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DENDROCTONUS MICANS (KUGELANN, 1794) ESTABLISHMENT ON HOST: PARENTAL FEMALES ATTACK VIGOROUS ORIENTAL SPRUCE TREES IN ARTVIN, TURKEY

POJAVA ŽENKI VELIKOG SMREKINOG LIKOTOČA *DENDROCTONUS MICANS* (KUGELANN, 1794) NA VITALNIM STABLIMA KAVKASKE SMREKE U ARTVINU, TURSKA

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SUMMARY

Dendroctonus micans (Kugelann, 1794), which was first discovered in 1966 in Turkey, has established almost in all oriental spruce forests in the Eastern Black Sea region until the late 2000s. In its expanding front it is responsible for killing spruce trees representing millions of cubic meters of wood. In recent years, oriental spruce forests have endemic population of this pest. But extreme climatic conditions that cause extreme weather circumstances may trigger suitable environment that favors *D. micans* outbreaks. In this study, we aimed to examine tree vitality of naturally infested and uninfested trees in the forest. Field studies were performed at a pure spruce stand in Taşlıca Forest Sub-District, Artvin Directorate of Forestry in 2016. Both infested and uninfested trees were selected in the stand closure. One core per tree was extracted from 30 naturally infested and 30 uninfested oriental spruce trees at the same stand. Core samples were taken at the second week of September. Phloem thickness, recent tree growth rates, diameter of breast height and the average number of xylem cells in a radial file formed until the sampling date were studied. Phloem thickness, recent tree growth rates and number of xylem cells were higher in infested trees. Host selection of *D. micans* was discussed in relation to characteristics of infested and uninfested trees.

KEY WORDS: Tree vitality, growth and increment, *Picea orientalis*, phloem thickness, host selection

INTRODUCTION
UVOD

The appearance of bark beetles date back 280 million years (Ross, 1965). Conifer-inhabiting bark beetle species that infest and kill apparently healthy standing trees are the most economically important forest insects. The Greater European Spruce Bark Beetle, *Dendroctonus micans* (Kugelann, 1794), with the spruce bark beetle *Ips typographus* (Linnaeus, 1758) (Coleoptera: Curculionidae, Scolytinae) are the most economically important forest pests in their distribution area in Eurasia (Grégoire, 1984, 1985,1988; Wermelinger, 2004; Özcan et al., 2011a; Alkan Akıncı et al., 2014). *D. micans* has caused serious damages and tree deaths during outbreaks in spruce forests since the first half of the 19th century to date (Grégoire, 1988; Özcan et al., 2011b; Fraser et al., 2014; Alkan Akıncı et al., 2018; Büyükterzi et al., 2022). It attacks apparently healthy trees besides forked, wounded and overthrown spruce trees (Granet and Perrot, 1977; Grégoire, 1988; Alkan Akıncı et al., 2014).

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During *D. micans* swarming period solitary females infest trees and construct egg galleries (Grégoire, 1985, 1988). Wounded trees, forked trees and trees at the stand edges are the most infested individuals in the forest (Granet and Perrot, 1977; Alkan Akıncı, 2017). Successful establishment of the beetles to trees with wounds are documented in oriental spruce forests (Alkan Akıncı et al., 2014). In a stand, there may be uninfested trees besides trees with multiple attacks. Also, some trees may have a number of successful and unsuccessful attacks together on their trunk (Bevan and King, 1983; Grégoire, 1984, 1985; Wainhouse et al., 1998). The lack of aggregation pheromone results in solitary attacks on trees but there are also high number of attacks reported by Turkish researchers (Eroğlu, 1995; Alkan Akıncı et al., 2014). Beetles mainly infest root collars (Grégoire, 1984). Almost 70% of the entrances are below 1 m at the trunk of the trees in Turkey. The percentage of the aborted attacks is 25% (Alkan Akıncı et al., 2014). The percentage of the aborted attacks is higher in Europe; in Belgium it is 70% (72-78%) (Grégoire, 1984). Radial and vertical resin ducts are cut by females during the construction of maternal galleries that cause resin flow, and consequently result in aborted attacks by beetles (Lieutier et al., 1992; Lieutier, 2007). *D. micans* attacks do not kill its host in a short period of time so infested trees are alive throughout different beetle generations (Grégoire, 1988).

