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Outbreak of *Orthotomicus erosus* (Coleoptera, Curculionidae) on Aleppo Pine in the Mediterranean Region in Croatia

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ABSTRACT

Background and Purpose: *Orthotomicus erosus*, Mediterranean pine engraver, is widely distributed across the Mediterranean and southern Europe, Asia and North Africa. It is considered as secondary pest found on recently dead or felled trees, but can also attack weakened living trees. In high population levels this species can attack healthy trees and cause their dieback. Severe outbreaks occur after dry periods, or after fire in adjoining stands in warmer parts of the Mediterranean region, while this scenario has never happened in Croatia up to now. Bark beetles are important forest pests which have already been researched and discussed in relation to climate change, indicating that the predicted increase in temperature would lead to higher survival rates and faster development, thus directly influencing their population dynamics. Increase in temperature may stimulate changes in insects' rate of development, voltinism, population density, size, genetic composition, extent of host plant exploitation, longitudinal and latitudinal distribution. Since climate conditions might have changed in the last few years as predicted in the Mediterranean region, the aim of our research is to document the first outbreak with high population levels of *O. erosus* in Croatia.

Materials and Methods: The extent of dieback was evaluated by counting trees with dieback symptoms on diagonal transects plotted through each of 33 forest management sections of Marjan Forest Park (Split). Trunk sections from several trees with early stage symptoms were collected for further laboratory analysis, which consisted of incubation phase and subsequent morphological identification. During regular yearly surveys in forests of Croatia, the pest was observed on several sites and damages were recorded for both years 2017 and 2018. The records were entered into a map using QGIS version 3.2.1-Bonn. Spatial data was downloaded from DIVA-GIS server. Monitoring efforts were initiated in affected areas where 13 flight barrier pheromone traps (Theyson®) equipped with pheromone lure Erosowit® (Witasek, Austria) were set-up in late March in state-owned and privately owned forests across Dalmatia. Catches in the traps were collected and *O. erosus* adults were counted on a weekly basis in order to identify the abundance of the pest in monitored sites, as well as to obtain the first information about population dynamics and to assess voltinism.

Results: On-site survey and the evaluation of dieback extent included sampling of 5% of all trees in Marjan Forest Park ,and the results showed that 23% of all trees in the forest park were affected by dieback symptoms. Visual examination of trunks, branches and bark showed symptoms of bark beetle infestation, while preliminary on-site examination of the observed adults pointed out to *O. erosus*. After two weeks in controlled conditions, bark beetle adults started to emerge from trunk sections which were placed in several mesh cages for incubation. Morphological identification by using stereomicroscope and the key for European bark beetles resulted in identification of *O. erosus* species. Over the course of the year 2017 one more site was reported to be infested with *O. erosus*, and eight additional sites were reported over the course of year 2018. In total, 446 ha were reported as infested, varying in intensity, in several different management units of state-owned and privately owned forests. The total number of trapped beetles in pheromone traps varied largely among sites. Our data indicate that several generations (at least 5 generations per year) were present in the year 2018.

Conclusions: Sudden surge in observed damages, as well as the number of beetles trapped during monitoring, in years 2017 and 2018 throughout Aleppo pine forests in Dalmatia are the first record of *O. erosus* outbreak in Croatia. *O. erosus* is native to Croatia and so far it has been considered only as a minor pest whose outbreaks have never been recorded.

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Drought intensity and frequency and aridification trends in the research area (Dalmatia, Croatia) cause cumulative stress to trees and have increased *O. erosus* occurrence. *O. erosus* is expected to exhibit increased voltinism, better overwintering performance and earlier spring flights. Our first results confirm this epidemic stage of *O. erosus* with high abundances in Dalmatia in 2018 and at least 5 generations per year, which alter the population level of this pest. Finally, with high dispersal abilities of *O. erosus* through active flight and easy transportation with infested material (logs and branches with bark), *O. erosus* has the potential to become an important forest pest in Croatia. Thus, extensive studies on its biology, ecology, natural enemies and interaction with ophiostomatoid fungal species are needed in order to predict further spread and suggest viable and effective management measures.

