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EFFECTS OF FEEDING BY TWO FOLIVOROUS ARTHROPODS ON SUSCEPTIBILITY OF HYBRID POPLAR CLONES TO A FOLIAR PATHOGEN

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ABSTRACT

We investigated variation in folivore-induced effects on subsequent plant suitability to a foliar pathogen. We used a leaf disk assay to expose three clones of hybrid poplar, NC11382, NE332 and NM6, to colonization by a leaf spot pathogen, *Septoria musiva*. Undamaged leaf disks of NE332 were the most resistant to *S. musiva*, followed by NM6 and NC11382, respectively. To test the effects of prior herbivory on subsequent susceptibility to this fungal pathogen, we inoculated *S. musiva* on leaf disks taken from leaves which had been exposed to feeding by *Tetranychus* mites or cottonwood leaf beetles. Prior activity by mites and cottonwood leaf beetle affected the subsequent susceptibility of clones NC11382 and NE332 to *S. musiva*.

Herbivory may have many effects on host plants. One such effect, induced plant resistance to herbivores and pathogens, may result from biochemical and physiological changes initiated by contact with the invading herbivore (e.g., Clausen et al. 1989). Although herbivores and plant pathogens may be confronted with and affected by similar induced defenses (Schultz 1983, Krischik et al. 1991, Hammerschmidt 1993, Klepzig et al. 1996), these interactions have largely been studied separately. Studies examining reciprocal effects between herbivores and plant pathogens are conducted even less frequently, perhaps due to the complexity of these multispecies interactions (Wargo and Houston 1974, Karban et al. 1987, Krischik et al. 1991, Klepzig et al. 1996). In particular, we know little about how folivore feeding affects subsequent susceptibility to foliar pathogens.

The purpose of this study was to consider how prior herbivory by folivorous arthropods and host plant clone interact to affect subsequent suitability to a foliar pathogen. Our study system consisted of two folivorous arthropods—*Tetranychus* spp. (Acari: Tetranychidae) mites and cottonwood leaf beetles, *Chrysomela scripta* Fabr. (Coleoptera: Chrysomelidae), three hybrid

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poplar clones (*Populus* spp.), and one fungal foliar pathogen—*Septoria musiva* Peck.

MATERIALS AND METHODS

We grew plants from dormant hardwood cuttings from coppiced field grown hybrid *Populus* clones NC11382 (*P. nigra* X *P. berolinensis*), NE332 (*P. simonii* X *P. berolinensis*) and NM6 (*P. nigra* X *P. maximowiczii*) (Robison and Raffa 1994, 1996) at the University of Wisconsin Arlington Experiment Station. We stored the cuttings (5 cm long) frozen until use, and planted them in Redi-Earth Peat-Lite® soil mix. We regularly flood irrigated and fertilized with 15 g Osmocote® slow-release fertilizer (7-16-12, plus micronutrients), plants and grew them in a glasshouse at 16:8 L:D photoperiod, 18–29°C and 25–80% relative humidity.

We assayed leaves from three types of plants. Because, within clones, variability is greater between leaves of the same physiological age than it is between trees (Robison and Raffa 1997), we removed leaves 3 and 4 (downward from the apical fully expanded leaf) from each plant for use in assays the same day. We collected leaves from control (undamaged) plants of all three clones, exhibiting no visible signs or symptoms of arthropod damage or disease. We collected leaves from clones 11382 and NE332 that exhibited extensive mite feeding damage (approximately 30–50% damage on each leaf), or exhibited *C. scripta* feeding damage (approximately 10% defoliation on each plant).

We followed the *S. musiva* colonization assays developed by Ostry and Skilling (1988). Due to our incomplete understanding of the mechanisms underlying any systemic responses within poplar, and because multiple infections may occur on the same leaf we followed the method cited above and removed multiple leaf disks from each leaf and used leaf disk as our experimental unit. Leaf disks (18 mm diameter) were placed abaxial side up in wells cut into 2% water agar in glass petri plates. Each petri plate contained 6 disks taken from the same clone and treatment (n = 4 plates per clone/treatment combination). We inoculated five leaf disks in each chamber with 0.1 ml of a spore suspension (1×10^6 conidia/ml), and the sixth disk with 0.1 ml of sterile distilled water. The assay plates were then incubated in a growth chamber at 24°C under continuous light. We measured areas of the resulting necrotic lesions using a transparent dot grid. The effects of treatments, clones, and time since inoculation on lesion size were analyzed using a repeated measures ANOVA, followed by Fisher's protected least squares means comparisons where appropriate (Abacus Concepts 1989).