In various studies, trees are artificially forced to induce susceptibility to bark beetles (Moeck et al., 1981; Coulson et al., 1986; Miller and Berryman, 1986; Storer and Speight, 1996; Alkan Akıncı, 2016). Freezing the base of the host trees (Moeck et al., 1981), damaging trees by artificial lightning strikes (Coulson et al., 1986) and artificial mechanical wounds (Miller and Berryman, 1986; Storer and Speight, 1996; Alkan Akıncı, 2016) attracted bark beetles on these trees mostly followed by tree death or successful colonization by the bark beetles.

Some tree parameters affect bark beetle brood survival or reproductive success. Wagner et al. (1979) presents data that bark moisture content affects brood survival of *Dendroctonus frontalis*. Stressed trees such as windthrowns, wounded trees or trees at stand edges have lower host defenses and greater nutritional quality (Reid and Robb, 1999; Alkan Akıncı et al., 2018). When mean annual increment in the last year, phloem thickness and several parameters of recent tree growth rate were studied on freshly dead *Pinus banksiana* to assess the reproductive performance of *Ips pini*, the results showed that tree growth rate parameters were positively related to beetle reproductive performance (Reid and Robb, 1999). Relationships between *D. micans* successful establishment and survival in its host are investigated experimentally in some studies (Alkan Akıncı and Erşen Bak, 2016; Alkan Akıncı et al., 2018). The role of phloem thickness, tree size and recent tree growth rate were used as tree vigor parameters in the study that parental *D. micans* females were inserted on healthy oriental spruce trees. Parental females were established successfully mostly on thicker phloem and trees that are growing vigorously (Alkan Akıncı and Erşen Bak, 2016). In another experimental study again parental *D. micans* females were inserted on healthy oriental spruce trees. In the study, periodic growth, phloem thickness, tree size, crown length, phloem moisture and amount of C, N in phloem have been compared between trees that *D. micans* females successfully established in the host tree or aborted. Results demonstrated that *D. micans* parental females successfully established on the codominant trees that have grown vigorously and have decreasing growth in the last 10 years, shorter crown length and higher N in the phloem (Alkan Akıncı et al., 2018).

In this study, oriental spruce trees that were naturally infested by *D. micans*, and uninfested trees in the forest are investigated. Phloem thickness, recent tree growth rates (the mean annual increment in the past five and ten years, and annual increment in the last year until the sampling date, diameter of breast height (DBH) and the average number of xylem cells (tracheids) in a radial file formed in the last year were studied to figure out tree characteristics of naturally infested and uninfested trees in the forest. We have tried to reveal the nature of the *D. micans* attacks and the characteristics of the naturally infested trees. In addition, we would like to compare our results with the results of the experimental studies that were formerly performed on oriental spruce.

MATERIALS AND METHODS
MATERIJALI I METODE

Field studies were performed at a naturally regenerated pure spruce stand that was at 1683 m a.s.l. and in southwest aspect in Taşlıca Forest Sub-District, Artvin Directorate of Forestry in 2016. In the stand examined, the estimated tree age was 90, the tree heights were about 20 m, slope was 3% and crown closure was about 70%. The stand is located in the Eastern Black Sea Mountains that has a rainy and foggy climate during whole year and also snowy winters. Both naturally infested and uninfested trees were selected in the stand closure. In the infested trees, *D. micans* was established and broods were developed under the bark. The broods were at the larval or pupal stage or there were young adults under the bark.

