Screening Hydrolyzed Casein as a Deer Repellent for Reforestation Applications

Bruce A. Kimball, John H. Russell, Jeffrey P. DeGraan, and Kelly R. Perry

ABSTRACT

Three independent experiments were conducted to evaluate hydrolyzed casein deer repellent to minimize browse damage in reforestation efforts. In the first experiment, western redcedar seedlings were treated with 12% hydrolyzed casein and a latex sticker or one of two commercial deer repellents in the nursery prior to a 45-day cold storage period. Treated and control (sticker only) seedlings were then offered to captive deer, and browse activity was monitored for 20 days. Whereas all control trees were severely browsed by day 4, the three repellents offered browse protection (17 to 33% survivability at day 20). The second experiment was a field evaluation of 12% hydrolyzed casein, a commercial repellent, and a control. Western redcedar seedlings in nine reforested units were treated and monitored periodically for browse damage by free-ranging deer. After 17 weeks, browse damage to the repellent-treated seedlings (93 and 89% survivability) was significantly lower than the control trees (85%). In the final experiment, three different products were used to affix hydrolyzed casein powder to western redcedar seedlings prior to a 45-day cold storage period. Treated seedlings were offered to captive deer, and browse activity was monitored for 28 days. Hydrolyzed casein–treated seedlings sustained significantly less browse damage (more than 70% survivability at 28 days) versus the controls (all trees browsed by day 28). Nursery treatment with hydrolyzed casein may provide significant protection for conifer seedlings in reforestation operations.

Keywords: deer browse, conifer, herbivory, regeneration, wildlife damage management

Damage to agricultural, horticultural, and forest resources by deer has been recognized as a substantial economic problem for some time (Wywialowski 1998). Browsing may result in complete plant loss or reduce future value of commodities via decreased yields and plant deformities (Nolte 1998). Fear of browse damage may also result in reduced purchases of susceptible tree and shrub species by homeowners (Lemieux et al. 2000). In natural systems, deer can negatively affect ecosystem properties (Cote et al. 2004) and threaten rare understory herbaceous species (McGraw and Furedi 2005).

The extent, cause, and physiological impact of deer browse damage to plantation forests has been reviewed by Gill (1992). However, few studies have quantified the economic impacts of deer browse to forest crops (Ward et al. 2004). Mitchell (1964) calculated that severe deer browsing of Douglas-fir (*Pseudotsuga menziesii* [Mirbel] Franco) in a Vancouver Island (British Columbia, Canada) plantation reduced yield by a relatively insignificant 1,290 board feet per acre in an 80-year rotation. Conversely, a more recent study indicated that net present value (NPV) of Sitka spruce (*Picea sitchensis* [Bong.] Carr.) declined significantly if more than 55% of the crop was multistemmed at harvest as a result of early deer browse to the apical meristem (Ward et al. 2004). Even in the absence of deformed trees, delayed establishment (growth cost of regenerating lost tissues) resulted in precipitous declines of NPV.

Such potential economic impacts have encouraged the timber industry to use various methods to minimize ungulate damage to seedlings during reforestation. For example, in British Columbia, Canada, it is believed that nearly one-third of the 9–12 million western redcedar (*Thuja plicata* Donn ex. D. Don) seedlings planted each year are protected with physical barriers (Annette van Nuijenaus, Western Forest Products, Inc., personal communication, Aug 2006). This practice results in an investment of nearly US$5 per protected seedling to promote free-to-grow trees (out of the reach of browsing ungulates).

A number of commercially available repellents have been used to deter browsing of trees and shrubs by deer (Nolte and Wagner 2000). We propose that two mechanisms describe mammalian responses to herbivore repellents: altered palatability and neophobia. For example, the protein fraction of animal-based repellents (active ingredients such as blood, egg solids, urine etc.) negatively affects palatability of the treated forage item (Kimball and Nolte 2006a). At the same time, odors produced via protein and lipid degradation serve as cues signaling palatability by a process called associative learning. Because deer are afraid of unusual stimuli (i.e., they are neophobic), novel sights and smells may also deter herbivory. However, habituation to repellents that rely solely on neophobia to reduce browsing is typically rapid (Nolte 1999).

