

The Effect of Phloem Moisture on *Dendroctonus micans* (Kugelann, 1794) (Coleoptera: Curculionidae) for Successful Establishment to Mechanically Wounded Oriental Spruce Trees in Turkey

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ABSTRACT

The effect of moisture content of the phloem on successful establishment of *Dendroctonus micans* was determined on mechanically wounded oriental spruce trees in Artvin, Turkey. A standard mechanical wound was made on trees and six insertions, of which one of them was control, were made on each tree. Adult *D. micans* females were inserted to trees. Experiment was performed as ten replicates. Numbers of drowned beetles between sites were significantly different. Average moisture content of the phloem was significantly different between the sites where oviposition did and did not occur. Beetles successfully established at sites with higher phloem moisture. But average phloem moisture content was not significantly different between sites that were around wounds and control site.

Key words: *Dendroctonus micans*, oriental spruce, *Picea orientalis*, phloem moisture content, wounded trees.

INTRODUCTION

The greater European spruce bark beetle, *Dendroctonus micans* (Kugelann, 1794) (Coleoptera: Curculionidae) is one of the major pests of oriental spruce, *Picea orientalis* (L.) Link. in Turkey. It was first discovered in 1966 in Turkey (Acatay, 1968) and today, almost all the oriental spruce forests are infested by the beetle (Alkan-Akinci *et al.*, 2014). It has caused heavy losses during outbreaks on the edge of its expanding range throughout these years (Benz, 1984; Özder, 1984; Eroğlu, 1995; Alkan, 2000; Eroğlu *et al.*, 2005; Alkan-Akinci *et al.*, 2009).

Dendroctonus micans females leave natal trees after kin mating and attack new trees or unattacked parts of existing host tree solitarily (Fielding and Evans, 1997; Grégoire, 1985, 1988). Successful establishment of the beetle does not depend on tree death. Depending on attack density a host can survive throughout several generations of the beetle. Unsuccessful attempts are usually followed by new attempts (Grégoire, 1985, 1988).

Beetle attacks usually occur on wounded and forked trees, and under branch nodes (Benz, 1984; Evans *et al.*, 1984; Grégoire, 1988; Eroğlu, 1995; Fielding and Evans, 1997; Alkan-Akinci, 2006; Özcan *et al.*, 2011). Forestry activities such as felling,

logging, pruning cause mechanical wounds on trees (Grégoire, 1988; Alkan-Akinci, 2006; Özcan *et al.*, 2011). Alkan-Akinci (2006) reports 9.3% mechanically wounded trees in 3.6 ha of experimental plots area in Artvin, Giresun and Maçka (Trabzon) oriental spruce forests. In these stands, 578 spruce trees were attacked, of which 205 were wounded trees. A study that has been conducted on Norway (*Picea abies* (L.) Kar.) and Sitka spruce (*Picea sitchensis* (Bong.) Carr.) showed that *D. micans* adults excavated significantly larger galleries in wounded trees (Wainhouse *et al.*, 1998). On Norway spruce, higher larval survival, higher larval dry weights and higher oviposition rates were recorded around wounds that had higher phloem moisture content (Storer and Speight, 1996). The relationship between wounded trees and *D. micans* attacks could indicate attraction of beetles to monoterpenes in spruce resin exuding from wounds (Vasechko, 1978; Wainhouse *et al.*, 1998). Besides, wounding cause significant changes such as an increase in the concentration of nitrogen and starch (Wainhouse *et al.*, 1998).

In Turkey, there are field observations that indicate beetle's host selection choice towards wounded trees (Alkan-Akinci *et al.*, 2014). In this paper it's aimed to investigate effect of moisture content of the phloem on *D. micans* adults' successful establishment on mechanically wounded oriental spruce trees in Turkey. It will provide experimental data and contribute to the understanding of beetle's ecology.

MATERIALS AND METHODS

Experiment was conducted at an oriental spruce stand in Cerattepe-Artvin at 1500 m asl. at southeast aspect. Unwounded and unattacked trees were used in the experiment. Slope was 10%. Experimental trees were selected randomly and at the inner part of the stand that had closure and no stand edges exposed to sunlight.