Keywords: Mediterranean pine engraver, climate change, *Pinus halepensis*, *Pityogenes calcaratus, Tomicus destruens*

INTRODUCTION

Bark beetles (Coleoptera; Curculionidae; Scolytinae) have been documented as a relevant ecological and economic factor in ecosystem functioning and their activity can indicate the health condition and vitality of forests [1]. They are also very important forest pests that can cause serious dieback of forests on huge areas [2-4] and enormous wood volume loss [5]. Out of many species only a few are able of causing tree dieback, and usually they have fluctuations from low abundance for lengthy periods when they are limited to stressed trees [6]. However, their populations can rise suddenly and spread over large areas, sometimes killing huge numbers of healthy trees as well.

There are 42 bark beetle species recorded living on Mediterranean conifers and usually they have been registered on weakened trees only [1, 7]. According to Pfeffer [8] and Knižek [9], 11 bark beetle species have been listed for Croatia, developing on Aleppo pine trees (*Pinus halepensis* Mill.), out of which only 3 species have the potential to behave like a pest (*Tomicus destruens* Woll., *Orthotomicus erosus* Woll. and *Pityogenes calcaratus* Eich.).

Orthotomicus erosus, Mediterranean pine engraver, is widely distributed across the Mediterranean and southern Europe, Asia and North Africa. It has been introduced to Fiji, South Africa, Swaziland [10] and the USA [11]. It can occur in association with other bark beetle species (*Pityogenes calcaratus, Tomicus destruens* [12]) and in symbiotic relationship with blue stain fungi (Ophiostomatidae) [13]. It is considered as a secondary pest found on recently dead or felled trees, but can also attack weakened living trees (e.g. under drought stress). In high population levels this species can attack healthy trees and cause their dieback [14]. Severe outbreaks occur after dry periods, or after fire in adjoining stands in warmer parts of the Mediterranean region [15, 16].

Mediterranean forests are one of the world's biodiversity hotspots characterized by a variety of environmental conditions with an array of plant and animal species diversity. As such, they represent a major socio-economic component for tourists that visit the Mediterranean every year, with their unique biological richness and multiple values [17]. However, today they are under severe threat from climate change and it is predicted that Mediterranean forest ecosystems will suffer from its impacts by 2050 [17]. Mediterranean forests composed mostly of drought-tolerant Mediterranean oak species [18] have been over-used for their multiple resources. Huge amount (75%) of these ecosystems has been destroyed by humans already in the 19th century [19]. Aleppo pine has been widely planted in reforestation of the degraded areas as a fast-growing pioneer species. First reforestation efforts in Croatia were documented in 18th century [20]. Aleppo pine was chosen as the most suitable species because of its drought tolerance [18] and it was widely distributed on the coast of Croatia [21]. Today, Aleppo pine plays an important role in edaphic preparation for other native species (e.g. *Quercus ilex* L.) [22]. This pine species is also very important in preservation of multiple environmental services provided by Mediterranean forests (e.g. carbon sequestration, biodiversity, landscape quality, soil and water protection, landscape and recreational role). However, this widely growing species is endangered by prolonged drought periods that cause water deficit and weaken the trees, as well as by forest fires, pests and diseases [23-25]. New examples of climate change induced pest occurrences give rise to special concerns since these changes could have long-term effects on the vitality and health status of Aleppo pine forests in the coastal region of Croatia [25, 26]. Bark beetles have already been researched and discussed in relation to climate change, indicating that predicted increase in temperature would lead to higher survival rates and faster development, thus directly influencing their population dynamics [27].