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In a series of bioassays with captive subjects, we recently demonstrated that white-tailed deer (*Odocoileus virginianus* leucurus; Kimball et al. 2005) and black-tailed deer (*Odocoileus hemionus* columbianus; Kimball and Nolte 2006b) strongly avoid plants treated with hydrolyzed casein. We recommended that an aqueous formulation of 8 or 12% hydrolyzed casein with 0.26% latex-based sticker would be a cost-effective deer repellent for reforestation use (Kimball and Nolte 2006b). Hydrolyzed casein is a mixture of small proteins and peptides produced by enzymatic hydrolysis of the milk protein casein. Hydrolyzed casein is food safe and readily available from protein suppliers. It is a primary constituent of many cosmetics and foods, including baby formula. However, although it is exempt from US Environmental Protection Agency requirements for residue tolerance, it is not yet registered for use as a repellent.

To evaluate the proposed deer repellent for reforestation applications, we conducted three independent experiments using western redcedar seedlings. In the first experiment, seedlings were treated in the nursery with a 12% hydrolyzed casein solution or one of two ready-to-use repellents. Treated seedlings were lifted, stored for 45 days (to simulate operational practice), and offered to captive black-tailed deer. The purpose of this experiment was twofold. First, we wanted to demonstrate that repellents could be applied in the nursery to eliminate the cost of field application. In addition, we wanted to compare the performance of the proposed deer repellent versus commercially available ready-to-use products.

The second experiment was conducted by treating seedlings in reforested units located in the Pacific Northwest United States and Vancouver Island, British Columbia, Canada. As a means of comparison in this field study, a ready-to-use, commercially available liquid deer repellent was used in addition to the proposed hydrolyzed casein solution. The purpose of this experiment was to demonstrate efficacy of hydrolyzed casein in field applications.

In the third experiment, seedlings were treated with one of three different antitranspirant products, dusted with hydrolyzed casein powder, lifted, stored for 45 days, and offered to captive deer. The purpose of this experiment was to demonstrate that hydrolyzed casein could be delivered as a dry powder with several different commercially available stickers. In all experiments, browse damage to seedlings was monitored at regular intervals to determine the efficacy of the various treatments.

Materials and Methods

Treatments

Hydrolyzed casein (spray-dried powder; HCA-411; American Casein Company, Burlington, NJ), Deer-Away Big Game Repellent powder (BGR-P; IntAgra, Inc., Minneapolis, MN), and ready-to-use Plantskydd Deer Repellent (Tree World, Inc., Des Moines, IA) were used as deer repellents. Several products were tested as agricultural stickers for adhering hydrolyzed casein to conifer seedlings: Tactic (Loveland Products, Greeley, CO), Moisture Guard (Hot Pepper Wax, Greenville, PA), Antistress (Enviroshield Products, Houston, TX), and Vapor Gard (Miller Chemical and Fertilizer Corporation, Hanover, PA).

Experiment 1: Nursery Application of Repellents

Two-year-old western redcedar seedlings were treated with one of four treatments at the Washington Department of Natural Resources Webster Nursery on Feb. 18, 2005. Treatments consisted of hydrolyzed casein (12% [weight/volume]) applied with 0.26% (volume/volume) Tactic latex sticker in water, Plantskydd, BGR-P, and a control consisting of just the sticker at the same concentration as above. The commercial products were applied as directed on the labels. Liquids were applied by using tank-type garden sprayers. The following day, all trees were lifted from the nursery bed and stored in a cooler at 2°C for 45 days.

Following storage of treated conifers, four treatment plots were replicated in each of five 0.125-ha pens. Treatments were randomly assigned to a unique plot in each pen. Each plot consisted of 12 identically treated western redcedar seedlings planted in a 3 × 4 arrangement. Within each plot, individual trees were 1 m apart, and plots were separated by at least 3 m (to minimize potential olfactory effects of the treatments). For the bioassay, three black-tailed deer were placed in each pen after the treated trees were planted. Once herded into the pen, there was no human contact or handling. Test subjects were provided ad lib access to pelleted basal diet (Formula 135 deer feed; X-Cel Feeds, Tacoma, WA), water, and mineral block in addition to the test plots for the duration of the experiment. The number of bites observed on each individual tree was recorded on days 1, 2, 3, 4, 7, 9, 13, 16, and 20 or until sustaining severe browsing (defined as at least 10 cumulative bites, according to Kimball et al. 2005). Animal procedures were approved by the National Wildlife Research Center Institutional Animal Care and Use Committee (QA-1236).