Bark wounding

A standard wounding pattern, which was used by Storer and Speight (1996), was used in the experiment (Figs. 1a, 1b, 1c). This wounding pattern was modified by Storer and Speight (1996) from the patterns used by Miller and Berrymann (1986) in their study. According to wounding procedure, 9 mm wide bark strips were removed from trees by a chisel. Wounds were made at 1.7 m above the ground and away from branches or branch nodes (Storer and Speight, 1996). Wounds were made on August 24, 2012, three weeks prior to insertion of beetles. Wounds were made on 10 trees that had 24.8 cm (range: 18-32 cm) diameter of breast height on average. There were 6 insertion sites, of which site 2 was opposite side to wound on the trees. Site 2 was used as control site for making comparisons with other sites around wounds as explained by Storer and Speight (1996).

Origin of *Dendroctonus micans* adults and their insertion into trees

Beetles were collected from naturally infested oriental spruce trees in Artvin. They were collected on September 14, 2012. A total of 158 adults were collected from two trees. Larger females that were weighing more than 29 mg were used in

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the experiment. These beetles were assessed as females depending on Robinson *et al.* (1984). Insertion of *D. micans* adults were made three weeks after wounding, on September 17, 2012. Each adult were put in a plastic cap and nailed in the middle of each insertion point with two pins. Two weeks after beetle insertion, on October 01, 2012, two bark samples that were 5 cm in diameter were taken from bark sampling sites from each of insertion points. These fresh bark disks were used to designate percentage moisture content of the phloem. Five weeks after beetle insertion, on October 22, 2012, barks covering adult galleries were removed by a hatchet. Number of eggs, dead and alive beetles were recorded.

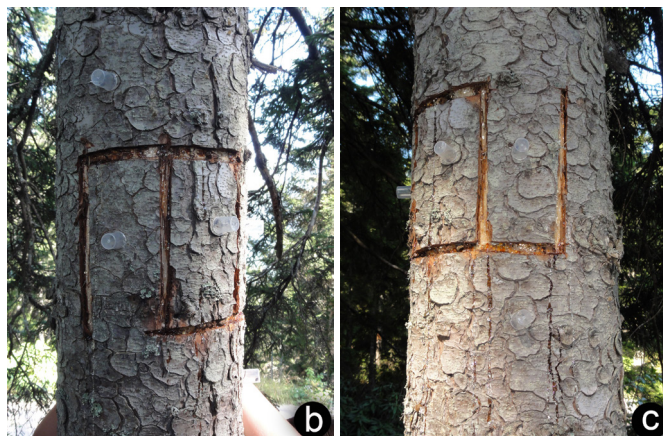
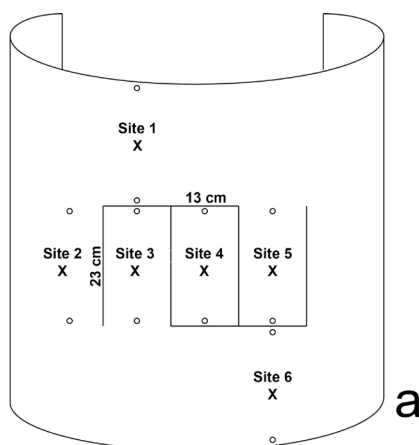


Fig. 1. a. Wounding pattern, which was used by Storer and Speight (1996), was performed on trees (site 2 is located on the opposite site of the tree as control site). b-c. Mechanical wound on an experimental tree.

Moisture content of the phloem samples

The whole procedure that was followed by Storer and Speight (1996) for determining percentage moisture content of the phloem was repeated in this work. Outer bark

of the fresh bark disks were removed from phloem tissue and put in hermetic glass boxes immediately after they were taken from trees. These boxes were settled in a cool box and brought to laboratory. Fresh disks were weighted and recorded. Then they were dried at 80°C for 48 hours. Dried disks weighted again and percentage moisture content was calculated for each insertion site.

Statistical analyses

Data were analyzed using IBM SPSS statistics version 19.0. Numbers of drowned beetles between sites were compared with Chi-square test. Independent Samples *t* test was performed to compare moisture contents of the phloem where females did and did not lay eggs. Analysis of variance (ANOVA) and Chi-square tests were performed to compare moisture contents of the phloem and proportions of adults that laid eggs between insertion sites, respectively. Standard error is given with the mean values.

