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Challenging Chinese Hemlock (*Tsuga chinensis*) with Hemlock Woolly Adelgid (*Adelges tsugae*) Ovisacs

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Abstract. Chinese hemlock (*Tsuga chinensis*) is a promising candidate for use as an ornamental tree in the mid-Atlantic region of the United States where native hemlocks have been devastated by hemlock woolly adelgid (*Adelges tsugae*). Published research has indicated that Chinese hemlock is adaptable to USDA Zone 6 and has some degree of resistance to hemlock woolly adelgid. Chinese hemlock has been observed to be resistant to hemlock woolly adelgid while growing in close proximity to infested eastern hemlocks, although a direct challenge of the species has not previously been documented. This study reports on the development of a procedure for challenging hemlocks with hemlock woolly adelgid ovisacs to determine host plant resistance and the use of the procedure to challenge Chinese hemlock. Chinese hemlock demonstrated complete resistance to hemlock woolly adelgid.

Key Words. *Adelges tsugae*; hemlock; hemlock woolly adelgid; host resistance; integrated pest management (IPM); plant health techniques; *Tsuga canadensis*; *Tsuga chinensis.*

Hemlock woolly adelgid (*Adelges tsugae*) poses a major threat to native hemlocks in eastern North America. Both eastern (*Tsuga canadensis*) and Carolina (*T. caroliniana*) hemlock are susceptible to this pest (McClure and Cheah 1999). Hemlock woolly adelgid (HWA) is native to Asia and was unintentionally introduced to eastern North America in the 1950s (McClure 1991). Since that time, the distribution of the pest has increased to cover approximately half of the native range of eastern hemlock (Bentz et al. 2002).

Effective management of HWA with pesticides can be achieved in landscapes and nurseries. On a large scale such as forests and park systems, the issue becomes more complicated. Chemical control is not economically, logistically, or environmentally practical. Because of this, research has been undertaken to develop a biologic control program for this key pest of hemlock (Bentz et al. 2002).

One of the most promising areas of research is the identification of resistant or tolerant species of hemlock. Once identified, these plant species may be used as substitutes or incorporated into breeding projects with eastern hemlock. Chinese hemlock (*T. chinensis*) deserves consideration for use in eastern North American landscapes. Chinese hemlock is native to central and western China. Outside of its native habitat, it is not commonly grown because of its slow growth habit (Swartley 1984; Vidakovic 1991). Resistance to HWA has been reported in its native range (Montgomery et al. 2000) and in North America (Bentz et al. 2002; Del Tredici and Kitajima 2004).

Del Tredici and Kitajima (2004) proposed Chinese hemlock as a suitable landscape substitute for eastern hemlock. They grew Chinese hemlocks in close proximity to HWA-infested hemlocks for 4 years. The Chinese hemlocks showed rapid growth in USDA Zone 6 conditions and did not develop any HWA infestations. Although the lack of infestation Del Tredici and Kitajima reported is a good indication of some level of resistance, the extent of this resistance was unknown. Furthermore, the level of interaction between Chinese hemlock and HWA in that study is unknown.

One method of gauging resistance in a host plant is to challenge the plant with pest inoculum. Cages or sleeves of netting may be used to protect the inoculum and assure that the pest and host come into direct contact.

This study was conducted to develop a procedure for challenging hemlocks with HWA ovisacs and to challenge Chinese hemlocks growing under field conditions in three different Pennsylvania, U.S. counties with HWA ovisacs. Eastern hemlocks were used to evaluate the effectiveness of the challenge procedure.

MATERIALS AND METHODS

The experiment was a multilocation experiment that was completely randomized at each location. The three Pennsylvania locations were Center County (USDA Zone 6A; elevation 368 m [1214 ft]), Berks County (USDA Zone 6B; elevation 74 m [244 ft]), and Lackawanna County (USDA Zone 5B; elevation 515 m [1700 ft]). At each location, there were multiple blocks of each hemlock species (Eastern and Chinese hemlock). Each block was an individual plant. At the beginning of the challenge procedure, the hemlocks used in this study had been field-planted for three full growing seasons and were pest-free.