RESULTS

The leaf disk area colonized by *S. musiva* varied significantly by clone, and clone X treatment interaction (Table 1a). On leaf disks from undamaged plants, areas colonized by *S. musiva* varied significantly by clone (Table 1b), and were consistently largest on clone NC11382. Lesions were generally smallest on NE332, and intermediate on NM6 (Figure 1).

Clonal effects on amount of fungal colonization were also detected. Significant necrotic lesions formed on NC11382 by day 19, but not until days 26 and 33 on clones NM6 and NE332, respectively. By the experiment's end lesions on NM6 occupied up to 69% of the leaf disk surface area, nearly equivalent to those on NC11382. Uninoculated control disks (N=4 for each treat-

Table 1. Analyses of variance for size of *S. musiva* lesions measured after inoculation of hybrid poplar leaf disks. (A) Two-way, repeated-measures ANOVA of clone (NC11382 and NE332) by treatment (mite-damaged, beetle-damaged and undamaged) effects. (B) One-way, repeated-measures ANOVA of clonal (NC11382, NE332 and NM6) effects (undamaged tissues). Greenhouse-Geisser (G-G) and Hunyh-Feldt (H-F) estimated p-values are given.

A.

Source	df	SS	MS	F	P	G-G	H-F
clone	1	64.78	64.78	52.28	.0001	—	—
treatment	2	4.43	2.22	1.79	.1714	—	—
clone *treatment	2	6.91	3.45	2.79	.0659	—	—
Subject (Group)	114	141.27	1.24	—	—	—	—
time	5	9.64	1.93	6.86	.0001	.0002	.0001
time * clone	5	29.61	5.92	21.09	.0001	.0001	.0001
time * treatment	10	2.78	0.28	0.99	.4501	.4307	.4336
time * clone * treatment	10	8.40	0.84	2.99	.0011	.0076	.0061
time * Subject (Group)	570	160.07	0.28	—	—	—	—

B.

Source	df	SS	MS	F	P	G—G	H—F
clone	1	20.11	20.11	11.26	.0014	—	—
subject	58	103.62	1.79			—	—
time	5	40.71	8.14	26.53	.0001	.0001	.0001
time * clone	5	6.15	1.23	4.01	.0016	.0108	.0091
time * subject	290	88.99	0.31			—	—

ment within each clone) did not develop any necrotic lesions during the course of this experiment.

The relationship between prior herbivory and subsequent fungal growth varied between clones. Lesions on leaf disks from undamaged leaves of clone NC11382 began to form after 2 weeks, but did not form on trees with prior herbivory by either mites or beetles until about 23 days. Lesions were larger than those formed on leaf disks from mite- or *C. scripta* damaged leaves until 26 days, and were significantly larger until 33 days post inoculation (Figure 1a). Average lesion size was significantly larger on mite damaged than on *C. scripta* damaged leaf disks at 33 days post inoculation. By the end of the experiment (37 days post inoculation), up to 91% of the leaf disk surface had become necrotic. Using the curve fitting option in SuperANOVA (Abacus Concepts 1989), we found significant linear relationships between lesion size and time since inoculation for undamaged ($y = 0.08 \times - 0.86$, $r^2 = 0.96$), mite damaged ($y = 0.12 \times -1.98$, $r^2 = 0.92$), and *C. scripta* damaged ($y = 0.09 \times -1.60$, $r^2 = 0.93$) leaf disks of clone NC11382.

Lesions began to form earlier on inoculated leaf disks from NE332 trees with prior herbivory than on undamaged trees (Figure 1b). At 37 days post inoculation, fungal colonized areas accounted for approximately 54% of the surface area. The average final colonized area on NE332 leaf disks was approximately equal to the average colonized area on leaf disks from clone NC11382 eleven days previous. Using the curve fitting option in SuperA-