RESULTS

A total of 60 insertions were made on the trees. Of these, 10 were control sites and 50 sites were located around wounds. A total of 120 bark samples were used to determine percentage moisture content of the phloem.

Twenty-two (36.7%) adults laid eggs, 20 (33.3%) were drowned in resin (Fig. 2) and 18 (30%) were dead in plastic caps (Table 1). There were no drowned beetles at the control site. There were 1, 6, 6, 4 and 3 drowned beetles at sites 1, 3, 4, 5 and 6, respectively. Chi-square test showed that numbers of drowned beetles between sites were significantly different ($\chi^2 = 17.282$, $df = 5$, $p < 0.05$). Proportions of adults that laid eggs were 0.6, 0.5, 0.3, 0.1, 0.3 and 0.4 at the sites, respectively. Beetles were more successful at the sites with higher phloem moisture (Table 2). Average phloem moisture was 49.2% at the sites where females laid eggs and 44.2% at the sites with no oviposition. Values were significantly different between these sites (*t* test, $t = 6.274$, $p < 0.05$).



Fig. 2. *Dendroctonus micans* female drowned in resin.

Average phloem moisture content of the sites were $48.0\% \pm 1.213$, $47.4\% \pm 1.001$, $45.8\% \pm 1.037$, $44.7\% \pm 1.293$, $44.8\% \pm 0.725$ and $45.7\% \pm 1.374$, respectively. Site

3, 4, 5, and 6 had relatively but not-significantly lower and site 1 had relatively but not-significantly higher phloem moisture content than control site 2 (Table 1). But phloem moisture contents of the sites were not significantly different (ANOVA, $df = 5$, $F = 1.428$, $p = 0.229$). Sites around the wounds and control sites did not differ in terms of average phloem moisture content.

Between sites, site 1 had the highest moisture content and control site 2 was the second. In site 4 and 5 moisture content was the lowest. Proportion of adults that laid eggs was highest in site 1 and 2, respectively, and lowest in site 4. Chi-square test showed that proportions of adults that laid eggs between sites were not significantly different ($\chi^2 = 7.139$, $df = 5$, $p = 0.210$).

DISCUSSION

Moisture content of the phloem at site 1 was higher than the control site 2. Proportion of adults that laid eggs at site 1 was the highest. Counts at site 1 were also higher than the expected counts in Chi-square test. Higher moisture content of the phloem at site 1 may explain the higher oviposition proportion. Phloem moisture content of site 6 was lower than control site, but counts were also higher than expected counts at site 6. Other factors that are not considered in this experiment may be appropriate at this site for beetles' establishment. Storer and Speight (1996) found that adult *D. micans* are more likely to oviposit at phloem areas with lower astringin and total stilbene contents.

Beetles were more successful at the sites with higher phloem moisture. Average phloem moisture content of the sites where oviposition occurred was 49.2% which is higher than average values of all sites. Oviposition is higher at sites with higher moisture content in the experiment of Storer and Speight (1996). Moisture and nitrogen content that is important in insect nutrition and favor beetle development are known to correlate positively (Scriber and Slansky, 1981; Storer and Speight, 1996).

Of the inserted adults, 20 were drowned in resin and 18 were dead in plastic caps after some initial tunneling attempts. *D. micans*' attacks can succeed at one place, but can fail at other places of the same tree. Beetles have to find a suitable spot on the host (Vouland, 1991).

Former studies in the north-eastern Black Sea Region in Turkey reported high numbers of attacks such as 40-160 attacks on a tree in heavily infested stands with some trees having only one attack. In the stands, there were some trees with a number of aborted attacks on them, as well as some unattacked trees (Eroğlu, 1995; Alkan-Akinci *et al.*, 2014). This is also reported in Norway and Sitka spruce in Europe (Bevan and King, 1983; Grégoire, 1984, 1985; Wainhouse *et al.*, 1998). Presence of lignin and its distribution throughout the bole of trees also affects beetles' establishment and survival. Its indirect effects in slower development of broods are also reported to be important in increasing the chance of predators such as its specific predator *Rhizophagus grandis* (Gyllenhal, 1827) (Coleoptera: Monotomidae) and woodpeckers to find their prey, and being exposed to induced defensive reactions (Wainhouse *et al.*, 1990, 1998).