Climate change could indirectly negatively influence forest ecosystems through range expansion and changing of seasonal phenology of insect pests, resulting in faster development and higher feeding rates of phytophagous insects [27]. This negative influence is likely to be accelerated with increased temperatures and frequency and intensity of droughts with extended growth period of vegetation predicted for Mediterranean forests [28]. Other negative influences of climate change on forests might be seen in physiological changes in tree defense mechanisms and indirect effects through changes in abundances of natural enemies and competitors [29]. Temperature may stimulate changes in insects' rate of development, voltinism, population density, size, genetic composition, extent of host plant exploitation, longitudinal and latitudinal distribution [14, 30].

Among bark beetles, *O. erosus* is considered as a secondary pest which infests already fallen and/or stressed trees [31]. Attacks of *O. erosus* and *P. calcaratus* have already been recorded in warmer Mediterranean regions such as Israel [32], Iran [33], Morocco [34], Turkey [35] and Tunis [36]. According to written records, this species has not yet been observed in Croatia [25, 37]. When it comes to bark beetles in the Mediteranean part of Croatia, only very local outbreaks of *T. piniperda* L. or *T. destruens* have been recorded [24, 38, 39]. However, it was predicted that climate change increases

the possibility that the outbreaks will spread and increase in frequency, and that Mediterranean forests could become more vulnerable to their attacks than temperate and boreal regions [1]. In outbreaks of pests correlated to climatic change outside their historical ranges, northwards spreading or elevation expanding have already been well documented [e.g. 36, 41-42].

According to Lieutier *et al.* [1], *O. erosus* infesting Aleppo pine may develop up to 4 generations per year or even up to 7 generations [15, 40], while in Croatia only 2 to 3 generations have been described [43]. Males of *O. erosus* bore through the bark into the cambium where they mate with several females. Females construct egg galleries and can oviposit up to 75 eggs in the niches along the galleries [12]. Larvae pass through three instars and gnaw their galleries perpendicularly to maternal ones. The adults perform maturation feeding beneath the still moist bark and if the bark is too dry, they move to another tree [12]. Insect overwinters as an adult and beetles fly in spring at temperatures of 12-14 °C [15, 41]. The beetles choose rough-barked tree trunk and branches larger than 5 cm in diameter for breeding. Smooth barked sections of the trunk are used for maturation feeding, while lower trunk parts of old trees with too thick bark are not suitable for attack [12].

Considering that climate conditions for multiple generations of *O. erosus* described by Mendel [15, 40] were not fulfilled could be the reason why outbreaks of this insect have never been documented in Croatia [24]. Though, these conditions might have changed in the last few years as predicted [28].

The aim of our research is to document the first outbreak with high population levels of *O. erosus* in Croatia.

MATERIALS AND METHODS

First Record of Symptoms and Identification of the Pest

In 2017, unusual wilting and drying of Aleppo pine trees was reported in Marjan Forest Park, Split (43°30´33˝N; 16°24´38˝E). The report triggered intensive on-site survey in order to determine the extent and cause(s) of dieback of Aleppo pine trees. The extent of dieback was evaluated by counting trees with dieback symptoms on the diagonal transects plotted through each of 33 forest management sections of the forest park. The identification of dieback causative agent was performed by visual examination of trunks and branches (Figure 1). Trunk sections from several trees with early stage symptoms were collected for further laboratory analysis. Samples were taken to entomological laboratory of Croatian Forest Research Institute, Jastrebarsko, Croatia.

Laboratory analysis consisted of incubation phase and subsequent morphological identification. Incubation was performed by distributing trunk sections (collected during field survey) into mesh cages and subjecting them to controlled temperature of 20(±2)°C and L:D=16:8. Trunk sections were sprayed with distilled water across the entire surface once per day in order to maintain bark moisture. Upon emergence, adult beetles were collected from mesh cages and morphologically identified. Identification was performed by visual examination, using stereoscopic light microscope Olympus SZX7 and the key for bark beetle identification [8, 9].