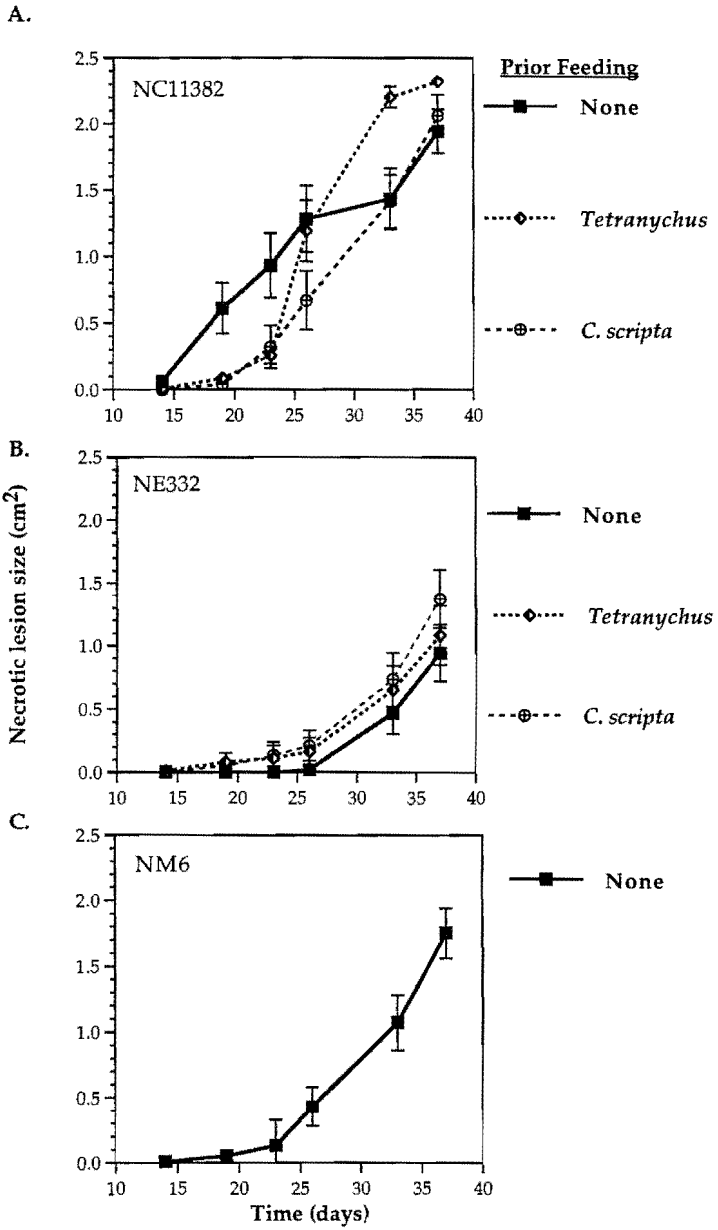

Figure 1. Mean (± s.e.) size of *S. musiva* lesions on leaf disks removed from undamaged, *Tetranychus* mite damaged and cottonwood leaf beetle (*C. scripta*) damaged hybrid poplar trees at various sampling times. Data analyzed by repeated measures ANOVA. (A) Clone NC11382, (B) Clone NE332, (C) Clone NM6.

NOVA (Abacus Concepts 1989), we found significant exponential relationships between lesion size and time since inoculation on undamaged [$y=(1*10^{-7})*10^{0.19X}$, $r^2=0.94$], mite damaged [$y=(1*10^{-3})*10^{0.08X}$, $r^2=0.95$], and *C. scripta* damaged [$y=(2*10^{-4})*10^{0.11X}$, $r^2=0.89$] leaf disks of clone NE332.

DISCUSSION

Undamaged tissue from clone NC11382 was more susceptible to *S. musiva* than was similar tissue from NE332. The two clones also differed in the patterns of response with a more pronounced effect in clone NE332 than in clone NC11382. We cannot say for certain at this time, however, whether the alterations we saw in host susceptibility to a pathogenic fungus represent physiological induction due to herbivory. Other possible explanations may involve alterations in phylloplane flora (Wilson 1995).

On undamaged plants from all three clones lesions spread progressively faster on clone NE332, NM6, and NC11382, respectively. These relative levels of leaf symptom severity differ somewhat from field rankings of severity of stem cankers caused by the same fungus, in which clone NM6 was the most, clone NE332 was intermediate, and clone NC11382 was the least, resistant (Robison and Raffa 1996).

The interactions of hybrid poplar with its suite of potential plant parasites are highly variable. Each inducing agent and subsequent herbivore may elicit different responses depending upon the host genotype. Although a uniform theory on inducibility among artificial clones is not likely (Robison and Raffa 1994), the variation that is present can be very useful for tree improvement and clonal deployment strategies. Moreover, inducibility needs to be an important component in understanding pest impacts and population changes in managed and natural ecosystems (Haukioja and Neuvonen 1988).

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