The experiment was designed to examine five treatments (Table 1). HWA inoculum rate was the first consideration, with treatments of low (10 to 20 ovisacs) and high (25 to 40 ovisacs)

Table 1. The five inoculation treatments.

Treatment number	HWA inoculation rate	Sleeve	Abbreviation
1	Low (10–20 ovisacs)	100 µm sleeve	L100
2	High (25–40 ovisacs)	100 µm sleeve	H100
3	Low (10–20 ovisacs)	1000 µm sleeve	L1000
4	High (25–40 ovisacs)	1000 µm sleeve	H1000
5	Intermediate (20–25 ovisacs)	No sleeve	CNS

HWA = hemlock woolly adelgid.

Table 2. The total number of blocks and replications at each experiment site.

County	Blocks (trees)	L100	H100	L1000	H1000	CNS
Eastern hemlock						
Berks	6	6	6	6	6	6
Center	5	5	5	5	4[z]	5
Lackawanna	6	6	6	6	6	6
Totals	17	17	17	17	16	17
Chinese hemlock						
Berks	5	5	5	5	5	5
Center	5	5	5	5	5	5
Lackawanna	4	4	4	4	4	4
Totals	14	14	14	14	14	14

[z]One sleeve was destroyed by a dog several weeks into the study.

rates. The use of a protective sleeve was also considered to reduce potential predation and general loss of inoculum resulting from wind or animal contact. Sleeves of 100 μm aperture and 1000 μm aperture Nitrex® Bolting Cloth (Wildlife Supply Co., Buffalo, NY) were constructed. White Duragrip™ (Fastech Inc., Jacksonville, FL) strips of 1.6 cm (0.64 in) were sewn along two longer edges of 38 cm × 30 cm (15.2 in × 12 in) pieces of cloth to create the sleeves. The enclosed sleeves had a length of 38 cm (15.2 in) and a diameter of 9 cm (3.6 in). The fifth treatment was considered a control and consisted of an intermediate inoculum rate (20 to 25 ovisacs) with no protective sleeve. The purpose of the control treatment was to test the ability of HWA inoculum to establish on a host in a nonprotected setting.

Inoculation of Eastern Hemlock

The developmental status of HWA spring eggs was monitored to enable the harvesting of inoculum from infested trees shortly before hatch occurred. On 18 April 2006, 20 cm (8 in) long lateral branch tips of eastern hemlock infested with HWA ovisacs were collected. The branches were harvested from a natural forest edge in Center County, Pennsylvania. After cutting the branches, the numbers of HWA ovisacs were counted. The branches were then placed in a 10 mL (0.3 fl oz) aquatube filled with water and stored in a cool location for transport.

The branches were then attached to the non-HWA-infested eastern hemlocks at three sites in Pennsylvania. Treatments 1 through 4 (Table 1) were randomly assigned to the four cardinal directions of the plant, whereas Treatment 5 was placed in a location representative of the placement of the other treatments as determined on a plant-by-plant basis. The point of application was in the middle to upper third of the vertical height of the plant. Florist wire was used to loosely attach the inoculum branches to the hemlocks before the sleeves were applied. The two applications covered by 1000 μm mesh were sealed on both ends of the sleeve with ultraviolet-resistant duct tape. The two applications covered in 100 μm mesh were sealed on both ends with plastic pull ties. Weather data loggers were used at each site. Water levels in the tubes were monitored regularly and refilled whenever noticeable depletion occurred.

Table 3. The least squares means of nymphs on eastern hemlock at each experiment location.

Location	Least squares mean
Berks	7.33 a
Center	9.15 a
Lackawanna	20.33 b

Table 4. Successful establishment of hemlock woolly adelgid on eastern hemlock at each experiment location.