Measurements – *Mjerenja*

To determine the vitality of the naturally infested trees, we designated 30 naturally infested and 30 uninfested oriental spruce trees at the same stand in the field. Diameter of the breast height (DBH) was 33.20 cm ± 0.57 and 33.77 cm ± 0.62 at infested and uninfested trees, respectively. On each tree, cores were extracted. Annual growth ring widths were

Table 1. Values of the measured characteristics at the sampled infested trees
Tablica 1. Vrijednosti izmjerenih značajki na uzorkovanim napadnutim stablima

No. of trees *Broj stabala*	DBH *Prsni promjer stabla* (cm)	Phloem thickness *Debljina floema* (mm)	MAI-5 *Srednji godišnji prirast u zadnjih pet godina* (mm)	MAI-10 *Srednji godišnji prirast u zadnjih deset godina* (mm)	PGR *Omjer periodičnog rasta*	Annual increment in the last year *Godišnji prirast u posljednjoj godini* (mm)	Number of xylem cells Broj stanica ksilema
1	33	6.47	0.76	1.47	0.29	1.46	16
2	35	4.53	1.57	1.57	1.00	1.53	51
3	34	5.13	0.91	1.01	0.82	1.16	39
4	38	3.40	2.75	2.75	1.00	3.40	112
5	38	3.33	0.40	0.35	1.30	2.43	13
6	26	5.37	1.02	1.03	0.98	0.74	25
7	35	3.77	0.78	0.72	1.19	0.79	35
8	32	3.53	1.21	1.20	1.05	1.16	46
9	36	8.80	1.40	1.32	1.24	1.33	50
10	29	7.33	1.71	1.71	1.00	1.66	51
11	32	4.17	1.83	1.72	1.13	1.87	66
12	32	3.00	0.61	0.63	0.94	1,37	13
13	34	5.29	1.13	1.22	0.87	0.96	24
14	31	6.21	3.36	3.54	0.90	2.42	79
15	35	2.63	1.92	1.91	1.01	1.37	47
16	39	3.68	2.11	2.02	1.09	1.89	47
17	36	1.63	1.62	1.52	1.14	1.63	51
18	34	4.74	2.65	1.91	2.27	2.89	85
19	36	2.37	1.82	1.68	1.18	2.26	76
20	30	2.83	0.92	0.99	0.86	0.88	35
21	30	1.95	1.09	1.06	1.07	1.37	51
22	34	3.95	2.92	3.06	0.91	2.63	88
23	30	3.00	1.60	1.68	0.90	1.53	58
24	37	2.21	0.98	0.95	1.03	1.16	40
25	36	5.33	0.20	0.32	0.46	0.24	13
26	31	3.37	0.60	0.68	0.78	0.57	30
27	29	2.16	0.91	0.92	0.99	0.77	24
28	30	6.32	0.60	0.95	0.46	0.68	29
29	33	5.16	1.65	1.56	1.13	1.42	48
30	31	4.00	3.01	2.71	1.25	1.37	46

measured in the last ten years to provide increment for the last five (MAI-5) and ten years (MAI-10) (Reid and Robb, 1999). Mean annual increment in the past five years divided by the mean annual increment of the preceding five years to determine periodic growth ratio (PGR) (Mahoney, 1978; Reid and Robb, 1999; Wainhouse, 2005). The number of xylem cells (tracheids) in a radial file formed in the last year until the sampling date were studied on cross sections. Phloem thickness was measured on core samples. Leica M60 stereomicroscope was used to make measurements and Olympus BX53 light microscope to take photographs. Values of the measured characteristics are presented in Table 1-2.

Analysis – *Analiza*

Data were analyzed using IBM SPSS statistics version 19.0. Measured variables were compared between naturally infested and uninfested trees by t test. Standard error of mean (SEM) and minimum and maximum values were given with means.

RESULTS
REZULTATI

Phloem thickness, MAI-5, MAI-10, mean annual increment in the last year until the sampling date and number of xylem cells were higher in infested trees (Table 3).

Phloem thickness was 4.19 mm ± 0.31 (1.63 – 8.80 mm) and 3.01 mm ± 0.23 (1.43 – 7.07 mm) in infested (Figure 1d) and uninfested trees, respectively. The difference between infested and uninfested trees was significant (t test, $t = 3.071$, $df = 58$, $p < 0.05$).