Localities and Years of Record

Similar symptoms were observed also in other Aleppo pine forests in Dalmatia, and on-site training was organized

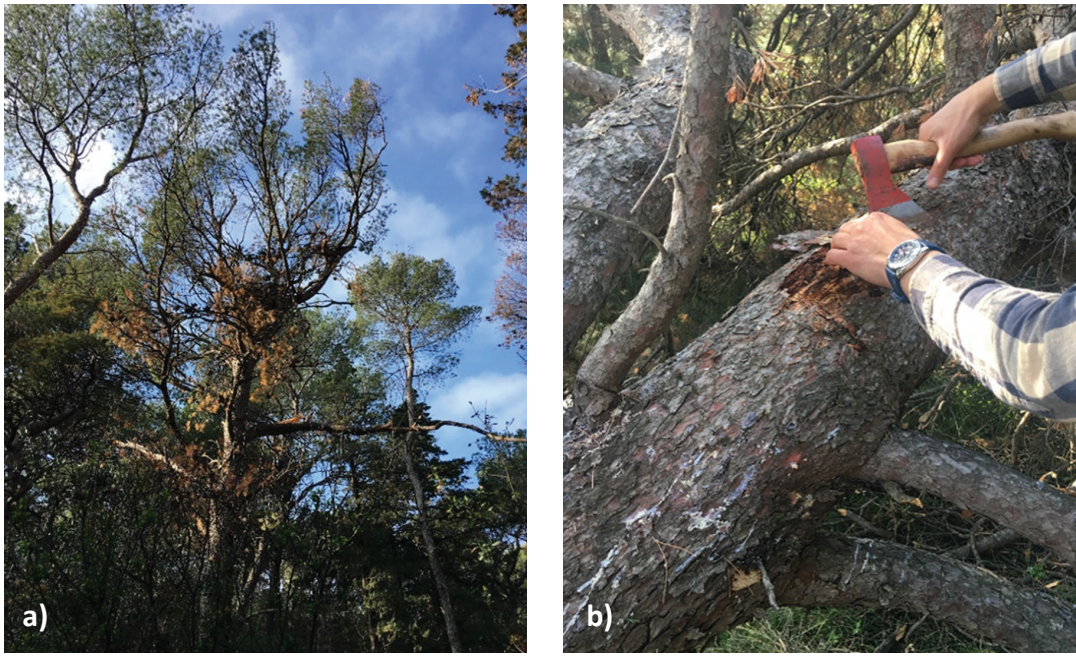

FIGURE 1. a) Green crown of freshly infested Aleppo pine tree; **b)** Examination of the trunk and branches for infestation symptoms.

in the summer of 2017 in Biograd Forestry Office in order to familiarize regional foresters with new threats posed by the potential outbreak of *O. erosus* and to teach them how to identify damages caused by this bark beetle. During regular yearly surveys in forests of Croatia, the pest was observed on several sites and damages were recorded for both years 2017 and 2018. The records were entered into a map using QGIS version 3.2.1-Bonn. Spatial data was downloaded from DIVA-GIS server.

Monitoring of *O. erosus* in Dalmatia

After surprising record of damage done by *O. erosus* in 2017, monitoring efforts have been initiated. Thirteen flight barrier pheromone traps (Theyson®) equipped with pheromone lure Erosowit® (Witasek, Austria) were set up in late March in state-owned and privately owned forests across Dalmatia. Catches in the traps were collected and *O. erosus* adults were counted on a weekly basis in order to identify the abundance of the pest in monitored sites, as well as to obtain the first information about population dynamics and to assess voltinism.

RESULTS

First Report of Symptoms and Identification of the Pest

On-site survey and evaluation of dieback extent included sampling of 5% of all trees in Marjan Forest Park , and the results showed that 23% of all trees in the forest park were affected by dieback symptoms by the end of 2018. Visual examination of trunks, branches and bark showed symptoms of bark beetle infestation, while preliminary on-site examination of the observed adults pointed out to *O. erosus* (Figure 2).