A Kaplan-Meier survival analysis was performed to compare survivability distribution functions among treatments, using the Wilcoxon test of equality (PROC LIFETEST; SAS 2002). Failure time was defined as the first experimental time period when severe browse (10 cumulative bites) was measured on an individual tree. Trees that survived to the end of the experiment (i.e., trees that did not meet definition of failure) were assigned an arbitrary failure time of 25 days and censored according to the standard application of survival analysis (SAS, Cary, NC).

Failure data were also analyzed by ranking failure time among treatments (1 = shortest failure time) and subjecting the rank data to Kruskal-Wallis analysis (Iman 1982). The values of failure day were ranked in ascending order within pens. Rank was the response for the nonparametric analysis, with treatment the fixed effect. Multiple comparisons of mean ranks were made using Fisher's least significant difference test (LSD option; SAS, Cary, NC).

Experiment 2: Field Application of Repellents

At each of nine sites located in Oregon, Washington, or British Columbia, 300 2-year-old western redcedar seedlings were treated in an alternating fashion at the time of planting with hydrolyzed casein solution (12%), Plantskydd, or control solution (100 seedlings per treatment) during February and March 2005. A sticker (Tactic) was used at a concentration of 0.26% (volume/volume) in both the hydrolyzed casein formulation and control solution (sticker only). Sites were selected on the basis of recent browse history, evidence of local deer populations, and property access. Seedlings were planted by cooperators as part of normal operational planting regimes.

Treatments were applied in continuous transects across the site (i.e., there were no untreated trees among individuals treated). Trees were sprayed until wet, but not dripping, with garden-type tank sprayers. Treatments were identified by color-coded stake-wire vinyl flags placed next to each tree. Color-treatment combinations were counterbalanced among sites. During a period of 17 weeks post-treatment, each tree was inspected for browse damage two to four

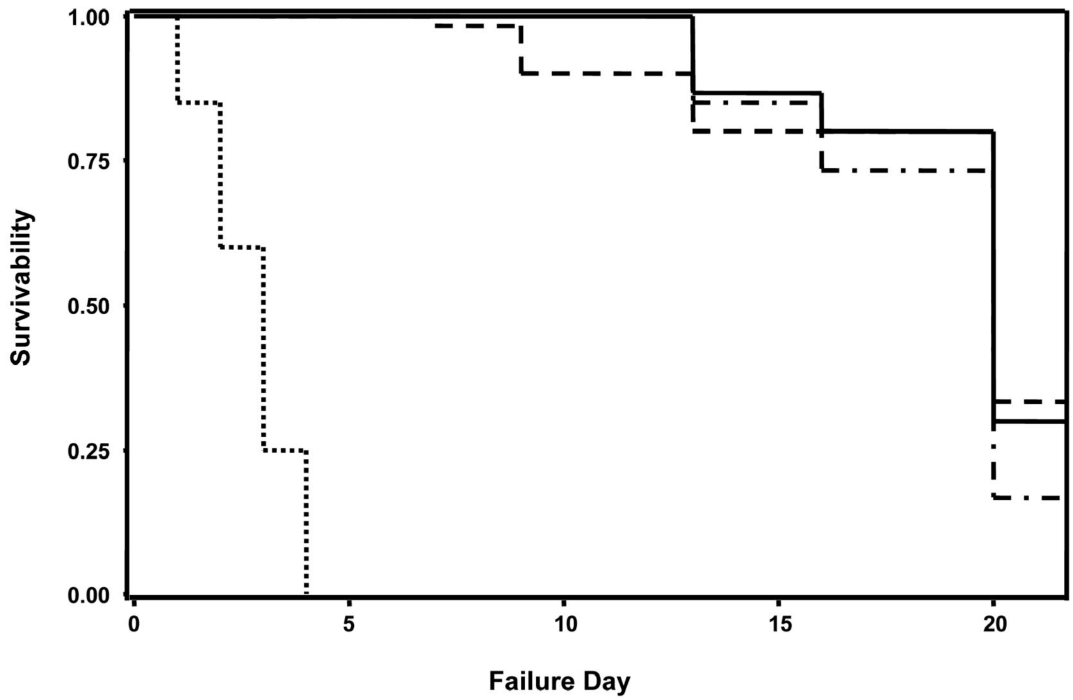

Figure 1. Survivability functions of western redcedar (*T. plicata*) seedlings treated in the nursery with three different repellents and a control in experiment 1. Treated seedlings were stored for 45 days prior to being offered to captive black-tailed deer (*O. hemionus*). Failure of an individual seedling was indicated by sustaining 10 or more bites. Deer had ad libitum access to the treated seedlings for 20 days. ······ = Control; - - - - - - - = Plantskydd; – · – · – · = hydrolyzed casein; ——— = Big Game Repellent.

times. Evidence of browse to the apical meristem (i.e., leader damage) was recorded (yes or no).