MAI-5 and MAI-10 was 1.47 mm ± 0.15 (0.20 – 3.36 mm) and 1.47 mm ± 0.14 (0.32 – 3.54 mm) in infested, and 1.08

Table 2. Values of the measured characteristics at the sampled uninfested trees
Tablica 2. Vrijednosti izmjerenih značajki na uzorkovanim nenapadnutim stablima

No. of trees *Broj stabala*	DBH *Prsni promjer stabla* (cm)	Phloem thickness *Debljina floema* (mm)	MAI-5 *Srednji godišnji prirast u zadnjih pet godina* (mm)	MAI-10 *Srednji godišnji prirast u zadnjih deset godina* (mm)	PGR *Omjer periodičnog rasta*	Annual increment in the last year *Godišnji prirast u posljednjoj godini* (mm)	Number of xylem cells *Broj stanica ksilema*
31	30	3.00	1.33	1.15	1.16	1.60	49
32	30	3.07	0.27	0.42	0.46	0.57	17
33	32	4.58	0.61	0.68	0.80	0.71	22
34	33	4.17	0.55	0.46	1.50	0.50	24
35	38	5.46	1.64	1.55	1.23	2.27	79
36	28	3.17	0.37	0.32	1.37	1.50	17
37	31	2.67	0.80	0.81	0.98	0.75	24
38	38	7.07	1.24	1.34	0.82	0.92	33
39	37	3.94	1.03	0.94	1.20	1.20	32
40	33	3.04	0.63	0.68	0.88	0.54	17
41	30	3.21	3.26	2.61	1.67	2.42	88
42	34	1.57	1.23	1.08	1.33	1.23	38
43	32	1.87	1.39	1.34	1.08	1.17	49
44	35	1.43	0.93	0.90	1.05	0.73	34
45	28	2.08	2.05	2.04	1.01	2.04	77
46	38	3.16	0.34	0.38	0.81	0.34	17
47	35	2.03	0.90	0.85	1.12	0.89	35
48	34	2.27	1.80	1.93	0.87	1.17	39
49	38	3.16	1.93	1.47	1.91	1.04	67
50	36	1.58	1.20	0.84	2.53	1.16	37
51	36	2.42	0.46	0.55	0.71	0.46	19
52	34	2.50	1.38	1.36	1.03	1.09	44
53	39	2.50	1.28	1.26	1.03	1.57	55
54	30	3.13	1.07	1.21	0.79	0.83	31
55	37	2.92	1.77	1.89	0.88	1.02	68
56	38	1.54	0.76	0.84	0.83	0.58	20
57	37	2.13	0.22	0.25	0.79	0.16	11
58	29	2.17	0.33	0.36	0.84	0.30	9
59	31	4.17	0.69	0.75	0.86	0.67	27
60	32	4.17	0.93	0.88	0.86	0.80	21

Table 3. Mean ± SEM values of characters measured on infested and uninfested trees
Tablica 3. Srednje ± SEM vrijednosti značajki izmjerenih na napadnutim i nenapadnutim stablima

Measured characters *Izmjerene značajke*	Infested trees *Napadnuta stabla*	Uninfested trees *Nenapadnuta stabla*	*p*
Phloem thickness (mm) *Debljina floema (mm)*	4.19 ± 0.31	3.01 ± 0.23	0.003*
MAI-5 (mm) *Srednji godišnji prirast u zadnjih pet godina (mm)*	1.47 ± 0.15	1.08 ± 0.12	0.0049*
MAI-10 (mm) *Srednji godišnji prirast u zadnjih deset godina (mm)*	1.47 ± 0.14	1.04 ± 0.10	0.016*
PGR *Omjer periodičnog rasta*	1.01 ± 0.06	1.08 ± 0.07	0.455^ns
Mean annual increment in the last year until the sampling date (mm) *Godišnji prirast u prošloj godini (mm)*	1.50 ± 0.13	1.01 ± 0.10	0.005*
DBH (cm) *Visina prsnog promjera (cm)*	33.20 ± 0.57	33.77 ± 0.62	0.504^ns
Number of xylem cells *Broj stanica ksilema*	46.27 ± 4.39	36.67 ± 3.89	0.107^ns

*Significant at 0.05 level, ^ns Non-significant at 0.05 level.