After two weeks in controlled conditions, bark beetle adults started to emerge from trunk sections which were placed in 10 mesh cages for incubation. Morphological identification by using stereomicroscope and the key for European bark beetles resulted in positive identification of the species as *O. erosus*.

Localities and Years of Record

Over the course of the year 2017, aside from Marjan, one more site was reported to be infested with *O. erosus*, and eight additional sites were reported over the course of the year 2018 (Figure 3). In total, 446 ha and about 9.000 m^3 of wood mass were reported as infested, varying in intensity, in several different management units of state-owned and privately owned forests in Šibenik-Knin County in 2018 (Table 1).

Monitoring of *O. erosus* in Dalmatia

Pheromone traps for monitoring the abundance of *O. erosus* in Aleppo pine forests in Dalmatia were set up on 13 sites in total (both in state-owned and privately owned forests). The total number of trapped beetles varied largely among sites (from 21,442 specimens to 140,448 specimens trapped in Šibenik) and fluctuated substantially over the course of the year (Figure 4 and 5). Our data indicate that several generations (at least 5 generations per year) were present in the year 2018. These data need further validation in the years that follow.

FIGURE 2. *O. erosus* bark beetle, observed on the bark of Aleppo pines in Marjan Forest Park: **a)** Bark beetle galleries and **b)** Adult stage of beetles.

TABLE 1. Area and wood mass infested by *O. erosus* reported for private, protected and state-managed forests in 2018.

Forest Office	Forestry	Management unit	WGS coordinates		Infestation		
			N	E	Area (ha)	Wood mass (m^3)	Intensity (%)
Private forests	Šibenik-Knin County	Šibenske šume	43.7448	15.9695	10.5	n/a	1-20
State-protected forests	Split	Marjan	43.5139	16.4092	196.0	5,900	21-40
	Korčula	Ošjak	42.9606	16.6794	18.0	1,800	21-40
	Makarska	Osejava	43.2886	17.0138	70.0	150	1-20
	Dubrovnik	Lokrum	42.6276	18.1112	70.0	100	1-20
State-managed forests Split	Biograd	Biograd	43.9308	15.4802	25.0	400	21-40
		Pašman-Vrgada	43.9540	15.3494	31.0	250	41-60
		Turanj	43.9978	15.4266	3.0	25	1-20
	Korčula	Šaknja Rat	42.9438	16.7932	17.5	200	1-20
	Zadar	Nin-Kožino	44.1996	15.2144	2.0	10	1-20
		Starigrad	44.3499	15.3502	3.0	20	1-20
Totals					**446.0**	**8855.0**	**1-60**