These data were also subjected to Kaplan-Meier survival analysis. Failure time was defined as the first observation of leader browse on an individual tree. Trees that survived to the end of the experiment (did not meet definition of failure) were censored according to the standard application of survival analysis (SAS, Cary, NC).

Experiment 3: Comparison of Formulation Stickers

Treatments consisted of three antitranspirant products diluted in water (1:30): Moisture Guard (paraffin), Antistress (acrylic copolymer), and Vapor Gard (*p*-menthene polymer). Two-year-old western redcedar seedlings were treated at the Webster Nursery on Feb. 9, 2006, with the appropriate sticker solution and dusted with hydrolyzed casein using a powder mill duster until finely coated. Control trees were untreated. On the day following treatment, seedlings were lifted from the nursery beds and subjected to 45-day storage prior to planting. Following seedling storage, the bioassay with captive deer was conducted in a manner similar to that of experiment 1.

Four treatment plots (Moisture Guard, Antistress, Vapor Gard, and control) were replicated in each of five 0.125-ha pens. Treatments were randomly assigned to a unique plot in each pen. Each plot consisted of 12 identically treated western redcedar seedlings planted in a 3 × 4 arrangement. Following planting, three black-tailed deer were placed in each pen and provided ad lib access to pelleted basal diet, water, and mineral block in addition to the test plots for the duration of the experiment. Browsing of each seedling was assessed on multiple days (1, 2, 3, 4, 7, 10, 15, 21, and 28).

As in experiment 1, data were subjected to Kaplan-Meier survival analysis, with failure time defined as the first experimental time period when severe browse (10 cumulative bites) was measured on an individual tree. Trees that survived to the end of the experiment (i.e., trees that did not meet definition of failure) were assigned an arbitrary failure time of 30 days and censored for analysis. Ranked failure time among treatments (1 = shortest failure time) was also subjected to Kruskal-Wallis analysis. Multiple comparisons of mean ranks were made using Fisher's least significant difference test (LSD option; SAS, Cary, NC).

Results and Discussion

Experiment 1

The proposed hydrolyzed casein repellent formulation and two commercially available repellents were tested in experiment 1. When seedlings were offered to captive deer after 45 days of storage, survivability of western redcedar seedlings treated in the nursery differed between the three treatments and control ($P < 0.0001$). Kruskal-Wallis analysis revealed that the three repellents (BGR-P, Plantskydd, and hydrolyzed casein) produced greater seedling survivability versus the control ($P = 0.001$), but there were no differences among the three repellents ($\alpha = 0.05$ for least significant difference test). Visual inspection of the survivability functions indicated that significant browsing of repellent-treated seedlings occurred between days 16 and 20 of the experiment (Figure 1).

Among the commercially available products was the powdered form of Big Game Repellent. Although a two-step process (sticker followed by powder) may be difficult to use in remote field locations (versus a premixed liquid formulation), delivery of powders is quite feasible in nursery applications. The two commercially available repellents used in this experiment (BGR-P and Plankskydd) were chosen for comparison with hydrolyzed casein because they have been demonstrated as two of the most effective deer repellents on the market (Nolte and Wagner 2000).