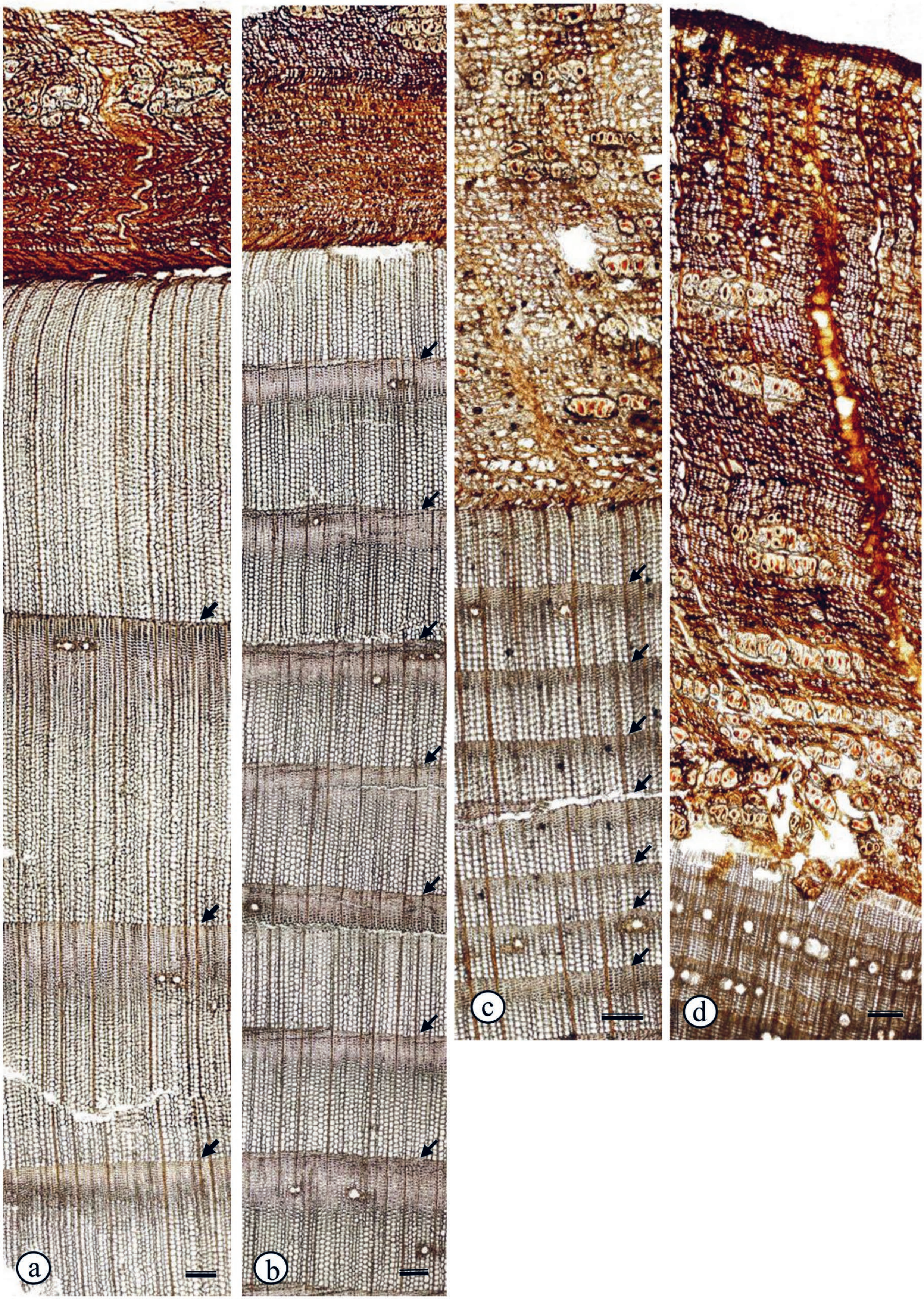

Figure 1. Cross sections. a-b: growth ring widths sampled from infested trees (no. 19 and 27) and c: uninfested tree (no. 46); d: phloem thickness from an infested tree (no. 25). Scale bars = 200 μm.