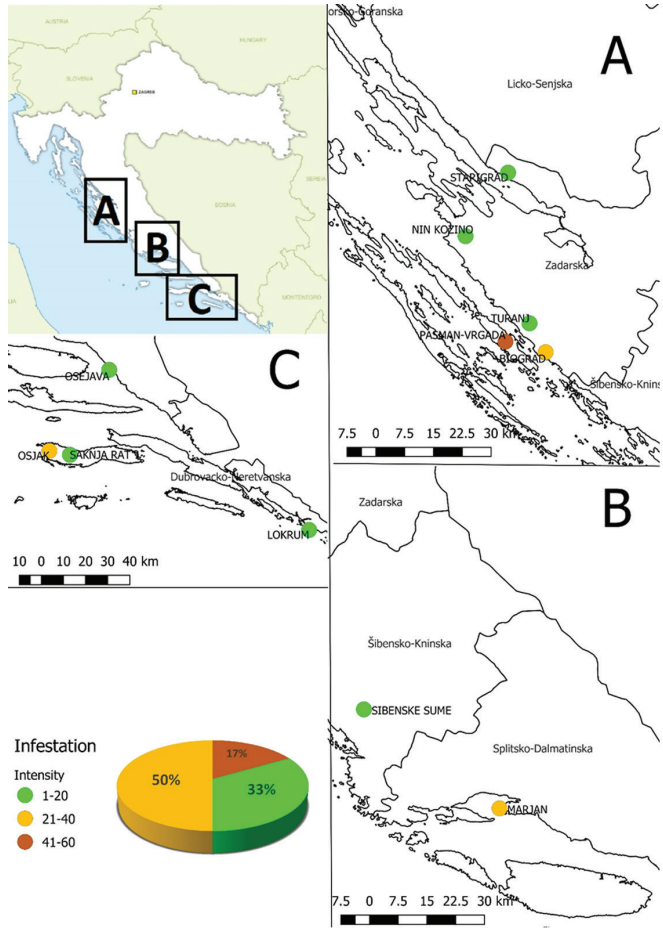

FIGURE 3. Map of management units reported as infested by *O. erosus* in 2018. Coloration according to the reported infestation intensity. The pie chart represents area ratios of the reported infestation intensities.

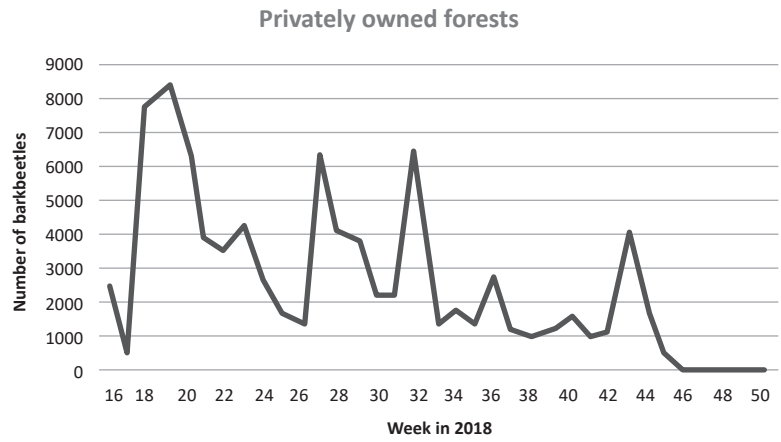

FIGURE 4. Weekly distribution of the total number of trapped *O. erosus* beetles per trap in 2018 in privately owned forests.

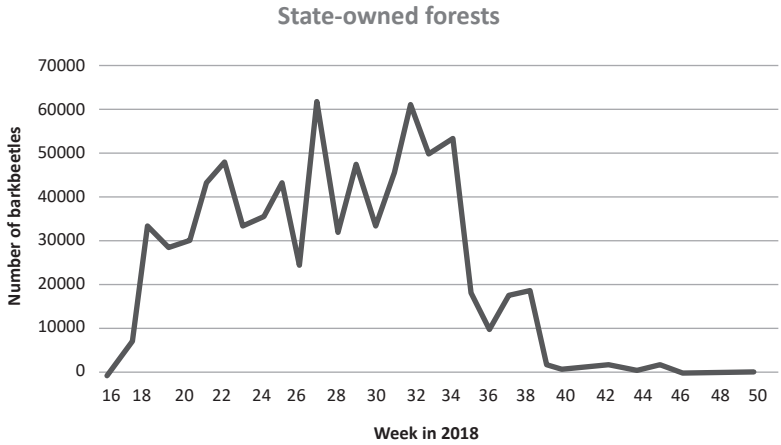

FIGURE 5. Weekly distribution of the average number of trapped *O. erosus* beetles per trap in 2018 in state-owned forests.