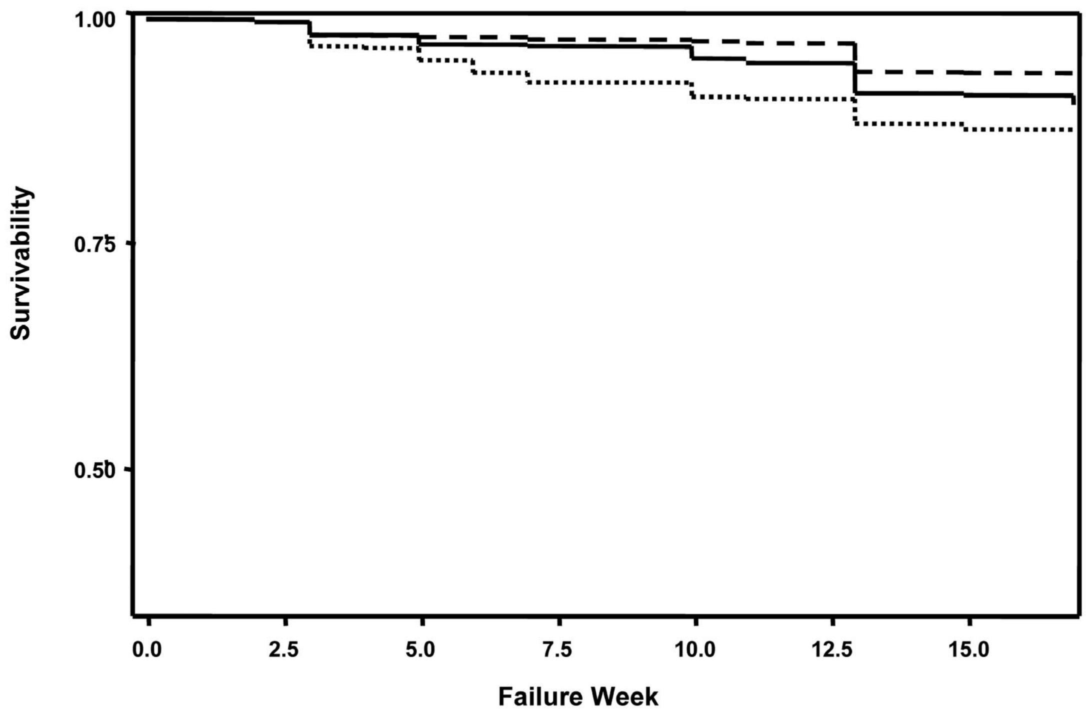

Figure 2. Survivability functions of western redcedar (*T. plicata*) seedlings treated with hydrolyzed casein, Plantskydd, or control as part of a field study in experiment 2. Failure of an individual seedling was indicated by browsing of the apical meristem (leader). · · · · · · = Control; - - - - - - - = Plantskydd; ——— = Hydrolyzed casein.

Although all three repellents provided protection from browse damage versus the control, a large proportion of seedlings failed by day 20 of the bioassay (study termination date). In contrast, 100% survival was observed for western redcedar seedlings treated immediately prior to the bioassay with hydrolyzed casein and BGR-P in an earlier experiment (Kimball and Nolte 2006b). However, not all control trees failed by termination of that prior study, whereas all controls failed by day 4 in the present study (Figure 1). Thus, browse pressure may have been much greater on treated seedlings in the present study versus the prior one. Regardless, these results suggest that repellents applied in the nursery and stored for 45 days were effective in reducing browse damage relative to the controls.

Experiment 2

Although deer browse activity was evident at only five of the nine study sites, data from all sites were included in the analysis. Survivability of western redcedar seedlings differed among the three treatments ($P < 0.0001$). Inspection of the log-rank statistics indicated that survivability of Plantskydd-treated seedlings was greater than that of hydrolyzed casein–treated seedlings, which in turn, was significantly greater than the control seedlings (Figure 2).

The field study demonstrated that both Plantskydd and hydrolyzed casein reduced browse damage relative to the control. However, Plantskydd provided significantly better browse protection for the entire 17 weeks of the experiment. A previous comparison of 12% hydrolyzed casein and Plantskydd conducted with captive deer indicated that both repellents equally protected western redcedar seedlings in a 3-week bioassay (Kimball and Nolte 2006b). Inspection of the results from the field data further indicate that 12% hydrolyzed casein and Plantskydd provide equal browse protection for approximately 5 to 10 weeks, but Plantskydd was more effective at 17 weeks. Assuming degradation of hydrolyzed casein to occur at a similar rate as the blood proteins present in Plantskydd, this observation suggests that the sticker used to affix hydrolyzed casein to seedlings may be a limiting factor for prolonged effectiveness.

Experiment 3

Survivability of seedlings treated with hydrolyzed casein and each of three stickers was significantly greater than that of control seedlings ($P < 0.0001$). Visual inspection of survivability functions indicated minimal browsing of hydrolyzed casein–treated seedlings until after day 25 of the experiment (Figure 3). Despite this late browse activity, seedling survivability was still high (>70%) for all three sticker treatments after 4 weeks.