Slika 1. Poprečni presjeci. a-b: širine godova uzorkovanih s napadnutih stabala (br. 19 i 27) i c: nenapadnuto stablo (br. 16); d: debljina floema napadnutog stabla (br. 25). Mjerila = 200 μm.

mm ± 0.12 (0.22 – 3.26 mm) and 1.04 mm ± 0.10 (0.25 – 2.61 mm) in uninfested trees, respectively (Figure 1a, b, c). Values were significantly different between infested and uninfested trees (t test, $t = 2.013$, $df = 58$, $p < 0.05$ for MAI-5 and $t = 2.484$, $df = 58$, $p < 0.05$ for MAI-10).

In the infested and uninfested trees PGR was 1.01 mm ± 0.06 (0.29 – 2.27 mm) and 1.08 mm ± 0.07 (0.46 – 2.53 mm), respectively. The difference was not significant (t test, $t = 0.752$, $df = 58$, $p > 0.05$).

Mean annual increment in the last year until the sampling date was 1.50 mm ± 0.13 (0.24 – 3.40 mm) and 1.01 mm ± 0.10 (0.16 – 2.42 mm) in infested and uninfested trees, respectively. The difference in mean annual increment in the last year until the sampling date for infested and uninfested trees was significant (t test, $t = 2.934$, $df = 58$, $p < 0.05$).

DBH was 33.20 cm ± 0.57 (26 – 39 cm) and 33.77 cm ± 0.62 (28 – 39 cm) in infested and uninfested trees, respectively. The difference was not significant (t test, $t = 0.672$, $df = 58$, $p > 0.05$).

In the infested and uninfested trees number of xylem cells was 46.27 ± 4.39 (13 – 112) and 36.67 ± 3.89 (9 – 88), respectively. The difference between the infested and uninfested trees was not significant (t test, $t = 1.637$, $df = 58$, $p > 0.05$).

DISCUSSION
RASPRAVA

As a whole, oriental spruce trees that had higher phloem thickness, MAI-5, MAI-10, mean annual increment in the last year until the sampling date and number of xylem cells were infested successfully by *D. micans*. Within the scope of our study, we can remark that spruce trees with higher recent tree growth rates in the same stand are more likely to be infested by *D. micans* than others.

Phloem thickness of the infested trees was 4.19 mm on average, and it was 1.18 mm thicker than uninfested trees. There are other studies that have indicated the positive effect of phloem thickness for bark beetles (Amman, 1972; Berryman, 1976; Haack et al., 1987a, b; Alkan Akıncı and Erşen Bak, 2016; Alkan Akıncı et al., 2018). Storer and Speight (1996) expresses that *D. micans* host selection may be determined in part by the suitability of the substrate for larval development. So, by attacking trees with thicker phloem, *D. micans* parental females may assure their larvae to have enough nourishment throughout development stages. There was a contrasting result that phloem thickness had no unique effect on a bark beetle species -pine engraver- reproduction reported by Reid and Robb (1999). In their case, the mean thickness of phloem in the focal stand was 1.07 mm that is less than 1.45 mm the body width of pine engravers, and required beetles also to consume outer bark and xylem of the trees (Reid and Robb, 1999).