DISCUSSION

Sudden surge in the observed damages, as well as the number of beetles trapped during monitoring, in years 2017 and 2018 throughout Aleppo pine forests in Dalmatia, as presented in this paper, is the first record of *O. erosus* outbreak in Croatia. *O. erosus* is native to Croatia and so far it has been considered only as a minor pest and its outbreaks have never been recorded [23, 24, 39, 43, 44]. Thus, the outbreak came as a surprise, rising questions about causes of this phenomenon, predictions of further expansions, as well as the availability and efficacy of control measures. Drought intensity and frequency have increased in the region of *O. erosus* occurrence (Dalmatia, Croatia) and aridification trends cause cumulative stress to trees [43]. More evidence shows that population dynamics of forest insects have already been affected by climate change [14, 26, 44, 46, 47]. Temperature is the primary climatic factor affecting insects since they are poikilothermic organisms and respond quickly to temperature changes [29]. The responses manifest through changes in population density and distribution range of the affected species [48]. Generally, in mid-latitude regions temperature increases associated with climatic changes could result in changes of beetle ecology, such as extension of geographical range, increased overwintering, increased number of generations, extension of development season and so on [49]. It seems to be very important that the beetles jointly overcome tree defense mechanisms, and temperature positively influences such synchronization [50]. Furthermore, reduced production of active defenses could reduce tree resistance to bark beetle attacks [51].

According to IPCC [28], intensity, duration and frequency of drought have increased in semi-arid areas, e.g. in the Mediterranean region, which manifests as aridification process capable to trigger forest diebacks [52]. Trees stressed by aridification processes are becoming more prone to insect infestations, which leads to an increase of injury and eventually to tree death [53, 54]. On the other

hand, longitudinal and altitudinal range shifts caused by climate change have already been documented for various species of forest insects (as reviewed in [14]). Furthermore, temperature increase has been predicted to influence the distribution of Mediterranean species of bark beetles (e.g. *T. destruens*), enabling them to spread northwards and feed on new hosts in new areas [54].

O. erosus can establish up to 7 generations per year [40] and it exhibits accelerated development at high temperatures with no thermic limitations for mass production, which enables it to pass from endemic to epidemic stage within months [12, 15, 40]. With predictions for temperature change in the Mediterranean region towards warmer winters and warmer and drier summers [14, 28], *O. erosus* is expected to exhibit increased voltinism, better overwintering performance and earlier spring flights. This might probably ultimately increase its population size, resulting in more severe and widespread damages as reported for other bark beetle species [56, 57]. Our first results confirm this epidemic stage of *O. erosus* with high abundances in Dalmatia in 2018 and at least 5 generations per year, which alter the population significance of this pest.

It is very hard to discriminate direct from indirect events of climate change [30], which is why the overall impact of all interacting factors should be considered. With this in mind, natural enemies are also influenced by climate change as the host and parasitoid synchronization may be disrupted under altered climate conditions [27]. Unexpected events, e.g. storms which are more frequent and are predicted to increase (as reviewed in [14]), can provide an abundant source of fresh food for bark beetles, increasing the build-up of populations. High availability of food source (stressed trees, freshly felled trees) together with increased voltinism can lead to permanently high bark beetle populations that are able to attack healthy trees, enabling them to become primary pests [58].

Furthermore, *O. erosus* is associated with several ophiostomatoid species [13, 59]. Some ophiostomatoid fungal species in association with bark beetles show increased virulence and it is assumed that they help their vectors, bark beetles, to break the defensive mechanisms of the host plant. This is a successful strategy for weakening the host plant and plays an important role in the attack strategy of bark beetles [60, 61]. How and to what extent could the temperature increase affect fungal species remains unknown, making this issue one of the questions that should be addressed in future studies. Our first preliminary research has shown several species of ophiostomatoid fungal species (Pernek and Matek, unpublished data) isolated from samples taken from the same localities shown in Table 1.

Finally, with high dispersal abilities of *O. erosus* through active flight and easy transportation with infested material (logs and branches with bark), *O. erosus* has the potential to become a serious forest pest in Croatia. Thus, extensive studies on its biology, ecology, natural enemies and interaction with ophiostomatoid fungi are needed in order to predict its further spread and to suggest viable and effective management measures.

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