Location	Least squares mean[z]
Berks	1.50 a
Center	1.60 ab
Lackawanna	1.77 b

[z]Data coded as 1 = noninfested; 2 = infested.

Inoculation of Chinese Hemlock

On 27 April 2006, HWA-infested branches were harvested in the same location in Center County, Pennsylvania. The same procedure was followed; however, the 10 mL (0.3 fl oz) aquatubes were replaced with 50 mL (1.5 fl oz) centrifuge tubes to increase available water. A 6 mm (0.24 in) diameter hole was drilled in the cap, allowing the stem to be inserted. Two silicone-based caulks were used to seal the opening between the branch stems and the cap.

Chinese hemlocks in Center County were inoculated on 27 April 2006. The remaining branches were placed in cool storage, and trees in Berks County and Lackawanna County were inoculated the next day (Table 2). Water levels in the tubes were monitored regularly, and the tubes were refilled whenever noticeable depletion occurred.

Data Collection and Evaluation

On 31 October 2006, the sleeves were removed from both the eastern and Chinese hemlocks at the Lackawanna County and Berks County sites. A 16× hand lens was used to examine the branches for live first instar nymphs. When present, nymphs were quantified by number per linear 30 cm (12 in) of host branch rounded to the nearest five. The next day, the same procedure was used to collect data at the Center County site.

The data from the eastern hemlocks indicates that the challenge procedure was effective. Analysis of variance was conducted using the GLM procedure (SAS Institute, Inc., Cary, NC). The means were compared with the probability of difference (P = 0.05) with the least significant differences modified for the unequal sample size. A relationship existed between the experiment sites and the establishment of HWA. When establishment was quantified by the nymph least squares mean, the

Table 5. The least squares means of nymphs on eastern hemlock by treatment.

Treatment	Least squares mean
L100	19.33 c
H100	15.69 bc
L1000	8.67 ab
H1000	16.00 bc
CNS	1.67 a

Table 6. Successful establishment of hemlock woolly adelgid on eastern hemlock by treatment.

Treatment	Least squares mean[z]
L100	1.77 bc
H100	1.83 c
L1000	1.57 b
H1000	1.81 bc
CNS	1.13 a

[z]Data coded as 1 = noninfested; 2 = infested.

highest establishment occurred at the Lackawanna County site (Table 3).

To detect differences in the percentage of applications with successful HWA establishment, each inoculation site was classified as noninfested (1) or infested (2). Establishment was highest at Lackawanna and Center Counties (Table 4).

The least squares means of nymphs for the inoculation treatments had some differences (Table 5). The lowest numbers of nymphs occurred on the control (CNS) and L1000 treatments. Treatments with the high rate of inoculum or 100 μm mesh had higher levels of infestation than the CNS treatment.

When quantified by the percentage of applications with successful HWA establishment, the CNS treatment had a lower least squares mean than the other treatments (Table 6).

No live nymphs were found on any of the Chinese hemlocks.

RESULTS AND DISCUSSION

Eastern Hemlock

The fact that HWA will readily feed on eastern hemlock is well established (Gouger 1971; McClure 1987). Failure of the HWA to establish on some eastern hemlocks in this study may have been the result of host resistance, handling and disturbance, environmental stress, or inoculum levels.

One factor that supports the role of environmental stress in mortality is the difference in survival rates by location, which may be explained by temperature trends at the experiment sites. The temperatures at the Lackawanna County site were consistently lower than the other two sites, and prevailing winds contributed a wind chill factor to lower the temperature even more. The mesh cages provided enough protection from the winds to prevent the ovisacs from being damaged or desiccated, and the lower temperatures relieved stress. This cool environment may have contributed to the high level of infestation.