Table 1. Phloem moisture contents and oviposition at insertion sites.

No. of trees	Site																	
	1			2			3			4			5			6		
	M (%)	Adult		M (%)	Adult		M (%)	Adult		M (%)	Adult		M (%)	Adult		M (%)	Adult	
		D	A		D	A		D	A		D	A		D	A		D	A
		Oviposition			Oviposition			Oviposition			Oviposition			Oviposition			Oviposition	
		Yes	No		Yes	No		Yes	No		Yes	No		Yes	No		Yes	No
1	45.25	+	+	49.54	+	+	47.28	+	+	43.87	+	+	43.85	+	+	52.01	+	+
2	54.52	+	+	52.35	+	+	49.98	+	+	47.96	+	+	46.74	+	+	53.64	+	+
3	45.95	+	+	48.19	+	+	43.95	+	+	44.79	+	+	47.11	+	+	46.54	+	+
4	46.73	+	+	45.31	+	+	49.96	+	+	45.97	+	+	47.36	+	+	44.21	+	+
5	45.72	+	+	42.78	+	+	39.76	+	+	42.22	+	+	42.71	+	+	42.77	+	+
6	43.97	+	+	45.63	+	+	47.07	+	+	53.18	+	+	44.92	+	+	45.35	+	+
7	44.42	+	+	45.85	+	+	44.99	+	+	41.12	+	+	41.31	+	+	40.34	+	+
8	48.55	+	+	44.91	+	+	45.73	+	+	37.92	+	+	45.82	+	+	41.56	+	+
9	51.25	+	+	52.19	+	+	41.92	+	+	44.64	+	+	46.49	+	+	43.15	+	+
10	53.61	+	+	47.03	+	+	47.41	+	+	45.55	+	+	41.59	+	+	47.47	+	+

M: Moisture content of the phloem, D: Dead, A: Alive

Table 2. Phloem moisture contents of the sites where females did and did not lay eggs. Values are significantly different (*t* test, *p* < 0.05)

Moisture content of phloem (%)	Oviposition (mean ± SE)	
	Yes	No
	49.2a ± 0.695	44.2b ± 0.384

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In the current experiment, numbers of drowned beetles between sites were significantly different. And all the drowned beetles were around wounds. Wainhouse *et al.* (1998) reported no overall effect of wounding on resin content and lower resin concentrations in new wounds, and also decrease in the moisture content of the wounded bark. In another experiment on Norway spruce, the induced defense reaction of the host is reported not to play a role in the success or failure of the attack, because few beetles are killed by resin during attacks (Lieutier *et al.*, 1992). In the current experiment, plastic caps that were used during insertion of females were adjacent to tree bark. So caps were filled by exuding resin and females had no chance to avoid it. Proportion of aborted attacks (63.3%) in this experiment may be affected by the insertion method. Because field observations indicate that 25% of the attempts are aborted in oriental spruce trees in Turkey (Alkan-Akinci *et al.*, 2014). In Europe, higher aborted attacks were reported. *D. micans* has more than 70% (72-78%) abortive attacks in Belgium (Grégoire, 1984). Further investigations on the abortive attacks of *D. micans* can contribute to these results.

Within this experiment, females do preferred the sites with higher moisture content. Positive correlation between moisture content and insect nutrition substances (Scriber and Slansky, 1981; Storer and Speight, 1996) may be the reason of beetles' successful establishment or preference of these sites. Storer and Speight (1996) conclude this relationship as suitability of substrate for larval development.

In conclusion, this study presents that average phloem moisture content did not differ between sites on newly wounded trees. Numbers of drowned beetles between sites were significantly different. And all the drowned beetles were around wounds. Proportions of adults that laid eggs between sites were not significantly different. But average phloem moisture of the sites where females laid eggs were significantly different from the sites where no oviposition occurred. Beetles were more successful at the sites with higher phloem moisture.

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