow the damage, i.e. the progress of the pest in the tree, however, this test cannot be performed on site. Accordingly, the sample must always be delivered to the imaging equipment, therefore the image acquisition can only be performed on stored trees or freshly felled ones.

The penetration into the cypress is caused by larvae, consequently the depth of the larvae from the surface depends on the diameter of the branch. The explanation for this phenomenon is the special location of the developing larvae in the bark tissues, which is responsible for the water and nutrient transport in the tree. The wet conditions prevailing in this external layer of the branch ensure the optimal water supply for the development of the larvae. Similar biological features associated with other buprestid species have been determined by ecologists (Evans et al. 2007; Vuts et al. 2016). The destruction of the transition zone of intensive nutrient and water of the tree which is triggered by special arthropods occurs through the spectacular withering of the branch or other parts of the trunk (Rabl et al. 2017; Volkovitsh & Karpun 2017). According to the study of Jendek et al. (2018), the infested tree perishes within 1–3 years. *L. festiva* is frequently accompanied by longhorn *Semanotus russicus* (Fabricius, 1776) (Col.: Cerambycidae) with similar destructive efficiency. Unlike the shallow galleries of *L. festiva*, the tunnels bored by larva of *S. russicus* lies distinctly deeper in the wood.

Based on our results, it can be ascertained, that the larvae of *L. festiva* can develop in branches of various diameters, but we could not explore them in branches less than 3 cm in diameter (Volkovitsh et al. 2019). This observation is in agreement with the results of Jendek et al. (2018), according to whom the diameter of the injured parts of the tree are always larger than 2 cm. It follows that the juvenile, thin branches cannot provide the optimal conditions for the developing larvae.

The larvae keep certain distances from each other because of the egg-laying ethology and the intraspecific competition of the species (Ruicănescu & Stoica 2019). The explanation for this observation is the maintenance of the quality of adequate living space and nutrient content necessary for larval development.

The main benefit of our imaging method is that it allows for a detailed study of the developmental stages of the hidden-developing insects living in plant tissues. The *in vitro* laboratory conditions create an opportunity for the observation of fine biological details, which are to be compromised when employing traditional diagnostic methods such as plant tissue dissection (Gibb 2014). Moreover, dissection-based methods pose stress conditions for the target organisms which can make it impossible to assess the natural conditions. Consequently, in certain cases the data acquired by traditional diagnostic methods may lead to drawing false biological and ecological conclusions as opposed to employing non-destructive imaging techniques.

The additional advantage of CT imaging is that it permits hidden pest-identification based on the predetermined pest-specific damaging charac-

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ters (e.g. volume, position of cavity) in an indirect way. Its future application paves the way for early detection of damage triggered by organisms in the case of seedling trees (due to their sizes) raised in nurseries.

In summary, our results stemming from employing a non-invasive approach provide new information to the biological data and the damaging properties of this invasive buprestid species, which can contribute to a better understanding of the extent of the damage, as well as to the realisation of integrated, cross-border protection efforts against *L. festiva*.

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