Inoculum level and mesh size were not as critical as the presence of a sleeve. The L1000 treatment resulted in less HWA establishment than the L100 treatment, although all sleeved treatments had higher percentages of applications with HWA establishment than the nonsleeved CNS treatment. The unprotected ovisacs were exposed to more direct sunlight, wind, and potential predators. The sleeved applications had a higher degree of protection, and subsequently a higher percentage of applications resulted in HWA establishment.

Chinese Hemlock

No HWA nymphs were found on the Chinese hemlocks in these field studies. All three sites yielded the same result. This suggests Chinese hemlock has substantial resistance to HWA.

CONCLUSION

This study demonstrates a procedure for successfully challenging hemlock species with HWA ovisacs. Use of this procedure allows direct interaction between HWA and the hemlock species being evaluated. The use of protective sleeves was observed to be a major factor in challenging hemlocks with HWA. This challenge procedure could be used to test additional hemlock species for resistance to HWA.

Chinese hemlock has demonstrated considerable resistance to HWA and may be useful as a replacement for eastern hemlock in ornamental landscapes. Further research into the genetic factors involved in this resistance may also offer clues for breeding projects with eastern hemlock.

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Résumé. La pruche de Chine (*Tsuga chinensis*) est un candidat prometteur pour son usage à des fins d'arbre ornemental dans la région Atlantique centrale des États-Unis là où les pruches indigènes sont dévastées par la cochenille laineuse de la pruche (*Adelges tsugae*). Les recherches publiées ont indiqué que la pruche de Chine est adaptable en zone de rusticité 6 (selon l'index du USDA) et qu'elle a un certain degré de résistance à cette cochenille. La pruche de Chine a été observée comme étant résistante à la cochenille laineuse de la pruche alors qu'elle poussait à proximité de zones infestées où les pruches du Canada étaient présentes, quoique une comparaison directe entre les espèces n'avait pas encore été documentée auparavant. Cette étude traite du développement d'une procédure pour mettre à l'épreuve les pruches indigènes face à la cochenille laineuse de la pruche afin de déterminer la résistance de la plante et ainsi mettre par la suite à l'épreuve de la même manière la pruche de Chine. La pruche de la Chine a démontré une complète résistance à la cochenille laineuse de la pruche.

Zusammenfassung. Tsuga chinensis ist ein vielversprechender Kandidat für Pflanzungen in den mittleren Atlantikregionen der USA, wo die einheimischen Hemlocktannen durch die Wollschildlaus drastisch reduziert wurden. Die veröffentliche Literatur hat die chinesische Hemlocktanne als anpassungsfähig für die Zone 6 des USDA klassifiziert. Sie zeigt einigen Widerstand gegen die Wollschildlaus. Die chinesische Hemlocktanne wurde als resistent gegenüber der Wollschildlaus in der Nähe von infizierten Hemlocktannen gefunden, obwohl eine direkte Ablehnung der Art bislang nicht dokumentiert werden konnte. Diese Studie berichtet über die Entwicklung einer Prozedur, Hemlocktannen mit den Eiern der Wollschildlaus zu versehen, um den widerstand der Pflanzen zu testen und diese Methode bei chinesischen Hemlocktannen anzuwenden. Die chinesische Hemlocktanne zeigte einen kompletten Widerstand gegen die Wollschildlaus.

Resumen. *Tsuga chinensis* es un candidato para usarse como árbol ornamental en la región del Atlántico medio de los Estados Unidos, donde los árboles nativos han sido devastados por el aldégido (*Adelges tsugae*). La investigación publicada ha indicado que el Tsuga chino es adaptable a la Zona 6 del USDA y tiene algún grado de resistencia al aldégido. El Tsuga se ha observado resistente al aldégido cuando crece en la proximidad de Tsugas del este infestados, aunque un desafío directo de las especies no ha sido documentado previamente. Este estudio reporta el desarrollo de un procedimiento para tsugas con aldégidos para determinar la resistencia de la planta hospedera y el uso de este procedimiento para el tsuga chino. El tsuga chino demostró completa resistencia al aldégido.