Hydrolyzed casein delivered as a powder with antitranspirant stickers effectively reduced deer browse damage to the seedlings for nearly 4 weeks, despite 45-day storage of the treated seedlings. Antitranspirant products were tested because they are specifically formulated to withstand storage and transport conditions. Results of experiment 3 suggest that these products may have withstood storage and/or environmental conditions better than the latex sticker used in experiment 1. However, this conclusion should be confirmed with an experiment using all stickers in the same bioassay. The antitranspirant products were also used at a much higher concentration than the latex sticker of experiment 1. Following the label instruction for typical use, the latex sticker was used at a concentration of 0.26%, whereas the antitranspirant products were each used at a concentration of 3.3% (approximately the label recommendation for each product).

Implications

Predicting the likelihood and extent of browse damage to reforested plantations is a difficult task. Despite accounting for local knowledge and history of browse damage, nearly half (four of nine)

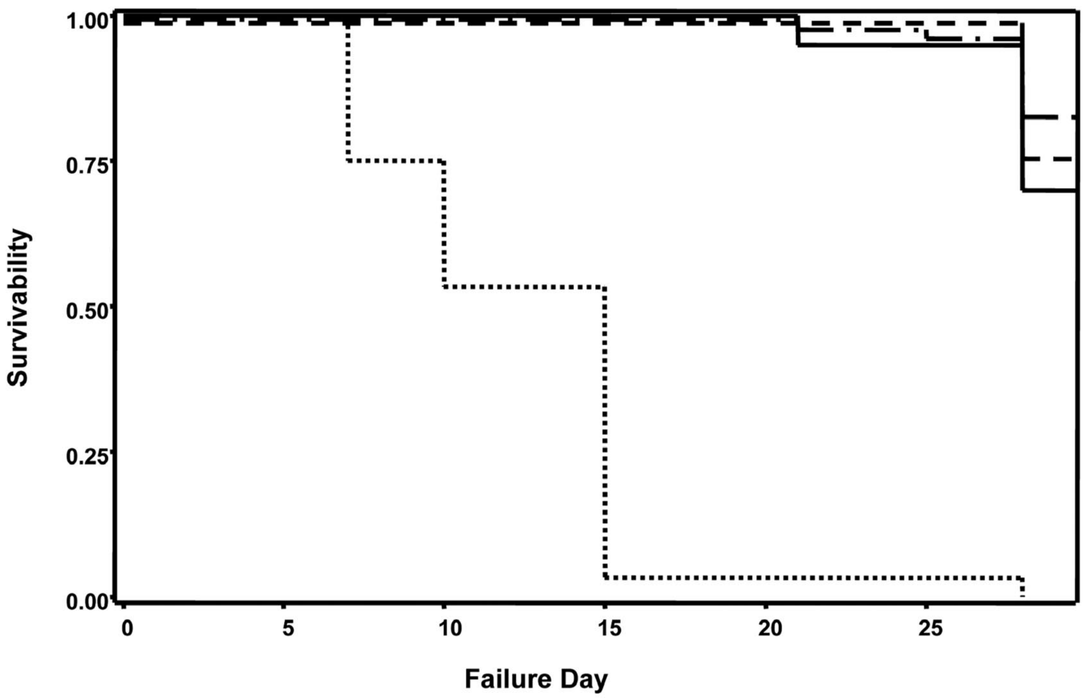

Figure 3. Survivability functions of western redcedar (*T. plicata*) seedlings treated in the nursery with hydrolyzed casein and one of three different stickers, as well as a control, in experiment 3. Treated seedlings were stored for 45 days prior to being offered to captive black-tailed deer (*O. hemionus*). Failure of an individual seedling was indicated by sustaining 10 or more bites. Deer had ad libitum access to the treated seedlings for 28 days. · · · · · · = Control; - - - - - - - = Vapor Gard; – · – · – · = Moisture Guard; ——— = AntiStress.

of the treatment sites in experiment 2 did not sustain any browse damage during our study. Thus, time and resource investments for browse reduction must be carefully considered. An effective deer repellent that is inexpensive and easy to apply can make protecting forest resources more cost-effective.

Results from the three experiments reported here demonstrate that hydrolyzed casein can be used to minimize browse damage in reforested plantations. Furthermore, hydrolyzed casein can be applied as either a powder or in solution directly in nursery beds—depending on nursery practices and equipment availability. Hydrolyzed casein applied in the nursery effectively reduced browsing when applied in association with several antitranspirant products, even after a 45-day cold storage period.

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