MAI-5 and MAI-10 in the infested trees was higher than the uninfested ones. *D. micans* preferred vigorously growing trees during attacks. In the former experimental studies that *D. micans* females were inserted on healthy oriental spruce trees, parental females were tended to be successful on vigorously growing trees (Alkan Akıncı and Erşen Bak, 2016) or codominant trees that have decreasing growth in the last 10 years (Alkan Akıncı et al., 2018). These trees provide thicker phloem and larger phloem surface area to *D. micans* larvae for feeding. And being stressed in some way also benefits beetles. Reid and Robb (1999) have reported comparable results for *Ips pini*. Their results showed that tree growth rate parameters are positively related to beetle reproductive performance on freshly dead *Pinus banksiana* (Reid and Robb, 1999).

There was also a significant difference between mean annual increment in the last year until the sampling date in infested and uninfested trees. Mean annual increment in the last year until the sampling date in infested trees was 0.49 mm higher than the uninfested ones. Infested trees have grown vigorously until the sampling date in the last year as well.

The difference between the DBH of the trees and number of xylem cells in infested and uninfested trees was not significant. The number of xylem cells was higher in infested trees (Figure 1a, b, c). Infested trees have higher cambium activity so they have higher wood and phloem formation.

Oriental spruce is one of the dominant conifer species in Artvin and Eastern Black Sea region, and natural spruce forests are of great economic and ecological importance in its distribution area. Tree mortalities associated with outbreaks cause reductions in tree density that cause changes in microclimate that favor beetles (Fettig and Hilszczański, 2015). Such a microclimate alteration in a stand involves changes in reproduction of beetles such as phenology, and voltinism (Fettig and Hilszczański, 2015) and trees' exposure to direct sunlight. Death of larger trees in a stand creates neighboring trees to get more sunlight. Trees at the stand edges and in sun-exposed patches are preferably infested by bark beetles (Schopf and Köhler, 1995; Mezei et al., 2012). Eventually, loss of vigorously growing larger trees reduces resistance of remaining living trees in the stand.

CONCLUSION
ZAKLJUČAK

In our study scale, we can conclude that parental females that start attacks on new hosts have preferred vigorously growing oriental spruce trees. *D. micans,* during its natural attack behavior, has established on trees with thicker phloem and higher recent tree growth rates that had higher cambium activity. Our results contribute to the results obtained from former experimental studies (Alkan Akıncı and Erşen Bak, 2016; Alkan Akıncı et al., 2018) in oriental spruce forests.

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SAŽETAK

Dendroctonus micans (Kugelann, 1794), koji je prvi put otkriven 1966. godine u Turskoj, do kasnih 2000-ih zahvatio je gotovo sve šume kavkaske smreke u istočnoj crnomorskoj regiji. Tijekom svog širenja, uništio je milijune kubičnih metara šume stabala smreke. Posljednjih godina šume kavkaske smreke sadrže endemsku populaciju ovih štetnika. No, ekstremni klimatski uvjeti koji uzrokuju ekstremne vremenske uvjete, mogu stvoriti uvjete koji pogoduju naglom razvoju *D. micans*. Ovim se istraživanjem željelo ispitati vitalnost stabala prirodno zaraženih i nezaraženih stabala u šumi. Terenska istraživanja obavljena su 2016. godine na čistoj sastojini smreke u šumskom podokrugu Taşlıca, Uprave za šumarstvo Artvin. U sklopu sastojina odabrana su i zaražena i nezaražena stabla. Jezgre su uzete iz 30 prirodno zaraženih i 30 nezaraženih stabala smreke u istom sklopu. Uzorci jezgre uzeti su u drugom tjednu rujna. Proučavana je debljina floema, stopa (debljinskog) prirasta stabala, prsni promjer stabla i prosječan broj ksilemskih stanica u radijalnom redu formiranom do dana uzorkovanja. Debljina floema, nedavna stopa prirasta stabala i broj stanica ksilema bili su veći kod zaraženih stabala. O izboru domaćina *D. micans* raspravljalo se u odnosu na značajke zaraženih i nezaraženih stabala.

KLJUČNE RIJEČI: vitalnost stabla, rast i prirast stabala, *Picea orientalis*, debljina floema, izbor domaćina